Horticulture, Viticulture and Viniculture

VITIS

Biology

and Species

António Manuel Jordão
Renato V. Botelho
Editors

NOVA

HORTICULTURE, VITICULTURE AND VINICULTURE

VITIS

BIOLOGY AND SPECIES

HORTICULTURE, VITICULTURE AND VINICULTURE

Additional books and e-books in this series can be found on Nova's website under the Series tab.

HORTICULTURE, VITICULTURE AND VINICULTURE

VITIS

BIOLOGY AND SPECIES

ANTÓNIO MANUEL JORDÃO
AND
RENATO V. BOTELHO
EDITORS

nova
science publishers
New York

Copyright © 2020 by Nova Science Publishers, Inc.

All rights reserved. No part of this book may be reproduced, stored in a retrieval system or transmitted in any form or by any means: electronic, electrostatic, magnetic, tape, mechanical photocopying, recording or otherwise without the written permission of the Publisher.

We have partnered with Copyright Clearance Center to make it easy for you to obtain permissions to reuse content from this publication. Simply navigate to this publication's page on Nova's website and locate the "Get Permission" button below the title description. This button is linked directly to the title's permission page on copyright.com. Alternatively, you can visit copyright.com and search by title, ISBN, or ISSN.

For further questions about using the service on copyright.com, please contact:
Copyright Clearance Center
Phone: +1-(978) 750-8400 Fax: +1-(978) 750-4470 E-mail: info@copyright.com.

NOTICE TO THE READER

The Publisher has taken reasonable care in the preparation of this book, but makes no expressed or implied warranty of any kind and assumes no responsibility for any errors or omissions. No liability is assumed for incidental or consequential damages in connection with or arising out of information contained in this book. The Publisher shall not be liable for any special, consequential, or exemplary damages resulting, in whole or in part, from the readers' use of, or reliance upon, this material. Any parts of this book based on government reports are so indicated and copyright is claimed for those parts to the extent applicable to compilations of such works.

Independent verification should be sought for any data, advice or recommendations contained in this book. In addition, no responsibility is assumed by the Publisher for any injury and/or damage to persons or property arising from any methods, products, instructions, ideas or otherwise contained in this publication.

This publication is designed to provide accurate and authoritative information with regard to the subject matter covered herein. It is sold with the clear understanding that the Publisher is not engaged in rendering legal or any other professional services. If legal or any other expert assistance is required, the services of a competent person should be sought. FROM A DECLARATION OF PARTICIPANTS JOINTLY ADOPTED BY A COMMITTEE OF THE AMERICAN BAR ASSOCIATION AND A COMMITTEE OF PUBLISHERS.

Additional color graphics may be available in the e-book version of this book.

Library of Congress Cataloging-in-Publication Data

Names: Jordão, António Manuel, editor. | Botelho, Renato V. (Renato
Vasconcelos), editor.
Title: Vitis : biology and species / António Manuel Santos Tomas Jordão,
Renato Botelho.
Other titles: Horticulture, viticulture and viniculture.
Description: Hauppauge, New York : Nova Science Publishers, [2020] |
Series: Horticulture, viticulture and viniculture | Includes
bibliographical references and index. |
Identifiers: LCCN 2020032376 (print) | LCCN 2020032377 (ebook) | ISBN
9781536183085 (hardcover) | ISBN 9781536184242 (adobe pdf)
Subjects: LCSH: Grapes--Varieties.
Classification: LCC QK495.V55 V58 2020 (print) | LCC QK495.V55 (ebook) |
DDC 583/.79--dc23
LC record available at https://lccn.loc.gov/2020032376
LC ebook record available at https://lccn.loc.gov/2020032377

Published by Nova Science Publishers, Inc. † New York

Complimentary Contributor Copy

This book is a fascinating journey into the viticultural world and biodiversity, jealously guarded by man starting from the Mediterranean, in the oceans, islands and continents. The authors narrate the symbiosis of man and Vitis, the most generous genus, in which rural and scientific knowledge are admirably reflected and fruitful technological innovations ripen. The reading invites, with a look at the past, to appreciate the inestimable values preserved in the wine-growing landscapes and, at the same time, look with confidence to the future, inspired by the search for harmony between man and nature.

Prof. Dr. Adamo Domenico Rombolà
Professor of Viticulture
University of Bologna, Italy

The book "Vitis: Biology and Species" provides an interesting and comprehensive overview of viticulture in different producing regions of the world, including different species and new technologies applied to characterize them. Addressing scientific questions for experts and professionals, but in a clear and accessible way, this book will also help technicians, producers and the general public to find answers to several aspects involving the modern cultivation of vines.

Prof. Dr. Regina Vanderlinde
Institute of Biotechnology - University of Caxias do Sul, RS, Brazil
President of O.I.V. - International Organisation of Vine and Wine

CONTENTS

PREFACE

The genus *Vitis,* one of the 16 genera in the family *Vitaceae,* according to the Plant List (2013) includes 68 species and is distinguished from the other genera in *Vitaceae* by its petals that remain coalescent at the top and separated at the base (calyptra), falling as cap. All *Vitis* are lianas, or woody, climbing vines.

The taxonomy of the genus *Vitis* has been the subject of discussion, however it is generally considered to comprise two distinct sections: *Muscadinia* and *Euvitis,* differing in basic morphological, anatomical, and cytological characters. The *Muscadinia* section comprises three species. The best known, *V. rotundifolia*, originated in the southeastern United States and was domesticated by European settlers. It serves as a rooststock to cope with the high sensitivity of European grapevines to *Phylloxera* disease. This section also includes *V. munsoniana* J.H. Simpson ex Planch. and *V. popenoei* J.L.Fennell. The second section, the *Euvitis*, includes most of the wine producing species. *Vitis vinifera*, the primary cultivated *Vitis* species, produces the most famous quality wines. Native to Asia near the Caspian Sea, it has been imported to Europe since before recorded history.

The most cultivated grapevines belong to the *Euvitis* sub-genus. These fall under three groups: The first group is the American group and is made up of more than 20 species, including *V. berlandieri*, *V. riparia* and *V. rupestris*, which were used as rootstock to address the *phylloxera* crisis. In this group there are also species, such as *Vitis labrusca*, more resistant to fungal diseases in humid climates, important for juice production in many Countries. The second group is the East Asia group and consists of around 55 species, which are currently considered to be of limited importance to viticulture. Finally, the third group is the Eurasian group that is made of one single species, *Vitis vinifera* L., which accounts for most of the world's *Vitis* varieties. There are two sub-species of *Vitis vinifera: sylvestris*, which corresponds to the wild form of the vine, and *vinifera*, referring to the cultivated form.

According to the Report of International Organization of Vine and Wine published in 2017, there are 21.045 different names of varieties, including 12.250 for *V. vinifera*, but it is important to note that this includes a considerable number of synonyms and homonyms. Thus, the actual number of vine varieties for the *V. vinifera* species in the world is estimated at 6.000. Therefore, its is clear that genus *Vitis* shows an impressive genetic variability for many agronomic characteristics being strongly conditioned by the climatic and soil conditions where the plants are implanted. The significant increase in genetic diversity leading to the creation of several varieties more resistant to different diseases, while allowing the production of grapes with specific characteristics for the production of several products, either for the consumption of fresh and dried grapes, or for the production of other processed products, namely juices, wines and distillate drinks.

Therefore, the goal of this book is to summarize in a concise manner the accumulated information about the most recent developments in *Vitis* species characterization, biology and composition from different origins to fulfill the need for accurate state-of-the art information and perspectives regarding to the most recent studies on different dimensions of *Vitis* plants production.

This book is composed by thirteen chapters that provide current research on different topics of recent knowledge about native grape varieties from different origins, the impact of different climatic and soil conditions on vine managements, the description of the main grapevines disease and their control, grape varieties composition and the use of modern digital technologies on viticulture.

So, the first chapter reviews about *Vitis labrusca* and its products, benefits of the intake of grape-based products and the solutions that industry has found in the face of production problems. The second chapter aims to present a characterization of several native and created vine varieties from Serbia, while the third chapter discuss the biological specificity of the native varieties of Crimea region (Russia) using the phenology and agrobiology indicators and the use of these native grapevines in winemaking. Chapter 4 discuss and describes some of the most important biotic and abiotic stresses suffering the Mediterranean vineyards, followed by the chapter 5, where is shows a brief review of specific viticulture and grape varieties found in Azores islands, an archipelago formed by nine islands of volcanic origin in the middle of the north Atlantic Ocean. From Brazil, other researcher describes in chapter 6, the main characteristics of the three Brazilian different winegrowing zones, according to climate conditions and vine management. In chapters 7, 8 and 9 the authors review the most important topics related with grapevines diseases, their control by the use of different strategies. The introduction and the practical application of remote sensing in viticulture by the use use of unmanned aerial vehicles and satellite imagery in discuss in chapter 10. Other researchers in chapters 11 and 12 introduce a characterization of the volatile and phenolic composition found in different grape varieties. Finally, in chapter 13 the authors discuss the most important practices related to the modern vitiviniculture.

These chapters are written by a group of international researchers of vitiviniculture, in order to provide up-to-date reviews, overviews and summaries of current research on the vine species and their biology. This book is not only for technicians actively engaged in the field, but also for students attending technical schools and/or universities and other professionals that might be interested in reading and learning about some fascinating areas of vine plants and discovery the most recent tendencies and knowledge.

Finally, it was with great pleasure that we accepted the opportunity offered by Nova publishers to assemble and edit this book. We are greatly indebted to the authors, that generously to share their knowledge and experience with others thought their contribution to this book.

Editors

ANTÓNIO M. JORDÃO (M.Sc, PhD)
Professor of Oenology
Polytechnic Institute of Viseu,
School of Agriculture, Portugal.
Researcher of Chemistry Research Centre of
Vila Real, Portugal.
email: antoniojordao@esav.ipv.pt

RENATO V. BOTELHO (PhD)
Professor of Fruit Crops and Viticulture
State University of Mid-Western of
Paraná, Department of Agronomy -
Research Group in Fruit Crops and
Post-harvest, Brazil.
email: rbotelho@unicentro.br

In: Vitis: Biology and Species
Editors: António M. Jordão et al.

ISBN: 978-1-53618-308-5

Chapter 1

VITIS LABRUSCA AND ITS DERIVATES

Amanda Stolzenberg Blembeel[1,2]**,**
Isabel Cristina Teixeira Proença[2] ***and Caroline Dani***[1,2,*]
[1]Laboratório de Bioquímica, Centro Universitário Metodista - IPA,
Porto Alegre/RS, Brazil
[2]Programa de Pós Graduação Farmacologia e Terapêutica,
Universidade Federal do Rio Grande do Sul - UFRGS, Porto Alegre/RS, Brazil

ABSTRACT

In this chapter, we will discuss *Vitis labrusca and* its products; benefits of the intake of grape-based products; and the solutions that industry has found in the face of production problems. *Vitis Labrusca* grapes represent more than 80% of Brazilian wine and grape juice production and have significant importance as table grapes and their derivatives. In 2017, for example, the best grape harvest in years was registered with more than 753,000 tons of grape processed, showing an increase of 6.15% when compared to the previous record in 2011. This increase could be explained because the population attributed the consumption to a healthy choice. This affirmation could be due to richness in phenolic compounds with high antioxidant activity generating benefits to human health. On the other hand, the wine industry generates substantial volumes of solid organic waste (pomace), which cannot be used in the manufacture of juices and wines. This pomace, also known as by-products, consists of skins, seeds, and pulp residues, which contain a large number of phenolic compounds and significant levels of dietary fiber. In this sense, the industry has tried to reuse the waste to reduce economic losses and environmental impacts, launching new products derived from the production of beverages on the market. Based on this context, the number of scientific publications increases, relating the benefits of grapes and its derivatives with health and improvement in the quality of life in different pathologies.

---

[*] Corresponding Author's Email: carolinedani@yahoo.com.br.

Keywords: grape, grape pomace, phenolic compounds, *Vitis labrusca*, *Vitis* sp.

1. THE HISTORY OF VITICULTURE ON BRAZIL

Historically, the first relevant data show that the first vines were brought to Brazil by Martim Afonso de Souza, in 1532, from Portugal, to disseminate agriculture in the new colony. The first seedlings of *Vitis vinifera* were planted in the state of São Paulo, formerly known as captaincy of St. Vincent. However, according to Leão (2010), due to the wet climatic conditions, there was no development of the vines. Then, the cultivation of vines was shifted to the Atlantic plateau. In 1551, there is the first record of the juice extraction of *Vitis vinifera* grapes, producing the first Brazilian wine, however, as occurred before, the production did not resist, due to climatic and soil conditions (Ibravin, 2020).

The arrival of the Jesuits in the Missions region (Figure 1), in the state of Rio Grande do Sul, in 1626, boosted viticulture in southern Brazil. According to Souza (1969), Priest Roque Gonzalez de Santa Cruz, with the Guarani indigenous community helping, introduced the vines in the state, producing wine, a key element in religious celebrations. In 1789, the Portuguese court prohibited the cultivation of grapes from protecting their wine production, this decision inhibited the sale of the wine in Brazil and restricted the activity to the domestic sphere (Ibravin, 2020). In 1808, even with the prohibition of the cultivation of grapes, there is a development of the habits of wine consumption. However, with the Portuguese King Famly arriving in Brazil, the wine was incorporated into meals, social meetings, and numerous religious festivities (Ibravin, 2020).

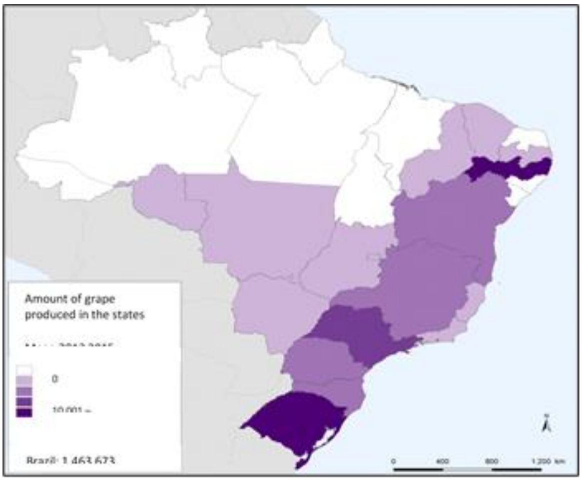
Source: Ibravin (2020).

Figure 1. Elaboration of wine in 1881 in the Rio Grande do Sul.

The interest in growing grapes increased with German immigration in 1824. With the permission of Dom Pedro I, the Italian João Batista Orsi started the cultivation of European grapes in the Serra Gaúcha, became one of the pioneers of the wine production industry of the region (Ibravin, 2020). In 1840, Thomas Messiter, an English merchant, presented the *Vitis labrusca* and *Vitis bourquina,* a grapevine species, to the Rio Grande do Sul, both native to the USA, more resistant to pests and diseases. Their seedlings were initially planted on Sailors Island, located in Lagoa dos Patos, but soon spread throughout the state. Records dating from 1860 showed that the cv. Isabella, one of the species of *Vitis labrusca*, was accepted by farmers, mainly by its resistance to diseases (Ibravin, 2020).

The leap in the national wine production occurred with Italian immigrants' arrival in the Rio Grande do Sul in 1875. Italians improved the quality of Brazilian wine because they had technical knowledge of wine production and culture, attributing greater importance in economic activity. The oldest winemaking recorded in the Rio Grande do Sul shows that 500,000 liters of wine were produced in Garibaldi. These data were reported in 1883 by the consul of Italy, Enrico Perrodo, after visiting the region (Ibravin, 2020).

Due to intense disordered competition, variation in quality, and the growing importance of activity, in 1928, a Brazilain company was formed, called “União de Vinhos,” an association established in an attempt to organize the sector, coordinated by Oswaldo Aranha, changing in 1929 to grape producers. Over ten years, 26 cooperatives were founded, including some that continue to exist today. With this model, small producers became competitive, gaining a more stable position in the next decade (Ibravin, 2020).



Source: Adapted from Porto Alegre (2020).

Figure 2. Grape juice map from Brazilian regions.

Table 1. Annual evolution of the planted area and the produced quantity of grape 2000-2015 in Brazil and in the Rio Grande do Sul State

Year	Brazil		Rio Grande do Sul	
	Area intended for harvesting (ha)	Quantity produced (t)	Area designed for harvesting (ha)	Quantity produced (t)
2000	59,838	1,024,482	34,156	532,553
2001	63,325	1,058,590	34,682	498,219
2002	66,308	1,148,648	36,681	570,181
2003	68,461	1,067,422	38,533	489,015
2004	71,640	1,291,382	40,351	696,599
2005	73,222	1,232,564	42,450	611,868
2006	75,385	1,257,064	44,298	623,878
2007	78,325	1,371,555	45,379	704,176
2008	81,286	1,421,431	47,206	776,964
2009	81,677	1,365,491	48,259	737,363
2010	81,534	1,355,461	48,753	694,518
2011	81,840	1,495,336	49,198	830,286
2012	82,897	1,514,768	50,180	840,251
2013	79,759	1,439,535	50,056	807,693
2014	78,779	1,454,183	50,007	812,517
2015	78,026	1,497,302	49,739	876,215

Source: Adapted from the Porto Alegre (2020).

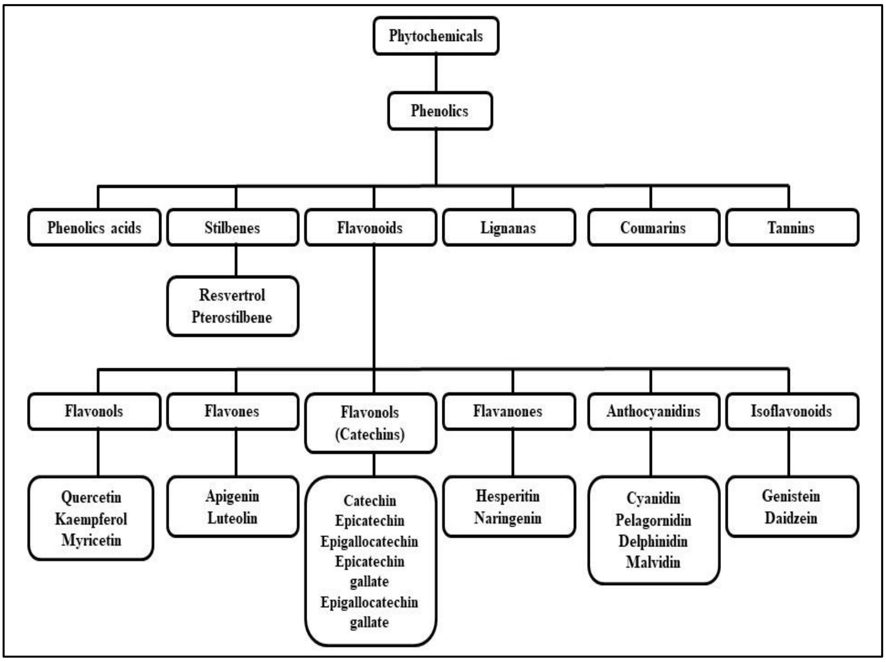
In 1951, the Georges Aubert winery moved from France to Brazil, marking the beginning of a cycle that promoted the national viticulture. The interest of foreign companies in the country brought new techniques to vineyards and wineries and increased the quality of production, in addition to expanding the areas of grape cultivation (Ibravin, 2020).

Today, grape production in Brazil has an area of approximately 82,000 hectares, divided primarily between six regions, Figure 2 (Porto Alegre, 2020). There are more than 1,100 wineries spread throughout the country, most of them installed on small farms. “Serra Gaúcha” is the largest wine region in the country, with about 40,000 hectares of vineyards. In this region, more than 80% of the production originates from the production of American grapes (*Vitis labrusca* and *Vitis bourquina*) and hybrid interspecific (Ibravin, 2020), where 330 million liters of wines, juices, and other grape products are elaborated annually (Brasil 2016).

Average production has increased sharply from 533,651 tonnes/year in the period 2000 to 2002 to 832,142 tonnes per year from 2013 to 2015 (Porto Alegre 2020) (Table 1). In 2017, grape production showed a record of production, approaching one million tons, an amount higher than national production until 1999, of which about 752,495,050 tons were destined for wine production. These are better results than the 2016 crop, which had a production drop of 52.79% compared to 2015 due to climate problems. Moreover, it was increased by 9.21% compared to 2015 (usual crop). Grape production in 2018 and 2019 was lower than in 2017, demonstrating a decrease in planted area. However, the quality of these last harvests was higher, showing that the wine production is increasing in quality too, and this is one of the causes to explain that the number of wine consumers in Brazil is expanding (Brasil, 2016; Sidra, 2020).

2. GRAPE COMPOSITION

Grape is a fruit rich in mineral salts such as calcium, iron, sodium, and potassium and has vitamins B and C. Moreover, it stands out in the national and international market because it is one of the fruits that most contain phenolic compounds when compared to other fruits. However, due to its nitrogen diversity, culminates in different characteristics, as to taste and coloration, being directly linked to the concentration of phenolic compounds (Abe et al., 2007; Kato et al., 2012; Toaldo et al., 2015). According to Bravo et al. (1998), the grape has a higher concentration of phenolic compounds than any other fruit such as orange, pear, and/or peach (Bravo, 1998). Rho and Kim (2006) reported that grape juice has a three times higher concentration of phenolic compounds than orange juice.



Source: Adapted from Liu [2013].

Figure 3. Classification of polyphenols within the class of phytochemicals.

Phenolic compounds are divided into flavonoids (catechin, hesperidin, anthocyanidins, and isoflavones) and non-flavonoids (phenolic acids, stilbenes among them resveratrol, lignans, and tannins) (Liu, 2013), as described in Figure 3.

Anthocyanins are essential compounds for the production of red wines. In addition to conferring the coloring of wine, anthocyanins contribute to sensory attributes. Catechin and epicatechin are mainly present in grape seeds, being responsible for the flavor and quality of wines and grape juices. In a smaller amount, we find the quercetin, kaempferol, and myricetin acting as co-pigments together with anthocyanins in the production of wines. Phenolic acids, on the other hand, are found in low concentrations, represent one of the main compounds in white grapes, influencing the aroma and taste of wines (Abe, 2007).

The study conducted by Abe et al. (2007), quantified phenolic compounds and antioxidant capacity of cultivars of *Vitis labrusca* L. and *Vitis vinifera* L grown in Minas Gerais in the 2005/2006 harvest. The results showed that the darkest coloring grapes (leaf on the rootstock 420A) presented higher content of anthocyanins and, consequently, higher phenolic content and antioxidant capacity. In contrast, the grapes of the cultivar Moscato Embrapa, free of anthocyanins, presented low values of total phenolic contents and antioxidant capacity (Abe, 2007).

In addition to their characteristics, all these compounds contribute to increasing the health benefits, whether from juices or wines. However, among all phenolic compounds, resveratrol has attracted particular attention in recent decades due to epidemiological studies that show a beneficent correlation between moderate wine consumption and the decrease in the incidence of cardiovascular diseases, conferring a health protection factor (Abe, 2007).

Table 2. Total phenolic content, total anthocyanins, and antioxidant capacity of different vine cultivars

	Cultivar/rootstock	Total phenolic	Total Anthocyanins	Antioxidant capacity
V. Labrusca L.	Niagara rose/IAC 766	208 ± 12c	12.8 ± 0,1d	7.6 ± 0.4e
	Niagara rose /196-17	214 ± 13c	18 ± 2d	7.6 ± 0.5e
	Ives/ 420A	391 ± 30a	248 ± 24a	19 ± 2a
	Ives / 196-17	390 ± 28a	198 ± 16b	13.8 ± 0.9b
V. Vinifera L.	Syrah	385 ± 30a	111.8 ± 0.2	10.3 ± 0.3d
	Merlot	337 ± 21b	97 ± 7	12.1 ± 0.6c
	Moscato Embrapa	65 ± 1d	n.d.	2.7 ± 0.1f

Total phenolic content (mg.100 g -1 gallic acid equivalents), total anthocyanins (mg.100 g -1 dicyanidine equivalents3-glycoid) and antioxidant capacity (μmol equivalent to Trolox.g -1 sample) of vine cultivars produced in Minas Gerais in the 2005/2006 crop.

Source: Adapted from Abe et al. (2007).

Resveratrol is found in large quantities in grape skins, the main of the varieties of *Vitis labrusca* L., and has been gaining prominence due to its proven benefits. Resveratrol decreases lipid levels in blood serum, can increase HDL (Lipoprotein of high Density), and contributes to the remotion of LDL (Low D Lipoproteinensity) of the bloodstream, thus preventing obstruction of the arteries (David, 2007). Also, inhibits the synthesis of thromboxins, consequently acting as an anticoagulant (Jang, 1997); has chemopreventive effect by inhibition of c-kinase protein and repression of the cascade of the chemical araquidonic acid, metabolic responsible for inducing the genesis of tumors (Subbaramaiah, 1998).

The grape 'Isabella' (*Vitis labrusca* L.) is the primary cultivar producing grapes in southern Brazil due to high productivity, diversity of use (wine, juice, and table) and tolerance to diseases such as oidium and anthracnose (Rizzon, 2000).

3. *VITIS LABRUSCA* SPECIE

There are numerous varieties of grapes for the preparation of wines and by-products; within all, we can highlight the genus *Vitis*, which belongs to the *Vitaceae* family, being composed for more than 60 species. Its geographical distribution covers the Asian, European, and American continents. The Asiatic continent has 29 species described, while America has 34 species. Both continents, Asiatic and America, have high genetic diversity, with species adapted to different environmental conditions. On the European continent, there is the presence of only two species, *Vitis vinífera* and *Vitis silvestris* (Brasil, 2020), and the southeast region of North America is especially rich in wild *Vitis* species (Olmo, 1979).

The most cultivated species in the world is *Vitis vinifera*, popularly known as European grapes or fine grapes, being used for the production of wine as well as table grapes and grapes for the production of raisins. The second most cultivated species in the world is *Vitis labrusca* (Toaldo, 2015).

The varieties of *Vitis labrusca* grapes are limited to a few dozen. *V. labrusca* grapes are used for fresh consumption and processing, in particular for the preparation of grape juice, as well as in some regions for the preparation of wines. The grapes of this species are all classified in Brazil as "rustic grapes" or "common grapes." In general, this class of cultivars is characterized by presenting high productivity and high resistance to diseases. Also, the varieties of *Vitis labrusca* has the remarkable flavor and aroma characteristics, being preferred by consumers both for fresh consumption and in the production of wines and juices (Camargo 2010).

In Brazil, there are different varieties of *Vitis labrusca*. The main types of cultivars for industrial purposes differ by the climate influence, for example, the varieties Bordo, Concord, Concord clone 30 and Rubea has grown better in hot regions in subtropical climates (Maia and Camargo, 2005) (Table 3). Also, around the world, different kinds of cultivation choices are producing different characteristics in grape products. For example, in Brazil, there are two types of vineyards, organic and conventional production. The difference is the use or not of pesticides. Therefore, the main characteristic of organic production is the exclusion of synthetic fertilizers and pesticides and therefore require a longer ripening time (Melo, 2015). Dani et al. (2007) observed, for example, that grape juice from organic production presented higher concentrations of phenolic compounds, including resveratrol, anthocyanidins, and tannins. This is because non-use the pesticide makes plants more susceptible to the action of pathogens causing it to increase their production of phenolic compounds as a means of protection (Soleas et al., 1997; Dani et al., 2007).

Table 3. Types of cultivation of *Vitis labrusca* and their hybrids

Cultivar	Description
Ives	Cultivation of purple grape, cultivated in Minas Gerais, Paraná, Rio Grande do Sul and Santa Catarina, resistant to fungal diseases, originates wine and juice, being widely used for color improvement in products based on Isabel and Concord.
BRS Cora	It is an ink grape of the 'Muscat Belly' x 'BRS Rubea' crossing for cultivation in tropical regions, recommended for color improvement in 'Isabella' and 'Concord' based products.
BRS Lorena	White grape, recommended for the elaboration of Asti-type sparkling wine, originally from the intersection of 'Malvasia Bianca' x 'Seyval', adapted very well the climatic conditions of southern Brazil.
BRS Rúbea	Purple grape, from the crossing of 'Niagara Rose x Ives, recommended to compose 'Isabella' and 'Concord' grapes in the preparation of juice, adapts the conditions of Serra Gaúcha.
Concord	Purple grape, quality reference for juice preparation, intensely cultivated in the southern states.
Concord Clone 30	It is an early clone of the cultivar Concord, with early maturation, intensely cultivated in Paraná.
Isabella	Purple grape, very rustic and highly fertile, with abundant crops, is consumed as a table grape, preparation of white, rosy and red wines, preparation of vinegar and provides high-quality juices, also, to be used for sweets and jams, a primary cultivar of Rio Grande do Sul and Santa Catarina.
Early Isabella	Purple grape 'Isabella' natural mutation, recommended for the elaboration of juice, wine, and fresh consumption, it is a cultivar with wide adaptability.
Moscato Embrapa	White grape, alternative to produce table wines, widespread in the Rio Grande do Sul, but, behaves well in subtropical and tropical regions, consumers well accept its wine.
Niagara	White grape, very rustic, intensely cultivated in the Rio Grande do Sul, Santa Catarina, and Minas Gerais, widely used in winemaking.

Source: Adapted from Maia and Camargo (2005).

4. GRAPE JUICE PRODUCTION AND COMPOSITION

According to Brazilian law, grape juice is a drink extracted from the grape that can be presented clean or cloudy, which undergoes adequate technological processes, not fermented, non-alcoholic, flavor, color and aroma well characteristic, subjected to treatment that ensures their conservation and presentation until the time of consumption. In Brazil, about 10% of the production is intended for the processing of grape juice (Rizzon et al., 1998). According to the latest report, CONAB (National Supply Company), the increase in grape juice consumption proved to be a market tendency in Brazil, with the expectation of the weight of consumption of vines products in Brazil in particular grape juice 100% natural, ready for consumption (Conab, 2019). Moreover, according to SISDEVIN (Vinicola Registration System), in 2019, there was a 48% increase in grape juice production compared to 2018. These data demonstrate the importance of research conducted influencing the consumption of grape juice to reduce the intake of sweetened beverages and improve the eating habits of the entire population.

Grape juices, according to Dani et al., (2007), available on the market are red and rose, white, organic, or conventional. These can come from the reconstitution process where the juice water is removed, for transport and storage, and then the same amount of water is added. This juice is also known as 100% grape but is precisely equal to natural juice.

Unlike wine, grape juice is a non-alcoholic beverage that can be consumed freely, favoring its consumption for all ages. As for the nutritional aspect, it can be compared with the fruit itself, simply because in its final composition are present the main constituents such as sugars, acids minerals, vitamins, and phenolic compounds that are responsible for its color and structure (Rizzon et al., 1998). Grape juice produced from the species *Vitis labrusca* L. has numerous nutrients and bioactive compounds, including phenolic compounds (Dani et al., 2007; 2008; 2009) that have antioxidant, antimutagenic, anticancer, anti-atherogenesis and aid in immune response (Bub et al., 2003; Al-Ahmadi et al., 2014; Miglio et al., 2014). Because of this, grape juice has become a food of high scientific relevance, which could be used as a therapeutic adjuvant in several diseases. The differences in the concentrations of total polyphenols and isomer forms of resveratrol are related to the different processing stemming from industry (Table 4). For example, integral juice is obtained through processes, it is not fermented, without adding sugars and their natural concentration. Reprocessed juice is obtained by dilution of concentrated and or dehydrated juice until its natural concentration (Sautter et al., 2005).

The consumption of grape juice, compared to wine, as a source of phenolic compounds can be advantageous since the absence of alcohol content allows the individual freedom in consumption and prevents the consumption of substances such as biogenic amines, present in wine, toxic when there is indiscriminate consumption (Malacrida et al., 2005). As previously mentioned, the main grape species for juice production are ‘Isabella’, ‘Ives’, and ‘Concord’ (*Vitis labrusca* L.), and southern Brazil is the largest producer. Toaldo et al., (2015) verified the phenolic composition of organic and conventional grape juices produced in Brazilian Southern (Toaldo et al., 2015). However, Lima et al., (2014), investigated the amount of polyphenols present in grapes produced in northeastern Brazil. This tropical semi-arid climate region has been receiving considerable investments from the winery sector. therefore, it is already the second region with the highest production of juice and refined wines, representing more than 95% of the national grape export (Lima et al., 2014). The vineyards planted for juice production in this region are ‘Early Isabella’ (*Vitis Labrusca*) and the hybrid BRS Cora and BRS Violeta. Both studies demonstrated the variety of phenolic compounds found in the juice. Regarding the results referring to the southern region of Brazil, the authors observed that organic juice presents a higher concentration of polyphenols when compared to conventional. Moreover, despite the varieties planted in northeastern Brazil, they observed that the varieties have an acceptable amount of polyphenols standing out BRS Violeta (Table 5).

Table 4. Total polyphenols, *trans*-resveratrol, and *cis*-resveratrol content in different industrial grape juices Grape juice

	N	Polyphenols (gallic acid mg.L-1)				
		Min	Max	Average	SD	C.V.%
Whole grape juice (NFC)	2	1617.4	2213.2	1915.3	421.3	11.0
Reconstituted (FC)	2	1551.7	1615.9	1583.8	45.4	1.4
Reconstituted with sugar added (FC)	6	205.4	933.4	607.0	259.4	42.7
Grape juice	N	Trans-resveratrol (mg.L-1)				
		Min	Max	Average	SD	C.V.%
Whole grape juice (NFC)	2	0.39	0.44	0.41	0.03	4.3
Reconstituted (FC)	2	0.61	0.90	0.75	0.20	13.6
Reconstituted with sugar added (FC)	6	0.19	0.32	0.25	0.05	84.0
Grape juice	N	Cis-resveratrol (mg.L-1)				
		Min	Max	Average	SD	C.V.%
Whole grape juice (NFC)	2	0,07	0,26	0,16	0,13	40,7
Reconstituted (FC)	2	1,22	1,59	1,40	0,26	9,3
Reconstituted with sugar added (FC)	6	0,07	0,67	0,38	0,33	86,8

NFC: not from concentrated; FC: from concentrated grape juice; SD: standard deviation; CV: coefficient of variation; N: number of samples. Source: Adapted from Sautter et al. (2005).

Table 5. Phenolic compounds of *V. labrusca* organic grape juices produced in 2 different Brazilian regions by the use of several cultivars

Phenolic Composition	Southern Region		Northeast Region		
	Types the production		Grape cultivar		
	Organic	Conventional	IP	BC	BV
Total phenolics (GAE mg. L-1)	3378.33 ± 50.08	2015.00 ± 21.79	779 ± 27	1944 ± 6	2712 ± 3
Total monomeric anthocyanins (mg. L-1)	1592.44 ± 33.70	420.01 ± 7.24	29 ± 1	225 ± 1	464 ± 6
Flavanols (mg. L-1)					
(+)-Catechin	500.52 ± 12.33	79.89 ± 30.19	4.7 ± 0.1	12.4 ± 0.3	19.8 ± 0.4
(-)-Epicatechin	53.48 ± 19.78	14.40 ± 0.77	1.0 ± 1.0	1.4 ± 0.5	0.6 ± 0.1
Phenolic acids (mg. L-1)					
Gallic acid	16.96 ± 0.39	11.51 ± 0.10	1.8 ± 0.1	13.6 ± 0.1	10.5 ± 0.8
Caffeic acid	29.95 ± 1.57	14.08 ± 0.17	8.6 ± 0.1	35.8 ± 0.5	28.9 ± 0.4
p-Coumaric acid	11.23 ± 0.16	10.73 ± 0.51	2.6 ± 0.1	4.5 ± 0.4	9.0 ± 0.1
Anthocyanins (mg. L-1)					
Malvidin 3,5-diglucoside	721.26 ± 20.99	189.43 ± 1.29	1.8 ± 0.0	0.7 ± 0.0	11.7 ± 0.0
Malvidin 3-glucoside	23.91 ± 2.59	47.42 ± 0.73	0.9 ± 0.1	ND	1.6 ± 0.2
Cyanidin-3,5-diglucoside	785.53 ± 39.56	152.02 ± 6.98	ND	11.8 ± 0.1	38.0 ± 0.6
Cyanidin 3-glucoside	21.72 ± 4.17	7.17 ± 0.59	3.0 ± 0.0	1.4 ± 0.1	32.7 ± 0.5
Delphinidin 3-glucoside	17.79 ± 1.01	12.15 ± 0.09	ND	11.7 ± 0.2	73.7 ± 1.2

IP = Isabel Precoce; BC = BRS Cora; BV = BRS Violeta; ND= not detected.

Source: Adapted from Lima et al. (2014) and Toaldo et al. (2015).

5. GRAPE JUICE AND RESEARCH

As has been mentioned before, grapes are rich in polyphenols. Phenolic compounds, in turn, are a class of phytochemicals, that is, they are compounds present in foods such as fruits and vegetables capable of acting in chemical reactions of the human organism (Liu, 2013).

Given this important role of grape and its derivatives in human health, in recent years, we have observed an increasing number of scientific publications about that. Theses publication is trying to elucidate the benefits of these compounds to prevent various pathologies. In addition, we can highlight its already recognized neuroprotective effect (Dani et al., 2008; 2010; Gabardo et al., 2018), hepatoprotective, observing a protective effect of grape juice in the face of a hyper lipidic diet or induction of liver damage provoked by carbon tetrachloride (CCl4) in experimental models (Dani et al., 2008b; Buchner et al., 2014; Schaffer et al., 2016; Gonçalves et al., 2018; Kovaleski et al., 2019) of epigenetic modulation (Gonçalves et al., 2017); improving biochemical parameters (Lacerda et al., 2018). In addition to generating benefits to descendants in transgenerational models (Hilger et al., 2015; Wohlenberg et al., 2017).

Apart from these, numerous publications in experimental models also increase the number of researches with humans to increasingly strengthen the importance of including this drink in the consumption of the population to reduce risks of various comorbidities. Zuanazzi et al., (2019) showed that white grape juice consumption in women reduced the abdominal circumference CA, increased HDL, and there was no change in blood pressures suggesting consumption as an adjuvant in feeding (Zuanazzi et al., 2019). In contradiction with Neto et al., (2017) reported a decrease in resting BP and improvement of post-exercise hypotension (HPE) in a study that evaluated the effect of these parameters before and after the consumption of red grape juice in hypertensive individuals (Neto et al., 2017). Finally, all these and all-study demonstrate the importance of the insertion of phenolic compounds in food and the impact they make on the organism.

6. GRAPE POMACE

The food industry is responsible for producing large amounts of waste that can represent high costs when unused. In addition to the use of waste and consequently new forms of consumption, this material has been reported as a natural source rich in bioactive compounds. Grape production is one of the most abundant agroeconomic activities, yielding more than 60 million tons produced each year globally. This production is mainly directed to fresh consumption, such as table fruits, juices, and raisins (Teixeira et al., 2014). Wine production implies the generation of large quantities of waste consisting mainly of organic, wastewater, greenhouse gas emissions, and inorganic waste (Musee et al., 2007). Among these residues, we find the unused remains of the grape, such as pomace, made up of seeds, pulp and skin, stems, and grape leaves. Grape pomace represents approximately 16-20% of the total volume, which is equivalent to the generation of about 210,000 tons per year, being directed mainly to composting or discarded in open areas, causing environmental problems (Rockenbach et al., 2011; Rondeau et al., 2013; Bender et al., 2016). The marketing of grape pomace products are relatively new to the market, and there are not many studies on the biological effects of this product, which has low cost and potentially beneficial effect on health.

Grape skin and seeds can be separated and dried through physical methods, being used in the production of flours and oils to obtain functional ingredients such as natural antioxidants and dietary supplements (Monrad et al., 2010). They represent, on average, 82% of the dry weight of the grape pomace, containing a large number of phenolic compounds, such as anthocyanins, hydroxykinetic acids, catechins, and flavonoids (Rockenbach et al., 2011). Besides, they have significant levels of dietary fiber (65-80%), containing high nutritional value (Llobera and Cañellas, 2008) (Table 6).

The distribution of phenolic compounds in different parts of the grape was reviewed by Xia et al., (2010). The skin and components of the pomace are rich in anthocyanins, hydroxykinetic acids, flavanols, and flavonol glycosides, while in the seeds are present gallic acid and flavonols (Kammerer et al., 2004; Xia et al., 2010). On the other hand, the concentration of phenolic compounds in grape pomace varies according to grape varieties. It is influenced by the place of cultivation, climate, maturity, and fermentation time (Fuleki and Ricardo-da-Silva 1997; Kennedy et al., 2000; Shi et al., 2003; Montealegre et al., 2006).

Grape pomace is being reused as an ingredient for new industrial interest products, such as flours and oils, considering the rich nutritional and functional composition. It is favoring the commercialization of healthier foods, in addition to new experimental research. Studies with grape seed flour are generally scarce but show promising results. Variety of Chardonnay grape seed flour showed an antioxidant capacity, three to twelve times higher than seed flours from other selected fruits such as raspberry, blueberry, and cranberry (Parry et al., 2006). Besides, grape seed flour metabolites are related to modulation of the intestinal microbiota. They may regulate lipid metabolism fatty acids (Kim et al., 2015).

Grape seeds are composed of 13-19% oil. This oil is rich in essential fatty acids, proteins, non-digestible carbohydrates, and non-phenolic antioxidants such as tocopherols and beta-carotene that can exert an anti-arteriosclerotic activity (Yu et al., 2012). The use of grape flour is being used as an alternative in dietary supplementation of hemodialysis patients since the fluid restriction is an essential element in the diet control of these patients.

Table 6. Composition of different grape pomace obtained from *Vitis vinifera* and *Vitis labrusca* species

Determinations	Cabernet Sauvignon (V. vinifera)	Merlot (V. vinifera)	Mix* (V. labrusca)	Terci (V. labrusca)
Moisture	13.63 ± 0.14	6.59 ± 0.21	2.85 ± 0.08	8.52 ± 0.15
Protein	5.32 ± 0.19	13.23 ± 0.71	11.58 ± 0.10	13.99 ± 0.09
Total lipids	4.83 ± 0.11	9.48 ± 0.27	7.61 ± 0.27	9.02 ± 0.47
Ash content	2.87 ± 0.06	5.10 ± 0.12	4.97 ± 0.43	6.32 ± 0.12
Total dietary fiber	26.42 ± 1.30	58.99 ± 0.92	56.57 ± 0.48	57.73 ± 0.66

*The Mix sample was composed of 3 red grape cultivars: Ives (65%), Isabella (25%) and BRS Violet (10%).

Source: Adapted from Ribeiro et al., (2015).

CONCLUSION

Vitis Labrusca is the second most cultivated species in the world, and this variety of grapes, mainly ‘Isabella’, ‘Concord’, and ‘Ives’, can be used for consumption in nature and processing, originating wines, juices, and other by-products. In Brazil, this species is very important, representing more than 80% of the grape production. This specie results in important products to this Country, such as table wine and, more recently, the grape juice. Grape juice production is increasing a lot and is a goal for the export market. In general, wines containing the *Vitis labrusca* grape showed more intense aroma/flavor notes described as sweet, grape, blackberry and roses.

The grape pomace is by-products of the winemaking process, which consist of mixtures of seeds and grape skins, and the conversion of these by-products into ingredients of value is of great interest to the industry, to minimize environmental impacts and boost new research for healthier foods and clarify their benefits in the face of human health.

The benefits of grapes are associated with the presence of phenolic compounds and their action as antioxidant potential, and because of their ability to prevent oxidation of biological substrates. If your nutritional value is calculated, you will see a vital fruit that can be included in the population's diet. Moreover, studies have shown that regular consumption of its derivatives, such as juice and or wine, is associated with a reduction in mortality and morbidity due to some chronic non-communicable diseases, preventing the action of free radicals, helping to combat premature aging.

REFERENCES

Abe, L. T., Da Mota, R. V., Lajolo, F. M. Genovese, M. I. (2007). Phenolic compounds and antioxidant activity of *Vitis labrusca* and *Vitis vinifera* cultivars. *Ciência e Tecnologia de Alimentos*, 27:394-400.

Al-Ahmadi, A. A., Ali, S. S., Ayuob, N. N., Ansary, A. K. A. (2014). Amelioration of hypercholesterolemia induced hepatic changes with red grape juice: A histopathological study. *Histology and histopathology*, 29:1169-1183.

Bender, A. B. B., Luvielmo, M. De Mello., Loureiro, B. B., Speroni, C. S., Boligon, A. A., Da Silva, L. P., Penna, N. G. (2016). Obtaining and characterization of grape skin flour and its use in an extruded snack. *Brazilian Journal of Food Technology*, 19:e2016010.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Empresa Brasileira de Pesquisa Agropecuária (Embrapa). Desempenho da vitivinicultura brasileira em 2015 [Performance of Brazilian vitiviniculture in 2015]. (2016). https://www.embrapa.br/busca-de-noticias/-/noticia/9952204/artigo-desempenho-da-vitivinicultura-brasileira-em-2015. Accessed 11 Feb 2020.

Brasil. Agencia Embrapa de informação tecnológica. *Cultivares* [*Cultivars*]. https://www.agencia.cnptia.embrapa.br/gestor/uva_para_processamento/arvore/CONT000g5f8cou802wx5ok0bb4szwyx060i6.html. Accessed 11 Feb 2020.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, 56: 317-333.

Bub, A., Watzl, B., Blockhaus, M., Briviba, K., Liegibel, U., Müller, H., Pool-Zobel, B. L., Rechkemmer, G. (2003). Fruit juice consumption modulates antioxidative status, immune status and DNA damage. *The Journal of Nutritional Biochemistry*, 14:90-98.

Buchner, I., Medeiros, N., Lacerda, D. S., Normann, C. A., Gemelli, T., Rigon, P., Wannmacher, C. M. D., Henriques, J. A. P., Dani, C., Funchal, C. (2014). Hepatoprotective and antioxidant potential of organic and conventional grape juices in rats fed a high-fat diet. *Antioxidants*, 3:323-338.

Camargo, U. A., Maia, J. D. G., Ritschel, P. S. (2010). Novas cultivares brasileiras de uva. Bento Gonçalves [New Brazilian grape cultivars. Bento Gonçalves]. *Embrapa Uva e Vinho.*

Companhia Nacional de Abastecimento (CONAB) (2019). *Uva Industrial* [*Industrial Grape*]. Análise Mensal. Brasília. https://www.conab.gov.br/info-agro/analises-do-mercado-agropecuario-e-extrativista/analises-do-mercado/historico-mensal-de-uva. Accessed 11 Feb 2020.

Dani, C., Oliboni, L. S., Vanderlinde, R., Bonatto, D., Salvador, M., Henriques, J. A. P. (2007). Phenolic content and antioxidant activities of white and purple juices manufactured with 59 organically- or conventionally-produced grapes. *Food and Chemical Toxicology,* 45:2574-2580.

Dani, C., Pasquali, M. A. B., Oliveira, M. R., Umezu, F. M., Salvador, M., Henriques, J. A., Moreira, J. C. (2008a). Protective effects of purple grape juice on carbon tetrachloride-induced oxidative stress in brains of adult Wistar rats. *Journal of Medicinal Food*, 11:55-61.

Dani, C., Oliboni, L. S., Pasquali, M. A. B., Oliveira, M. R., Umezu, F. M., Salvador, M., Moreira, J. C., Henriques, J. A. (2008b). Intake of purple grape juice as a hepatoprotective agent in Wistar rats. *Journal of Medicinal Food*, 11:127-132.

Dani, C., Oliboni, L. O., Umezu, F. M., Pasquali, M. A. B., Salvador, M., Moreira, J. C., Henriques, J. A. (2009). Antioxidant and antigenotoxic activities of purple grape juice - organic and 453 conventional - in adult rats. *Journal of Medicinal Food*, 12: 1111-1118.

Dani, C., Oliboni, L. S., Agostini, F., Funchal, C., Serafini, L., Henriques, J. A., Salvador, M. (2010). Toxicology *in vitro* phenolic content of grapevine leaves (*Vitis labrusca* var. Bordo) and its neuroprotective effect against peroxide damage. *Toxicology in Vitro*, 1:148-153.

David, J. M. P., David, J. P., Santos, V. L. C. S., Santos, M. de L. S., Mota, M. D. (2007). Resveratrol: ações e benefícios à saúde humana [Resveratrol: actions and benefits to human health]. *Diálogos da Ciência: Revista da Rede de Ensino FTC*, 10:1-11.

Fuleki, T., Ricardo-da-Silva, J. M. (1997). Catechin and procyanidin composition of seeds from grape cultivars grown in Ontario. *Journal of Agricultural and Food Chemistry*, 45:1156-1160.

Gabardo, T., Frusciante, M. R., Marinho, J. P., Dos Santos, M., Abujamra, A. L., Dani, C., Funchal, C. (2018). *In vitro* antioxidant activity of purple grape juice (Vitis Labrusca) against temozolamide treatment in rat brains. *Beverages*, 4:1-10.

Gonçalves, L. K., da Silva, I. R. V., Cechinel, L. R., Frusciante, M. R., de Mello, A. S., Elsner, V. R., Funchal, C., Dani, C. (2017). Maternal consumption of high-fat diet and grape juice modulates global histone H4 acetylation levels in offspring hippocampus: A preliminary study. *Neuroscience letters*, 661:29-32.

Gonçalves, L., Bortolato, G., Neto, R. D. B., Frusciante, M. R., Funchal, C., Dani, C. (2018). Grape juice consumption with or without high fat diet during pregnancy reduced the weight gain and improved lipid profile and oxidative stress levels in liver and serum from Wistar rats. *Beverages*, 4:78.

Hilger, D. K., Wohlenberg, M. F., Schaffer, T.K., Machado, F. D. S., Gonçalves, L. K., Bortolato, G., Dani, G., Rodrigues, A., Funchal, C., Dani, C. (2015). Purple grape juice, an important flavonoids source, influence in biochemical parameters in offspring of Wistar rats. *Food and Nutrition Science*, 6:683-691.

Instituto Brasileiro do Vinho (IBRAVIN). *História do Vinho no Brasil* [*History of Wine in Brazil*]. https://www.ibravin.org.br/Historia-do-Vinho-no-Brasil. Accessed 11 Feb 2020a.

Instituto Brasileiro do Vinho (IBRAVIN). *Panorama Geral* [*Overview*]. https://www.ibravin.org.br/Panorama-Geral. Accessed 11 Feb 2020b.

Jang, M., Cai, L., Udeani O. G., Slowing, K. V., Thomas, C. F., Beecher, C. W. W., Fong, H. H. S., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., Moon, R. C. Pezzuto, J. M. (1997). Cancer chemopreventive activity of resveratrol, a natural product derivated from grapes. *Science*, 275:218-220.

Janiques, A. G. De P, R., Leal, V. De O., Stockler-Pinto, M. B., Moreira, N. X., Mafra, D. (2014). Effects of grape powder supplementation on inflammatory and antioxidant markers in hemodialysis patients: A randomized, double-blind study. *Brazilian Journal of Nephrology*, 36:496-501.

Kammerer, D., Claus, A., Carle, R., Schieber, A. (2004). Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. *Journal of Agricultural and Food Chemistry*, 52:4360-4367.

Kato, C. G., Tonhi, C. D., Clemente, E. (2012). Anthocyanins in grapes (*Vitis vinífera* L.) grown in conventional systems. *Revista Brasileira de Tecnologia Agroindustrial*, 6:809-821.

Kennedy, J. A., Matthews, M. A., Waterhouse, A. L. (2000). Changes in grape seed polyphenols during fruit ripening. *Phytochemistry*, 55:77-85.

Kim, H., Kim, D. H., Seo, K. H., Chon, J. W., Nah, S. Y., Bartley, G. E., Arvik, T., Lipson, R., Yokoyama, W. (2015). Modulation of the intestinal microbiota is associated with lower plasma cholesterol and weight gain in hamsters fed chardonnay grape seed flour. *Journal of Agricultural and Food Chemistry*, 63:1460-1467.

Kovaleski, E. S., Gonçalves, L. K., Bortolato, G., Marinho, J. P., Silva, L. F. L., Russo, M. K. B., Agostini, F., Funchal, C., Dani, C. (2019). Effects of the ingestion of different kinds of white grape juice (*Vitis labrusca*) during adolescence on body weight, biochemical parameters and oxidative stress in liver of adult Wistar rats. *Food Chemistry*, 291:110-116.

Lacerda, D. S., Almeida, M. G., Teixeira, C., Jesus, A., Júnior, E. S. P., Bock, P. M., Henriques, J. A. P., Gomez, R., Dani, C., Funchal, C. (2018). Biochemical and physiological parameters in rats fed with high-fat-diet: the protective effect of chronic treatment with purple grape juice (bordo variety). *Beverages*, 4:100.

Leão, P. C. De S. (2010). Breve histórico da vitivinicultura e a sua evolução na região semiárida brasileira [Brief history of viticulture and its evolution in the Brazilian semiarid region]. *Anais da Academia Pernambucana de Ciência Agronômica*, 7:81-85.

Lima, M. S., Silani, I. S. V., Toaldo, I. M., Correa, L. C., Biasoto, A. C. T., Pereira, G. E., Bordignon-Luiz, M. T., Ninow, J. L. (2014). Phenolic compounds, organic acids antioxidant activity of grape juices produced from new Brazilian varieties planted in the Northeast Region of Brazil. *Food Chemistry*, 161:94-103.

Liu, R. H. (2013). Health-Promoting Components of Fruits and Vegetables in the diet. *Advances in Nutrition*, l:384S–392S.

Llobera, A., Cañellas, J. (2008). Antioxidant activity and dietary fiber of Prensal Blanc white grape (*Vitis vinifera*) by-products. *International Journal of Food Science & Technology*, 43:1953-1959.

Maia, J. D. G., Camargo, U. A. (2005). Sistema de produção de uvas rústicas para processamento em regiões tropicais do Brasil [System of production of rustic grapes for processing in tropical regions of Brazil.]. *Embrapa Uva e Vinho*. Versão eletônica.https://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Uva/UvasRusticasParaProcessamento/cultivares.htm. Accessed 11 Feb 2020.

Malacrida, C. R., Motta, S. (2005). Total phenolics and anthocyanins in grape juice. *Food Science and Technology*, 25:659-664.

Melo, G., Botton, M., Garrido, L. (2015). *Produção orgânica de uva para mesa* [*Organic table grape production*]. 1.

Miglio, C., Peluso, I., Raguzzini, A., Villaño, D. V., Cesqui, E., Catasta, G., Toti, E., Serafini, M. (2014). Fruit juice drinks prevent endogenous antioxidant response to high-fat meal ingestion. *The British Journal of Nutrition*, 111:294-300.

Montealegre, R. R., Peces, R. R., Vozmediano, J. L. C., Gascuen, J. M., Romero, E. G. (2006). Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. *Journal of Food Composition Analysis*, 19:687-693.

Monrad, J. K., Howard, L. R., King, J. W., Srinivas, K., Mauromoustakos, A. (2010). Subcritical solvent extraction of anthocyanins from dried red grape pomace. *Journal of Agricultural and Food Chemistry*, 58:2862-2868.

Musee, N., Lorenzen, L., Aldrich, C. (2007). Cellar waste minimization in the wine industry: A systems approach. *Journal of Cleaner Production*, 15:417-431.

Neto, M. M., Silva, T. F., Lima, F. F., Siqueira, T. M. Q., Toscano, L. T., Moura, S. K. M. S. F., Silva, A. (2017). Whole red grape juice reduces blood pressure at rest and increases post-exercise hypotension. *Journal of the American College of Nutrition*, 36:533-540.

Olmo, H. P. Grapes. In: Simmonds, N. W. (Ed.). *Evolution of crop plants*. New York: Longman Group, 1979.

Parry, J., Su, L., Moore, J., Cheng, Z., Luther, M., Rao, J. N., Wang, J. Y., Yu, L. L. (2006). Chemical compositions, antioxidant capacities, and antiproliferative activities of selected fruit seed flours. *Journal of Agricultural and Food Chemistry*, 54:3773-3778.

Porto Alegre. *Atlas socieconimico Rio Grande do Sul*. O RS é o maior produtor nacional de uva e o segundo maior produtor de maçã [*Socieconomic Atlas Rio Grande do Sul*. RS is the largest national grape producer and the second largest apple producer]. https://atlassocioeconomico.rs.gov.br/uva-e-maca. Accessed 11 Feb 2020.

Rho, K. A., Kim, M. K. (2006). Effects of different grape formulations on activity capacity lipid peroxidation and oxidative DNA damage in aged rats. *Journal of Nutritional Science and Vitaminology*, 52:33-46.

Rockenbach, I. I., Rodrigues, E., Gonzaga, L. V., Caliari, V., Genovese, M. I., Gonçalves, A. E. S. S., Fett, R. (2011). Phenolic compounds content and antioxidant activity in pomace from selected red grapes (*Vitis vinifera* L. and *Vitis labrusca* L.) widely produced in Brazil. *Food Chemistry*, 127:174-179.

Rondeau, P., Gambier, F., Jolibert, F., Brosse, N. (2013). Compositions and chemical variability of grape pomaces from French vineyard. *Industrial Crops and Products*, 43:251-254.

Ribeiro, L. F., Ribani, R. H., Francisco, T. M., Soares, A. A., Pontarolo, R., Haminiuk, C. W. (2015). Compounds from grape pomace (*Vitis vinifera* and *Vitis labrusca*) by spectrophotometric, chromatographic and spectral analyses. *Journal of chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 1007:72-80.

Rizzon, L. A., Manfrói, V., Meneguzzo, J. (1998). *Elaboração de suco de uva na propriedade vitícola* [*Elaboration of grape juice on the wine estate*]. Bento Gonçalves: Embrapa Uva e Vinho.

Rizzon, L. A., Miele, A., Meneguzzo, J. (2000). Evaluation of cv. Isabel to elaborate red table wine. *Ciência e Tecnologia de Alimentos*, 20:115-121.

Sautter, C. K., Denardin, S., Alves, A. O., Mallmann, C. A., Penna, N. G., Hecktheuer, L.H. (2005). Determination of resveratrol in grape juice produced in Brazil. *Food Science and Technology*, 25:437-442.

Schaffer, T. K., Wohlenberg, M. F., Medeiros, N., Martins, J. B., Agostini, F., Funchal, C., Dani, C. (2016). Evaluation of antioxidant activity of grapevine leaves extracts (*Vitis* *labrusca*) in liver of Wistar rats. *Anais da Academia Brasileira de Ciências,* 88:187-196.

Shi, J., Yu, J., Pohorly, J. E., Kakuda, Y. (2003). Polyphenolics in grape seeds-biochemistry and functionality. *Journal of Medicinal Food,* 6:291-299.

Sistema IBGE de Recuperação Automática (SIDRA). *Levantamento Sistemático da Produção Agrícola* [*Systematic Survey of Agricultural Production*]. https://sidra.ibge.gov.br/tabela/1618. Accessed 11 Feb 2020.

Soleas, G. J., Diamandis, E. P., Goldberg, D. M. (1997). Resveratrol: a molecule whose time has come? And gone? *Clinical Biochemistry,* 30:91-113.

Sousa, J.S.I. de (1969). *Uvas para o Brasil* [*Grapes for Brazil*]. São Paulo: Melhoramentos.

Subbaramaiah, K., Chung, W. J., Michaluart, P., Telang, N., Tanabe, T., Inoue, H., Jang, M., Pezzuto, J. M., Dannenberg, A. J. (1998). Resveratrol inhibits cyclooxygenase-2 transcription and activity in phorbol ester-treated human mammary epithelial cells. *The New York Academy of Sciences*. 273:21875-21882.

Teixeira, A., Baenas, N., Dominguez-Perles, R., Barros, A., Rosa, E., Moreno, D. A., Garcia-Viguera, C. (2014). Natural bioactive compounds from winery by-products as health promoters: a review. *International Journal of Molecular Sciences*, 15:15638-15678.

Toaldo, I. M., Cruz, F. A., Alves, T. L., De Gois, J. S., Borges, D. L. G., Cunha, H. P., Da Silva, E. L., Bordignos-Luiz, M. (2015). Bioactive potential of *Vitis labrusca* L. grape juices from 95 southern region of Brazil: phenolic and elemental composition and effect on lipid peroxidation in healthy subjects. *Food Chemistry*, 173:527-535.

Toaldo, I. M., Cruz, F. A., Silva, E. L., Bordignos-Luiz, M. T. (2016). Acute consumption of organic and conventional tropical grape juices (*V. Labrusca* L.) increases antioxidants in plasma and erythrocytes, but not glucose and uric acid levels in healthy individuals. *Nutrition Research*, 36:808-817.

Wohlenberg, M. F., Machado, F. S., Schaffer, T. K., Bortolato, G., Marinho, J. P., Medeiros, N. S., Mello, A., Agostini, F., Gerson, S., Funchal, C., Dani, C. (2017). Effect of chronic treatment with grape juice during pregnancy and nursing in central nervous system of female Wistar rats. *Journal of Nutritional Health and Food Engineering*, 6:75-81.

Xia, E., Deng, G., Guo, Y., Li, H. (2010). Biological activities of polyphenols from grapes. *International Journal of Molecular Science*, 11:622-646.

Yu, J., Ahmedna, M. (2012). Functional components of grape pomace: their composition, biological properties, and potential applications. *International Journal of Food Science and Technology*, 48:221-237.

Zuanazzi, C., Maccari, P. A., Beninca, S. C., Branco, C. S., Theodoro, H., Vanderlinde, R., Siviero, J., Salvador, M. (2019). White grape juice increases high-density lipoprotein cholesterol levels and reduces body mass index and abdominal and waist circumference in women. *Nutrition*, 57:109-114.

In: Vitis: Biology and Species
Editors: António M. Jordão et al.

ISBN: 978-1-53618-308-5

Chapter 2

NATIVE AND CREATED VINE VARIETIES AND WINES FROM SERBIA

Uroš Miljić*[*] *and Vladimir Puškaš
Faculty of Technology, University of Novi Sad, Novi Sad, Serbia

ABSTRACT

The Republic of Serbia has a long tradition of vine growing and wine production. The preservation of indigenous and regional vine varieties is important for the development of viticulture and winemaking in each country, through the preservation of the wine tradition and the promotion of wine-growing areas. Although in the past these varieties were rather suppressed by the world-famous varieties, especially in the smaller and less known wine countries such as Serbia, there is an aspiration to revive them and put them on the world wine map. Isolation and cultivation of the best clones of native vine varieties, as well as the application of traditional techniques in wine production are prerequisites to preserve and promote the tradition of viticulture and winemaking in a particular region. Clonal selection is a complex long-standing process based on scientific and experimental research that improves the characteristics of existing varieties. Indigenous and regional vine varieties are usually characterized by greater resistance to adverse agro-ecological conditions in the specific terroir than introduced varieties. In response to the increasingly visible impacts of global climate change on vine and wine production, the creation of new varieties is becoming very important. Breeders all around the world have been working on making new resistant varieties which can ensure vine quality as high as the most remarkable and internationally grown varieties. As a result, a significant number of resistant table and wine varieties were also released in less known wine countries. This chapter introduces the most important native and created vinevine varieties grown in Serbia and sums up the key characteristics of the plant, bunch and wine.

Keywords: created varieties, vine, native varieties, wine, Serbia

---

[*] Corresponding Author's Email: urosmiljic@yahoo.com.

1. VINE GROWING AND WINE PRODUCTION IN THE REPUBLIC OF SERBIA

The Republic of Serbia has a long tradition of vine growing and wine production which is primarily based on favorable agro-ecological conditions and the effort and skill of local producers. Geographically, the territory of Serbia is located between 42^o and 46^o of the northern latitude and 19^o and 22^o northern longitude and it is mainly characterized by a temperate climate. According to the Winkler Index, vine growing regions in Serbia belongs to II and III bioclimatic zone. Serbian wine regions receive between 1500-2100 average annual hours of sunshine and 650 mm of annual precipitation (rainfall). Ground air currents are largely conditioned by orography. In the warmer part of the year, northwest and west winds prevail. The colder part of the year is dominated by east and southeast winds. In the hilly-mountainous areas of southwestern Serbia, southwest winds prevail. Furthermore, vineyards in Serbia are grown on various soil types including loam, loess sand, clay, alluvium or more precisely vertisols, eutric cambisols, fluvisols, podzols, dystric cambisols etc. The vineyards in northern parts of the country have more lime in the soil, while the soil in central and southern wine regions is more acidic. The vineyards in the northern plain parts of Serbia are at slightly lower altitudes, 80 -150 m, while in the central and southern hilly parts, they are usually positioned at up to 500 m.

According to the official statistical data from 2019, there are around 22,000 hectares of vineyards currently in the Republic of Serbia. From that surface, around 17,500 ha are used for growing of vines for wine production (75% of the total area under vineyards) while the remaining vineyards (around 4,500 ha) are under varieties used for table vines production (Ivanišević et al., 2015). In the last 10-year period (2009–2018), the average total annual production was around 170,000 tons, while the average vine yield was 7.9 t/ha (Jakšić et al., 2019a). The average wine production was around 1.3 million hl (period 2008-2017).

Consumption of fresh vines in Serbia in the ten-year period (2008-2017) according to the Republican Bureau of Statistics data was 7.24 kg/household. Considering the average number of members in a Serbian household during the observed period (2.88 people), the average consumption of vines was 2.51 kg per capita. In the same period (2008-2017), the average wine consumption was 8.94 L/household, or 3.1 L per capita household member (Jakšić et al., 2019a).

The vine production is based on both modern and traditional viticultural practices. The spacing between rows in vineyards is usually between 2.2-2.7 meters while the distance between plans in a row is mostly 0.6-0.8 meters, giving the number of 4,000 to 6,000 vines per hectare. Both modern and traditional canopy management strategies applied in the vineyards are the result of years of monitoring and comparing both internationally accepted and traditional domestic principles. This includes common vine training systems such as simple and double Guyot, Cordon de Royat, Smart-Dyson, as well as traditional systems such as Župski and Karlovački (Vujović, 2013). The technology of vine processing and wine production is being improved and modern technological trends and procedures are being introduced. Moreover, the concept of integrated vine production is gradually being restored or introduced. A growing interest among vine growers in organic viticulture and wine production has been demonstrated in the recent decade.

Despite great diversity of native and regional vine varieties, Serbian wine industry is nowadays more oriented to the growing of world-famous international vine varieties. The main explanations of this trend are coming from the higher availability of foreign certified high-quality planting material and proven potential of the international varieties for production of high quality wines. Moreover, the placement and sale of these wines on domestic market is much easier compared to the wines from native varieties. The dominant international varieties among the white vines are Chardonnay, Sauvignon Blanc, Riesling and Graševina (Riesling Italico). While among red varieties, Cabernet Sauvignon, Merlot, Pinot Noir, Cabernet Franc and Frankovka (Blaufrankisch) are the most cultivated.. Among table vines, dominant varieties are Muscat de Hambourg and Cardinal. However, different adaptability of these widespread varieties to domestic agro-ecological conditions can be accompanied by the uncertainty in yield and quality.

In the previous period, there was a massive deforestation and decay of vineyards with domestic varieties. Moreover, there was no specific program for preservation and isolation of the best genetic material before the deforestation. Due to this situation, Serbia has been left without significant areas with native and regional varieties, which represents a permanent loss of domestic vinevine genetic database (Jakšić et al., 2019b). After the decades of stagnation Republic of Serbia is currently working on re-establishing of its viticulture and winemaking sector by creating a relatively new wine identity. The preservation and/or re-establishment of native and regional vine varieties and their greater representation in overall domestic vine production are becoming very important.

The idea is to revive native vine varieties and put them on the world wine map through the isolation and cultivation of the best clones. Clonal selection is a complex long-standing process based on scientific and experimental research that improves the characteristics of existing varieties. Indigenous and regional vine varieties are usually characterized by greater resistance to adverse agro-ecological conditions in the specific terroir than introduced varieties. The joint work of the members of scientific institutions and wine producers is directed to the investigation of both traditional and modern technological practices for the production of quality, unique and distinctive wines from these varieties.

The most important native and regional vine varieties grown in Serbia are Smederevka, Tamjanika, Bagrina, Kreaca, Muskat Krokan, Prokupac, Skadarka, Slankamenka, Začinak, Seduša and Vranac (Table 1).

Table 1. The most important native and regional vine varieties (*Vitis vinifera)* grown in Serbia for wine production

White varieties	Red varieties
Smederevka	Prokupac
Tamjanika	Skadarka
Bagrina	Slankamenka
Kreaca	Vranac
Muskat Krokan	Seduša
Sremska Zelenika	Začinak
	Crna (Black) Tamjanika
	Kevedinka

Table 2. The most important resistant vine varieties created in Serbia for wine production

White varieties	Red varieties
Neoplanta	Probus
Sila	Dionis
Panonia	Rumenika
Morava	Jagodinka
Petka	Župski Bojadiser
Lasta	Krajinski Bojadiser
Bačka	
Župljanka	

Breeders in Serbia, as those from all over the world, have been working on making new varieties resistant to biotic and abiotic stress factors and which can ensure similar vine quality as the most famous internationally grown varieties. Moreover, global climate changes have increasingly visible impacts on vine and wine production, so the creation of new varieties is becoming even more important. The variety grown in the vineyard is especially crucial for sustainable (organic) production of vines and wines. Due to susceptibility to diseases and pests and the minimal interventions allowed, the use of the most famous *Vitis vinifera* varieties in organic production can be risky and expensive and can lead to a decrease in the quality of the grapes (Korać and Cindrić, 2004). A significant number of resistant table and wine vine varieties (Table 2) was released in Serbia and grown even beyond the borders of the country. Apart from the already proven high tolerance of new Serbian vine varieties to fungal diseases, some of them still require the evaluation and identification of the most suitable agro-technical practices whose application will ensure the highest possible vine yield and quality. Moreover, determination of the optimal vinification parameters (full technological, phenolic and aromatic vine maturity, fermentation strategy, aging potential, etc.) is constantly going on for all newly created varieties. Thus, the aim of this chapter is to present viticultural and oenological characteristics of the most important native and regional vine varieties grown in Serbia. Moreover, the characteristics of resistant vine varieties created in Serbia and their potential for quality wine production will be also presented.

2. NATIVE VINE VARIETIES

As already mentioned, autochthonous and regional vine varieties in Serbia were being neglected and placed aside with respect to international varieties from the beginning of XXI century. It has only recently started with the emphasizing the importance of domestic varieties cultivation as part of a strategy for development of viticulture and winemaking sector and establishing a new wine identity of the country. The strategy implies work on clonal selection and certification of domestic varieties and propagation of their more intensive spread in Serbian vineyards.

It is also very important to mention rare (minor) wine and tablegrape varieties, varieties which are grown in non-commercial vineyards, endangered varieties and varieties described/mentioned/presented which can no longer be found in productive vineyards in Serbia (Table 3). However, it should have in mind that it is possible that some of the names of these varieties are actually synonymous of certain well-known autochthonous and regional vine varieties (Jakšić et al., 2019b). These varieties will not be described in more detail in this book chapter.

Table 3. Rare (minor) and endangered vine varieties found/previously grown in Serbia

Vine varieties	
Čavčica	Mirkovača
Crni Grašac	Ovči Repak
Volujaraka	Šljiva Grožđe
Volovsko Oko	Vrapčije Grožđe
Vrna Zelenika	Peršun Grožđe
Čađavica	Čauš Beli
Plavetni Drenak	Zlatava
Radovinka	

2.1. White Wine Varieties

This section introduces the key characteristics of the most commonly grown native and regional white varieties. The photos of these varieties are given in Figure 1.

Smederevka

Smederevka (*Vitis vinifera* L., synonyms: Dimyat, Szemendriai zold, Belina) is a domestic Serbian white grape variety. It is mostly grown in Serbia, but also in other countries in the region such as North Macedonia, Bulgaria and Romania. According to the classification of Negrulj (1956) it is classified as convarietas *Pontica*, subconvarietas *Balcanica*. This variety is mediumly vigorous and highly productive. Berries are large, oval, and usually yellow-green (Figure 1A). Its vegetation begins early while full maturity is accomplished very late, which classifies this variety in the 4th epoch according to Pulliat (1897). Due to the high vine yields, the variety requires short pruning, in order to limit the yield and produce high quality wine. The cluster is large, weighing usually around 200 to 300 g, medium dispersed. Smederevka is sensitive to powdery mildew but much more to black rot. On the other hand, the resistance to gray rot (*Botrytisciner*ea) is relatively high. Moreover, it is poorly resistant to low temperatures and winter/early spring frosts (Cindrić at al., 2000a).

Grape juice has a quite neutral flavor and usually contains 17-20% of total soluble solids (TSS) and 5 to 8 g/L of titratable acidity as tartaric acid. The content of nitrogen-based compounds is quite low in must and usually requires supplementation during alcoholic fermentation. The content of malic acid can be high and therefore, implementation of malolactic fermentation can be justified. Jordeva et al. (2016) showed that cold maceration (8 °C, during 12 and 24 hours) of pomace positively influenced the quality of Smederevka wines. A significant increase in the dry extract, ash, total phenols as well as a decrease in the content of tartaric acid and higher alcohols in Smederevka wines were recorded. Moreover, Smederevka wines produced by cold maceration at 8 °C during 24 hours were characterized by the best sensory properties. Clarification of the must from Smederevka grapes is significantly facilitated by the use of pectolytic enzymes. Mojsov et al. (2011) reported that after 4 hours of maceration at 18 to 20^0C with the addition of commercial pectolytic enzyme, free run juice yields were increased by approximately 7%. Moreover, a drastic reduction in the volume of gross lees (by almost 50%) compared to control trial was recorded. Characterization of the phenolic profile of different parts of Smederevka vines showed that total phenolic content was around 60 g/kg as gallic acid equivalent (GAE) in the seeds, around 6 g/kg GAE in the skin and around 0.5 g/kg in the pulp (Sredojević, 2018). Moreover, total phenolic content in Smederevka wine was 0.14 g/L GAE (Sredojević, 2018). A comparative analysis conducted in North Macedonia showed that Chardonnay had higher polyphenolic content in different parts of the vines (skins, seed and pulp) than Smederevka vines, even the seeds of Smederevka were richer in flavan-3-ols (Ivanova et al., 2011a). Furthermore, Chardonnay wines were shown to be richer in phenolic acids compared to Smederevka wines (Ivanova et al., 2010). The dominant phenolic component in Smederevka wines was *trans*-coutaric acid. The content of total phenolics, total flavonoids, and total flavan-3-ols in Smederevka wines during aging significantly decreases in the first two months of storage in bottles, while the content of these parameters stays quite stable during the following four-month aging (Ivanova et al., 2011b). Smederevka is used for production of light fresh wines with a pleasant aroma and taste. This variety has great potential for the production of sparkling wines. It is also used for making blends since it can improve the freshness of white wines from other varieties. The grapes of this the variety is also widely used for fresh consumption (Jakšić et al., 2019b).

Tamjanika

Tamjanika (*Vitis vinifera* L.) represent a variety whose origins have not yet been fully elucidated due to great diversity of very similar variants and clones. The extensive scientific research is currently going on with the goal to make differentiation (if it exists) among different synonyms used for this variety: Tamjanika, White Tamjanika, Muscat a Petit Grain, Muscat White, Muscat Yellow, Yellow Tamjanika or Muscat de Frontignan. One of the studies conducted by Bešlić et al. (2012) reported that major trait variations within 'Tamjanika group' are related to skin color, aroma intensity and sex of the flower. Skin can be yellowish ('Yellow Tamjanika', Figure 1C) and greenish ('White Tamjanika', Figure 1B). Moreover, 'Yellow Tamjanika', has female type of flowers, while 'White Tamjanika' has hermaphrodite flower. Until the finalization of the numerous investigations, all clones and varieties grown in Balkans are covered by the name ‘Tamjanika group’. Unquestionably, it is a very famous and widely represented white Muscat grape variety in Balkans. Also, the same authors reported two different genotype profiles for White Tamjanika samples collected in different vineyards (study used 10 SSR markers).

Tamjanika variety is characterized by a significant vigor and medium productivity. The berries are round, medium-sized and usually yellow-green, sprinkled with tiny white dots. The size of clusters is medium, rarely large, and their shape is usually cylindrical or cylindrical-conical. Furthermore, the structure of the clusters is medium compacted or compacted. Most of the Tamjanika clones fully mature at the end of the 3rd epoch according to Pulliat (1897) which classifies it as relatively late variety (Jakšić et al., 2019b). Spur and cane pruning is recommended and all common vine training systems that allow this type of pruning are possible. This variety is moderately resistant to powdery mildew and black rot. In general, it is moderately resistant to *Botrytis cinerea*, however longer rainy periods during autumn in combination with inappropriate viticultural practices, can significantly increase the susceptibility.

Grape juice usually contains 20-24% of TSS and 5-7 g/L of titratable acidity (as tartaric acid). The must is colorless with a pronounced Muscat aroma and taste. Jovanović et al. (2010) investigated the influence of pruning mode on grape yield and quality of Tamjanika vines grown in Župa wine region (South Serbia) during the period 2000-2002. Meteorological conditions were favorable for development of quality grapes during these three vintages. The applied training system was one-armed horizontal cordon. The average yield varied in the range 8500-13800 kg per hectare, depending on the conditions during vegetation and ripening. Sugar content varied within the range 18-20%, while titratable acidity was 5.9-6.5 g/L tartaric acid. Characterization of the phenolic profile of different parts of white Tamjanika vines showed that total phenolic content was around 65 g/kg GAE in the seeds, around 4 g/kg GAE in the skin and only around 0.5 g/kg in the pulp. Moreover, total phenolic content in Smederevka wine was 0.18 g/L GAE (Sredojević, 2018).

Bagrina

Bagrina (*Vitis vinifera* L., synonyms: Braghina, Crvena Dinka or Turska ružica) is believed to be an autochthonous vine variety of Balkan Peninsula. It is mostly grown in Serbia (the eastern part - Timočka krajina), Romania and slightly in Bulgaria. Although the color of the vine skin is light pink/violet, the variety is used almost entirely for production of white wines. In the past, the color of Bagrina wines was orange, light copper, and amber-like. Nowadays, the color is yellow, yellow-green (usual to conventional white wines). The name probably comes from the word 'bagrem', which is Serbian name for robinia tree (*Robinia pseudoacacia*). It is classified as convarietas *Pontica*, subconvarietas *Balcanica*. It matures in the 3rd epoch according to Pulliat (1987) and therefore, it is classified as late variety. It is characterized by a pronounced vigor. The productivity is greatly dependent on weather conditions. It is influenced by the fact that it has a morphologically hermaphrodite, but functionally female flower, so the fertilization may not be effective enough. Therefore, it is usually grown together with the pollinator variety (usually Smederevka or Prokupac) in order to improve the yield. It is sensitive to low temperatures. It is more susceptible to downy than to powdery mildew, and relatively resistant to *Botrytis* (Cindrić at al., 2000a).

Bagrina was mostly abandoned for international varieties because of its problematic characteristics (fertilization). However, the recent tendency for the preservation of native and regional grapevine varieties has resulted in a more intensive re-planting of this variety in the eastern Serbia. This part of Serbia is known for long, hot and relatively dry summers (better known area for growing of red varieties). However, Bagrina provides grapes with lower pH, high acidity and high overall quality under these agro-ecological conditions. It grows well on moderately fertile, warm and scattered soils (lime and sand) (Milosavljević and Jović, 1999).

The cluster is medium-sized, weighing usually around 100 to 200 g. The berries are rounded, medium-sized, with thin skin (Figure 1D). The yield varies with the degree of fertilization (usually 5-18 tones/ha) and accordingly, the yield influences the quality. Moreover, depending on the fruit load, Bagrina gives wide range of TSS contents (18-24%). Titratable acidity also reaches relatively high values (6-10 g/L tartaric acid) (Milosavljević and Jović, 1999; Cindrić at al., 2000a). The pulp (mesocarp) is soft, juicy and colorless. The must taste is neutral. The wine aroma is usually a combination of herbal, floral and fruity aromas (white flowers, apricots, peaches etc.). The taste of Bagrina wines is mineral, creamy, moderately full, and persistent. Finnally, there is still a lack of data describing phenolic and aromatic composition of this native variety.

Figure 1. Native white wine vine varieties from Serbia.
a) Smederevka; b) White Tamjanika; c) Yellow Tamjanika; d) Bagrina.
(adapted from www.vivc.de and www.grozd.rs).

2.2. Red Wine Varieties

This section introduces the key characteristics of the most commonly grown native and regional red varieties. The photos of these varieties are given in Figure 2.

Prokupac

Prokupac (*Vitis vinifera* L., synonyms: Kameničarka, Rskavac, Rekovačka crnka, Nikodimka, Prokupka, Niševka or Zarčin) is a very old domestic Serbian red vine variety with unspecified exact origin. It is classified as convarietas *Pontica*, subconvarietas *Balcanica*. It matures between the 3rd and 4th epochs, and therefore it is classified as late to very late variety. Prokupac is one of the most important Serbian autochthonous vine varieties. It was the most planted vinevine on Serbian territory around one hundred years ago. However, after the Second World War, its name was mainly associated with the production of low-quality bulk wines in big industrial wine cellars. Larger crop yields achieved in that times (even greater than 20 tones/ha) were only able to give light, low extract wines characterized by pale red color, unripe tannins and vegetative aromas. After the years of poor reputation and as a consequence of an extensive research conducted within the strategy for re-establishment of Serbian viticultural and winemaking identity from the beginning of 21st century, Prokupac is starting to show its full potential in quality wine production. In order to obtain vine populations with improved production properties such as vigor, bunch and berry mechanical composition, disease resistance, grape and wine quality, an extensive clone selection was carried out (Zdunić at al., 2019). Marković et al. (2008) allocated and described 25 Prokupac clones based on the most important ampelographic and technologic properties, while Ministry of Agriculture, Forestry and

Water Management of Serbia until now recognized 12 clones with the best technological properties (clones 40/5, 40/8, 41/1, 41/3, 41/4, 41/6, 42/1, 42/2, 43/2, 43/6, 43/7).

Figure 2. Native red wine varieties grown in Serbia.
a) Prokupac; b) Kadarka; c) Vranac. (Adapted from www.vivc.de)

Prokupac has strong vigor and high yielding capacity. Its shoots are strong and developed with upright growth so it can be grown on lower training systems. In older vineyards vines are grown in a single-pole system or even without support. It is suitable for spur pruning where it gives good yields. The most common are Župski and Krajinski vine training systems. In general, it is resistant to *Botrytis cinerea* and sensitive to low temperatures and winter/early spring frosts (Cindrić et al., 2000a, Jakšić at al., 2019b). It is optimal to be grown on dry, well-permeable and rocky soil with southern exposition (Zdunić at al., 2019). Nowadays, modern production of Prokupac limits the vine yields to around 5-7 tones/ha which allows production of wines with more intensive ruby red color, medium body, and expressed freshness. The cluster is medium-sized, weighing usually around 150 to 200 g, medium dispersed. The berries are medium sized, round, slightly flat – spheroidal. The skin of ‘Prokupac’ is dark-blue, with characteristic dots (Figure 2A).

Under appropriate agro-ecological conditions, TSS content in must usually varies between 18-22% while titratable acidity is in the range 5-7 g/L tartaric acid. Characterization of the phenolic profile of different parts of ‘Prokupac’ grapes showed that total phenolic content was around 100 g/kg GAE in the seeds, around 10 g/kg GAE in the skin and only around 0.1 g/kg in the pulp (Sredojević, 2018). According to Pantelić et al. (2016), who investigated phenolic composition in different parts of berries of 13 vine varieties grown in Serbia, the most dominant compound present in the seeds of cv. Prokupac was (+)-catechin (1111.66 mg/kg) followed by gallocatechin gallate (788.80 mg/kg). The most dominant phenolic compound in Prokupac vine skin was quercetin (44.7 mg/kg) and resveratrol (13.7 mg/kg). Furthermore, among phenolics of vine pulp the highest contents of gallocatechingallate, ferulic and gentisic acids were recorded.

Comparing ‘Cabernet Sauvignon’, ‘Merlot’, ‘Vranac’ (an autochthonous Balkans variety) and ‘Prokupac’ grapes from three vineyard blocks from the southern Serbia, Mitić at al. (2012) reported similar contents of total phenolic compounds in Vranac’ and ‘Prokupac’ grapes (156 and 158 mg GAE/100 g, respectively), while higher values (170 and 173 mg GAE/100 g, respectively) were recorded for ‘Cabernet Sauvignon’ and ‘Merlot’. Moreover, ‘Prokupac’ grapes contained significantly lower total flavonoid, and total anthocyanin contents than ‘Cabernet Sauvignon’, ‘Merlot’ and ‘Vranac’ grapes. The most dominant anthocyanins in ‘Prokupac’ grapes are malvidin-3-*O*-glucoside, peonidin-3-*O*-glucoside and malvidin-3-*p*-coumaroylglucoside. The contents of hydroxycinnamic acids (*t*-caftaric acid, *c*-coutaric acid, *t*-coutaric acid and *t*-fertaric acid) in ‘Prokupac’ grape extracts were similar as the ones determined in ‘Cabernet Sauvignon’, ‘Vranac’ and ‘Merlot’ grapes. The total antioxidant activity of ‘Vranac’ and ‘Prokupac’ grapes were similar ($p < 0.05$), but lower ($p < 0.05$) than that of ‘Cabernet Sauvignon’ and ‘Merlot’ (Mitić at al., 2012). The most dominant phenolic compounds in ‘Prokupac’ wines, according to Sredojević (2018) are: gallic acid (34 mg/L), (+)-catechin (6 mg/L) and (-)-epicatechin (6 mg/L). Furthermore, among macro and microelements in the wine, contents of K - 500 mg/kg, P - 132 mg/kg, S - 129 mg/kg, Mg - 53 mg/kg, Ca - 28 mg/kg, Na - 1.2 mg/kg and Fe - 0.7 mg/kg were recorded (Sredojević, 2018). The wines from this variety are usually light to medium-colored, light to medium-bodied with moderate acidity and gentle tannins when the production process does not include too prolonged post-fermentative maceration. The development of garnet and brick colors in early stages of aging (2nd and 3rd year) is specific for some clones. By the character and structure, ‘Prokupac’ wines are the most similar to ‘Blaufränkisch’ or ‘Pinot Noir’.

Kadarka

Kadarka (*Vitis vinifera* L., synonyms: Skadarka, Gamza, Cetereska or Cherna) is an old red grape variety of Balkan Peninsula, most probably originated from Skadar lake which is divided by Montenegro and Albania. Nowadays, it is mostly grown in Hungary and Bulgaria, but also in other countries in the region such Serbia (the northern parts of the country) and Romania. It is classified as convarietas *Pontica*, subconvarietas *Balcanica*. Many Kadarka clones and variants can be distinguished from each other based on the morphological and quality properties. It is classified as late variety since in achieves the full ripeness in 3rd epoch according to Pulliat (1897). The ripening season usually starts from the end of September to early October. The variety prefers cooler and dryer climate.

‘Kadarka’ vines develop strong trunk with upright shoots which allowed it to grow without the support in the past. The variety requires spur pruning. It mostly has hermaphrodite flower, but female or male types of flowers can also be encountered. It is a robust variety capable of giving high yields (12-16 tones/ha). However, the production of quality red wines requires reduction of the yield. Kadarka usually have medium sized, conical or cylindrical bunches, weighing usually around 150-200 g. These bunches often appear compact and single winged. The color varies from blue to black, with small and spherical berries, usually having a thin skin and a thick waxy cover (Cindrić et al., 2000a) (Figure 2B).

'Kadarka' vine is highly resistant to drought and is one of the varieties the best adapted to sandy soils. It is moderately susceptible to powdery and downy mildew. It is very sensitive to *Botrytis* and vine ripening in rainy autumn conditions usually causes decrease in the yield and quality due to rotting (a consequence of the thin skin of berries). If the conditions are adverse, the berries also tend to crack. Hence, this variety gives better results in airy, hilly areas with rich and relatively drained soils. Furthermore, it is highly sensitive to low temperatures. In the past, they protected the vine from low temperatures by hugging, which today is often not possible due to higher cultivation forms.

At moderate fruit load and good agro-ecological conditions during the growing season, the vines can reach about 18-21.5% of TSS and 6-9 g/L (as tartaric acid) of titratable acidity. Cultivar Kadarka typically produces a wine with a vivid, dense ruby color and a pronounced red fruit flavors (raspberry, blackberry and red cherry) along with typical spiciness, especially when the autumn is warm and dry. The wines are also characterized by freshness and delicate taste coming from good acidity and mild tannin structure. GC-MS description of the primary aroma profiles of two 'Kadarka' wines originating from different terroirs was carried out (Csóka et al., 2013). The data showed that, beside certain smaller similarity, the primary aroma profiles considerably differ due to the different agro-ecological conditions at the place of origin. The grape of 'Kadarka' from plants of the poorer sandy soil is more fragrant, wealthier in terpenes and relative compounds than the ones grown on the loess. The content of total phenolic compounds in 'Kadarka' wines is lower (500-1100 mg/L GAE) compared to the world-famous red varieties such as Cabernet Sauvignon, Cabernet Franc and Merlot (Pour-Nikfardjam and Pickering, 2008; Balga, 2014). The anthocyanin profile of this variety mainly includes the highest presence of malvidin-3-glucoside (250-450 mg/L), while the contents of delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside and peonidin-3-*O*-glucoside are similar and about ten times lower (25-45 mg/L) than of malvidin-3-*O*-glucoside (Pour-Nikfardjam et al., 2006; Avar et al., 2007). Furthermore, the same authors reported that the most dominant phenolic acid is caftaric (45 mg/L), while among flavan-3-ols the content of (+)-catechin was the highest. The content of *trans*-resveratrol in 'Kadarka' wines was around 1-2 mg/L.

The breeders sought to transfer the positive characteristics of Kadarka to new varieties. The four Hungarian new varieties are the offspring of Kadarka (Rubintos, Biborkadarka, Kurucver and Karmin), as well as one Serbian (Probus).

Vranac

Vranac (*Vitis vinifera* L., synonyms: Vranac Crnogorski, Vranac Crmnički or Vranec) is an ancient red wine vine originating from Montenegro but whose cultivation has expanded to Serbia and North Macedonia centuries ago, and where is also considered local and autochthonous. It is classified as convarietas *Pontica*, subconvarietas *Balcanica*. It matures in the 3rd epoch, and therefore it is classified as late variety.

Vranac is moderately vigorous and very productive vine variety. It is able to give larger crop yields (up to 15 tones/ha). It is moderately sensitive to powdery and downy mildew. On the other hand, the resistance to *Botrytis cinerea* is relatively high. Moreover, it is highly sensitive to low temperatures. It is suitable for both long and short pruning. It has hermaphrodite flower. The variety gives compactly structured and large bunches (200-300 g). Medium to large sized, thin skinned and deeply colored berries are characteristic for these blue-black grapes (Cindrić et al., 2000a) (Figure 2C).

Bogicevic et al. (2015) investigated the impact of several viticultural practices on the quality of 'Vranac' grapes. Early defoliation of Vranac vines reduces the vine yield compared to control untreated vines. Early leaf removal slightly reduces the cluster weight. This treatment also resulted in a slightly increased ratio of skin vs. berry weight. There were no recorded differences in the berry weight and in the number of berries per cluster. Cluster thinning does not affect the berry weight. However, early leaf removal followed by cluster thinning resulted in a reduction of the cluster weight, berry weight, and berry numbers per cluster. Furthermore, early defoliation and cluster thinning lead to better soluble solids, anthocyanins and proanthocyanidins accumulation compared to the vines from untreated vines. The highest content of anthocyanins and proanthocyanidins was determined in the skin extracts where both treatments were applied, followed by the extract from the treatment included only defoliation. Therefore, cultivar Vranac was proved to be less sensitive to the effects of early leaf removal and cluster thinning compared to cv. Cabernet Sauvignon (Bogicevic et al., 2015).

Depending on the agro-ecological conditions, TSS in must can reach even more than 25% while titratable acidity is usually in the range 6-7 g/L tartaric acid. Andjelkovic et al. (2013) investigated changes in polyphenolic content of 'Vranac' grapes from the southern Serbia during ripening. The average sugar content in grapes samples at the start of veraison was 114 g/L and it increased to 238 g/L after 50 days (at harvest). The average titratable acidity decreased from 32 to 5 g/L (expressed as tartaric acid) in the same period. Moreover, the total polyphenols content (expressed as mg GAE/g of fresh source) increased almost constantly in samples of grape seeds, skin and pulp during the period from the start of veraison to the harvest date. The authors also reported that (+)-catechin, (-)-epicatechin and procyanidin dimer B2 are the most prevalent phenolic compounds in the seeds, while anthocyanis such as malvidin, petunidin and delphinidin glucosides are dominant in the grape skin. All grape extracts were shown to have high radical-scavenging activity. Furthermore, Pajović et al. (2014) conducted extensive varietal characterization discrimination of Montenegrin red wines (Vranac, Kratošija, and Cabernet Sauvignon) based on the polyphenol content. According to the results obtained, 'Vranac' grapes showed lower polyphenol content (in average 1750 mg/kg of fresh berries, expressed as (+)-catechin) in comparison to 'Cabernet Sauvignon' (in average 2360 mg/kg of fresh berries, expressed as (+)-catechin). On the other hand, ‘Vranac’ grapes had the highest anthocyanin content in the skins. However, the larger berry size (almost twice) of ‘Vranac’ grapes in comparison to ‘Cabernet Sauvignon’ produces a dilution effect resulting in the lowest content of polyphenols in the grapes and wines. The average content of anthocyanins in ‘Vranac’ grapes was 1100 mg/kg of grape fresh mass or 830 mg/L in the corresponding four-month-old wine. The average content of low-molecular mass proanthocyanidins was 1100 mg/kg of grape fresh mass or 500 mg/L in the four-month-old wine. On the other hand, the average contents of anthocyanins in fresh grape and four-month-old wine for cv. Cabernet Sauvignon were 950 mg/kg and 730 mg/L, while the contents of low-molecular mass proanthocyanidins in the same sources were 1850 mg/kg and 630 mg/L, respectively.

Radović et al. (2015) investigated resveratrol concentration variability among four ‘Vranac’ types (3 clones and the population) during three harvests. Results showed the highest average concentration of the following composts: *trans*-piceid (1.1 mg/L), *cis*-piceid (1.2 mg/L), of *trans*-resveratrol (0.6 mg/L), *cis*-resveratrol (0.4 mg/L) and total resveratrol (3.1 mg/L). Similar results were obtained by Pajović-Šćepanović et al. (2018) who reported that the average content of total resveratrol in ‘Vranac’ wines (1.9 mg/L) was almost twice as high as the average determined in ‘Cabernet Sauvignon’ (1 mg/L). Young ‘Vranac’ wines have a bright purple hue with aromas reminiscent of red berries and fruit jams. The young wines have robust tannin structure which in combination with fruity acidity provides crispness and richness. After a couple of years of aging, the intensive purple hue develops into an intense dark ruby and inky wines. More complex aromas are being developed with aging. Moreover, the taste becomes subtler, round, and full, while sharpness significantly decreases, giving a longer and smoother finish.

3. DOMESTIC CREATED VINE VARIETIES

Effects of global climate changes on vine and wine production are becoming more and more evident. Breeders all around the world have been working on the creation of new resistant varieties which can ensure vine quality as high as the best internationally grown varieties. A significant number of resistant table and wine varieties released in less known wine countries is being increasingly grown in the vineyards all around the world.

From the seventies of the twentieth century, reputable scientific research centers located in Serbian, namely in cities of Belgrade, Novi Sad and Niš, focused their attention on plantation crossing and breeding, which resulted in creation of significant number of new vine varieties. The most important Serbian research institutions are experimental fields for viticulture in Sremski Karlovci (University of Novi Sad) where 21 new vine varieties and 3 clones of Riesling Italico (Welschriesling) were bred, and experimental fields in Radmilovac (University of Belgrade), which created 23 new vine varieties. Both of these institutions are among the biggest Vitis germplasm in this part of Europe. The photos of some domestic created vine varieties are given in Figure 3.

Depending on the plant breeding objectives, three phases in creating new vine varieties were pointed out: *Phase 1*- improvement of the quality of cultivated autochthonous grapevine varieties (Smederevka, Kevidinka, Prokupac, Kadarka, etc) by its cross-breeding with top quality Western-European vine varieties; *Phase 2*- improvement of the resistance to low winter temperatures, while preserving grape and wine quality by cross-breeding with Eastern Asian species (*Vitis amur*ensis) known for its high resistance to low temperatures, tolerance to fungal diseases and short vegetation; *Phase 3* - improvement of the fungal disease resistance and grape and wine quality by cross-breeding with North American species highly resistant to powdery and downy mildew.

Resistant cultivars (Table 2) have been more often given the advantage, especially within the concept of ecological production. Ecological production of vines promotes production systems that respect the environment and protect the human health. Thus, reducing pesticide use in vine production in order to preserve the environment and human health is possible by growing cultivars tolerant or resistant to fungal diseases. However, high grape and wine quality is also required. The most represented Serbian vine varieties grown in domestic, but also in international wine regions are: Neoplanta, Sila, Župljanka, Morava, Panonija, Petka, Bačka, Petra and Probus. The latest unofficial data from 2019 reported that several of these varieties are grown in Hungary, covering the area larger than 100 ha. Moreover, the planting of vine varieties created in Serbia has started in Ukraine and Russia. Dergunov (2017) reported that based on the physico-chemical and biochemical properties, Serbian vine varieties Cosmopolita, Petra, Bačka and Panonia were proven as promising for the Russian winemaking and vinevine-growing industry.

Neoplanta

Neoplanta vine variety is created by cross-breeding of Smederevka x Red Traminer. The author of this variety is professor Dragoslav Milisavljević. It is most represented in Serbian Fruška Gora wine region. 'Neoplanta' grapes ripen between the 2nd and 3rd epochs. It was named of by old Roman name of Novi Sad, the capital city of Vojvodina region in the northern Serbia. This vine variety is highly vigorous and is able to bear high yields (12 tones/ha). It requires cane pruning, and it tends to form a thick canopy. It has hermaphrodite flower. The cluster is medium-sized and the berries are small or medium-sized mostly green-yellow (Figure 3A). This cultivar shows relatively high susceptibility to low temperatures and frost, as well as to *Botrytis* and powdery mildew. The content of sugar is relatively high, and TSS content usually reaches up to 22-23%. On the other hand, the titratable acidity is in the range 5-8 g/L tartaric acid, but in the most cases in it does not exceed 6 g/L (Milosavljević and Jović, 1999; Cindrić at al., 2000a). This sometimes causes the lack of wine freshness. This variety is mostly used for production of high-quality dry wines with strong Muscat aroma. In particularly favorable years, grapes can be left on the vine for longer and can be used to produce natural semi-sweet or sweet wines (late harvest and ice wine). Marković et al. (2001) pointed out good production potential of this variety based on basic biological features of fertility and vine quality.

Figure 3. Domestic created vine varieties in Serbia.
a) Neoplanta; b) Sila; c) Morava; d) Panonia; e) Petra; f) Probus. (Adapted from www.vivc.de and www.grozd.rs).

Sila

Sila is a variety created by cross-breeding of Kevedinka (Kövidinka, Ružica) x Chardonnay. The authors of this variety are professors P. Cindrić, S. Lazić and V. Kovač. It is a late variety, since it matures in the 3rd epoch. It is mostly grown in Vojvodina region (the northern Serbia), however, it is also present in Hungary. This is a variety with medium lushness and high productivity. It has hermaphrodite flower. It can be pruned to spur, but it is more suited to pruning on arches. The cluster is loose, medium-sized (around 200 g) and the berries are small, rounded, mostly pale green (Figure 3B). Mesocarp is juicy and skin is thick and firm. It gives high yields (10-12 tones/ha). Sila is characterized with high resistance to gray mold (*Botrytis*) mainly because of loose cluster and thick skin. It is relatively sensitive to low temperatures and frost (Milosavljević and Jović, 1999; Cindrić at al., 2000a).

The content of TSS is usually around 20% and while titratable acidity is 5-8 g/L tartaric acid. Ružić and Jazić (1998) investigated effect of some winemaking procedures and conditions on the quality of 'Sila' wine. They reported that the pomace maceration should be short and followed by quick and gentle pressing. Alcoholic fermentation should be carried at lower temperatures (around 15 ^{0}C). The most dominant phenolic acids in 'Sila' wines are 2,5-dihydroxybenzoic, caffeic and ellagic acid (0.8-1.6 mg/L) while among flavonoids, the largest amounts of (+)-catechin, (-)-epicatechin and resveratrol are present (0.5-1 mg/L) (Beara et al., 2017). The same authors reported significant antioxidant activities of 'Sila' wine against DPPH• and •NO free radicals (IC50 values 0.2 and 0.98 mg/mL, respectively) which were, in some cases, comparable with the results obtained for red wines. Sila gives quality wines, light, refreshing (with plenty of acids), with discreet pleasant varietal aroma and harmonic taste.

Morava

Morava is a variety created by interspecies crossing of domestic high-quality genotype SK 86-2/293 (SK 77-7/4 x Bianca) (genotype containing Kunbarat and Red Traminer) and Riesling. In its hereditary basis it contains: around 3% of *Vitis amurensis*, around 6% of four North American species (*V. rupestris, V. berlandieri, V. labrusca* and *V. lincecumii*), and more than 90% of *V. vinifera*. The authors of this variety are Professors P. Cindrić and N. Korać from Faculty of Agriculture, University of Novi Sad. It matures in the 3rd and 4th epoch, so it is a very late variety. Morava is one of the most commonly grown Serbian created vine varieties. According to Serbian National Wine Register, this resistant variety is grown on more than 200 hectares in Serbia. It is mostly grown in central Serbia and Vojvodina region (the northern Serbia). However, vineyards under this variety more and more extended beyond the borders of the country (mostly in Hungary). Morava is presently recommended as one of the most suitable varieties for organic viticulture in Serbia.

This variety is characterized by high productivity (around 10-12 tones/ha), medium to high vigor and an upright shoot growth. The bunch is loose, conical and medium sized (around 130-160 g), with medium, rounded and green berries with characteristic aroma (Figure 3C). The berries have a strong thick skin and a juicy pulp. It is highly resistant to downy mildew and gray mold, while tolerance to powdery mildew is moderate (more common on leaves than on the fruit). It is highly resistant to low winter temperatures. It is more fruitful compared to cv. Riesling. 'Morava' grapes are characterized by TSS contents around 20% and high titratable acidity (usually 7-10 g/L tartaric acid). The content of total phenolic compounds is around 0.3 g/L GAE (Ivanišević et al., 2012). Wines produced from

‘Morava’ grapes are of very high quality with a characteristic aroma similar to Sauvignon Blanc.

Panonia

Cultivar Panonia was created by the same cross-breeding (hereditary basis) as cv. Morava. It was created by professors P. Cindrić, N. Korać and V. Kovač. It is an early ripening variety, classified in the 1st epoch. According to Serbian National Wine Register, Panonia is grown on around 30 hectares in Serbia (mostly northern parts). Current knowledge indicates that it is also grown in Hungary. It is characterized by medium productivity (around 8 tones/ha) and upright shoot growth with few or no laterals, since they stop growing after several leaves are formed (the characteristic of variety). The cluster is medium sized (100-150 g), loose, widely cone and wing-shaped. Berries are round, small, and green-yellow (Figure 3D). Panonia is a variety with high tolerance to powdery mildew and downy mildew. The susceptibility to *Botrytis cinerea* is relatively low, especially if optimal harvest times were met. A significant tolerance to low winter temperatures is present (Ivanišević et al., 2012). The variety is very suitable for the organic production since it can mostly be successfully grown without pesticide application. Usually contains high sugar amounts (TSS > 20%) and plenty of acids (titratable acidity above7 g/L tartaric acid). The content of total phenolic compounds is around 0.4 g/L GAE (Ivanišević et al., 2012). Dergunov (2017) reported that the content of total aromatic compounds in ‘Panonia’ wine is around 480 mg/L.

Petra

Petra is a vine variety created by cross-breeding of Kunbarat x Pinot Noir. The authors of this variety are professors P. Cindrić, and V. Kovač. East Asian species *Vitis amurensis* was included in the hereditary stock through the introduction of Hungarian variety Kunbarat. Petra includes 12.5% of *V. amurensis* and 87.5% of *V. vinifera* (Cindrić et al., 2000b). As late variety, it ripens in the 3rd epoch. It Petra develops strong trunk. It usually bears medium yields (around 8 tones/ha). The bunch is small (120-140 g) and compact while berries are small, rounded, aromatic and usually greyish green (Figure 3E). It is very resistant to low temperatures but the vegetation starts early, so there is a risk of late spring frosts. Furthermore, ‘Petra’ grapevines are characterized by significant resistance to downy mildew and *Botrytis cinerea* and pronounced sensitivity to powdery mildew (Cindrić et al., 2000a).

Sugar contents in vines can reach high values (TSS content is usually 22-25%, but sometimes even higher) while titratable acidity is mostly in the range 6-9 g/L tartaric acid.

High contents of sugar and acids, as well as a muscat scent, makes the grapes of this variety suitable for the production of dessert wines. The production of aromatic wine spirits from ‘Petra’ grapes gives very good results. According to the characterization of polyphenols in different parts of ‘Petra’ grapes done by Sredojević (2018), total phenolic content was around 50 g/kg GAE in the seeds, around 1 g/kg GAE in the skin and around 0.1 g/kg GAE in the pulp. Moreover, total phenolic content in young ‘Petra’ wine was 0.2 g/L GAE while, the antioxidative activity was 1.2 mmol ET/L (ET - trolox equivalent). The most dominant phenolic compounds in ‘Petra’ wines determined by Sredojević (2018) are: gallic acid (0.4 mg/L), caffeic acid (0.6 mg/L), (+)-catechin (0.9 mg/L) and arbutin (1 mg/L). Moreover, the following mineral contents were reported by Sredojevic (2018): K - 190 mg/kg, P - 65 mg/kg, S - 90 mg/kg, Mg - 40 mg/kg, Ca - 30 mg/kg, Na - 12 mg/kg and Fe - 0.75 mg/kg. According to Dergunov (2017), the content of total aromatic compounds in ‘Petra’ wine is around 625 mg/L.

Probus

Probus is a vine variety created by cross-breeding from Kadarka x Cabernet Sauvignon. The variety was created by professors D. Milisavljević, S. Lazić and V. Kovač. It was named of the Roman emperor Markus Aurelius Probus who planted the first vinevines in Fruška Gora hill (the northern Serbia) in 3rd century. It is late to very late variety since it matures between 3rd and 4th epoch. The sensitivity to low winter temperatures is quite high. It is relatively sensitive to *Botrytis* (Cindrić et al., 2000a). Probus variety bears medium yields (around 8 tones/ha). The cluster is medium sized (140-180 g), cylindrical, and compact while, the berries are small, rounded, usually dark blue/black and juicy (Cindrić et al., 2000a) (Figure 3E). When agro-ecological conditions allow (warm and dry autumn), it accumulates even up to 25% of sugar. The pronounced titratable acidity (usually 6-9 g/L) contributes to the good aging potential of Probus wines. This variety has the ability to accumulate high contents of anthocyanins in skin, reaching almost twice compared with Cabernet Sauvignon (Kalajdžić et al., 2015). Similar relationship was also determined for the content of total phenolic compounds in these two varieties. Moreover, the extractability of the anthocyanins is also very high. Cvejić et al. (2016) described the extraction rates of total and individual polyphenols during traditional maceration and fermentation of ‘Probus’ wine. The obtained results also confirmed high extractability of the anthocyanins during pre-fermentation maceration and begening phases of alcoholic fermentation. Moreover, the average values for total phenolics, total flavan-3-ols and total anthocyanins content in young ‘Probus’ wines were 2 g/L GAE, 900 mg/L and 450 mg/L, respectively. The most dominant anthocyanins in young wines were malvidin-3-*O*-glucoside (average values 200-230 mg/L), petunidin-3-*O*-glucoside (average values 20-25 mg/L) and delphinidin-3-*O*-glucoside (average values 15-20 mg/L).

Among other polyphenols, higher contents of gallic and syringic acids, catechin and resveratrol were highlighted. The influence of aging for 4 years on the phenolics of 'Probus' wine was also assessed by comparison with the values determined for the same 1-month old wines (Atanacković-Krstonošić et al., 2019). The results showed significant increase in the contents of gallic and *p*-coumaric acids and (+)-catechin after four year of aging as a result of the hydrolysis of gallotannins and corresponding complexes of these compounds and e.g., anthocyanins (Puškaš and Miljić, 2012; Cvejić Hogervorst et al., 2017). The content of monomeric anthocyanins decreased for more than 90% due to participation in the formation of polymeric pigments. Moreover, the content of resveratrol stayed relatively unchanged. 'Probus' wines are intensely colored (dark red hue), rich in extract, with harmonious taste and pleasant red fruits aroma

CONCLUSION

Considering the potential of the wines from native and regional vine varieties from Serbia, as well as from domestic created varieties, introduced through this chapter, it is necessary to continue with the preservation and further improvement of these varieties. This includes the additional work on clonal selection and creation of new resistant vine varieties, as well as extensive research on the most suitable viticultural and winemaking techniques which can ensure high grape and wine quality. The main goal of these activities should be directed to the improvement of competitiveness of domestic wine producers on local and international market.

REFERENCES

Atanacković Krstonošić, M.T., Cvejić Hogervorst, J.M., Torović, Lj.D., Puškaš, V.S., Miljić, U.D., Mikulić, M.P., Gojković Bukarica, Lj.C. (2019). Influence of 4 years of ageing on some phenolic compounds in red wines. *Acta Alimentaria*, 48(IV):449-456.

Andjelkovic, M., Radovanović, B., Radovanović, A., Andjelkovic, A.M. (2013). Changes in polyphenolic content and antioxidant activity of vines cv Vranac during ripening. *South African Journal of Enology and Viticulture*, 34(II):147-155.

Avar, P., Pour Nikfardjam, M.S., Kunsági-Máté, S., Montskó, G., Szabó, Z., Böddi, K., Ohmacht, R., Márk, L. (2007). Investigation of phenolic components of Hungarian wines. *International Journal of Molecular Sciences*, 8:1028-1038.

Balga, I. (2014). *A review of the effect of wine technology procedures on the polyphenolic compounds and sensory analysis in red wines*. PhD Thesis, Corvinus University of Budapest, Deparment of Oenology, Hungary.

Beara, I.N., Torović, Lj. D., Pintać, D.Đ., Majkić, T.M., Orčić, D.Z., Mimica-Dukić, N.M., Lesjak, M.M. (2017). Polyphenolic profile, antioxidant and neuroprotective potency of vine juices and wines from Fruška Gora region (Serbia). *International Journal of Food Properties*, 20(SIII): S2552–S2568.

Bešlić, Z., Todić, S., Korać, N., Lorenzi, S., Emanuelli, F., Grando, M.S. (2012). Genetic characterization and relationships of traditional vine cultivars from Serbia. *Vitis,* 51(IV):183-189.

Bogicevic, M., Maraš, V., Mugoša, M., Kodžulović, V., Raičević, J., Šućur, S., Failla, O. (2015). The effects of early leaf removal and cluster thinning treatments on berry growth and vine composition in cultivars Vranac and Cabernet Sauvignon. *Chemical and Biological Technologies in Agriculture,* 2(XIII):1-8.

Cindrić, P., Korać, N., Kovač, V. (2000a). *Sorte vinove loze (Vine varieties).* 3rd Edition. Faculty of Agriculture, University of Novi Sad & Prometej Novi Sad.

Cindrić, P., Korać, N., Kovač, V. (2000b). *Vine breeding in the Vojvodina province*. VII International Symposium on Vinevine Genetics and Breeding, doi: 10.17660/ActaHortic.2000.528.72.

Cvejić, J., Puškaš, V., Miljić, U., Torović, Lj., Rakić, D. (2016). Varietal phenolic composition of Probus, Rumenika and Frankovka red wines from Fruška Gora (Serbia) and changes in main compounds during maceration. *European Food Research and Technology,* 242:319-1329.

Cvejić Hogervorst, J., Atanacković Krstonošić, M., Bursać, M., Miljić, U. (2017). Polyphenols, in: Galanakis, C.*, Nutraceutical and Functional Food Components, 1st Ed., Effects of Innovative Processing Technique,* Elsevier, Academic Press, 203-258.

Dergunov, A. (2017). The prospect of production of quality white wines in Russia from vines varieties of Serbian breeding. *Fruit growing and viticulture in the South of Russia,* 48(VI):13-28.

Ivanišević, D., Korać, N., Čabilovski, R., Manojlović, M., Paprić, Đ., Kuljančić, I., Medić, M. (2012). Wine vine cultivars suitable for organic production. *International Symposium for Agriculture and Food, IV Macedonian Symposium for Viticulture and Wine Production,* Skopje, North Macedonia, 171-181.

Ivanišević, D., Jakšić, D., Korać, N. (2015). *Vinogradarski atlas (Winegrowing Atlas*). Republican Bureau of Statistics, Republic of Serbia.

Ivanova, V., Meudec, E., Souquet, J-M., Vojnoski, B., Cheynier, V., Stefova, M. (2010). HPLC analysis of hydroxycinnamic acid derivatives in Smederevka and Chardonnay wines. *XXI Congress of Chemists and Technologists of Macedonia,* Ohrid, North Macedonia, 126.

Ivanova, V., Stefova, M., Vojnoski, B., Dörnyei, Á., Márk, L., Dimovska, V., Stafilov, T., Kilár, F. (2011a). Identification of polyphenolic compounds in red and white vine varieties grown in R. Macedonia and changes of their content during ripening. *Food Research International,* 44:2851-2860.

Ivanova, V., Vojnoski, B., Stefova, M. (2011b). Effect of the winemaking practices and aging on phenolic content of Smederevka and Chardonnay wines. *Food and Bioprocess Technology,* 4:1512-1518.

Jakšić, D., Bradić, I., Beader, M., Ristić, M., Popović, D., Mošić, I., Dodok, I. (2019a). *Vinogradarstvo i vinarstvo Srbije – Studija: Analiza sektora proizvodnje i prerade grožđa i proizvodnje vina (Viticulture and wine production of Serbia - Analysis of the graphic production and wine processing sectors).* Center for Viticulture and Winemaking in Niš, Republic of Serbia.

Jakšić, D., Bradić, I., Beader, M., Milić, R., Ristić, M. (2019b). *Autohtone i regionalne sorte vinove loze Srbije (Autochthonous and Regional Vine Varieties of Serbia).* Center for Viticulture and Winemaking in Niš, Republic of Serbia.

Jordeva, S.N., Mojsov, K.D., Andronikov, D., Janevski, A., Gaber, S. (2016). The effects of the duration of cold maceration on the composition and sensory properties of Smederevka wines. *Advanced Technologies,* 5 (II):60-63.

Jovanović, Z., Garić, M., Ćirković, B. (2010). The effect of pruning mode on vine yield and quality of the cultivar White Tamjanika in Župa vine district. *Agroznanje - Agro-knowledge Journal,* 11(II):93-99.

Kalajdžić, M., Ivanišević, D., Drenjančević, M., Korać, N., Jukić, V., Kokovic, J. (2015). The accumulation of anthocyanins and total phenols during ripening in cv Probus and Cabernet Sauvignon vine berries in Fruska Gora wine region. *Contemporary Agriculture*, 64(III-IV):217-222.

Korać, N., Cindrić, P. (2004). Table vine cultivars convenient for ecological wine production. *Contemporary Agriculture*, 1-2:23-33.

Marković, N., Nakalamić, A., Todić. (2001). Yield and quality of new wine-vine varieties and clones in the Belgrade area. *Journal of Agricultural Sciences,* 46(I):41-48.

Marković, N., Todić, S. Jović, S., Bešlić, Z., Petrović, A. (2008). Biological and technological properties of cv. Prokupac clones. *XIII Serbian Congress of Fruit and Vine Ggrowers,* 89.

Milosavljević, M., Jović, S. (1999). *Grožđe i vino (Vines and wine).* Znanje Imanje edition, Agena, Belgrade, Serbia.

Mitić, M., Souquet, J.-M., Obradović, M., Mitić, S. (2012). Phytochemical profiles and antioxidant activities of Serbian table and wine vines. *Food Science and Biotechnology,* 21(VI):1619-1626.

Mojsov, K., Ziberoski, J., Božinović, Z., Petreska, M. (2011). Effects of pectolytic enzyme treatments on white vine mashs of Smederevka on vine juice yields and volume of lees. *46th Croatian and 6th International Symposium on Agriculture,* Opatija, Croatia, 968-971.

Pajović, R., Raičević, D., Popović, T. Sivilotti, P., Lisjak, K., Vanzo, A. (2014). Polyphenolic characterization of Vranac, Kratosija and Cabernet Sauvignon (*Vitis* *vinifera* L. cv.) vines and wines from different vineyard locations in Montenegro. *South African Journal of Enology and Viticulture,* 35(I):139-148.

Pajović-Šćepanović, R., Wendelin, S., Eder, R. (2018). Phenolic composition and varietal discrimination of Montenegrin red wines (*Vitis vinifera* var. Vranac, Kratošija, and Cabernet Sauvignon). *European Food Research and Technology,* 244:2243-2254.

Pantelić, M.M., Dabić Zagorac, D.Č., Davidović, S.M., Todić, S.R., Bešlić, Z.S., Gašić, U.M., Tešić, Ž.Lj., Natić, M.M. (2016). Identification and quantification of phenolic compounds in berry skin, pulp, and seeds in 13 vinevine varieties grown in Serbia. *Food Chemistry,* 15:243-252.

Pour-Nikfardjam, M.S., Márk, L., Avar, P., Figler, M., Ohmacht, R. (2007). Polyphenols, anthocyanins, and trans-resveratrol in red wines from the Hungarian Villany region. *Food Chemistry,* 98:453-462.

Pour-Nikfardjam, M.S., Pickering, G.J. (2008). Influence of variety and commercial yeast preparation on red wine made from autochthonous Hungarian and Canadian vines. Part I: phenolic composition. *European Food Research and Technology*, 227:1077-1083.

Pulliat, V. (1897). *Early wine and table grapes (Les resins precoces pour le vin et la table).* Coulet, Montpellier.

Puškaš, V., Miljić, U. (2012). The application of D-optimal design for modeling the red wine aging process. *Food Control,* 28(II):362-367.

Radović, B., Tešević, V. Kodžulović, V., Maraš, V. (2015). Resveratrol concentration in Vranac wines. *Vitis,* 54 (Special Issue): 169-171.

Ružić, N., Jazić, Lj. (1998). Effect of some winemaking conditions on the quality of Sila wine. *Poljoprivreda (Agriculture),* 47:201-205.

Sredojević, M. (2018). *Hemijska karakterizacija grožđa i sortnih vina iz različitih vinogorja u Srbiji* (*Chemical characterization of vines and varietal wines from different vineyards in Serbia*). PhD Thesis, Faculty of Agriculture, University of Belgrade, Republic of Serbia.

Vujović, D. (2013). *Vinogradarstvo (Viticulture).* Faculty of Agriculture, University of Belgrade, Republic of Serbia.

Zdunić, G., Gođevac, D., Šavikin, K., Krivokuća, D., Mihailović, M., Pržić, Z., Marković, N. (2019). Vine seed polyphenols and fatty acids of autochthonous Prokupac vine variety from Serbia. *Chemistry and Biodiversity,* 16(VII) doi: 10.1002/cbdv. 201900053.

www.vivc.de, date of access 15.01.2020.

www.grozd.rs, date of access, 15.01.2020.

In: Vitis: Biology and Species
Editors: António M. Jordão et al.

ISBN: 978-1-53618-308-5

Chapter 3

NATIVE GRAPE VARIETIES OF THE EURO-ASIAN ECO-GEOGRAPHICAL REGION OF RUSSIA: TAXONOMIC, BIOLOGICAL AND AGROECONOMIC SPECIFICITY OF CULTIVARS FROM CRIMEA

Vladimir Volynkin*[*], *Vladimir Likhovskoy, Alla Polulyakh, Svetlana Levchenko, Elena Ostroukhova, Irina Vasylyk and Irina Peskova

Russian National Research Institute of Viticulture and Winemaking “Magarach”,
Yalta, Crimea, Russian Federation

ABSTRACT

Each eco-geographical region is characterized by specific spectra of species, forms, and cultural grapevine varieties that are obtained in the process of natural evolution and as a result of artificial selection in the process of domestication of grapes. Certain species of grapevine have historically been lost and were discovered only as a result of paleontological studies, and some grow nowadays as relict forms and endemics of certain eco-geographical regions. Such varieties, as cultivars, are considered autochthonous if they come directly from forms of wild grapes that grow only in this region or native if they do not come from these wild forms. The differentiation of 80 native grapevine varieties of Crimea according to a complex of 84 ampelographic signs indicates that they belong to the species *Vitis vinifera* and various eco-geographical groups of Eurasia. The obtained results confirm the hypothesis about the origin of native grapevine varieties of Crimea. A comparison of some cultivated native grapevine varieties and identified varieties of *Vitis vinifera sylvestris* of Crimea proves that a number of native varieties are truly

---

[*] Corresponding Author’s Email: volynkin@ukr.net.

autochthonous. The biological specificity of the native varieties of Crimea on the phenology and agrobiology indicators was established. The traditional and promising areas of the use of native grapevines cultivars in winemaking are presented.

Keywords: agrobiology, cultivars, eco-geographical group, grapes, wild forms, wine.

1. INTRODUCTION

On Earth, there are regions occupied by individual countries, combining several eco-geographical regions of origin and cultivation of crops, in particular grapevines. In such countries, the forms of grapevines grown belong to various botanical taxa. The loss of genetic resources and the elimination of genes of any plant can be both global and local, if we consider the process in individual regions and in the whole Planet. Anyway, the loss of a genetic sample at any point of our Planet is global, since it is irreplaceable for the entire genetic diversity of a crop on Earth, and, consequently, for all humanity.

At each historical evolutionary point of development and variability of plant crop, it is important for the entire world scientific community to answer the question of the past state of the genetic diversity of individual cultures and flora as a whole, to compare it with the state at the current time. No less urgent is the possibility at the present time of searching for the preserved relict forms of plants and endemics so that it would be possible to clarify the origin, formation and variability of individual botanical taxa of each separately considered crop of the entire diversity of the planet's flora.

In this chapter, the emphasis is on the research of Russian scientists in the search and historical preservation of existing speicies of grapevines in Russia; search and study of relict and endemic forms preserved to our time. It is considered what they have done over the last time, beginning with the XIX century, in revealing the issues of variability in the plant world, identification and taxonomy of samples, their preservation, using the example of viticulture. The world conservation of plant genetic resources for their further use is of great importance for all humanity (FAO, 2017).

Genetic resources of cultivated plants with valuable economic characteristics used for the production of food products and the creation of raw materials for industry ensure stable development and functioning of the environmentally safe agricultural economy in the conditions of constant changes in the natural and climatic conditions and social circumstances. Population growth and economic development of countries make significant changes in the living conditions of all organisms and ecological systems of our planet. The introduction of new intensive technologies for cultivating crops using introductions, the reconstruction of old plantations, the reduction in the number of varieties in industrial plantations (the introduction of monocultures), the disappearance of wild relatives of cultivated plants in many places under the influence of anthropogenic factors; all lead to a real threat of a significant loss number of plant diversity (FAO, 2017).

Starting from the XIX century, expeditions were organized in Russia to study the flora of various eco-geographical regions, conduct paleontological studies, and search for preserved growing relict forms of vegetation, including grapevines. It was revealed that various species of grapevines grew in this territory. Nevertheless, as a result of natural and artificial selection, domestication of wild-growing forms of grapevines, only a few species have been preserved in the natural nature. Varieties of either one species of *Vitis vinifera* or breeding varieties, in the genome of which the genes of this species and other species of the *Vitis* genus are combined, are cultivated industrially.

The multifaceted nature of this problem also lies in the fact that no country in the world can independently provide itself with plant diversity (Cooper, 2002; FAO, 2014; 2017). Therefore, the conservation of genetic resources of grapevines is of great importance both for modern science, and for future generations (Polulyakh et al., 2017).

2. SYSTEMATIC OF GRAPEVINES AS A REFLECTION OF THE EVOLUTIONARY TRANSFORMATION OF THIS CROP

The change in the ecological conditions of plants habitat and the associated evolutionary transformation of any plant crops, including grapevines, is reflected in modern concepts of the taxonomy of crop varieties. The process of evolutionary transformation of grapevines leads not only to the formation of more adapted genotypes, but also the loss of individual genotypes and the possible elimination of genes, which we can observe at the present time. Therefore, in order to preserve the evolutionary history of a crop, it is necessary to regularly review the current state of its systematic. This chapter contains data on the taxonomy of one grapevine species, *Vitis vinifera* of the *Vitaceae* family, the interrelationship of various subspecies within this species, the interrelationship between the commercial cultivars and wild varieties of this species, the formation of eco-geographical groups and the possibility of specifying the centers of the species origin.

2.1. The Theory of the Centers of Origin of Grapevines and Their Ancient Species that Have Not Survived to the Present Day

Until the XIX century naturalists and botanists conducted a tremendous hard work on the identification and systematics of flora samples, having formed botanical taxa of each separately considered crop. At this initial stage, it was necessary to single out independent crops from the diversity of flora. An indisputable contribution to the theory of the evolution of grapevines and the theory of centers of origin of crops was made by Russian scientists N.I. Vavilov (Vavilov, 1926; 1931) and P.M. Zhukovskyi (Zhukovskii, 1964), and later on in the history of form formation and classification of pre-existing and extant genera and species of grapevines made a fundamental contribution to A.M. Negrul (Negrul, 1946).

2.2. The Extinct Primitive Genus *Cissites*

The oldest species among the known representatives of the family of vine-trees belong to the number of primitive flowering plants, which first appeared on the Earth. In the fossil state only plant remains are found, so they have to be studied in separate parts. In most cases, the remains of leaves, seeds, fruits, stems and, rarely, flowers are found. On these remains, found in those or other layers, whole plants are restored, giving an idea of the vegetation of past eras.

The oldest species from the family of grapevine (as, for example, *Cissites*), are known since the time of the Lower Cretaceous. Many types found in the Cretaceous sediments of the Northern Hemisphere are similar to modern representatives of the grape family, but it is possible that they belong to some closely related groups of flowering plants, which are now extinct.

The structure of the seeds found, can provide quite convincing evidence of their belonging to the *Vitaceae* family. This is the most reliable criterion for the recognition of fossil plants similar to grapevines. However, fossil grapevines, recognized by seeds and fruits, is known only from the end of Cretaceous time; they become numerous in the sediments from the beginning of the Tertiary period.

Among the oldest types of grape plants is the genus *Cissites* (a likely ancestor of the modern genus *Cissus*). During the Cretaceous period, North America was the center of development of representatives of this genus, which had a great variety of species. This genus was first established by O. Heer, who characterizes his development of finger-lobed leaves and other features that bring him closer to the modern genus *Cissus* L. (Heer, 1878).

Ancient species of the genus, according to modern ideas, is *Cisseteus parvifolius* (*C. obtusilobus* Sap.), referred to the genus *Cissites*, which occurs in the Lower Cretaceous sediments of the eastern part of North America and in Western Europe. The deeply dissected leaves of *Cissites parvifolius* with blunt lobes (Figure 1) resemble leaves of some species of grapes that appeared later. Apparently, North America and Portugal were at that time still bound by land, as a result of which the same type of primitive grapevine plant was widely spread, including in Russia (Krishtofovich, 1938).

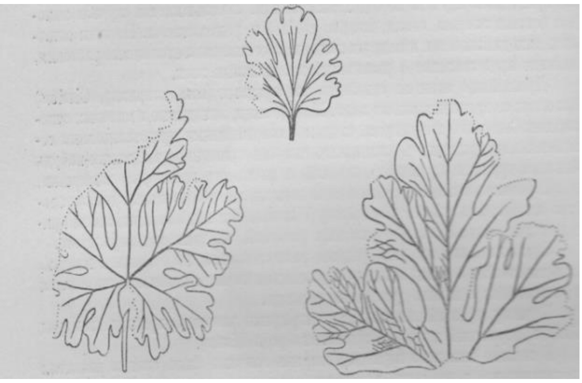


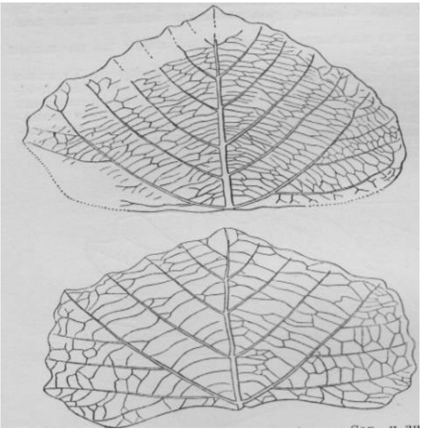
Figure 1. Leaves of *Cissites parvifolius*.

In the Upper Cretaceous deposits of North America belonging to the later centuries of the Cretaceous, representatives of the genus *Cissites* are found in Dakota sandstones, distributed not only in the state of Dakota, but also in Canada. These areas are an important center for the development of a significant number of species of the genus *Cissites*, whose representatives were distributed in a large number of species and varieties from Canada through Dakotas and; Nebraska to Kansas in the south. In this vast area there were many different types of primitive grapevine plants. In the literature, there are indications of finding the remains of the same plants in the polar regions (Greenland). In Central Asia, in Russia, on the western side of the Urals, in the Upper Cretaceous sediments, a special species was discovered: *Cissites Kryshtofovichianus* Jarm., whose typical shape is represented by a broadly ovate leaf with deep notches and pointed lobes somewhat resembling a maple leaf. Another species was found in the south of the Urals, along the Kulden-Temir River, the tributary of the Emba River, and named *Cissites uralensis* Krysht. In this species the leaf blade is broadly ovoid, with five major veins and deep notches and blunt teeth, than it differs from *Cissites Kryshtofovichianus* Jarm (Krishtofovich, 1938).

2.3. Grapes Belonging to the Ancient and Modern Genus *Cissus*

In the higher horizons of the Cretaceous system, types of leaves with more complex venation appear, representing a transition to a pinnate form. These types bring together the genus *Cissites* with the modern genus *Cissus*, although it is difficult to establish a definite relationship of fossil forms with the modern representatives of the genus *Cissus* already because there are no data on the nature of flowers in representatives of the genus *Cissites* and fossil *Cissus*. This includes, for example, the species *Cissus coloradensis* Knowlt. et Cock. (*S. laevigata* Lesq.), which has integral leaves with three main veins and a well-defined secondary and tertiary venation (Krishtofovich, 1938).

There are a number of transitional types between the form of *Cissus primaeva* Sap., which has a broad ovoid leaf blade, and forms with elongated lanceolate, apparently, triple leaves, provided with small denticles along the edges (for example, *Cissus ampelopsidea* Sap. From the Lower Eocene of France); such leaves are similar to the leaves of some species of *Ampelopsis* and *Cissus* (Figure 2). In Russia, apart from the Pacific coast, no fossils belonging to the genus *Cissus* have been found to this day. On the western coast of Sakhalin, *C. spectabilis* Heeg was found, which had ovoid, slightly cordate, uneven finely toothed leaves and well-defined venation. Findings of representatives of the genus *Cissus* on Sakhalin A.N. Krishtofovich tend to be Cretaceous (Krishtofovich, 1938).

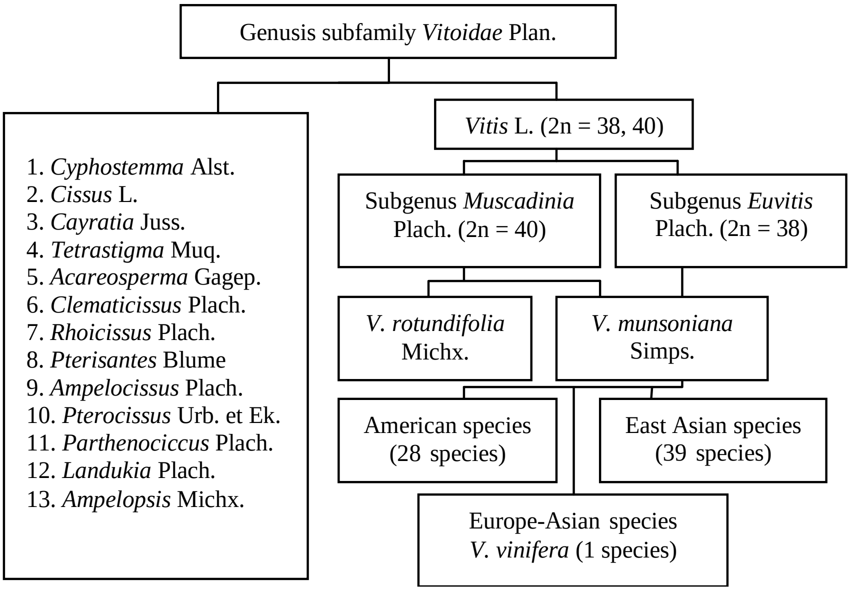


Source: Negrul (1946).

Figure 2. Fossil leaf *Cissus primaeva* Sap. (top) and a leaf of modern *Cissus tomentosa* Lam (bottom).

Other representatives of the family of grapevines, having complex leaves and attributed to the genus *Ampelopsis* Michx. and *Parthenocissus* L., are described in the fossil state much less frequently. This is because the preservation of leaves completely in the complexity of their form is possible only in rare cases. Finding the same lobules of a leaf cannot lead to a correct definition, and the leaves or their imprints found in this state can be taken for completely different plants. From the Eocene sediments of Wyoming (the Grinriver suite), *Ampelopsis tertiaria* Lesq was described with leafy leaves, with four or five elongated elliptical denticles, reminiscent of the modern “wild grapes” of North America, *Parthenocissus quinquefolia* Michx.

In Europe, the genus *Tetrastigma* Planch, existed before the upper Oligocene, as shown by Kirchheimer’s studies in Germany) in Saxony (Kirchheimer, 1939). Finally, recently, the *Tetrastigma shantungensis* Hu et Chaney has been described from the Miocene flora of Shanwan in Shandong Province, China. The modern classification of the subfamily *Vitoideae* Planchon is as follows (Figure 3) (Planchon, 1864).

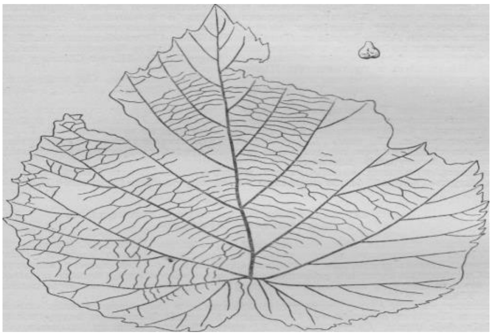


Source: Planchon (1864).

Figure 3. Classification of the subfamily *Vitoideae* Planchon.

2.4. The History of The Genus *Vitis* And The Modern Preserved Forms

The appearance of the first representatives of the family *Vitaceae* belonging to the genus *Vitis*, undoubtedly must be attributed to the Upper Cretaceous period, when there were already types of plants very similar in leaves with vines. The absence of reproductive organs and, in particular, of seeds does not allow to carry on the plants classification. However, in many cases it’s possible to have complete confidence in their belonging to the genus *Vitis*.

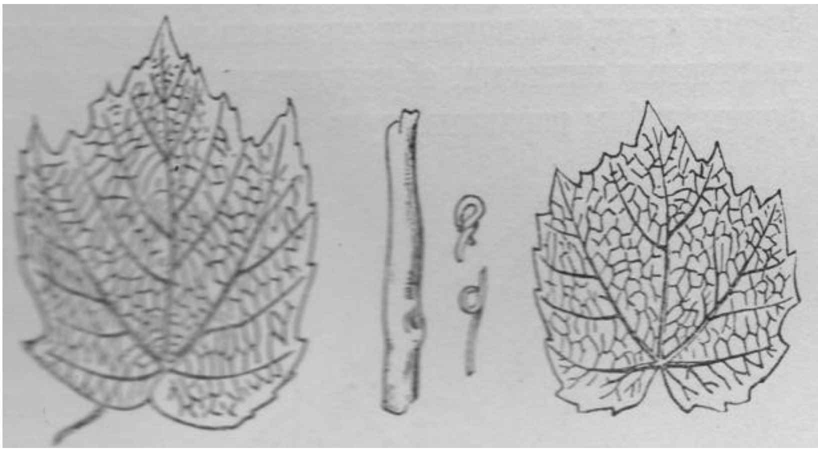


Source: Negrul (1946).

Figure 4. Leaf and seed of *Vitis Olrikii* Negev.

In the Eocene epoch, representatives of the genus *Vitis* were widely distributed not only in Eurasia, but also in the Far North. At this time in West Greenland, near Atanikerdliuk, in the community with bird Scott (Primus Scottii Neeg), a special species of grape, *Vitis arctica* Neeg, was grown, which is found in leaf and seed form. Acording to Negrul (1946), in the paleogene (of the Northern Hemisphere, *V. Olrikii* Neeg was very widespread - from western Greenland to northeastern Siberia (the Anadyr River), Kamchatka, Sakhalin, Alaska, British Columbia, Canada and the United States (in the south to the states of Colorado and New Mexico) (Figure 4).

The oldest authentic traces of the existence of the grapevine of the genus *Vitis* are known from the Palaeogene of Southern Europe. In the Lower Eocene of France, on the Marne, in the traverses of Cezanne, the undisputed remains of the grapevine were discovered by the geologist of Munier-Chalmas; here, not only the remains of leaves were found, but also the remains of stems and antennae of grapevines. The species has been extensively studied and described by Saporta (1888), called *Vitis sazannensis* Sap. (Figure 5), and it turned out that it is represented in Sezana with two subspecies: *V. Dutaillyii* Mun.-Chalm., having larger leaves with sharp teeth curved inwards; *V.Balbianii* Lem. - with a shorter leaf with blunt teeth. *Vitis sazannensis* Sap. is the oldest European species of the genus *Vitis*, represented by vegetative organs. In the Paleogene of the Far East of Russia, one of the best preserved fossil grape species, *Vitis sachalinensis* Krysht, was found and described in the sediments of Sakhalin Island, having a leaf blade broadly ovate with five main veins and strongly branched secondary and tertiary veins forming a frequent network with cells of various shapes; teeth unequal, bluntly pointed (Krishtofovich, 1938). In general, this species is also close to *Vitis Heeriana* Knowlt. (*V. crenata* Neeg) from Alaska.



Source: Negrul (1946).

Figure 5. Leaves, shoots and tendrils of *Vitis sezannensis* Sap.

Only from the second half of the Tertiary period, or from the Neogene, in Europe, were find a number of forms that are of closest interest, characterizing the stage of development of the genus *Vitis* in Europe before the emergence of modern races of the grapevine.

Very common in Eurasia is *Vitis teutonica* A. Br. The leaves of this grapevine are deeply lobed, asymmetric, three- and five-sided, long-petioled. On young shoots, the leaves are generally unequal, rounded in shape, with long pointed teeth. Flowers in truncated inflorescences - on short, thickened pedicels. Sometimes there are mummified berries and pear-shaped seeds, about 5 mm long. In the northeast the *V. teutonica* area reached the Irtysh River basin in Russia, where the easternmost location of this species is known (Krishtofovich, 1938).

The oldest type of Miocene forms of grapes in France is *Vitis sequanensis* Sap., known from the tuffs of Versailles of the Lower Miocene in the Department of Upper Sona. The leaves of this grapes have a rounded shape and angular blunt-toothed edges reminiscent of the grapes of the south of North America, related to *Vitis rotundifolia* Michx. (Florida) and belonging to the *Muscadinia* section. In the Upper Miocene sediments in the department of Ardeche (France) on Mount Sharre a special type of *Vitis praevinifera* Sap. was discovered (Saporta, 1888). It has a deep-oval, trilobate, with five veins leaf plate with large sharp teeth bent forward, and a slightly heart-shaped base. This Miocene type is very close to the wild, modern race of grapevine from the same region (Oevena). It has only the middle lobe of the plate more narrowed than it is observed in the modern form, defined as *Vitis cebannensis* Jord. *Vitis praevinifera* Sap. was first indicated for the territory of Russia by Krishtofovich, but the remains of it are represented by only one sheet, which does not allow to be sure of the identity of the plant with the French one (Krishtofovich, 1938).

In the Pliocene epoch, *Vitis teutonica*, akin to the American species of grapes, is still a prominent representative of grapes in Europe, and appeared in Europe, as already noted, from the upper Oligocene. However, from the Middle Pliocene in Europe, the shape of the wild grape is widely spread, and it still exists in Europe and West Asia namely *Vitis sylvestris* Gmel (Reid and Reid, 1911). In the middle and lower sediments of Holland (in Revere) and then in Poland, seeds of the same type were found in the Pliocene. They are described by Reid (Reid and Reid, 1911) initially as *Vitis orientalis* Boiss., but at present they are referred to as *Vitis Ludwigii* A. Braun. Seeds of *Vitis Ludwigii* are similar to the seeds of the modern species of *Vitis rothundifolia* Michx. (from the *Muscadinia* section) from southern Florida and Carolina.

Close to *V. Teutonica* species *V. Braunii* Ludw., characterized by the roundness of the outline of the leaves, with broader and shorter lobes and the presence of radial grooves near the prominent nodule of the chalaza, appearing even in the Miocene of Salzgauzen and Rokenberg. It also belongs to the grapevines of the American species (*Aestivales* and *Cordifolia-Ripariae*), and thus, together with *V. teutonica* and *V. Ludwigii*, reaches the upper Pliocene where *V. sylvestris* or its immediate ancestor already appears. But the first named species is where they find their end, not passing into the Quaternary period, when in Europe there are only representatives of the species *V. sylvestris* and *V. vinifera* (Negrul, 1946).

2.5. Modern Systematics of The Genus *Vitis*

The formulated N.I. Vavilov theory about the centers of origin of plants, which is the scientific development of the theory of evolution, is closely associated with the processes of classification, the establishment of the site of individual biological, in particular plant, objects in the general aggregate of the living, considered as a system. The foundations of the domestic classification of the *Vitaceae* family were laid by A.M. Negrul, who continued to develop the theoretical heritage of Vavilov. Practical implementation of the approaches to the classification of grapevines in a harmonious unified system became possible due to the study of a greater diversity of varieties of the species *Vitis vinifera* in one place on a single ampelographic collection of the Institute “Magarach”. Over the period of more than 190-year existence, the employees who worked for the collections made a significant contribution to the development of the systematics of *Vitis vinifera sativa*. Such an extension of the classification was undoubtedly the research carried out by P.M. Gramotenko, L.P. Troshin and other scientists at the end of the 20th century (Figures 6 and 7). At present, this trend of research continues in the breeding, genetics and ampelography department of the National Institute of Viticulture and Winemaking “Magarach” on the taxonomy of grape varieties of the eco-geographical groups on the signs of leaf and seed.

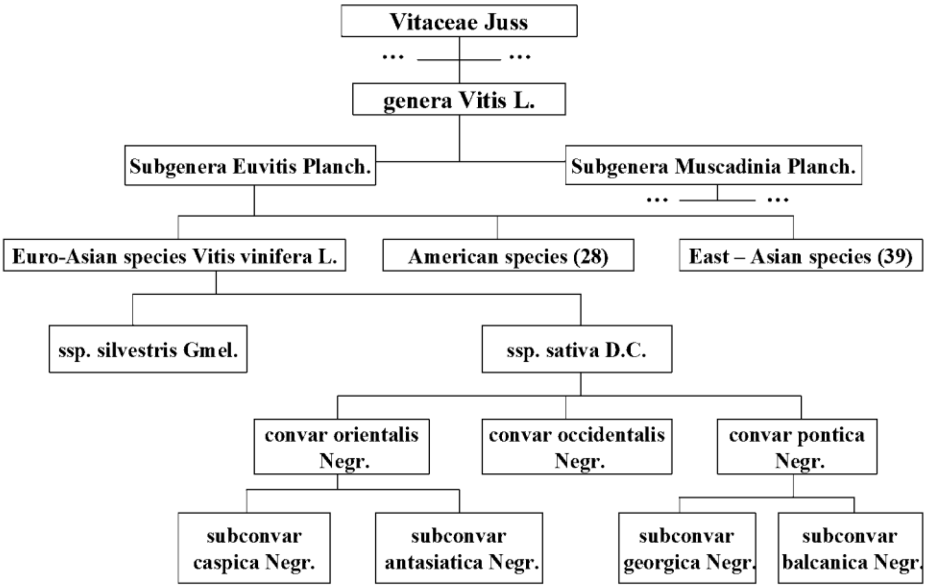


Figure 6. Grapevine classification suggested by A.M. Negrul (1946).

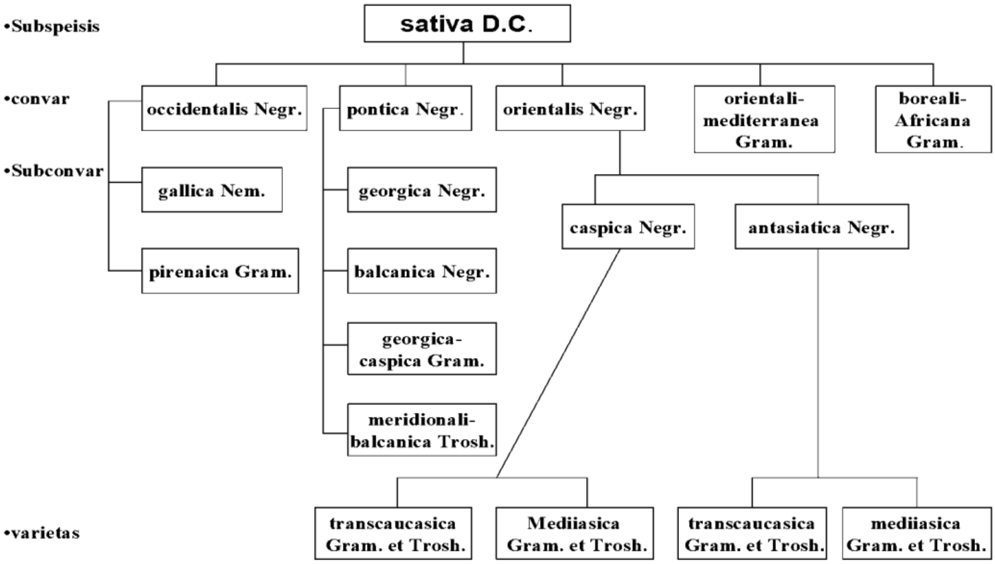


Figure 7. Classification of *Vitis vinifera* ssp. *sativa* D.C. updated in Institute "Magarach".

2.6. Prospects for Grapevine Taxonomy

Botanical diversity of grapevines, reflected in the classification and taxonomy of varieties and forms of the family *Vitaceae* L., reflects the process of natural evolution, natural and artificial selection. Scientists and naturalists in various countries of the world in the 18-20th centuries carried out a grandiose and laborious work, which made it possible to differentiate representatives of the *Vitaceae* L. family of botanical taxa. Studies have established that all this botanical diversity of grapevines was formed in various centers of origin. Depending on the abiotic conditions in the centers of origin, a genome developed in the forms of individual botanical taxa, in particular within genera and species differentiated in resistance to abiotic stress factors. Biotic conditions in these same centers of origin developed a genome in the forms of the same individual botanical taxa, within the genera and species of grapes, as a host plant, differentiated in resistance to biotic stress factors and the pathogen gene in the process of conjugate evolution. In the final result, samples of grapevines were formed in each separate center of origin, differentiating not only on botanical grounds, but also on a set of biological characteristics, in particular, resistance to biotic and abiotic stress factors of the biosphere.

Studying the origin and speciation in grapevines, it is established that all the centers of origin of groups of individual species are in different latitudes only on the northern hemisphere of the planet. They can be designated as follows: East Asia with subcentres in different latitudes; Eurasia (Europe and Central Asia), Asia Minor and North Africa, Southern latitudes of North America and Northern latitudes of North America. However, grape varieties have been successfully introduced to the countries of the southern hemisphere, where they are industrially cultivated and bear fruits, which ensures the production of table grapes and high-quality wine.

Although samples of botanical species belonging to different genera of the *Vitaceae* L. family have survived to this day. Species of the genus *Vitis* L., belonging to the subgenus Euvitis, and to a lesser extent related to the subgenus Muscadinia, are most common on the planet. In turn, among the species of the genus *Vitis* L. the most widespread form is *Vitis vinifera* L., which belongs to the subgenus *Euvitis*. Samples of grapes belonging to this species come from Eurasia, Asia Minor and North Africa.

Speciation, specifically the formation of the species *Vitis vinifera* L., is directly related to the existence of wild forest grapes, belonging to the relics of Eurasia. The studies carried out at the end of the 20th and the beginning of the 21st century revealed a significant difference, including morphological, morphometric and molecular-genetic markers, between forms of wild forest grapevines from various parts of Eurasia. Consequently, it may be considered necessary to continue these studies in order to isolate in the centers of origin of the grapevines individual foci or subfoci of origin, in which, among other things, there are samples of relict wild forest grapevines and autochthonous varieties that have come from them as a result of natural and artificial selection.

3. AUTOCHTHONOUS AND NATIVE GRAPEVINE SUBSPECIES, VARIETIES AND CULTIVARS OF *VITIS VINIFERA* L. SPECIES OF CRIMEA REGION

3.1. Ancient Subspecies of *Vitis Vinifera* ssp. *sylvestris*

The study of evolutionary transformation of plant organisms involves the collection of samples at different stages of this process and the comparison of these samples with previously collected ones. The change in the structure of the genome, the set of genes that can be observed in the course of evolution, leads to a change in the morphological features. Therefore, as a consequence, at the present stage of evolution of each specific plant species, it is necessary to continue collecting samples that exist at this stage of the temporal evolutionary period (Vavilov, 1926; 1931; 1987; Lyubishchev, 1982). Based on this concept, expeditions were organized to collect wild-growing forms and native varieties of grapevines in the Crimea region.

Crimea located in northern part of Black sea, and from the point of view origins of grapevines the Black sea eco-geographical region is concerns the centre of an culture origin. In 1812, in Crimea, the Nikitsky botanical garden was organized where the first ampelographic collection has been compiled in Russia. In 1828, from structure of the Nikitsky botanical garden, the Magarach school of viticulture and winemaking, further developed to Institute of Viticulture and Winemaking “Magarach” to which the ampelographic collection has departed and organized. Thus Institute “Magarach” is considered the first and oldest scientific institution of Russia in the field of viticulture and winemaking.

The material of the research was samples of the flora of the Crimea, which were identified by morphological features and referred to grapevine classification according to A.M. Negrul (Wulff, 1939). The identified samples were photographed and described in accordance with the requirements for identification of varieties and species of grapevines according to the Descriptor of IPGRI, UPOV and OIV (OIV, 2009). In order to preserve the forms of wild grapes in situ, in places of their natural growth, information on the samples found was transferred to the appropriate services of the reserves (Figure 8).

Source: Volynkin and Polulyakh (2011).

Figure 8. Bunches of the Crimean sample *Vitis vinifera* ssp. *sylvestris* Gmel (Hegi).

Analysis of information about the history of the Crimea region made it possible to distinguish two stages. The first reflects the state of historical processes in the Crimea according to archaeological research before our era, and; the second – in the Middle Ages of our era. According to the main habitats, different peoples were identified. It was natural to assume that it was proved as a result of earlier conducted archaeological studies that the existence of people in the Crimea was accompanied by the development of the grapevine crop. The search for the forms of grapevines, which could be classified as belonging to either *Vitis vinifera* ssp. *sativa* D.C. or to *Vitis vinifera* ssp. *sylvestris* Gmel.

In earlier conducted expeditionary surveys in the Crimea region, samples of wild grapevines were collected, which are centrally stored in a special herbarium of the Nikita Botanical Garden. Analysis of the available data made it possible to establish that to date, there are no collections in the territory of the Crimea where the populations of wild grapevines belonging to *Vitis vinifera* ssp *sylvestris* Gmel. would have been collected. The data of the herbarium indicate that individual samples of grapes in the Crimean forests were periodically collected.

Of the herbarium specimens that have survived to the present day, the samples collected in the years 1885-1886 are the oldest. Later, other collections of samples were carried out in 1901-1917, 1926-1929, 1950-1962, 1972-1982 and 1989-1997. Previously conducted expeditions made it possible to determine the main places in the Crimea region, where one might expect to find wild grapes at the present time. The main areas can be considered forest areas near the cities of Sevastopol, Yalta, Alushta, Sudak and Feodosia. In order to preserve the wild grapes in situ, in places of its natural growth, it was proposed to include it in the list of protected crops in Crimea.

Expeditions for the collection of wild grapevines in the Crimea since 2005 have been undertaken in the regions of the valleys of the Wuchang-Su rivers (Yalta), Ulu-Uzen (Alushta) and the Karadag Nature Reserve (Feodosiya). The greatest number of samples was collected in the catchment basin of the Ulu-Uzen river, which represents the right bank of the Alushta Valley. All collected samples were described and documented on the map of wild grapevines (*Vitis vinifera* ssp. *sylvestris* Gmel.). The found forms with a male and female flower are separated in the ratio 2:1. Samples of grapevines were collected in several high-altitude forest zones and belts from 50 to 700 m above sea level. In the area of the Wuchang-Su River and its tributary Yauzlar, samples of grapes are collected at an altitude of 250-700 m above sea level.

As a result of the works on the classification of *Vitis vinifera* ssp. *sylvestris* Gmel., it was possible to form a scheme of interrelation of individual groups of wild grapevines. Each group of forms isolated in the Crimea region is characterized by specific morphological features. Summing up, we can state that significant results have been obtained in the search for and detection of the genetic diversity of *V.v. sativa* and *V.v. sylvestris* in the Crimea and they are brought into line with previously conducted studies and previously obtained data. All this, taken together, made it possible to describe the state, from our point of view, of the classification of *V. v. sativa* and *V. v. sylvestris* up to subspecies and varietas levels.

3.2. Modern Taxonomy The Grapevine of Crimea Region

Grapevines, as a botanical crop, refer to the oldest species of the Earth's flora. The archaeological and botanical research carried out revealed the most ancient types of grapevines on the whole planet, some of which have been preserved only as archaeological finds, and some have survived to our time as relic vegetation in various centers of its origin on the Planet (De Lorenzis et al., 2015).

Studying the origin of grapevines, scientists come to the conclusion that there are separate centers of origin in which one, there are certain types of grapevines (This at al., 2006; Forni, 2012). These include East Asia, Central Asia, the Middle East, North Africa, Europe, northern North America and southern North America (Péros et al., 2011). The most common Eurasian species of grapes is the species *Vitis vinifera* L. (Musayev et al., 2015).

Studying the samples of grapevines attributed to the species *Vitis vinifera* L., the scientists conducted their systematic and established that within this species, subspecies can be distinguished (Zdunić et al., 2017). In particular, the ancient forest grapes *Vitis vinifera* ssp. *sylvestris* Gmel. (Kullaj et al., 2012; Ocete et al., 2015; Pipia et al., 2015) and another, combining all the cultivars of this species that were selected as a result of artificial selection, *Vitis vinifera* ssp. *sativa* DC. (Biagini et al., 2012; Doulati Baneh et al., 2015).

The Institute "Magarach" carried out research on the study and taxonomy of samples of grapevines attributed to the species *Vitis vinifera* L., which were selected as a result of expeditions in the Crimea, as a specific eco-geographical area for grapevines. Some of the results of these studies were presented earlier by several authors (Volynkin et al., 2012; Volynkin and Polulyakh, 2011; 2015) and a continuation of which is reflected in this chapter.

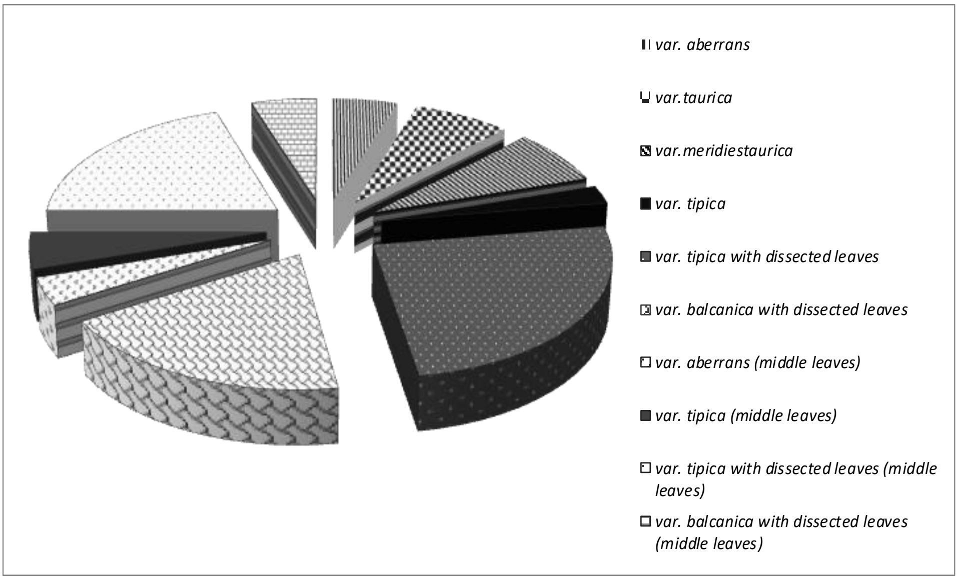
The material for research was 160 samples of grapevines selected in the forests of the Crimea, which were assigned to the subspecies *Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi) and 80 native grape cultivars of Crimea on ampelographic collection of the "Magarach" Institute. Search and selection of samples of wild forest grapevines of Crimea was carried out in places of its natural habitat. Samples of grapevines were collected in several high-altitude forest zones from 50 to 700 meters above sea level - in the Valley of the Uchan-Su river (in Yalta) and in the basin of the catchment of the Ulu-Uzen river (Alushta), representing the right bank of the Alushta Valleys. As a result, 160 different forms of *Vitis vinifera* ssp *sylvestris* Gmel. (Hegi) were identified, which were collected and planted on the ampelographic collection "Magarach", and later served as a material for research. Local cultivars of grapevines were isolated from the old plantings of the Southern coast of Crimea - the Yalta, Alushta, Sevastopol regions, but most (more than 60) cultivars were isolated from the old vineyards of the Sudak region.

In order to differentiate 80 different Crimean cultivars according to the basic morphobiological features, 84 features were identified that were previously identified as taxonomically significant for the identification and certification of cultivars of the eco-geographical group of the Black Sea basin *V.v. pontica* Negr. (Volynkin and Polulyakh, 2015). To differentiate the forms of wild grapes *Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi), 30 features of the leaf were selected. The description, identification and classification of samples of wild grapevines and native cultivars of Crimea was carried out according to generally accepted international methods (Schneider et al., 2015; Benito et al., 2017; Zdunić et al., 2017), including the OIV Descriptor (OIV, 2009).

Every crop plant originated from a wild ancestor, according to which it is possible to determine the place of its origin and trace its evolution. Wild relatives of crop grapevines belonging to the species *Vitis vinifera* L., occupy a large area of Eurasia. In each region, under the influence of local conditions, a variety of native cultivars developed: by selecting from wild vines or by importing cultivars that quickly crossed with native, previously selected cultivars. Since Crimea is one of the recognized foci of the primary origin of grapevines, the identification and study of wild forms and native cultivars is very important for clarifying issues of origin, the formation of local assortment and the evolution of culture as a whole.

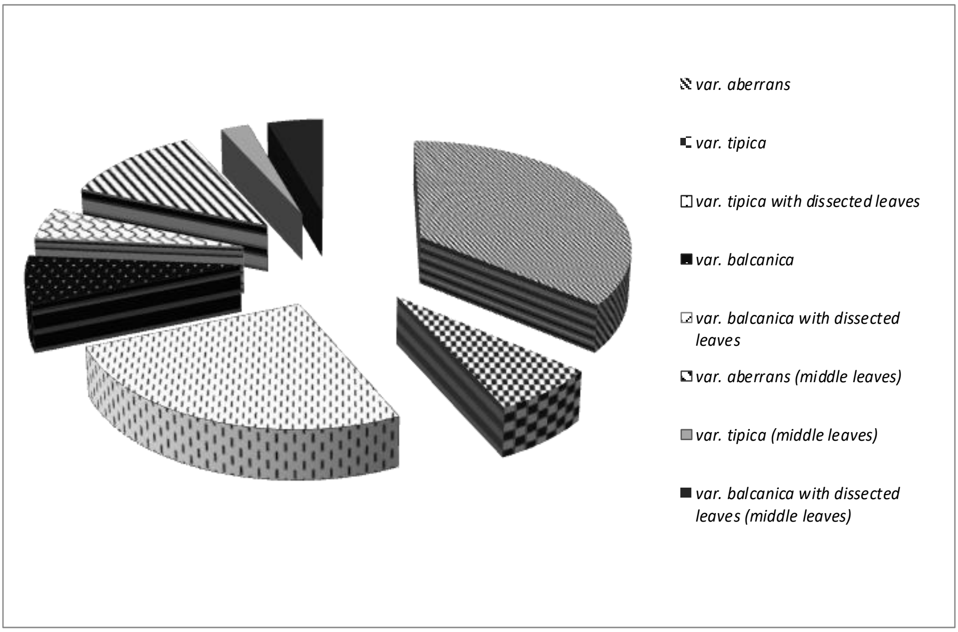
Study of morphological features of forms of wild forest grapes *Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi) populations of Yalta and Alushta, isolated in two different habitats, showed that all the selected forms belong to *Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi), the main distinguishing feature of which is dioeciousness, determined by the presence of plants with true male and female flower types (in the commercial grapevine cultivars of the subspecies *Vitis vinifera* ssp. *sativa* D.C., the flower type is bisexual or functionally female). It is established that the forms with the male type of flower are in a ratio of 3:1 with plants that have a female type of flower. As a result of a cluster analysis of *Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi) on 30 signs of an adult leaf, it was established its differentiation. In the Yalta population (Figure 9), varieties are identified of *Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi), which correspond to the groups described earlier in the study of the wild forest grapes of the Crimea: var. *aberrans* Negr. (5% of the total number of population forms); var. *taurica* Bol. et Mal. (7.5%); var. *tipica* Negr. (2.5%); var. *tipica* with dissected leaves Bol. et Mal. (25%); and var. *balcanica* with dissected leaves Bol. et Mal. (18.75%).

In a population of Alushta (Figure 10) are marked species: *Vitis vinifera* ssp. sylvestris var. aberrans Negr. - 35.7%; var. tipica Negr. - 7.2%; var. tipica with dissected leaves Bol. et Mal. - 26.2%; var. balcanica Negr. - 7.2% and var. balcanica with dissected leaves of Bol. et Mal. - 4.7%. In addition to these groups, varieties with a larger leaf within var. *aberrans*, var. *tipica*, var. *tipica* with dissected leaves and var. *balcanica* with dissected leaves groups are also distinguished in the Yalta population. The number of which from the total number of forms of the population of Yalta was 3.75; 5; 20% and 5%, respectively. In the Alushta population, varieties with a larger leaf are distinguished within the var. *aberrans*, var. *tipica*, and var. *balcanica* with dissected leaves groups, the number of which was 11.9; 2.4 and 4.7%, respectively. These forms, on the basis of the dissection of the leaf and the pubescence of its lower surface, are identical with the basic varieties, but differ from them by the size of a leaf, that exceeds the main varieties by 2-3 times; the shape of the upper cuttings and; the shape of the petiolate cavity.



Source: Volynkin et al. (2019).

Figure 9. Yalta population of Crimea *Vitis vinifera* ssp. *sylvestris* Gmel (Hegi).

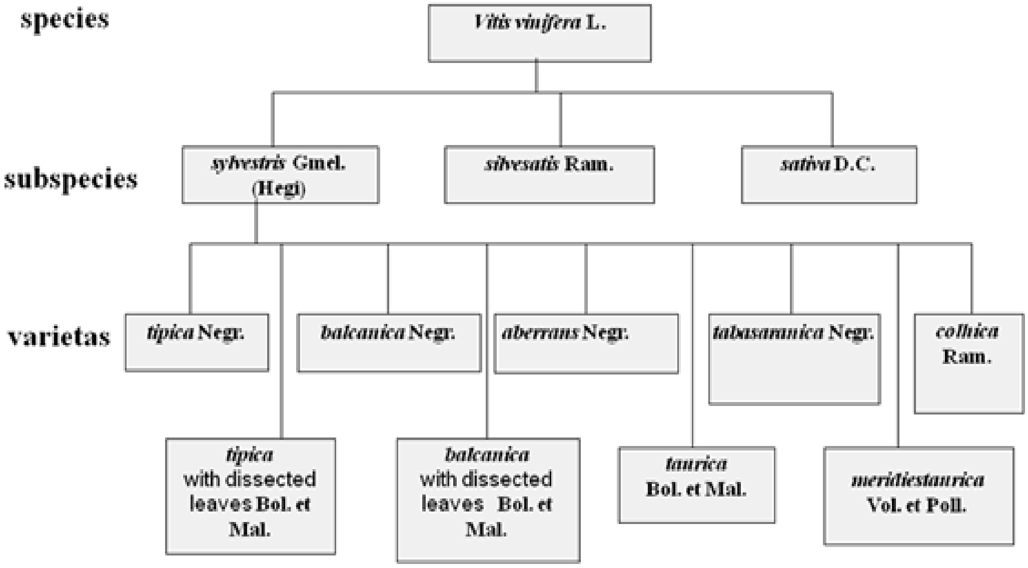


Source: Volynkin et al. (2019).

Figure 10. Alushta population of Crimea *Vitis vinifera* ssp. *sylvestris* Gmel (Hegi).

According to the described features, the isolated varieties with a larger leaf are similar to the cultivars of different eco-geographical groups. A significant group is represented by forms that are close in a number of features of the leaf to cultivars of the West European eco-geographical group: var. *aberrans*, var. *tipica*, var. *tipica* with dissected leaves. Forms belonging to var. *balcanica* with dissected leaves, have a great similarity with the cultivars of the eco-geographical group of the Black Sea basin. In addition, only the presence of dioecious plants in isolated groups with large leaves testifies to their true belonging to the wild forest grapes *Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi). Each group can be considered as an intermediate between known varieties of wild grapes and eco-geographical groups of cultivars. In general, this confirms the view of R.M. Ramishvili (Volynkin et al., 2019) on the existence of an intermediate between *V.v. sativa* and *V.v. sylvestris* group *V.v. silvesatis*.

In the population of *V.v. sylvestris* Yalta identified the forms, which by the studied features are defined as a species of *Vitis vinifera* ssp. *sylvestris* var. *taurica* Bol. et Mal. (Figure 11). However, within the limits of this group, plants with a dark green color of the upper surface of the leaf, more pronounced upper cuttings (shallow, arched, with pointed or rounded bottom) and the shape of the petiolate recess (wide open, pointed, with a bottom, limited veins). The ratio of the amounts of identified forms in the varieties of var. *taurica* is the same, and in total they make up - 7.5% of the total population of Yalta. The established differences give grounds for isolating an additional variety of *Vitis vinifera* ssp. *sylvestris* var. *meridiestaurica* Vol. et Pol. and to provide a classification of the grapes *Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi) of the Crimea (Figure 11).



Source: Volynkin and Polulyakh (2015).

Figure 11. Modern classification of the relict wild forest grapes of Crimea *Vitis vinifera* ssp. s*ylvestris* Gmel (Hegi).

Thus, as a result of studying the native forms of the Crimea, *Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi) it is established that in the Crimea varieties of wild grapes are found that are unique to this region, which can be attributed to relics and endemics. This statement gives grounds to consider this region (Crimea) as an independent sub center of origin of the grapevines.

Each vineyard district has its own native cultivars that have existed since time immemorial. The obtained differentiation of 80 native cultivars of Crimea according to the complex of 84 ampelographic features (Figure 12) testifies to their belonging to different eco-geographic groups: the Black Sea basin, the Western European and Eastern (Figure 13). As can be seen from the data obtained, about half (45%) are the cultivars of the Eastern eco-geographic group, 38% are the cultivars of the eco-geographical group of the Black Sea basin, and 17% are the cultivars of the West European eco-geographical group. Since the Crimea belongs to the Black Sea basin, it should be assumed that all native grape cultivars should belong to this eco-geographical group. However, the presence of cultivars belonging to other groups indicates that some of them do not originate from native wild grapevines.

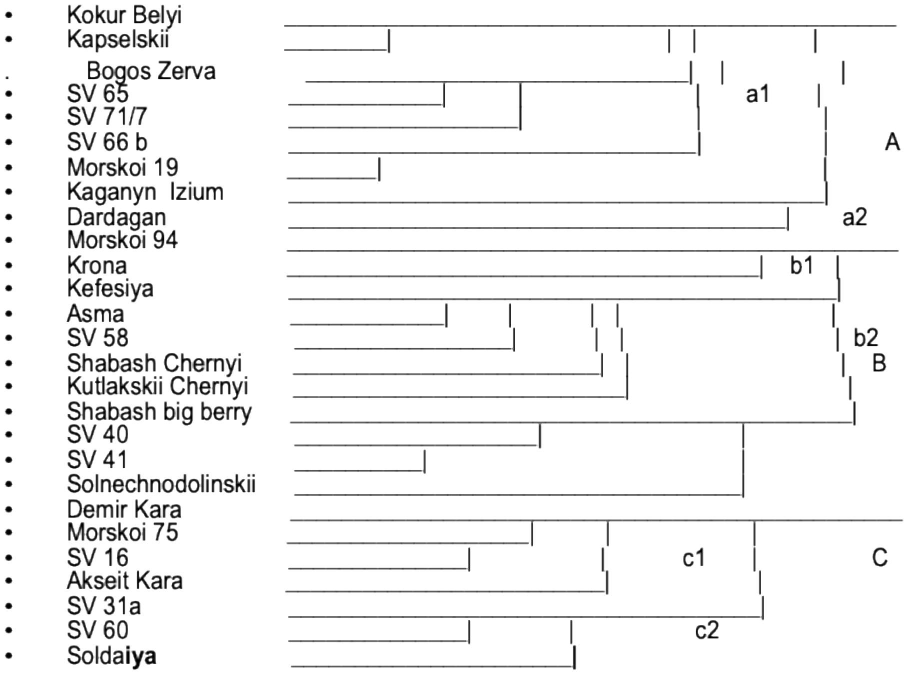
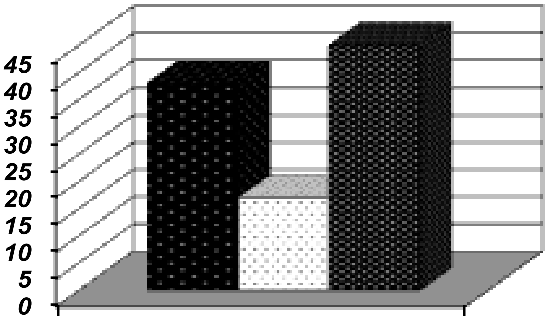


Figure 12. Systematic of native grape cultivars of the Crimean (SV - Sun Valley). Source Polulyakh and Volynkin (2005).

Archaeological evidence indicates that the grapevines were known to the local tribes of the mountainous Crimea long before the arrival of the Greeks in the tenth century BC. At first, wild grapevines were used in the culture, and later, with the development of the ancient Greek colonies on the Crimean coast in VII-VI centuries BC, new cultivars were imported. This is evidenced by archaeological data: all the seeds found from the excavations of wineries and other places belonged to cultivated cultivars or wild grapevines of the species *Vitis vinifera* L. There is a reason to believe that the primary formation of native cultivars occurred on the basis of selection of wild grapes of the Crimea. The process of forming cultivated cultivars of the Crimean grapevines is very complex. It is unlikely that only natural selection played a decisive role here, and artificial selection and hybridization of truly native and imported cultivars had a big impact.

It is possible that during the decay of viticulture in the Crimea, the cultivated grapevine cultivars were lost, and with the change of the dominant culture, new cultivars were introduced that acquired new names on the spot. Columella also wrote that “every district, every corner of it has grape cultivars peculiar to it, to which it gives its name. The cultivars transferred to other regions get their names there and change their quality to such an extent that sometimes they cannot be recognized” (Forni, 2012). A number of authors believe that many native cultivars of the Crimea are imported from Europe and Transcaucasia. Quite a lot of native cultivars of the Sudak region after careful study of their Sushkov and Katz (Volynkin et al., 2019), were in most cases Western European cultivars. It is known that about 40 cultivars were imported by the Genoese and the Turks. So, for example, the cultivar ‘Kovalevka’, according to its morphological features, has a similarity to the cv. ‘Gamay black’ or is a seedling of one of the representatives of the ‘Gamay’ group. Cultivar ‘Dardagan’ has a similarity with the Palestinian cultivars introduced into the Crimea by Tartar pilgrims. Cultivars of ‘Sary Kokur’, ‘Sary Pandas’, “Kokur belyi’ and ‘Kandavasta’ originate from Greece, as evidenced by their Greek names.



	1
■ *Vitis vinifera sativa convar. pontica Negr.*	*38*
□ *Vitis vinifera sativa convar. occidentalis Negr.*	*17*
▨ *Vitis vinifera sativa convar. orientalis Negr.*	*45*

Figure 13. Distribution of native grapevine cultivars of the Crimea according to eco-geographic groups. Source: Volynkin and Polulyakh (2015).

Our differentiation of 80 Crimean grapevine cultivars was performed into three separate groups, which, according to ampelographic characteristics, correspond to the cultivars of three eco-geographic groups (the Black Sea basin, Western European and

Eastern), once again confirms the hypothesis about the origin of the Crimean cultivars from different regions of the formation of cultural grapevines (Volynkin et al., 2019; 2020). Comparison of the data of a number of researchers of morphobiological features of the cultural native grapevine cultivars of Crimea and identified varieties of *Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi) allowed drawing some parallels and proving that a number of native cultivars were selected by a man in antiquity here, in place, in the Crimea from a natural forest fund. Basically, these are the cultivars of wine used: ‘Kovalevka’, ‘Khersonesskii’, ‘Lapa-Kara’, ‘Kastel Chernyi’, ‘Ekim Kara’, ‘Chernyi Krymskii’, ‘Djevat Kara’, ‘Kefesiya’ and others. Thus, for example, ‘Manjil Al’, according to old-timers, was introduced from the thickets of wild grapevines, which has been preserved till now on the slopes of Mount Manjil on the site of the former ancient Greek colony. Cultivar ‘Khersonesskii’, considering several morphological features, is similar to wild grapevines of Crimea and distributed in a limited area, covering the area adjacent to Sevastopol.

According to a number of researchers, the proof of the local origin of grapevine cultivars is their similarity in a number of morphological characters with wild forest grapevines growing in this region, which indicates the existence of a common ancestor from which, in the course of evolution, formed two subspecies.

3.3. Conclusion About Crimean Grape Taxonomy

Studies conducted by many scientists indicate the origin of the commercial grapevine cultivars (*Vitis vinifera* ssp. *sativa* DC.) comes from the forms of wild grapes (*Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi)). Our studies of autochthonous wild forest grapes form of Crimea *Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi) from various areas of Crimea on 30 signs of an adult leaf has allowed to reveal the varieties, previously described and to allocate an additional variety of *Vitis vinifera* ssp. *sylvestris* var. *meridiestaurica* Vol. et Pol. The differentiation of 80 native cultivars of the Crimean grapes along a complex of 84 ampelographic signs indicates the belonging of the native Crimean cultivars of the *Vitis vinifera* ssp. *sativa* DC. to different eco-geographic groups of Eurasia. The obtained results confirm the hypothesis about the origin of cultivars of the Crimean grapes from various regions of the formation of commercial grapevine cultivars. However, a comparison of the morphological signs of some of the native cultivars of the Crimean grapes and the previously identified varieties of *Vitis vinifera* ssp. *sylvestris* Gmel. (Hegi) proves that a number of native cultivars were select by man in antiquity in the Crimea from the natural forest fund and are truly autochthonous. Thus, the existence of relic endemic forms of autochthonous wild grapes at the present time in Crimea, the existence of transitional forms that are considered to be an intermediate link between wild grapes and cultivars, the availability of autochthonous cultivars that have originated from wild forest grapes of

Crimea, makes it possible to consider this region as an independent sub center origin of the commercial grapevines, which is valuable for studying the evolution of culture.

3.4. Modern Native Grapes Cultivars of Crimea: The Agrobiological Characteristics and Use in Winemaking

In the process of evolution, native cultivars of Crimea developed the properties to grow and produce good quality crops in the conditions of arid climate on poor rocky soils and on soils with a high level of salinity and liming (Ivanov, 1947; Polulyakh, 2007). Changes of climate on our planet lead to the modification of adaptability of plants to the effects of biotic and abiotic environmental factors. In its turn it is expressed in changes of phenology, agrobiology and crop quality parameters (Polulyakh and Volynkin, 2005; Ayba et al., 2014; Levchenko and Vasylyk, 2015; Likhovskoi et al., 2016; Polulyakh et al., 2017). We studied native grapevines cultivated in the eastern region of South Coast zone of Crimea viticulture (collection and production vineyards of “Solnechnaya Dolina” in Sudak) in the areal of their natural growth with the existing spectrum of grapevine cultivars developed over a long time.

The studies of agrobiological parameters, economically valuable traits, as well as the calculation of the economic efficiency of the cultivation of native grape cultivars were carried out in accordance with generally accepted methods (Guidelines for agricultural research in the viticulture of Ukraine, 2004). Methods of mathematical statistics were also used to process the primary material (Tukey, 1981). According to the scale of productivity of grape cultivars it was established that its level by the parameter of wet raw bunch weight in cultivars ‘Gevat Kara’ (167.2 g/shoot) and ‘Kokur Belyi’ (180 g/shoot) is characterized as average and do not significantly differ from the control. In cv. ‘Krona’, the parameter of shoot productivity is very poor. Low level of shoot productivity in the range from 147 g/shoot (cv. ‘Kapselski’) to 75.5 g/shoot (cv. ‘Kefesiya’) was noted in all other native cultivars under study. The highest crop yield among the black-berried cultivars belong to cvs. ‘Gevat Kara’ (62.2 c/ha) and ‘Cabernet Sauvignon’ (58.7 c/ha). In the group of white-berried cultivars the highest yield was observed in ‘Kokur Belyi’ cultivar (48.9 c/ha). For the weight of the bunch, all the studied black-berried cultivars are inferior to the control cv. ‘Cabernet Sauvignon’ (176.9 g) and cv. ‘Gevat Kara’ (177.9 g). In the group of white-berries, the cultivars ‘Kapselski Belyi’, ‘Solnechnodolinskii’, ‘Kokur Belyi’ and ‘Rkatsiteli’ do not differ from the average weight of the bunch. During the onset of technological ripeness, with almost same total sugars from 20.6 to 22.1 °Brix, the content of titratable acids significantly decreases from 7.5 to 8.4 g/100 cm^3, in black cultivars compared to the control (9.7 g/100 cm^3). In white-berries cultivars, the sugar content significantly exceeded their concentration in the control cultivar ‘Rkatsiteli’ (20.0 °Brix), except in ‘Shabash’ cultivar (19.7 °Brix). Similar difference, but in direction of decreasing the index of titratable acidity from 6.8 to 7.7 g/100 cm^3, was observed in all studied cultivars in comparison with the control (10.0 g/100 cm^3), except for the same cultivar 'Shabash' (10.2 g/100 cm^3).

Due to the fact that protective measures were taken on the breeding closes, only a comparative analysis of the resistance of cultivars was carried out. The cultivars 'Gevat Kara', 'Ekim Kara', 'Kefesiya' and 'Krona' were less effected by fungal diseases. Despite the complex of measures of chemical protection, a strong progression of fungal diseases was observed on susceptible cultivars 'Kokur Belyi', 'Sary Pandas' and 'Kok Pandas'.

It is known that the higher the parameter of the structure (the ratio of the weight of berries to the weight of the stems), the higher the economic value of the cultivar. To determine this parameter during the study period, the mechanical composition of the crop was studied. Content of pulp and juice in berries differs between cultivars, from 82.6 to 91.6%. The highest content of pulp and juice in berries was observed in 'Kapselski Belyi' cultivar (91.6%). The highest structural parameter was observed in cultivars 'Kefesiya' (46.7%), 'Gevat Kara' (33.4%) and 'Krona' (32.2%), the lowest - in the cultivar 'Ekim Kara'- 22.3.

Main parameters characterizing the economic value of the cultivar are: crop yield, cost of production, net income of the product obtained and level of production profitability. According to the indexed calculation of the above parameters, all native cultivars are profitable. Due to the low yield and high net cost of the cultivated grapes, the cultivars 'Ekim Kara' and 'Kefesiya' have a low profitability of 33.2%. The most profitable cultivars are 'Gevat Kara' (273.1%), 'Kokur Belyi' (144.6%), and 'Kapselski Belyi' (122.0%).

The most productive cultivars are 'Gevat Kara' (62.2 c/ha), 'Kokur Belyi' (48.9 c/ha) and 'Kapselski Belyi' (44.4 c/ha). Cultivars 'Kefesiya' (22.2 c/ha), 'Ekim Kara' (22.2 c/ha), 'Kok Pandas' (24.4 c/ha), 'Krona' (28.9 c/ha) and Sary Pandas (28.9 c/ha) have low crop capacity due to their functionally female type of flower. It was found that the highest level of productivity among native cultivars of Crimea is typical for the cultivars 'Gevat Kara' (167.2 g/shoot) and 'Kokur Belyi' (180.0 g/shoot). Analysis of the mechanical composition of the bunch showed that the highest parameter of structure is observed in cultivars 'Kefesiya' (46.7), 'Gevat Kara' (33.4), 'Krona' (32.2) and the lowest in cultivar 'Ekim Kara'(22.3). These features of native grape cultivars were factors in the historically established traditions of the authentic winemaking of the Crimea. A major role in discovering the winemaking potential of Crimean native cultivars was played by the employees of the Institute "Magarach" A. A. Ivanov, A. A. Preobrazhensky, K. S. Popov, M. I. Vdovkin and J. F. Katz. Thanks to their research, the valuable qualities of the cultivars 'Sary Pandas', 'Sary Habah', 'Christopher Sary', 'Kokur Belyi', 'Ekim Kara', 'Kefesiya', 'Lapa Kara' and 'Sale Aga' and others were established.

Traditionally, Crimean native cultivars are used to produce premium class liquor wines, the technology of which involves insisting or fermenting pulp, stopping the fermentation of must/pulp by adding grape or ethyl alcohol, and long-term aging in oak barrels. The historical leader in the production of liquor wines from native cultivars is the oldest (since 1888) winery ‘Sun Valley’, located in v. Solnechnaya Dolina. Here, from the old vines grapes (over 35 years old) ‘Ekim kara’ (70-80%), ‘Kefessia’ (15-30%) and ‘Gevat kara’ (5-10%) unique “Black Doctor” liquor is produced - the wine with an actual volume fraction of alcohol of 16% vol., and a sugar concentration of 160 g/L.

For better extraction of phenolic and aroma-forming components from the skin of grape berries, the introduction of alcohol into the fermenting pulp is practiced, followed by exposure of the alcoholized pulp for several days. “Black Doctor” is characterized by a rich ruby color, a developed complex bouquet with berries (prunes, black currants) tones with shades of chocolate, full velvety taste and a long aftertaste. From the native cultivars ‘Sary Pandas’, ‘Kok Pandas’, ‘Solnechnodolinskii’, ‘Kapselski Belyi”, ‘Kokur Belyi’ with the addition of white muscat grape cultivars ‘Gars Levelu’, ‘Furmint’, ‘Pinot gray’ produce the “Solnechnaya Dolina”, white liquor wine with its original taste and bouquet, in which the spicy tones of meadow herbs and flowers are combined with shades of dried fruit, melon. In ‘Sun Valley’ winery native grape cultivars are part of blends (up to 55%) of highly alcoholic (17.5% v/v) long-aging wines. Since 1945, “Massandra” has been producing easily recognizable liqueur wine “Kokur Dessert Sourozh” from ‘Kokur Belyi’. All these wines are highly appreciated by both experts and consumers.

At the same time, modern winemaking in Crimea is focused on expanding the production of dry and semi-dry white wines. ‘Kokur Belyi’ is widely used for the production of these wines. However, due to the indicated chemical and technological features of the native grape cultivars, their production into dry or semi-dry wines, especially monosorted grapes, requires additional measures, both in the cultivation of grapevines and in its processing.

To prevent the oxidation of dry white wines from native cultivars, the same measures are recommended as in the processing of classical ones. Their main task is to limit the extraction of phenolic substances from the solid parts of grapes and to block oxidases of must.

‘Ekim Kara’, ‘Kefessia’ and ‘Gevat Kara’ red grape varieties used for experimental studies were characterized by total sugars ranging from 18.9 to 25.5 Brix, titratable acids (expressed in terms of tartaric acid) from 3.8 to 5.7 g/L, pH from 3.19 to 4.08 and technological reserves of phenolic compounds (with Folin-Ciocalteu reagent) between 954 and 3369 mg/L. Using a variety of methods and parameters of pomace maceration, we assessed the impact of technological factors on formation of the phenolic complex and organoleptic characteristics of dry wines from each batch of grapes. A promising way to improve the technological parameters of grapes of native black and white cultivars should be considered clonal, classical selection.

CONCLUSION

Grapevines as an agricultural crop are known and developed along with the humanity from ancient times BC. However, scientists of the world have always been interested in the question of how the grapevines crops evolved from era to era. At the same time, aspects of the natural evolution of grapevines and experimental evolution were considered, when domestication of wild grapes occurred as a result of artificial selection as an anthropogenic factor. At the same time, in the process of natural evolution, not only the shaping of new species and varieties of grapevines took place, but also the elimination of certain species that survived only as relict forms found in the process of paleontological research but ceased to exist in their natural form in the flora of the planet took place.

This chapter presents material that demonstrates the results of research by Russian scientists in comparison with the results of research by European scientists, showing which species of grapevines once existed in Russia. In parallel, it is demonstrated that some species of grapevine have survived to our time, and which are relict and endemic, in particular in Russia, in the Crimea, growing in the natural habitat of the flora. It is proved that on the basis of these studies, Crimea, on the one hand, can be attributed to the subcenters of the origin of the grapevines, and, on the other hand, grape varieties domesticated from this gene pool are still being cultivated. It is reported that some grapevine varieties are truly autochthonous or as native, as they are used in modern viticulture and winemaking in Russia and Crimea in particular.

ACKNOWLEDGMENTS

We thank Mrs Svetlana Baranchuk and Mrs Maria Ostroukhova for technical translation of the book chapter.

REFERENCES

Ayba, V.Sh., Troshin, L.P., Kravchenko, R.V. (2014). The gene pool of native varieties and introducers of grapes in Abkhazia. *Polythematical Mathematical Network Electronic Scientific Journal of the Kuban State Agrarian University.* 100, 831-842, in Rus.

Benito, A., Munoz-Organero, G., De Andres, M.T., Ocete, R., Garcia-Munoz, S., Lopez, M.A., Arroyo-Garcia, R., Cabello, F. (2017). Ex situ ampelographical characterization of wild Vitis vinifera from fifty-one Spanish population. *Australian Journal of Grape and Wine Research*, 23:143-152. https://doi.org/10.1111/ajgw.12250.

Biagini, B., De Lorenzis, G., Scienza, A., Failla, O., Imazio, S., Maghradze, D. (2012). Wild grapevine (*Vitis vinifera* L. subsp. sylvestris (Gmelin) Hegi) in Italy: distribution and preliminary genetic analysis. *Acta Horticulturae,* 948:211-216. https://doi.org/10.17660/ActaHortic.2012.948.23.

De Lorenzis, G., Simone Di Lorenzo, G.B., Failla, O., Musayev, M.K., Salimov, V., Maghradze, D., Chipashvili, R. (2015). Study of genetic diversity in V. vinifera subs.sylvestris in Azerbaijan and Georgia and relationship with species of the cultivated compartment. *Acta Horticulturae,* 1074:49-53. https://doi.org/10.17660/ActaHortic.2015.1074.6.

Doulati Baneh, H., Mohammadi, S.A., Labra, M., Shafaie, F. (2015). Genetic diversity of wild grape (*Vitis vinifera* spp. *sylvestris*) populations in Zagros forests as revealed by SSR markers. *Acta Horticulturae,* 1082:189-194. https://doi.org/10.17660/ActaHortic.2015.1082.25.

FAO (2014). *Genebank Standards for Plant Genetic Resources for Food and Agriculture.* Food Agric. Org. United Nations (FAO). Rome. URL: http://www.fao.org/agriculture/crops/thematic-siemap/theme/seeds-pgr/gbs/en/.

FAO (2017). *The future of food and agriculture – trends and challenges.* Food Agric. Org. United Nations (FAO). Rome.

Forni, G. (2012). The origin of "Old World" viticulture. In: Caucasus and Northern Black Sea Region Ampelography/D. Maghradze, L. Rustion, J. Turok, A. Scienza and Faila, O., eds. *Vitis - Journal of Grapevine Research.*

Guidelines for agricultural research in the viticulture of Ukraine (2004). Yalta: IViV "Magarach". 264p.

Heer, O. (1878). *S*akhalin primitive fossil flora. Flora Miocene Sakhalin Island. *Memoirs Academy of Science, Sankt-Petersburg, ser. 7, 25 (7): 1-61.*

Ivanov, A.A. (1947). Crimean native grape varieties. *Simferopol,* 79 p, in Russ.

Kirchheimer, F. (1939). Ober die fossilen Reste der Rebengewachse [About the fossil remains of the vines]. W*ein und Rebe*. Mainz, 4:85-105.

Krishtofovich, A.N. (1938). Paleontological history of grapes, *Botanical Journal of the USSR,* M.-L., 23 (5-6):365-374, in Rus.

Kullaj, E., Simon, S., Pejić, I., Çakalli, A. (2012). Molecular characterization of wild grapevine (*Vitis vinifera* ssp. *sylvestris*) populations of Northern Albania. *Acta Horticulturae,* 948:129-133. https://doi.org/10.17660/ActaHortic.2012.948.14.

Levchenko, S.V., Vasylyk, I.A. (2015). A study of Tashly and Shabash populations and selection of highly-productive protoclones. *Development Problems of Regional Agro-industrial Complex.* 2 (22):17-22, in Rus.

Likhovskoi, V.V., Volinkin, V.A., Oleinikov, N.P., Vasylyk, I.A. (2016). Agrobiological and economic assessment of Crimean indigenous grape varieties. *Development Problems of Regional Agro-industrial Complex.* 25, 1-1 (25):44-49. in Rus.

Lyubishchev, A.A. (1982). *Problems of the form of systematics and the evolution of organisms.* - Moscow: Nauka. - 277 p., in Rus.

Musayev, M.K., Akparov, Z.I., Mammadov, S.H.S.H., Kuliyev, V.M. (2015). Results of the study of genetic resources of grapes in Azerbaijan. *Acta Horticulturae*, 1074:15-22.

Negrul, A.M. (1946). *Ampelography of USSR.* V. I, Moscow (in Russ).

Ocete, R., Fevereiro, P., Failla, O. (2015). Proposal for the wild grapevine (*Vitis vinifera* L. subsp. *sylvestris* Gmelin (Hegi) conservation in the European countries. *Vitis* 54:281-282.

OIV (2009). *OIV Descriptor List for Grape Varieties and Vitis Species* (2nd ed.). OIV (Off. Int. Vigne Vin), Paris, France.

Péros, J.-P., Berger, G., Portemont, A., Boursiquot, J.-M., Lacombe, T. (2011). Genetic variation and biogeography of the disjunct *Vitis* subg. *Vitis* (*Vitaceae*). *Journal of Biogeography,* 38:471-486. https://doi.org/10.1111/j.1365-2699.2010.02410.x.

Pipia, I., Gogniashvili, M., Tabidze, V., Kotorashvili, A., Kotaria, N. (2015). Plastid DNA sequwnced diversity in wild grapevine samples (*Vitis vinifera* ssp. *Sylvestris*) from Caucasus Region: complete plastid genome sequences of three Georgian samples. *Acta Horticulturae,* 1082:213-216. https://doi.org/10.17660/ActaHortic.2015.1082.29.

Planchon, J. (1864). *Study of Montpel'e tufa stone from point of view geology and paleontology*. Paris, p. 73.

Polulyakh, A.A. (2007). Adaptive potential of native Crimean grape varieties to extreme winter frosts of 2006. "Magarach". *Viticulture and Winemaking*, 4:5-8, in Russ.

Polulyakh, A.A., Volynkin, V.A. (2005). The study of local varieties of Crimea on a set of ampelographic features. "Magarach". *Viticulture and Winemaking,* 4:4-6, in Russ.

Polulyakh, A.A., Volynkin, V.A., Likhovskoi, V.V. (2017). Problems and prospects of grapevine genetic resources preservation at "Magarach" Institute. Vavilov *Journal of Genetics and Breeding*, 21:6, c. 606-614. https://doi.org/10.18699/VJ17.000.

Polulyakh, A.A., Volynkin, V.A., Zarmaev, A.A., Vasylyk, I.A. (2017). Ampelographic and agrobiological characteristics of Crimean native grape variety Krowna. Magarach. *Viticulture and Winemaking*, 3:12-15, in Rus.

Reid, C., Reid, E.M. (1911). *The lignite of Bovey Travey. Philosophical Transactions of the Royal Society*. London, Vol. 201, p. 161-178.

Saporta G., (1888) *Origine pale'ontologique des arbres cultive's ou utilise's par l'homme* [*Paleontological origin of trees cultivated or used by humans*]. Paris, p. 360.

Schneider, A., Boccacci, P., Ruffa, P., Torello Marinoni, D., Cavallo, L., Festari, I., Rotti, G., Raimondi, S. (2015). Identification and characterization of *Vitis vinifera* subsp *sylvestris* populations in north-western Italy. *Vitis,* 54:223-225.

This, P., Lacombe, T., Thomas, M. (2006) Historical origins and genetic diversity of wine grapes. *Trends in Genetics,* 22:511-519. https://doi.org/10.1016/j.tig.2006.07.008.

Tukey, J. (1981). *Analysis of the results of observations.* M Moscow: Mir, 693 p.

Vavilov, N.I. (1926). Centres of origin for cultivated plants. *Proceedings of Applied Botany, Genetics and Breeding*, 16(2), in Rus.

Vavilov, N.I. (1931). Wild relatives of fruit trees of “Asia part of the USSR and the Caucasus and problems of fruit trees origin. *Proceedings of Applied Botany, Genetics and Breeding*, 26(3), in Rus.

Vavilov, N.I. (1987). *Theoretical selection basics,* in Rus.

Volynkin, V.A., Polulyakh, A.A. (2011). Research of wild woody grapevine Vitis vinifera ssp. sylvestris origin. In: *1st International Symposium on Wild Relatives of Subtropical and Temperate Fruit and Crops*. March 19-23. Davis, California, USA.

Volynkin, V., Polulyahk, A. (2015). Origin of grapevine varieties in Crimea and *Vitis vinifera* subsp. *Sylvestris* classification. *Vitis,* 54 (Special Issue):227-228.

Volynkin, V., Polulyakh, A., Chizhova, A., Roshka, A. (2012). Ukraine: native varieties of grapevine. In: Caucasus and Northern Black Sea Region Ampelography D.Maghradze, L.Rustion, J.Turok, A.Scienza and O. Faila, eds. *Vitis,* p. 405-473.

Volynkin, V., Polulyahk, A. Kotolovets, Z. (2011). Improving the methodology for selecting the optimal grape variety. *Viticulture and Winemaking,* XLI, 15-18, in Russ.

Volynkin, V., Polulyakh, A., Levchenko, S., Vasylyk, I., Likhovskoi, V. (2019). Autochthonous grape species, varieties and cultivars of Crimea. *Acta Horticulturae,* 1259:91-98. https://doi.org/10.17660.

Volynkin, V., Polulyakh, A., Levchenko, S., Vasylyk, I., Likhovskoi, V. (2020). Aspects of the particular genetics of grapes prolonged for all horticulture crops, *IntechOpen.* https://doi.org/10.5772/intechopen.90566.

Wulff, E.V. (1939). About wild grapes *V. silvestris* Gmel. in the Crimea - *Soviet Botan*y. No. 2. - P.38-47, in Rus.

Zhukovskii, P. (1964). *Cultivated plants and their wild relatives.* 2nd edition. Leningrad. Publishing house “Kolos”. 790. in Rus.

Zdunić, G., Maul, E., Eiras Dias, J.E., Muňoz-Organero, G., Carka, F., Maletić, E., Savvides, S., Jahnke, G.G., Nagy, Z.A., Nikolić, D., Ivanišević, D., Beleski, K., Maraš, V., Mugoša, M., Kodzulovic, V., Radić, T., Hančević, K., Mucalo, A., Lukšić, K., Butorac, L. Maggioni, L., Schneider, A., Schreiber T., Lacombe, T. (2017). Guiding principles for identification, evaluation and conservation of *Vitis vinifera* L. subsp. *sylvestris*. *Vitis,* 56:127-131.

In: Vitis: Biology and Species
Editors: António M. Jordão et al.

ISBN: 978-1-53618-308-5

Chapter 4

RESPONSES OF THE EURASIAN WILD GRAPEVINE TO BIOTIC AND ABIOTIC STRESSES AND ITS IMPORTANCE AS A PHYTOGENETIC RESOURCE FOR BREEDING

Carlos Alvar Ocete Pérez[1], Miguel Lara Benítez[2],
Jesús Cambrollé Silva[3], Magdalena González-Maestro[4],
José Luis García Fernández[4], Rafael Ocete Rubio[1]
and Manuel Cantos Barragán[4,*]
[1]Freelance, Tirgo, La Rioja, Spain
[2]Department of Viticulture and Enology, IFAPA, Jerez de la Frontera, Cádiz, Spain
[3]Faculty of Biology, Seville University, Seville, Spain
[4]Department of Plant Biotechnology, IRNAS-CSIC, Seville, Spain

ABSTRACT

The present study describes some of the most important biotic, (parasites affecting grapevine) and abiotic (high lime and copper levels in soil) stresses suffering the Mediterranean vineyards. As a possible solution for alleviate these stresses, it is focused on the Eurasian wild grapevine, the dioecious parental of the present cultivars, and its importance as a plant genetic resource in the genetic improvement of existing varieties reducing the loss of agrodiversity in the vineyard. Greenhouse and *in vitro* tissue culture experiments were carried out in order to investigate the effects of a range of external calcium carbonate and also copper levels on growth, photosynthetic parameters and stem and root mineral concentrations. The results of our study indicate that plants of *V. vinifera*

---

[*] Corresponding Author's Email: cantos@irnase.csic.es.

ssp. *sylvestris* from two populations growing in a hypercalcic and a very metal polluted soils in its natural habitats are more tolerant to lime and Cu, respectively, than the commercial varieties of grapevine that have been studied and could constitute a basis for the genetic improvement in grapevine.

Keywords: anthropic impacts, calcareous soil, copper pollution, genetic erosion, phytogenetic resource, *vitis vinífera* l. subspecies *sylvestris*

1. INTRODUCTION

The four glacial periods during the Quaternary have influenced the distribution of the habitats of the botanical communities and the appearance of new species belonging to the same genus. In case of the *Vitis* L. genus, in the actual territory of the United States of North America, these vitaceae could go towards more southern latitudes. This migration was possible thanks to the fact that in this geographical area the mountain ranges have a North-South orientation. On the other hand, in the most oriental part of China, where the relief is slightly pronounced another proliferation of *Vitis* species took also place. So, in both geographical areas, some dozen of *Vitis* species can be found at present time (Zohary and Spiegelroy, 1975). In the rest of the Eurasian continent, the mountain ranges have an East-West orientation. Due to this fact, the migration capacity towards the South was restricted in the glacial periods. From the Iberian Peninsula up to Afghanistan (Indu Kush mountain-range) there is only one native grapevine, *Vitis vinífera* L. (Arnold, 2002), which sheltered principally in Transcaucasia, and in the South of the Mediterranean basin (Huglin et al., 1998). Some populations can also be found in the African Maghreb (Ocete et al., 2007). This autochthonous grapevine is dioecious and constitutes the wild parental of cultivars, belonging to the taxon, *Vitis vinifera* L. subspecies *sativa* (DC.) Hegi, generally hermaphrodite (Levadoux, 1956).

The Eurasian wild grapevine is a hydrophilic liana climbing on the accompanying vegetation, mainly on trees and shrubs, in order to access to an adequate lighting. It needs soils submitted to a constant renewal of substratum and variable periods of flood, according to the meteorological conditions of every year (Ocete et al., 2004) Its main habitats are azonal alluvial formations, mainly river- bank developed along rivers and creeks (Arnold et al., 1998) (Figure 1).

In Central Europe and in the South of the Caucasian region, some populations develop on floodplains of the big rivers, as in case of the Rhine or the Danube, between others (Terpó, 1969; Schumann, 1974). Some populations can be found in the mouth of some rivers, growing on arenosols, as it happens in the case of the Guadalquivir (Spain) and the Danube (Romania), as well as in the coastal area of the of Uccelina Park (Italy). Also they can develop in the shores of lakes and lagoons. In areas under humid climatology, some populations are situated in colluvial position, on the slopes of mountains and hills, as in case of several populations of the Cantabrian Mountain range (Spain), Jura (France), Piamonte (Italy) or the Alps (Switzerland). Some populations are also growing on cliffs of coastal areas, as in the case of the Cantabrian seaside, between Lapurdi (French Basque Country) up to Western Asturias (Spain) (Ocete et al., 2018).

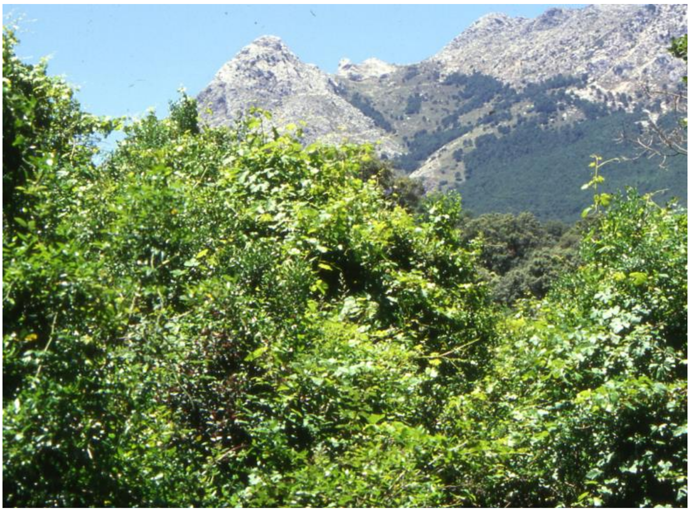


Figure 1. Wild grapevine population along river-bank forest.

Following the descriptors compiled by O.I.V. (OIV, 2009) and the ampelographical studies carried out by other authors (Meléndez et al., 2016 and Benito et al., 2017), the main differences between female and male exemplars are described below.

The aperture of the tip of the young shoot is half open in females and fully open in males; the intensity of anthocyanin pigmentation is of medium intensity with edged appearance. The size of the mature leave usually is medium in female and small or very small in male. The number of nodes is smaller in the case of the females. The petiolar sinus is more opened in the case of males. The male flowers generally present fully developed stamens and no gynoecium (flower Type I, according to O.I.V. (OIV, 2009) and the pollen is tricolporated (Gallardo et al., 2009). Less frequent are lianae with start of training of gynoecium (flower Type II, according to O.I.V. (OIV, 2009) (Figure 2a).

The female flowers show reflexed stamens and fully developed gynoecium and the pollen is acolporated (Gallardo et al., 2009) (Figure 2b). In the case of female vines, the size of the berries is lower than one centimeter of diameter (Figure 3). The colour of the skin is blue black. Only a few plants with white berries can be found in the wild.

Wild seed are morphologically different from cultivated ones. They are more rounded and show smaller measures. According to Stummer (1911), the value of the ratio width/length (W/L) ranges between 0.54 and 0.82 in the wild variety, corresponding the maximum frequency to the value 0.64, and between 0.44 and 0.74 in the cultivated variety, in this case the maximum frequency is 0.54. (Figure 4a and 4b). Several morphometric indexes were used to distinguish them, such as Stummer (1911); Logothetis (1974); Mangafa and Kotsakis (1996) and Terral et al. (2010).

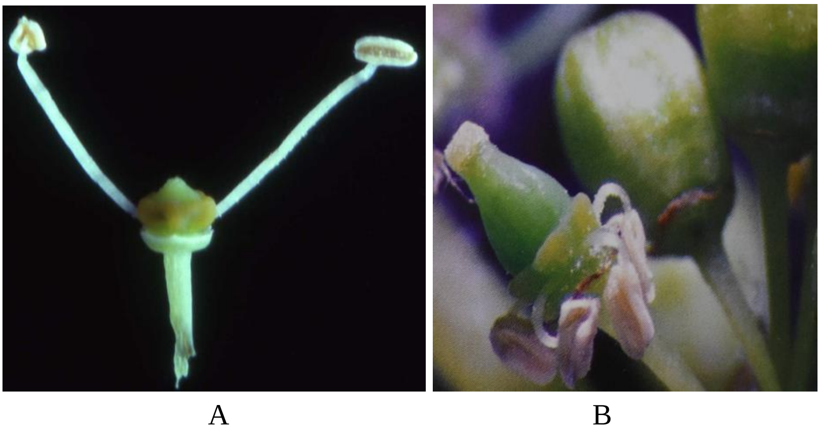


Figure 2. Male flower (A) and female flower (B).

Figure 3. Mature bunch.

Figure 4. A wild seed (A) and cultivated seed (B).

In very rare cases appear hermaphrodite wild vines with tricolporated pollen due to mutation of male exemplars. They were selected by man, due to his power of autofecundation and, in consequence, to produce bigger harvest than female ones. So, they were used to stablish plantations outside natural habitats (Forni, 2004). The cited differences between wild and cultivated seeds in archaeological investigations carried out in the Republic of Georgia (Southern Caucasus) indicate that this geographical area constitutes, until now, the first domestication focus in the tell of Shulaveri Gora dated around 8,000 BC. Also ceramic receptacles which have contained must or wine were found (Chilashvili, 2004; McGovern, 2004). There is a great genetic affinity between wild and cultivated grapevines in that country, so it confirms the origin of the domestication (Riaz et al., 2013). Diverse multidisciplinary works endorse the idea of that the grape cultivation radiated from the Triangle of the Fertile Grapevine, which has its central area in Ararat mountain, towards nearby regions, like Mesopotamia and Middle East (Vavilov, 1926). Later, it was moving towards Western Mediterranean areas (Negrul, 1938).

The wild grapevine is a plant of that the human being has used from different parts of its structure. Their berries were used as human food in diverse epochs, from the Paleolithic (Rivera and Walker, 1989). Their bunches were the material of the first vinifications. In fact, the vinification of wild berries has survived in some European areas, as it is the case of Sardinia, where the shepherds produced the naped vino de vulpa (fox wine) up to about twenty years ago.

The production of home-made vinegar from the fruit of the wild vine has been supported in Grazalema Mountain range (Cadiz, Spain) till the end of last century. It was with halite the main preservative of food for centuries.

The pharmacopeia is full of medicinal remedies that have its origin in the wild vine, principally in the sap, sheets and must (Laguna, 1566; Bustamante, 1971). The vine shoots have been used for the manufacture of ropes (Quer, 1784) and hoops for the construction of fishing traps (Ocete et al., 2011). The lianae have been used, also, as rootstock natives very well adapted to the area (Zimmermann, 1958). Finally, it is necessary to remark the presence of seeds wild or cultivated in burials of diverse epochs, because grapevine were part of funeral offering from the Bronze age up to the Paleochristian one (Torres-Vila and Mosquera, 2004; Ocete et al., 2011).

Several negative human impacts have reduced drastically the presence of wild grapevines in natural ecosystem (Arnold, 2002). So their geographical distribution at present is highly fragmented in small isolated populations (Arnold et al., 1998; Grassi et al., 2003; Ocete et al., 2011; Scali et al., 2018). Before the arrival of the American fungal parasitic, this subspecies was very common, as they were reflected in the great deal of uses indicated above. Logically, the arrival of the powdery and downy mildews was responsible for the death of the most sensitive vines of every population (Ocete et al., 2007).

The river-banks are growing on fertile soils, easily irrigable, that can devote themselves to diverse horticultural uses and forest developments. Also, it is necessary to add the transformation of the water courses and dams construction. Furthermore another causes are the cleanliness of the banks, the modernization of the road network, the construction of recreation areas and the use of weeding machines and herbicides, principally glyphosate, to keep clean the ditches of the highways (Martínez de Toda, 1991). Another problem of renewal of the wild grapevines happens in areas with high cattle growing and hunting use, where herbivorous massively eat the new plants emerged from seeds (Ocete et al., 2007). The massive employment of American rootstocks and direct producer hybrids (French hybrids) to re-constitute the vineyard destroyed by the phylloxera, brought another pernicious consequence. These *vitaceae* escaped from vineyards are invasive plants which have displaced to the wild grapevines of their natural habitats (Terpó, 1969; Arrigo and Arnold, 2007; Iriarte-Chiapusso et al., 2013). Some European countries, like France, Austria, Germany and Hungary have a specific legal figure for the wild grapevine. The reality is that its fulfillment is not real, as it was checked in the French Basque Country (Rodríguez-Miranda et al., 2016).

With the appearance of agriculture about 10,000 years ago, man begins to intervene in a decisive way the natural evolution of the plants that he grows, giving rise to the domestication process. Thus, the populations of cultivated plants begin to suffer strong selective pressures due to agricultural practices, migrations across commercial routes, finding diverse conditions of climate, soil, vegetation and other environmental factors. In this way, the populations of cultivated plants evolved differently according to the characteristics of the new zones and the different agricultural practices used and, in many cases, gene exchanges or hybrids with the wild species of the new localities were formed. Particularly, since 200 years ago, as a result of agricultural and industrial development and the progressive unification of cultural and nutritional habits, the number of crops and the heterogeneity within them have been progressively decreasing and, at present, 90% of the world's diet is based on only about 30 plant species and a few dozen varieties with an evident and dramatic loss of biodiversity. That is, the genetic base on which natural selection acts is being dangerously reduced (genetic erosion) increasing dramatically the vulnerability of different cultivars to situations such as environmental changes and/or the appearance of devastating diseases and to certain abiotic stresses, such as soil salinity or water deficit (Cambrollé et al., 2015).

Regarding to viticulture, traditional vineyards had miscellanies of different varieties and somatic variants of the same cultivar, so Ambrosi et al. (1994) estimate 10 to 20 thousands cultivars in existence. Today, in a world of 7.6 billion people with projection to be around 9.2 billion by 2050, viticulture is facing great challenges to maintain suitable productivity and high quality of both grape and wine. The grapevine is also strongly subject to significant genetic erosion or loss of variability (Vallecillo and Vega, 1995). Although, the problem of the genetic erosion of the vineyard begins since the first viticultural societies were selecting the plants; generally, hermaphrodites that were adjusted to the necessities of the same ones, in the last two decades there has been an important reduction of the number of cultivated varieties of each Registered Apellation of Origin Mark (RAOM). This was motivated by the introduction of vines of foreign origin due to technical reasons and/or market demand, with a clear detriment of other traditional minorities, following the “terroir” concept of the current viticulture.

At present, it is considered that there can be between 5,000 and 10,000 cultivated varieties, on a global scale, while literature indicates around 8,000. Approximately, 1,500 of them keep on maintained in the vineyards. Within, 16 varieties occupy 50% of the world surface (Anderson and Aryal, 2017). There is a heavy loss of diversity, because only some clones of the cited cultivars are planted. In consequence, the majority of the new plantations are constituted by only clones, diminishing of drastic way the agrodiversity, thus the genetic pool. It can constitute a serious problem facing the climate change and the possible appearance of new causative agents of biotic and abiotic stresses: viruses, phytoplasms, bacteria, fungi and pests (Ocete et al., 2018), drought (Serra et al., 2014; Rustinoni et al., 2016), soil salinity (Troncoso et al., 1999; Askri et al., 2017). However, the vineyard constitutes the most widespread fruit cultivation and of major economic yield of the planet (Vivier, 2002; Cambrollé et al., 2014; Susaj et al., 2014).

Wild grapevine populations maintain considerable genetic polymorphism and manifest wide variability (McGovern, 1996); the disappearance of these populations from their natural habitat would constitute an irreversible loss for the environment and for breeding programs (Grassi et al., 2003; Ocete et al., 2011). The wild vine, as a plant that has been evolving free of human artificial selection since the beginning of viticulture, can be used in the improvement of cultivated varieties, since wild populations still conserve an important global genetic diversity (Grassi et al., 2003) This rich genetic pool can be used to avoid the aforementioned loss of biodiversity that affects the current viticulture and confer to the cultivars, a greater resistance to certain pests, diseases and abiotic conditions. Some of the interesting characteristics of wild grapevines can be transferred through traditional improvement to commercial varieties, in accordance with the postulates of Integrated Production (IP) and, in this way, increase the level of diversity (Ocete et al., 2007).

For that reason, it is necessary to safeguard to the maximum the *in situ* and *ex situ* biodiversity of the wild populations, which lodge an important genetic pool (De Andrés et al., 2012; Zdunic et al., 2017), because it continues free of the human selection and its reproduction is mainly sexual. Logically, it is also necessary to conserve in germplasm collection the ancient traditional cultivars. Both kind of preservation play the most important strategic role in order to reinforce the props to a sustainable wine-growing process to produce by crossing new cultivars and rootstocks, taken into account the resistance to ponding water. On the other hand, microvinifications of wild grapevine produce red wines with good total acidity and color intensity (more than 25), two very important characteristics for Mediterranean areas where the cultivar Syrah is massively introduced, as in the case of Southern Spain, in order to increase the color of red wines (Lovicu et al., 2009). The top total polyphenols is about 92.6 and the maximum total anthocyanins is 562 mg L^{-1} (Meléndez et al., 2016).

Finally, it is important to indicate the high variety of arbuscular mycorrhizal symbiosis on roots of wild grapevine (Ocete et al., 2015) which could be transferred to ecological vineyards. On the other hand, the elevated native yeast biodiversity present in wild grapevines might help to face up the negative oenological consequences that climate change is producing in the vineyards (Puig-Pujol et al., 2016).

Majority of biotic stresses in grapevine cultivars are caused by arthropods, fungal diseases, bacteria and viruses. It is necessary to remark the importance of fungal disease and phylloxera imported in Europe in the 19-20 centuries. They are affecting to the practical totality of vineyards all over the world. On the other hand, wood fungal diseases constitute a very difficult problem to resolve at present.

Different abiotic stresses threat grapevine crop caused both by traditional viticulture and by a fast changing environment, possibly more severe in the future, but with clear signals in the present. Through the history of evolution, plants have developed a wide variety of highly sophisticated and efficient mechanisms to sense, respond, and adapt to a wide range of environmental changes. Among the abiotic stresses attributable to customary viticulture two of them, high levels of lime and copper in vineyard soils, are particularly interesting. In consequence, the knowledge of wild grapevine possibilities of exploitation for restore the genetic background related to tolerance of cultured grapevines to high lime or Cu contents in soil is a very important issue.

Calcareous soils are occupied by vineyards in some of the most important European viticultural areas as the Mediterranean region. Susceptible grapevines growing on such soils often suffer lime-induced Fe chlorosis (Bavaresco et al., 2006; Cambrollé et al., 2014). Despite of the diversity of responses to this abiotic stress according to grapevine variety (Tangolar et al., 2008), lime-induced chlorosis is a major problem for grapevine, because all cultivated varieties show important levels of susceptibility. The response of susceptible grapevines to lime-stress includes lower biomass in shoot and root, yield reduction and a characteristic leaf interveinal yellowing. The root growth reduction is due to soil bicarbonate presence and to a lower photosynthetic rate that also depends on decreased leaf chlorophyll under Fe stress conditions (Bavaresco and Poni, 2003). Lime-induced chlorosis caused by calcareous soil conditions has a great impact on fruit yield and quality in grapes (Tangolar et al., 2008), furthermore the fertilizers used for its control and prevention are often expensive, little efficient in the long term, and some are considered not environmentally friendly (Abadia et al., 2011; Álvarez-Fernández et al., 2011). Grafting grapevine varieties onto lime-tolerant rootstocks is the usual method in viticulture to overcome this stress. At low Fe-availability in soils, different grapevine rootstocks show considerable quantitative differences in terms of the root response reactions increasing iron uptake, proton extrusion, and reducing capacity (Varanini and Maggioni, 1982; Brancadoro et al., 1995) and rates of Fe uptake (Maggioni, 1980; Nikolic et al., 2000). Grapevine rootstocks used by viticulturists on calcareous soils worldwide are usually hybrids, mainly between *Vitis rupestris* and *Vitis berlandieri*, such as 333 EM (*Vitis* *vinifera* L., Cabernet Sauvignon x *Vitis berlandieri* Planchon.), 41B (*Vitis vinifera* L. cv. Chasselas x *Vitis berlandieri* Planch.), both with a tolerance level of 40% of lime in soil; or 140 Ruggeri (*Vitis berlandieri* cv. Rességuier nº2 x *Vitis Rupestris* cv. Lot) reaching only a 25% of tolerance (Chauvert and Reynier, 1984). However, despite the many studies that have been carried out (Bavaresco et al., 2000; Nikolic and Merk, 2000; Díaz et al., 2009), the optimal rootstock has not yet been obtained and the mechanisms involved in plant tolerance to calcareous soil conditions remain unclear.

In unpolluted soils, Cu levels are influenced mainly by the parent material from which the soils are formed and reach an average of 30 mg kg^{-1}. The maximum value of Cu proposed by the EU in soils treated with residual sludge is 200 mg kg^{-1} (Kloke and Einkmann, 1991; Galán and Romero, 2008). However, the long-term application of copper-based fungicides used intensively in European viticulture since the end of the 19th century to control vine fungal diseases, such as downy mildew caused by *Plasmopara viticola*, and additions of Cu compounds (such as $Cu(OH)_2$ and Cu_2O), have led to considerable accumulations of Cu in some vineyard soils (Komárec et al., 2010). Thus, Cu contents in some Croatian soils is above 700 mg kg^{-1} (Romić et al., 2004) and, specifically, in some vineyard soils in southern Brazil, values reaching 3200 mg kg^{-1} have been found (Mirlean et al., 2007). Phytotoxicity, yield losses and decreased wine quality are negatives consequences of this high Cu concentration in soil, which, in addition, cause deleterious effects on flora, fauna and human health (Ninkov et al., 2012).

The characterization of the largest possible number of wild specimens can be very useful when making trials to improve crop varieties together with maintaining genetic resources to guarantee biodiversity. Traditional breeding technologies and proper management strategies continue to play a vital role in crop improvement inducing stress tolerance. However, conventional breeding methods have limited success to provide desirable results, due to the plants often experience a wide variety of stress levels requiring a range of different response mechanisms. Therefore, it is necessary to arrange biotechnological techniques as tools for addressing the critical problems of crop improvement for sustainable agriculture. Various authors recommend the use of assays culturing *in vitro* differentiated tissue, nodal segments, in controlled conditions for screening tolerant or resistant lines to biotic (Santos et al., 2005) and abiotic (Troncoso et al., 1999; Rai et al., 2011) stresses. Further, *in vitro* cultures may be combined with conventional ones in breeding programs. Also, *in vitro* culture is an excellent tool to study the effect of increasing doses of a specific selection factor against a control because of individuals are grown in extremely controlled conditions. The shoot growth from apical or nodal buds is a very sensitive indicator to abiotic stress (Troncoso et al., 1999; Claeys et al., 2014). This methodology also allows a screening of a large number of genotypes in short periods and small areas. The more resistant lines could then be used for *in vivo* assays in the field.

2. METHODS

2.1. Biotic Stresses

From 1992, our research team carried out a systematic observation of pests and diseases on wild grapevine populations distributed within different countries of Europe, Asia and Africa. Data were taken from the concerning literature, such as Ocete et al. (1999) and Maghradze et al. (2009) and unpublished data. A total of 1,183 wild grapevines were studied in 12 countries from Europe, Asia and Africa (Table 1). To carry out the observation of biological stress, roots were unearthing up to a deep of 40 cm and aerial organs were examined to discover symptoms caused by parasitic species.

Table 1. Number of vines sampled in each country

Country	Number of vines sampled
Portugal	131
Spain	367
France	142
Italy	104
Germany	79
Switzerland	23
Hungary	84
Greece	27
Georgia	92
Azerbaijan	67
Armenia	21
Morocco	46
Total of grapevines sampled	1.183

2.2. Abiotic Stresses

2.2.1. Plant Material

2.2.1.1. Calcium Carbonate

Plant material was chosen from two natural populations from southern Spain; one of them growing in a hypercalcic calcisol soil with 62–67% calcium carbonate (“14/Rute/1” population) (37º 22’ 53’’ N - 40º 21’ 17’’ W), and the other growing in a humic fluvisol with 0% calcium carbonate (“14/Montoro/4” population) (38º 7’ 46’’ N – 40º 16’ 23’’ W). In addition, plants of the hybrid rootstock “41B” (*Vitis vinifera* L. cv. Chasselas x *Vitis berlandieri* Planch.) considered as a lime-tolerant rootstock (Bavaresco and Poni, 2003; Pavloušek, 2013) used by viticulturists on calcareous soils worldwide were used for comparison with the two wild grapevine populations.

2.2.1.2. Copper

For copper tolerance evaluation plant material was collected from a Cu-polluted site, located on the bank of the Agrio river ("Río Agrio" population) (Cambrollé et al., 2013), in Seville province (SW Spain) (37º 30' 45.7''N – 6º 13' 24.6'' W). The river flows through the Aznalcóllar mining zone, containing high levels of heavy metals. This situation was dramatically aggravated in 1998 when the river was suddenly flooded with 4-5 million cubic meters of mine wastes because of a holding dam that failed at a mine near Aznalcóllar (Cambrollé et al., 2013; 2015) contributing with very dangerous levels of several heavy metals (e.g., cadmium, copper and zinc). The other two accessions used for comparison with the first one were "14/Rute/1" population from non-contaminated site and "41B."

2.2.2. Treatments

2.2.2.1. Greenhouse Culture

2.2.2.1.1. Calcium Carbonate

Plants with around 20 cm in height were transferred to individual plastic pots (diameter 11 cm) filled with substrate performed mixing sterilized fine siliceous sand (Quality Chemicals, Ref. 7631-86-9) with 0, 20, 40 y 60% finely divided $CaCO_3$ (particle size < 5 µm in diameter) (Panreac Ref.141212.0416) irrigated with 20% Hoagland's solution (Hoagland and Arnon, 1950).

The pots were placed in a glasshouse with temperatures of 21–25°C, relative humidity of 40–60% and natural daylight - light flux: 200 and 1000 µmol m^{-2} s^{-1}). The experimental period was 30 days. Relative growth rate (RGR) of whole plants, dried at 80ºC for 48 h and then weighed, was calculated using the formula: $RGR = (\ln Bf - \ln Bi) \cdot D^{-1}$ ($g\ g^{-1}\ day^{-1}$) where Bf = final dry mass, Bi = initial dry mass (average of the three plants from each treatment dried at the beginning of the experiment) and D = duration of experiment (days).

2.2.2.1.2. Copper

Plants with 30 cm in height were allocated in pots with sterilized fine siliceous sand (Quality Chemicals, Ref. 7631-86-9) and five different Cu concentration treatments: 0, 50, 150, 600 and 1500 mg kg^{-1} Cu, applied in shallow trays within the same glasshouse (same climatic conditions as referred above). Cu treatments were prepared by mixing the 20% Hoagland's solution with $CuSO_4x5H_2O$ at the appropriate concentration. RGR was quantified. Also the experimental period was 30 days.

2.2.2.2. *In Vitro* Tissue Culture

2.2.2.2.1. Calcium Carbonate

"14/Montoro/1," "14/Rute/1" and "41B" plants were taken from IRNAS *in vitro* germplasm bank. From these plants uninodal explants (0.3-0.5 cm long) with one bud, were isolated and placed individually, under flow cabin, in sterilized test tubes containing four different calcium carbonate soil treatments: 0 (control), 20, 40 and 60% $CaCO_3$, prepared by mixing inert fine siliceous sand described above in the appropriate proportion. 17 g of de corresponding mix plus 5 ml of basal VD culture medium (Troncoso et al., 1990) plus 3% sucrose, 0.072 mg L^{-1} of BAP, 0.024 mg L^{-1} of ANA, pH 5.7 were added per tube. The fine, clay-sized fraction of $CaCO_3$, or active lime (Drouineau, 1942), is able to generate and maintain high levels of HCO_3 in the soil solution (Inskeep and Bloom, 1986) and is therefore a consistent indicator predicting the effect of lime-induced damage (Tagliavini and Rombolà, 2001). Culture was performed in a growth chamber at 23±2ºC, 30 µmol m^{-2} s^{-1} and 16 h photoperiod during 60 days. At the end of the culture period the survival percentage, shoot size and Fe contents in leaves were measured.

2.2.2.2.2. Copper

For studying *in vitro* copper tolerance ($CuSO_4$) plants of the two wild populations "Río Agrio" and "14/Rute/1" and of the grapevine rootstock ''41B' were chosen from IRNAS *in vitro* germplasm bank. From these plants similar explants to those used for *in vitro* calcareous soil assays, were isolated and cultured in sterilized tubes containing the same VD medium described before but gelled with bacto agar (0.6%) (Duchefa B010471.08) without Cu (control) or with 5, 50, 200 and 500 µM Cu using $CuSO_4x5H_2O$ at the appropriate concentration. Once closed and sealed, they were placed in culture room with the same culture conditions indicated for *in vitro* calcium carbonate assays during 60 days. At the end of the culture period the survival percentage, shoot size, rooting percentage, root number and Cu contents both in leaves and roots were measured.

2.2.3. Gas Exchange

For plants growing in greenhouse, gas exchange measurements were taken from randomly selected, fully expanded leaves (n=20, one measurement per plant plus eight extra measurements taken randomly), following 30 days of treatment, using an infrared gas analyzer in an open system (LI-6400, LI-COR Inc., Neb., USA). Net photosynthetic rate (A) and stomatal conductance to CO_2 (Gs) were determined at an ambient CO_2 concentration of 400 µmol mol^{-1} at 20-25°C, 50 ± 5% relative humidity and a photon flux density of 1600 µmol m^{-2} s^{-1}.Values of the parameters A and Gs were calculated using the standard formulae of Von Caemmerer and Farquhar (Von Caemmerer and Farquhar, 1981).

2.2.4. Photosynthetic Pigments

At the end of the greenhouse experimental period, photosynthetic pigments were extracted from fully expanded leaves of plants grown under each treatment, using 0.05 g of fresh plant material in 10 mL of 80% aqueous acetone (n = 12). After filtering, 1 mL of the suspension was diluted with a further 2 mL of acetone, and chlorophylls a (Chl a) and b (Chl b) contents were determined with a Hitachi U-2001 spectrophotometer (Hitachi Ltd, Japan), using two wavelengths (663.2 and 646.8 nm). Pigment concentrations (µg g^{-1} fwt) were calculated following the method of Lichtenthaler (Lichtenthaler, 1987).

2.2.5. Iron and Copper Contents

At the end of the experimental cycle, both in greenhouse and *in vitro* conditions, leaf (and roots in the case of copper *in vitro* experiment) samples were carefully washed with distilled water and then dried at 80 °C for 48 h and ground. Samples of 0.5 g each were then digested by wet oxidation with concentrated HNO_3, under pressure in a microwave oven to obtain the extract. Concentrations of Fe and Cu in the extracts for calcium carbonate and copper experiments respectively were determined by optical spectroscopy inductively coupled plasma (ICP-OES) (ARL-Fison 3410, USA).

2.2.6. Statistical Analysis

Statistical analysis was carried out using IBM SPSS Statistics version 22.0. Pearson coefficients were calculated to assess the correlation between different variables. Data were analyzed using one- and two-way analyses of variance (F-test). Data were tested for normality with the Kolmogorov-Smirnov test and for homogeneity of variance with the Brown-Forsythe test. Tukey tests were applied to significant test results for identification of important contrasts. Measured differences between fluorescence at dawn and midday were compared using the Student test (t-test). Differences in percentages cases were compared using z test.

3. RESULTS

3.1. Biotic Stress

On roots, no symptoms of Phylloxera (*Daktulosphaera vitifoliae*) (Fitch) (Homoptera, Phylloxeridae) were found. In natural habitats there is not presence of Phylloxera on roots, due to edaphical conditions, as swamped or sandy soils. But wild grapevine samples from different European regions are sensible to the insect when they are infested artificially with infested leaves of North American vitaceae in pots (Ocete et al., 2011) (Figure 5). Due to the cited absence of symptoms in natural habitats, it was used in France as rootstocks, as it was indicated by Camile Saint Pierre in De la Branchere (1876): "*We have thought on the possibility of grafting the cultivars on the wild grapevine outside river-bank forests, but after some months, this vitacea is not capable of escaping to the Phylloxera, and suffers its attacks on roots.*"

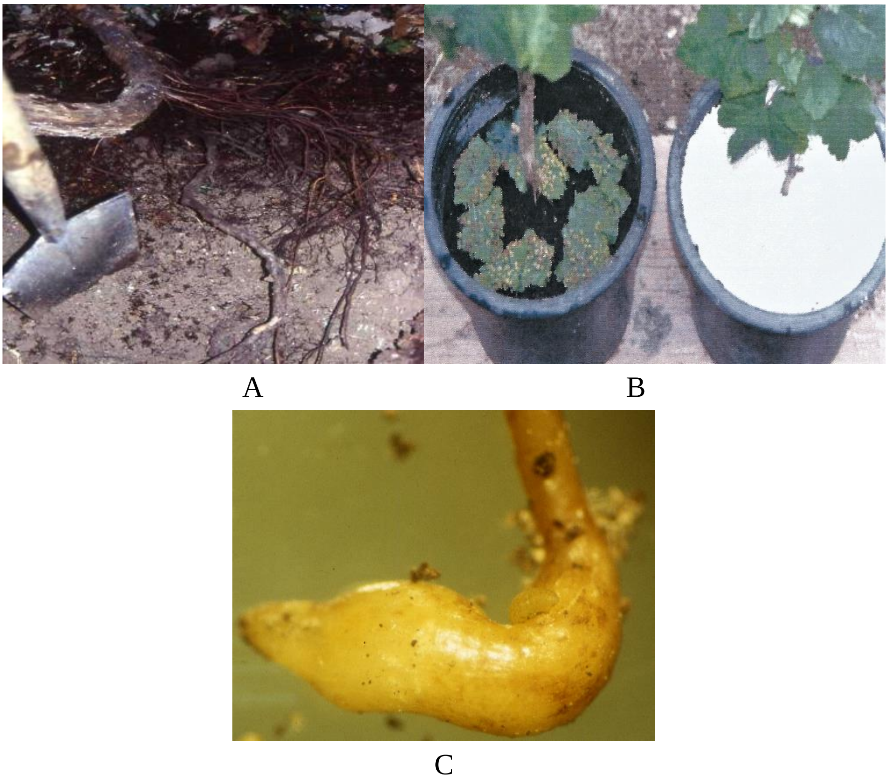
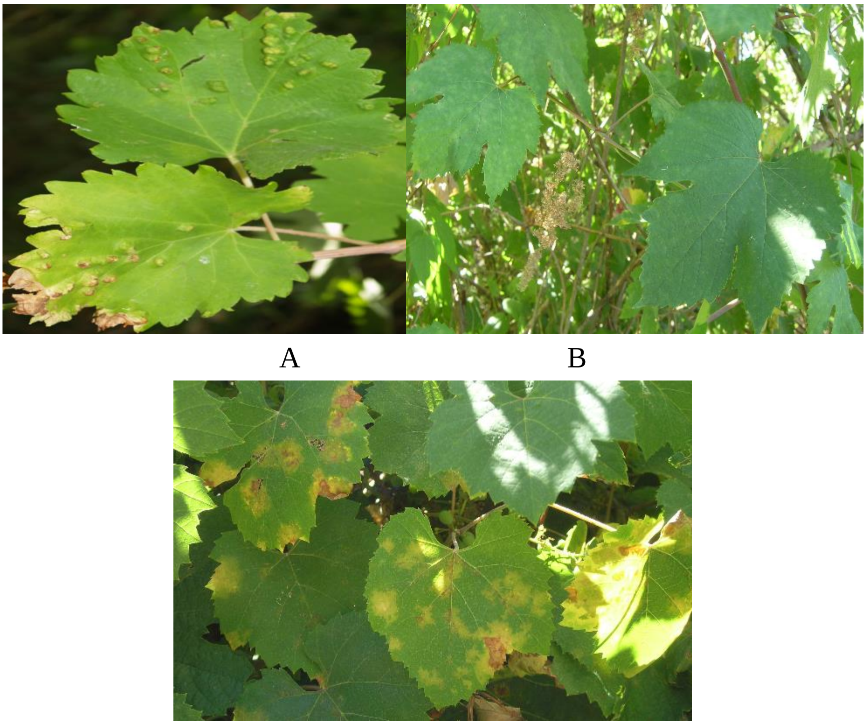


Figure 5. Absence of Phylloxera on roots in natural habitats (A); artificial infestation of wild grapevine with leaves of North American rootstock with Phylloxera galls (B) and symptoms of Phylloxera in grapevine after artificial infestation in pot (C).

On the other hand, no actions of dagger or needle nematodes, and no root rot were detected. It is necessary to remark that several roots of black poplar trees serving as a support to the vines in river-banks, showed several white mycelia plates of *Armillaria mellea* Vahl. On the aerial part of the vines, concretely on leaves the presence of erineum strain of *Colomerus vitis* (Pagenstecher) (Acari, Eriophyidae) is present in the practical totality of the vines, but there are several natural enemies of this pest, mites and insects (Farragut et al., 2008) (Figure 6a). Another mite, *Calepitrimerus vitis* (Nalepa) (Acari, Eriophyidae) provokes smaller level of infestation, situated between 37 and 17% of the vines sampled. In the case of insects *Empoasca vitis* (Goëthe) (Homoptera, Cicadellidae) and *Thrips angusticeps* Uzel (Thysanoptera, Thripidae) were observed in populations under a high humid climatology, as it was indicated in the case of the Basque country

(Spain, France). It maximum incidence is 26% in both cases. Data on the incidence of each parasitic species are compiled in Figure 6 and Table 2.


A B

C

Figure 6. Different levels of infestation caused by the erineum strain (A); infection caused by powdery mildew (B) and symptoms caused by downy mildew (C).

No malformations and syndromes caused by grapevine fan leaf virus (GFLV) were observed. The absence of the cited virus was confirmed in the laboratory by ELISA tests.

In reference to pathogenic fungi, the powdery mildew, *Erysiphe necator* (Schweinitz) Burrill, and the downy mildew, *Plasmopara viticola* (Berkeley and You Tan; Berlease and De Toni), their symptoms are frequent on leaves, sarments and bunches, mainly. These fungal diseases were imported from North America, like the Phylloxera, in the second half of the XIXth century. Both fungi can destroy completely the harvest and new small vines, depending on the intensity of the attack (Figure 6, B and C). However, the incidence of the cited pests and diseases varies from a vine to other one, inside the same population, due to genetic differences among the lianae. There are not usually frequent pathologies caused by wooden fungi, which are one of the main problems of the current vineyards (Armengol, 2017). Sometimes, they can appear sporadically around cuts provoked by human actions.

Percentages of infestation and infection detected in vines from different countries are shown in Table 2.

Table 2. Percentages of infestation/infection

	Colomerus vitis	*Calipitremus vitis*	*Thrips angusticeps*	*Empoasca vitis*	*Eysiphe necator*	*Plasmopara viticola*
Portugal	97	33	0	12	88	65
Spain	99	35	14	15	76	58
France	100	37	26	26	92	71
Italy	76	25	12	14	83	66
Germany	84	26	0	24	67	93
Switzerland	76	17	0	13	63	88
Hungary	96	23	17	30	81	95
Greece	89	16	0	0	92	67
Georgia	96	30	0	0	88	71
Azerbaijand	89	29	0	0	86	66
Armenia	76	17	0	0	69	58
Marocco	92	32	0	0	59	30

3.2. Abiotic Stress

3.2.1. Evaluating Wild Grapevine Tolerance to Calcareous Soil

3.2.1.1. Greenhouse

After 30 days at greenhouse conditions, a reduction of 70% in relative growth rate (RGR) of “41B” rootstock plants exposed to 20 and 40% $CaCO_3$ was found, whereas the RGR reduction in “14/Montoro/4” and “14/Rute/1” wild grapevine plants was around 40 and 30%, respectively. The 60% $CaCO_3$ treatment caused a similar growth reduction in the three studied plants (around 60–70% relative to the non-calcareous control) (Table 3).

Table 3. Relative growth rate ($gg^{-1}day^{-1}$) in plants from “14/Rute/1” and “14/Montoro/4” populations and *V. vinifera* x *V. berlandieri* “41B” in response to treatment with $CaCO_3$ for 30 days

Grapevines	$CaCO_3$ content (%)			
	0	20	40	60
14/Rute/1	0.0725 b	0.0517 c	0.0522 c	0.0300 b
14/Montoro/4	0.0572 a	0.0373 b	0.0331 b	0.0240 b
41B	0.0668 b	0.0211 a	0.2001 a	0.0166 a

Values represent the mean, n=12. In each column, means followed by different letter are statistically different ($p \leq 0.05$).

Net photosynthesis rate (A) declined significantly with increasing external $CaCO_3$ level (r = −0.87; r = −0.83; r = −0.87, for “14/Rute/1,” “41B” and “14/Montoro/4” plants, respectively). The most drastic decreased values of A in “14/Rute/1” plants occurred from 40 to 60% soil $CaCO_3$. Stomatal conductance (Gs) showed a decreasing trend with $CaCO_3$

content in “14/Rute/1” and “14/Montoro/4” wild grapevine plants. On the contrary, Gs in “41B” plants was drastically affected at 20% $CaCO_3$ and showed no response to further increases in external $CaCO_3$ content (Table 4).

Table 4. Net photosynthetic rate (µmol m^{-2} s^{-1}) (A), stomatal conductance (mmol m^{-2} s^{-1}) (Gs) in plants from “14/Rute/1” and “14/Montoro/4” populations and *V. vinifera* x *V. berlandieri* “41B” in response to treatment with $CaCO_3$ for 30 days

Grapevines	$CaCO_3$ content (%)							
	0		20		40		60	
	A	Gs	A	Gs	A	Gs	A	Gs
14/Rute/1	7.6 a	0.07 a	5.7 c	0.05 b	4.4 c	0.04 b	1.8 b	0.03 b
14/Montoro/4	7.9 a	0.07 a	4.0 b	0.03 a	2.4 b	0.02 a	1.3 ab	0.01 a
41B	7.8 a	0.11 b	2.5 a	0.03 a	1.5 a	0.02 a	0.7 a	0.02 ab

Values represent the mean, n=20. In each column, means followed by different letter are statistically different ($p \leq 0.05$).

Table 5. Chlorophyll a and b (µg g fwt $^{-1}$) in leaves of *V. vinifera* ssp. *sylvestris* from “14/Rute/1” and “14/Montoro/4” populations and *V. vinifera* x *V. berlandieri* “41B” in response to treatment with $CaCO_3$ for 30 days

Grapevines	$CaCO_3$ content (%)							
	0		20		40		60	
	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
14/Rute/1	10 a	4.4 a	8.6 ab	3.5 b	6.3 ab	3.5 b	3.3 a	1.7 a
14/Montoro/4	12 ab	5.3 b	9.3 b	4.0 b	7.6 b	3.5 b	2.6 a	2.2 a
41B	13 b	5.3 b	6.0 a	2.4	3.4 a	2.2 a	1.8 a	1.7 a

Values represent the mean, n=12. In each column, means followed by different letter are statistically different ($p \leq 0.05$).

Table 6. Total iron concentration (mg g^{-1}) in the leaves of plants of *V. vinifera* ssp. *sylvestris* from “14/Rute/1” and “14/Montoro/4” populations and *V. vinifera* x *V. berlandieri* “41B” in response to treatment with $CaCO_3$ for 30 days

Grapevines	$CaCO_3$ content (%)			
	0	20	40	60
14/Rute/1	89.6 a	93.0 b	85.4 b	72.0 b
14/Montoro/4	110.4 b	70.2 a	53.7 a	51.0 a
41B	101.1 ab	60.0 a	54.4 a	48.8 a

Values represent the mean, n=3. In each column, means followed by different letter are statistically different ($p \leq 0.05$).

Chlorophylls a and b decreased on exposure to increasing $CaCO_3$ (Chl a: $r = -0.99$; $r = -0.97$; $r = -0.95$, Chl b: $r = -0.92$; $r = -0.98$; $r = -0.87$, for “14/Rute/1,” “14/Montoro/4” and “41B” plants, respectively) affecting to a greater extent the “41B” plants in the 20 and 40% treatments, with significant less quantity of both chlorophylls than “14/Rute/1” and

"14/Montoro/4" wild grapevine plants. No significant differences between the pigment concentrations of both wild grapevine plants were found (Table 5).

Leaf Fe concentrations of both "41B" rootstock and "14/Montoro/4" wild grapevine plants decreased significantly with external $CaCO_3$ level (r = -0.87; r = -0.92, for "41B" and "14/Montoro/4" plants, respectively), whereas in the case of the "14/Rute/1," Fe concentrations showed little variation until the 40% $CaCO_3$ treatment and presented their lowest value at 60% $CaCO_3$ (Table 6).

3.2.1.2. *In Vitro* Tissue Cultures

After 60 days, in 60% $CaCO_3$, "14/Montoro/1" plantlets reduced significantly its survival in relation to the control up to 32.5%, whereas that for "14/Rute/1" and "41B" the reduction was 12% and 10.4%, respectively.

Shoot growth decreased with external $CaCO_3$ content (r = -0.76; r = -0.85; r = -0.86 for "14/Rute/1," "14/Montoro/1"; and "41B" plants, respectively). In the case of the grapevine rootstock "41B," shoot length was drastically affected at 20% $CaCO_3$ in relation to the control and showed very lower response to further increases in external $CaCO_3$ content with statistical differences regarding control and 20% $CaCO_3$. There were no significant differences between the shoot growth in the three study plants at 60% $CaCO_3$ 60 days after *in vitro* culture. However, at 40% $CaCO_3$ content, the shoot growth average was significantly lower in "41B" plants, which exhibited leaf chlorosis too, than in "14/Rute/1" wild grapevine plants (Table 7).

Total Fe content in leaves were very similar for "14/Montoro/4" *in vitro* plants after 60 days in the control (100.6 mg kg^{-1}) and at 60% $CaCO_3$ (112.9 mg kg^{-1}). However, total Fe content in leaves in "14/Rute/1" plants was increased from 233.3 mg kg^{-1} at the control to 453.1 mg kg^{-1} at 60% $CaCO_3$ and, on the contrary, in "41B" plants this content was reduced from 172.1 mg kg^{-1} at the control to 121.4 mg kg^{-1} at 60% $CaCO_3$.

Table 7. The effect of $CaCO_3$ concentrations on shoot growth (cm) of *V. vinifera* ssp. *sylvestris* from "14/Rute/1" and "14/Montoro/4" populations and *V. vinifera x V. berlandieri* "41B" in response to treatment with $CaCO_3$ for 60 days of *in vitro* culture

Grapevines	$CaCO_3$ content (%)			
	0	20	40	60
14/Rute/1	3.19 a	4.06 b	1.93 b	1.77 b
14/Montoro/4	3.68 a	3.64 ab	1.04 a	1.48 ab
41B	7.53 b	2.41 a	0.99 a	1.15 a

Values represent the mean, n=48. In each column, means followed by different letter are statistically different ($p < 0.05$).

3.2.2. Evaluating Wild Grapevine Tolerance to Copper in Soil

3.2.2.1. Greenhouse Experiment

According to Table 8, in the case of ‘‘41B’’ plants, relative growth rate (RGR) was drastically affected by external Cu concentrations of 600 mg Cu kg^{-1}. Relative to the control, reduction in RGR in the 600 and 1500 mg Cu kg^{-1} treatments was around 50%. On the contrary, the RGR of the ‘‘Río Agrio’’ and ‘‘14/Rute/1’’ wild grapevine populations were similar with increasing Cu concentrations up to 600 mg Cu kg^{-1}, but then decayed substantially on exposure to 1500 mg Cu kg^{-1}, with these values being significantly lower than those of the control treatment (Table 8). Plants from the ‘‘Río Agrio’’ and ‘‘14/Rute/1’’ wild populations treated with 1500 mg Cu kg^{-1} presented chlorosis at the end of treatment; in the case of the ‘‘41B’’ plants, leaf chlorosis was detected early in plants exposed to external Cu levels from 600 mg Cu kg^{-1}.

Table 8. Relative growth rate ($gg^{-1}day^{-1}$) in plants of *V. vinifera* ssp. *sylvestris* from “Río Agrio” and “14/Rute/1” populations and *V. vinifera* x *V. berlandieri* “41B” in response to treatment with external Cu for 30 days

Grapevines	Cu content (mg Cu kg^{-1})				
	0	50	150	600	1500
Río Agrio	0.0700 b	0.0724 b	0.0735 b	0.0615 b	0.0454 b
14/Rute/1	0.0557 a	0.0591 a	0.0585 a	0.0519 b	0.0323 a
41B	0.0755 b	0.0705 b	0.0774 b	0.0376 a	0.0357 a

Values represent the mean, n=12. In each column, means followed by different letter are statistically different *(p* = 0.05).

After 30 days of treatment, net photosynthesis rate (A) declined significantly under exposure to increasing external Cu level, reaching a minimum value at the highest external Cu concentration (r = -0.90, r = -0.87 and r = -0.82, for ‘‘Río Agrio’’, ‘‘41B’’ and ‘‘14/Rute/1’’ plants, respectively). In presence of 600 and 1500 mg Cu kg^{-1} in the nutrient solution, values of A in wild grapevine plants from the ‘‘Río Agrio’’ and ‘‘14/Rute/1’’ populations were significantly higher than those obtained in the ‘‘41B’’ plants (Table 9). In all three cases, stomatal conductance (Gs) was strongly correlated with A (r = 0.89, r = 0.96, r = 0.70, for ‘‘Río Agrio’’, ‘‘41B’’ and ‘‘14/Rute/1’’ plants, respectively; Table 9).

In the three cases, chlorophylls a and b decreased significantly on exposure to external Cu concentrations from 600 mg Cu kg^{-1}, but this decrease was considerably more pronounced for chlorophyll a in the leaves of “Río Agrio” (r = -0.93), “14/Rute/1” (r = -0.91) and “41B” (r = 0 -0.89) than for chlorophyll b (r = -0.74; r= - 0.65 and r = -0.80, respectively). On the other hand, both chlorophyll levels decrease was significantly more marked in the ‘‘41B’’ plants for both photosynthetic pigments (Tables 10 and 11).

Table 9. Net photosynthetic rate (µmol m^{-2} s^{-1}) (A), stomatal conductance (mmol m^{-2} s^{-1}) (Gs) in plants of *V. vinifera* ssp. *sylvestris* from the "Río Agrio" and "14/Rute/1" populations and *V. vinifera* x *V. berlandieri* "41B" in response to treatment with external Cu for 30 days

Grapevines	Cu content (mg Cu kg^{-1})									
	0		50		150		600		1500	
	A	Gs	A	Gs	A	Gs	A	Gs	A	Gs
Río Agrio	7.9 a	0.06 b	7.7 a	0.05 b	7.6 a	0.06 b	4.6 b	0.03 c	1.3 b	0.01 b
14/Rute/1	7.1 a	0.08 c	7.0 a	0.07 a	6.9 a	0.07 b	4.2 b	0.05 b	3.5 b	0.04 c
41B	8.4 a	0.05 a	8.1 a	0.05 b	7.9 a	0.04 a	1.0 a	0.01 a	0.2 a	0.01 a

Values represent the mean, n=12. In each column, means followed by different letter are statistically different ($p \leq 0.05$).

Table 10. Chlorophyll a (µg g fwt^{-1}) in leaves of *V. vinifera* ssp. *sylvestris* from "Río Agrio" and "14/Rute/1" populations and *V. vinifera* x *V. berlandieri* "41B" in response to treatment with external Cu for 30 days

Grapevines	Cu content (mg Cu kg^{-1})				
	0	50	150	600	1500
RíoAgrio	12.7±0.366 a	12.9±0.379 a	12.9±0.589 ab	9.3±0.184 b	6.60±0.310 b
14/Rute/1	13.0±0.724 a	13.3±0.655 a	12.5±0.519 a	9.0±0.599 b	5.53±0.661 b
41B	12.9±0.615 a	13.4±0.597 a	13.6±0.747 b	5.5±0.523 a	3.03±0.712 a

Values represent the mean, n=12. In each column, means followed by different letter are statistically different ($p \leq 0.05$).

Table 11. Chlorophyll b (µg g fwt^{-1}) in leaves of *V. vinifera* ssp. *sylvestris* from "Río Agrio" and "14/Rute/1" populations and *V. vinifera* x *V. berlandieri* "41B" in response to treatment with external Cu for 30 days

Grapevines	Cu content (mg Cu kg^{-1})				
	0	50	150	600	1500
RíoAgrio	5.25±0.168 a	5.40±0.461 a	5.64±0.290 a	4.23±0.159 b	3.06±0.255 b
14/Rute/1	4.97±0.511 a	5.21±0.733 a	5.38±0.361 a	3.78±0.350 b	2.79±0.328 b
41B	5.61±0.662 a	5.37±0.636 a	5.30±0.381 a	2.30±0.219 a	1.53±0.286 a

Values represent the mean, n=12. In each column, means followed by different letter are statistically different ($p \leq 0.05$).

Under exposure to 600 and 1500 mg Cu kg^{-1}, tissue copper concentrations in leaves were significantly higher in plants of the "41B" rootstock than in the "Río Agrio" and "14/Rute/1" wild grapevine plants. On the other hand, at these Cu levels, plants from the both wild grapevines presented significantly lower values of root Cu than plants of the studied rootstock. Besides, at lower levels of external Cu, from 150 to 600 mg Cu kg^{-1}, the "Río Agrio" roots presented significantly lower values of Cu than those of "14/Rute/1" (Tables 12 and 13).

Table 12. The effect of Cu concentrations on Cu contents in leaves (mg kg^{-1}) of *V. vinifera* ssp. *sylvestris* from “Río Agrio” and “14/Rute/1” populations and *V. vinifera x V. berlandieri* “41B” in response to treatment with external Cu for 30 days of culture

Grapevines	Cu content (mg Cukg^{-1})				
	0	50	150	600	1500
Río Agrio	0.010±0.001 a	0.014±0.001 a	0.017±0.0007 a	0.032±0.001 a	0.083±0.006 a
14/Rute/1	0.016±0.001 b	0.012±0.002 a	0.021±0.0004 b	0.036±0.001 a	0.101±0.006 a
41B	0.006±0.0007 a	0.014±0.00008 a	0.016±0.0002 a	0.075±0.003 b	0.142±0.006 b

Values represent the mean, n=48. In each column, means followed by different letter are statistically different ($p \leq 0.05$).

Table 13. The effect of Cu concentrations on Cu contents in roots (mg kg^{-1}) of *V. vinifera* ssp. *sylvestris* from “Río Agrio” and “14/Rute/1” populations and *V. vinifera x V. berlandieri* “41B” in response to treatment with external Cu for 30 days of culture

Grapevines	Cu content (mg Cukg^{-1})				
	0	50	150	600	1500
Río Agrio	0.02±0.003 a	0.09±0.006 a	0.25±0.0009 a	0.46±0.005 a	1.33±0.014 a
14/Rute/1	0.01± 0.008 a	0.30±0.005 b	0.39±0.024 b	0.93±0.071 b	1.37±0.058 a
41B	0.02±0.004 a	0.31±0.022 a	0.46±0.032 c	4.55±0.499 c	22.51±0.803 b

Values represent the mean, n=48. In each column, means followed by different letter are statistically different ($p \leq 0.05$).

3.2.2.2. *In Vitro* Experiments

After 60 days of culture in these conditions, all plants cultured reduced significantly its survival in an inverse relation to the Cu levels (r = - 0.98; r = - 0.97 and r= - 0.98 for “Río Agrio,” “41B” and “14/Rute/1,” respectively). On the other hand, in 50, 200 and 500 µM CuSO$_4$ “41B” plant survival (70.2, 35.4 and 10.4% respectively) was statistically lower than those of “Río Agrio” plants (93.75, 64.6 and 35.4%, respectively). In the case of “14/Rute/1” plants survival percentages in 200 and 500 µM (58.3 and 15.6%, respectively) were similar statistically to those of “Río Agrio” for these CuSO$_4$ concentrations and different to those of “41B” plants. In all cases, the stem growth (length and bud number averages) were drastically affected by the Cu level increasing in the culture medium (r = - 0.86; r = - 0.90 and r = - 0.84, respectively; for "Río Agrio," "41B" and "14/Rute/1" in the case of shoot length; and r = -0.88; r = -0.93 and r = - 0.83 for "Río Agrio," "41B" and "14/Rute/1," for bud number. Relative to the concentrations up to 50 µM, the evolution of both parameters were similar for each of the three considered grapevines. However, drastic reductions in both parameters in 200 and 500 µM Cu concentrations were found, and again, the lowest values in "14/Rute/1" and “41B” plants were obtained both for stem length and bud number (Tables 14 and 15).

Table 14. The effect of Cu concentrations on shoot growth (cm) averages of *V. vinifera* ssp. *sylvestris* from “Río agrio” and “14/Rute/1” populations and *V. vinifera* x *V. berlandieri* “41B” in response to treatment with external Cu for 60 days of *in vitro* culture

Grapevines	Cu content (µM)				
	0	5	50	200	500
Río Agrio	7.86±0.588 b	8.47±0.621 c	6.6±0.672 b	0.11±0.030 a	0.13±0.025 a
14/Rute/1	3.37±0.602 a	2.09±0.503 a	2.2±0.679 a	0.17±0.114 a	0.04±0.037 a
41B	6.27±0.614 b	5.91± 0.697 b	5.7±0.603 b	0.91±0.331 b	0.72±0.231 b

Values represent the mean, n=48. In each column, means followed by different letter are statistically different ($p \leq 0.05$).

Table 15. The effect of Cu concentrations on bud number averages of V. vinifera ssp. sylvestris from “Río agrio” and “14/Rute/1” populations and V. vinifera x V. berlandieri “41B” in response to treatment with external Cu for 60 days of in vitro culture

Grapevines	Cu content (µM)				
	0	5	50	200	500
Río Agrio	7.80±0.593 b	7.88±0.553 c	6.09±0.576 b	1.19±0.342 ab	0.82±0.196 ab
14/Rute/1	4.00±0.618 a	2.40±0.594 a	2.20±0.564 a	9.5±0.221 a	0.14±0.123 a
41B	6.13±0.596 b	5.37±0.544 b	5.67±0.483 b	2.41±0.717 b	1.25±0.421 b

Values represent the mean, n=48. In each column, means followed by different letter are statistically different ($p \leq 0.05$).

Root formation in the cases of “Río Agrio” and “14/Rute/1” explants was even more affected by $CuSO_4$ than the development of aerial parts of the plantlets (r = - 0.98 for plantlets of both wild grapevine). In the case of “41B” explants the rate of rooting was inversely related to the $CuSO_4$ concentrations (r = - 0.92). In any case, the percentages of root formation reached by “Río Agrio” explants in the substrates with 5 and 50 µM Cu (87.5 and 95.7%) were statistically higher than those obtained in “41B” (77.1 and 79.2%) or in “14/Rute/1 (72.9 and 67.4%). In the same way, in substrate with 200 µM Cu the rate of rooting registered in “Río Agrio” plantlets (54.2%) was also significantly higher than those obtained in “41B” (12.5%). Concentration of 500 µM Cu prevented rooting of the explants in all cases. The root number average per explant was less affected in “14/Rute/1” plantlets in relation to increasing $CuSO_4$ levels (r = 0.068) than the plantlets of the other two grapevine studied accessions (r = - 0.99 and r = - 0.85 for “Río Agrio” and “41B”) (Table 16).

Table 16. The effect of Cu concentrations on root number average of *V. vinifera* ssp. *sylvestris* from "Río Agrio" and "14/Rute/1" populations and *V. vinifera* x *V. berlandieri* "41B" in response to treatment with external Cu for 60 days of *in vitro* culture

Grapevines	Cu content (µM)				
	0	5	50	200	500
Río Agrio	2.64±0.1753a	2.69±0.1972a	2.60±0.1635a	1.92±0.2142a	1.13±0.125a
14/Rute/1	5.13±0.4465b	4.34±0.5575b	5.16±0.4871b	6.79±0.6472b	4.73±0.446b
41B	2.51±0.1716a	2.22±0.1895a	2.06±0.1624a	2.67±1.1155a	0 a

Values represent the mean, n=48. In each column, means followed by different letter are statistically different ($p \leq 0.05$).

According to Table 17, both leaf and root copper concentration increased significantly with Cu addition to the substrate (r = 0.99-stem; r = 0.99-root; r = 0.99-stem, r = 0.99-root and r = 0.97-stem, r = 0.99-root; for "Río Agrio," "41B" and "14/Rute/1," respectively).

Table 17. Influence of Cu concentration of the medium on the Cu composition (mg kg^{-1}) of the stems and roots of grapevines "Río Agrio," "41B" and "14/Rute/1" after 60 days of the onset of *in vitro* culture

		"Río Agrio"	"41B"	"14/Rute/1"
Part of plantlet	Cu level (µM)	Cu (mg Kg-1)		
Stem	0	14.53	21.36	16.24
Stem	5	31.53	17.35	18.62
Stem	50	96.39	106.23	75.71
Stem	200	-	766.27	1862.48
Stem	500	-	-	-
		"Río Agrio"	"41B"	"14/Rute/1"
Part of plantlet	Cu level (µM)	Cu (mg Kg-1)		
Root	0	19.85	15.03	13.81
Root	5	71.98	48.20	31.24
Root	50	407.61	284.67	225.10
Root	200	-	-	1050.28
Root	500	-	-	-

4. DISCUSSION

As general discussion, it is necessary to remark the absence, due to edaphical conditions, of infestation by Phylloxera, which was the destructor of vineyards and obligate to use North American resistant rootstocks for the re-construction of European vineyards. Moreover, it was noticed high percentage of symptoms caused by the erineum strain of *Colomerus vitis* mite and powdery and downy mildews in the wild (Table 2). Probably, it could be the main cause of the disappearance of those populations from Southern Caucasus including centenarian exemplars with large lods, some of them with the thickness of a ship's mast described by Pallas (1799-1801).

A characterization of the biometric and physiological responses of different species of the genus *Vitis* to the negative conditions of the vineyard is essential to provide ways to improve current classic cultivars through the incorporation of traits that are better adapted to the prevailing environmental and growing conditions. Nevertheless, the selection of plants with high tolerance to elevated levels of lime or copper in soil comparing wild subspecies and a commercial variety of grapevine has never been performed under the same experimental conditions; furthermore, to our knowledge, there are no studies to date evaluating the effects of lime soil contents above 20% in the genus *Vitis*.

The lower reduction in relative growth rate of "14/Rute/1" plants, (30%), growing in 20 and 40% $CaCO_3$, in relation to those of "14/Montoro/4" and, particularly, to those of "41B" (Table 3) indicates a good response to lime stress of the individuals of this population. Bavaresco et al. (1995) reported that shoot biomass reduction of the lime-tolerant species *Vitis cinerea* and *V.berlandieri* was around 41% and 35%, respectively, under exposure to a very lower active lime level (19%).

Evaluation of the photosynthetic performance, expressed as gas exchange, provides valuable information by which to rank the species according to its tolerance to high lime conditions. In all, "41B" rootstock plants and wild grapevines from both populations, increasing external $CaCO_3$ induced considerable decreases in net photosynthesis rate (A) and stomatal conductance (Gs) (Table 4). These recorded growth reductions are likely to be attributable to the decrease in photosynthetic carbon assimilation by reduction in net photosynthesis rate (A) in a 25% and 40%, relative to the control in plants exposed to 20% and 40% $CaCO_3$, respectively. However, it should be emphasized that the deleterious effects of 20 and 40% external lime on gas exchange parameters were considerably more pronounced in "41B" rootstock plants (Table 4). Moreover, at these soil $CaCO_3$ contents, wild grapevine plants from "14/Rute/1" population showed considerably higher values of A and Gs than the "14/Montoro/4" plants (Table 4). Three-year-old plants of *V. vinifera* L. cv Pinot Blanc, grafted onto the lime-susceptible rootstock "3309 C," experienced a reduction of around 50% in net photosynthesis rate under exposure to 16% active lime (Bavaresco et al., 2006), whereas the reduction in A found in *V. vinifera* grafted on the medium lime-tolerant rootstock "SO4" growing in a calcareous soil (17% active lime) was around 20%, relative to the control (Bavaresco and Poni, 2003).

Bioavailability of iron for plant requirement is strongly impaired under calcareous soil conditions and may often interferes with essential nutrient uptake and transport (Zancan et al., 2008; Bavaresco et al., 2013). In the case of "14/Rute/1," leaf Fe concentration was significantly higher at 40 and 60% $CaCO_3$ than those of "41B" and "14/Montoro/4" leaves, without statistical differences between them (Table 6). In consequence, "14/Rute/1" demonstrated a more efficient control of the nutritional status under $CaCO_3$ stress than that of the “41B” rootstock and wild grapevines from “14/Montoro/4” population. Thus, the notable decrease in the concentration of chlorophyll *a* recorded at 60% $CaCO_3$ in all studied plants (Table 5) may be partially related with the lower availability of Fe involved in chlorophyll synthesis found in this treatment (Lawlor, 2002). Phosphoenolpyruvate carboxylase (PEPC) activity is considered a physiological marker of Fe deficiency (Covarrubias et al., 2014). A significant decrease of PEPC activity was recorded in the Fe-chlorosis tolerant “140 Ruggeri” grapevine rootstock in presence of bicarbonate in the nutrient solution (Covarrubias and Rombolà, 2013). In this regard, the impairment of photosynthetic function detected in our study could be partly related to a decrease in the activities of certain enzymes implied in photosynthesis as PEPC. According to our results root Fe concentrations in “14/Rute/1” were maintained up to the 40% $CaCO_3$ treatment, indicating that this selected wild grapevine is capable of maintaining Fe uptake by the roots and translocation to leaves, even under high lime conditions. After 30 days of culture at greenhuose conditions, the substrate copper concentration resulting in 50% biomass reduction (EC50) of wild grapevine plants was higher than 1500 mg Cu kg^{-1}. On the contrary, in the ‘‘41B’’ grapevine rootstock, the EC50 value was around 600 mg Cu kg^{-1}. Toselli et al. (2009) reported that the Cu toxicity threshold in *Vitis vinifera* cv Sangiovese can be established at 200 mg kg^{-1} external Cu.

Relative to the control, the reduction of the net photosynthetic rate (A) in the 600 mg Cu kg^{-1} Cu treatment was around 42% in ‘‘Río Agrio’’ and ‘‘14/Rute/1’’ wild grapevine plants, and around 88% in ‘‘41B’’ plants (Table 9). It is known that external Cu concentrations greater than 20 mg kg^{-1} inhibit photosynthesis process (Baszynski et al., 1982; Vassilev et al., 2002). In our experiments, the reduction in photosynthetic carbon assimilation greatly induced growth reduction in plants exposed to the highest external Cu concentrations. The highest external Cu levels provoked considerable effects on net photosynthesis rate (A) and stomatal conductance (Gs). These results suggest that the reduction of A and chlorophylls in the leaves could be ascribed to the different effects of Cu on the integrity or function of the photochemical apparatus. However, it should be highlighted that the deleterious effects of Cu on photosynthetic function were more severe in the ‘‘41B’’ grapevine rootstock than in the wild grapevine plants, especially marked at 600 mg Cu kg^{-1}; since at this external Cu concentration, the reduction in net photosynthesis rate and pigment concentration of the ‘‘Río Agrio’’ and ‘‘14/Rute/1’’ wild grapevines was around half that of the ‘‘41B’’ plants (Table 9).

The roots of “Río Agrio” and “14/Rute/1” plants exhibit a stronger capability for actively avoiding Cu uptake from the nutrient solution, and/or excluding Cu from roots, than is the case of the ‘‘41B’’ grapevine rootstock (Table 13). Both systems of root defense against elevated Cu levels in soil, whether Cu exclusion (Llugany et al., 2003; Ke et al., 2007) for Cu tolerant ecotypes of different other species, or transference reduction to the aerial parts (Juang et al., 2012; Cambrollé et al., 2013; 2015) for grapevine, have been previously reported. In consequence, it is important to highlight the high tolerance capacity to copper sulfate in the culture medium presented by wild grape plants of the "Río Agrio" population under *in vitro* conditions, establishing the limit of tolerance at 50 μM. This response is according with the bibliography found for tobacco (60 μM) (Rout and Sahoo, 2007). Although in this case, besides being another species considered to be more tolerant, the material used is undifferentiated callus, compared to the starting material used in this experimental process, well-differentiated buds and, therefore, with less tolerance capacity and, however, it has similar levels of tolerance.

The tests carried out in the present project on tolerance to copper sulfate have allowed to obtain well-adapted plants at levels of 50 μM Cu (Tables 14 and 15) and with positive response even at four times higher levels (200 μM Cu) under *in vitro* conditions. These kind of experiments, until now was not described in the bibliography and, therefore, practiced for the first time in this work were then confirmed in greenhouse tests, as recommended by several authors (Skene and Barlass, 1988; Troncoso et al., 1999) obtaining again high survivals in these conditions, even in concentrations of 1500 mg Cu kg^{-1} (Table 8). That is, the wild grape plants of the population of “Río Agrio” manifest a clear tolerance to copper in both farming situations.

It should be highlighted the highest number of roots recorded in "14/Rute/1" plantlets (Table 16), that can be explained by an attempt on the part of the explant to colonize an area of the medium with lower copper content, because of plants from these wild grapevine population is considered with high productivity in radicular emission (Maghradze et al., 2015).

CONCLUSION

The main grapevine pest found was *Colomerus vitis,* affecting between 76-100% of the wild grapevine exemplars. Powdery and downy mildews fungal species would be one of the main causes of reduction of the wild grapevine populations from the second part of the 19th century to present.

As consequence of our results, plants from the population grown in hypercalcic calcisol soil (“14/Rute/1”) present a higher degree of lime tolerance and, compared to other wild grapevine population, could constitute an elite gene pool for the development of new lime stress-tolerant varieties of grapevine.

Under exposure to 600 mg Cu kg^{-1}, roots of both the ‘‘Río Agrio’’ and ‘‘14/Rute/1’’ wild grapevine plants were shown to be more efficient in controlling Cu uptake and/or excluding the metal, than was the case with the ‘‘41B’’ plants.

To our knowledge, there are not any study integrating grapevine and calcium carbonate under *in vitro* culture for selecting tolerant genotypes to lime conditions. In consequence, the similar response obtained in “14/Rute/1,” “14/Montoro/4 and “41B” plants for hypercalcic conditions and in “Río Agrio” “14/Rute/1” and “41B” for high levels of Cu in soil, in both culture conditions (greenhouse and *in vitro* technique) allows considering the *in vitro* culture as a reliable tool for rapid selection to these abiotic stresses in grapevines varieties.

The Eurasian wild grapevine constitutes a good phytogenetic resource for programs of selection of plants tolerant to calcium carbonate and copper in the soil and, definitively, it must be a very important reserve to be conserved and used in breeding programs of cultivars and rootstocks.

ACKNOWLEDGMENTS AND AUTHORS CONTRIBUTIONS

We thank the Seville University Glasshouse General Service for their collaboration. We are also grateful to María del Mar Parra Alejandre, Alberto González Moreno y José Manuel Romero Calderón for technical assistance. This research was funded by the Consejo Superior de Investigaciones Científicas (CSIC) (Project 201140E122). Authors' contributions: C.A.O.P. carried out the systematic observation of pests and diseases on wild grapevine populations. M.L.B. contributed determining ampelographical, pest and diseases characters. J.C.S. designed greenhouse experiments and control of ecophysiological variables. M.G.M. carried out *in vitro* experiments, contributed to greenhouse experiments and determinations in lab. J.L.G.F. contributed ran the laboratory works and collaborated drafting the paper. R.O.R. supervised wild grapevine population, pest and disease detections. M.C.B. contributed the design the study, processed the data and drafted the paper.

REFERENCES

Abadia, J., Vázquez, S., Rellan-Alvarez, R., El-Jendoubi, H., Abadia, A., Alvarez-Fernandez, A., López-Millán, A. F. (2011). Towards a knowledge-based correction of iron chlorosis. *Plant Physiology and Biochemistry*, 49:471-482.

Álvarez-Fernández, A., Melgar, J. C., Abadia, J., Abadia, A. (2011). Effects of moderate and severe iron deficiency chlorosis on fruit yield, appearance and composition in pear (*Pyrus communis* L.) and peach (*Prunus persica* (L.) Batsch). *Environmental and Experimental Botany,* 71:280-286.

Ambrosi, H., Dettweiler, E., Rühl, E., Schmid, J., Schumann, E. (1994). *Farbatlas Rebsorten. 300 Sorten und ihre Weine*; Ulmer Verlag: Stuttgar. [*Color Atlas of Grape Varieties. 300 varieties and their wines*. Ulmer Verlag: Stuttgart].

Anderson, K., Aryal, N. R. (2017). *Which winegrape varieties are grown where? A global empirical picture*. University of Adelaide Press.

Armengol, J. (2017). Enfermedades fúngicas de madera de la vid. Una visión general del problema. *Enoviticultura*, 46:6-15. [Fungal diseases of grapevine wood. An overview of the problem. *Enoviticulture*, 46:6-15].

Arnold, C. (2002). *Ecologie de la vigne sauvage en Europe* (Vitis vinifera L. subsp *silvestris* (Gmelin) Hegi). Naturelles, A.S.d.S., Ed. vdf Hochschulverlag AG: Zürich (Switzerland). 256 pp. [*Ecology of wild grapes in Europe*: Contributions to the geobotanical survey of Switzerland. 256 pp.].

Arnold, C., Gillet, F., Gobat, J. M. (1998). Occurrence of the wild vine Vitis vinifera ssp. silvestris in Europe. *Vitis*, 37:159-170.

Arrigo, N., Arnold, C. (2007). Naturalised Vitis Rootstocks in Europe and Consequences to Native Wild Grapevine. *PLoS ONE*, 2, e521.

Askri, H. Gharbi, F. Rejeb, S. Mliki, A., Ghorbel, A. (2017). RETRACTED: Physiological comparative study of six wild grapevine (*Vitis sylvestris*) accession responses to salinity (Retracted article. See vol. 10, Artn 402, 2017). *Arabian Journal of Geosciences*, 10:246.

Baszynski, T., Krol, M., Krupa, Z., Ruszkowska, M., Wojcieska, U., Wolinska, D. (1982). Photosynthetic apparatus of spinach exposed to excess copper. *Zeitschrift Fur Pflanzenphysiologie*, 108:385-395.

Bavaresco, L., Poni, S. (2003). Effect of calcareous soil on photosynthesis rate, mineral nutrition, and source-sink ratio of table grape. *Journal of Plant Nutrition*, 26:2123-2135.

Bavaresco, L., Bertamini, M., Iacono, F. (2006). Lime-induced chlorosis and physiological responses in grapevine (*Vitis vinifera* L. cv. Pinot blanc) leaves. *Vitis*, 45:45-46.

Bavaresco, L., Cantu, E., Trevisan, M. (2000). Chlorosis occurrence, natural arbuscular-mycorrhizal infection and stilbene root concentration of ungrafted grapevine rootstocks growing on calcareous soil. *Journal of Plant Nutrition*, 23: 1685-1697.

Bavaresco, L., Fregoni, M., Perino, A. (1995). Physiological aspects of lime-induced chlorosis in some *Vitis* species. 2. Genotype response to stress conditions. *Vitis*, 34:233-234.

Benito, A., Muñoz-Organero, G., De Andres, M. T., Ocete, R., Garcia-Muñoz, S., Lopez, M. A., Arroyo-Garcia, R., Cabello, F. (2017). *Ex situ* ampelographical characterisation of wild Vitis vinifera from fifty-one Spanish populations. *Australian Journal of Grape and Wine Research*, 23:143-152.

Brancadoro, L., Rabotti, G., Scienza, A., Zocchi, G. (1995). Mechanisms of Fe-efficiency in roots of Vitis spp. in response to iron-deficiency stress. *Plant and Soil*, 171:229-234.

Bustamante, J. (1971). *La Tierra y los Valles de Mena*; Bilbao, S. E., Ed. 606 pp. [*The Earth and the Valleys of Mena*; S. E., Ed. 606 pp.].

Cambrollé, J., García, J. L., Figueroa, M. E., Cantos, M. (2015). Evaluating wild grapevine tolerance to copper toxicity. *Chemosphere*, 120:171-178.

Cambrollé, J., García, J. L., Figueroa, M. E., Cantos, M. (2014). Physiological responses to soil lime in wild grapevine (*Vitis vinifera* ssp. *sylvestris*). *Environmental and Experimental Botany*, 105:25-31.

Cambrollé, J., García, J. L., Ocete, R., Figueroa, M. E., Cantos, M. (2013). Growth and photosynthetic responses to copper in wild grapevine. *Chemosphere*, 93:294-301.

Chauvet, M. and Reynier, A. (1984). *Manual de Viticultura* [*Viticulture Manual*]; Mundi-Prensa. Ed.

Chilashvili, L. (2004). *The vine, Wine and Georgians*; Petite Publisher: Tbilisi (Georgia). 363p.

Claeys, H., Van Landeghem, S., Dubois, M., Maleux, K., Inze, D. (2014). What Is Stress? Dose-Response Effects in Commonly Used in Vitro Stress Assays. *Plant Physiology*, 165:519-527.

Covarrubias, J. I., Rombolà, A. D. (2013). Physiological and biochemical responses of the iron chlorosis tolerant grapevine rootstock 140 Ruggeri to iron deficiency and bicarbonate. *Plant and Soil*, 370:305-315.

Covarrubias, J. I., Pisi, A., Rombolà, A. D. (2014). Evaluation of sustainable management techniques for preventing iron chlorosis in the grapevine. *Australian Journal of Grape and Wine Research*, 20:149-159.

De Andrés, M. T., Benito, A., Pérez-Rivera, G., Ocete, R., López, M. A., Gaforio, L., Muñoz, G., Cabello, F., Martinez-Zapater, J. M., Arroyo-Garcia, R. (2012). Genetic diversity of wild grapevine populations in Spain and their genetic relationships with cultivated grapevines. *Molecular Ecology*, 21:800-816.

Díaz, I., Del Campillo, M. C., Cantos, M., Torrent, J. (2009). Iron deficiency symptoms in grapevine as affected by the iron oxide and carbonate contents of model substrates. *Plant and Soil*, 322:293-302.

Drouineau, G. (1942). Dosage rapide du calcaire actif de sols. *Annales Agronomiques*, 12, 441-450. [Rapid determination of active limestone from soils. *Agronomic Annals*, 12:441-450].

Ferragut, F., Gallardo, A., Ocete, R., López, M. A. (2008). Natural predatory enemies of the erineum strain of *Colomerus vitis* (Pagenstecher) (Acari, Eriophyidae) found on wild grapevine populations from southern Spain (Andalusia). *Vitis*, 47:51-54.

Forni, G. (2004). Dall'origine della viticoltura alla sua introduzione in Italia.; *F. Del Zan, O.F.A.S.E.E.*, Gorizia., Ed..pp. 19-97. [From the origin of viticulture to its introduction in Italy. *F. Del Zan, O.F.A.S.E.E.*, Gorizia., Ed.19-97p.].

Galán, E., Romero, A. (2008). Contaminación de Suelos por Metales Pesados. *Revista Sociedad Española de Mineralogía*, 48-60. [Contamination of soils by heavy metals. *Journal of Spanish Society of Mineralogy*, 48-60].

Gallardo, A., Ocete, R., López, M. A., Lara, M., Rivera, D. (2009). Assessment of pollen dimorphism in populations of *Vitis vinifera* L. subsp *sylvestris* (Gmelin) Hegi in Spain. *Vitis*, 48:59-62.

Grassi, F., Labra, M., Imazio, S., Spada, A., Sgorbati, S., Scienza, A., Sala, F. (2003). Evidence of a secondary grapevine domestication centre detected by SSR analysis. *Theoretical and Applied Genetic*, 107:1315-1320.

Hoagland, D., Arnon, D. I. (1950). The water-culture method for growing plants without soil. *California Agricultural Experiment Station Bulletin*, 347:1-39.

Huglin, P., Schneider, Ch. (1998). *Biologie et écologie de la vigne*. Ed. Lavoisier Tec. et Doc., Paris, 370 pp. [*Biology and ecology of the grapevine*. Ed. Lavoisier Tec. et Doc., Paris, 370 pp.]

Inskeep, W. P. and Bloom, P. R. (1986). Calcium-Carbonate supersaturation in soil solutions of calciaquolls. *Soil Science Society of America Journal*, 50:1431-1437.

Iriarte-Chiapusso, M. J., Salinas, J. A., Pérez, M. A., Ocete, E., Ocete, R. (2013). La vid silvestre, un taxón amenazado en diversos ecosistemas ibéricos. *Cuad. Soc. Esp. Cienc. For.*, 38:143-148. [The wild vine, a taxon threatened in various Iberian ecosystems. *Notebooks of the Spanish Society of Forest Sciences*, 38:143-148].

Juang, K. W., Lee, Y. I., Lai, H. Y., Wang, C. H., Chen, B. C. (2012). Copper accumulation, translocation, and toxic effects in grapevine cuttings. *Environmental Science and Pollution Research*, 19:1315-1322.

Ke, W., Xiong, Z. T., Chen, S., Chen, J. (2007). Effects of copper and mineral nutrition on growth, copper accumulation and mineral element uptake in two *Rumex japonicus* populations from a copper mine and an uncontaminated field sites. *Environmental and Experimental Botany*, 59:59-67.

Kloke, A., Einkmann, T. (1991). *Nutzungund schutzbezogene Orientierungsdaten für (Schad) Stoffe in Böden*. Sonderdruck aus Heft, 1, 8. [*Use and protection-related orientation data for (harmful) substances in soils*. Special print from booklet, 1, 8.]

Komárek, M., Cadková, E., Chrastný, V., Bordas, F., Bollinger, J. C. (2010). Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. *Environment International*, 36:138-151.

Laguna, A. (1566). *Pedacio Dioscórides Anazarbeo, acerca de la materia medicinal y de los venenos mortíferos*; Salamanca (Spain); 616 pp. [*Pedacio Dioscórides Anazarbeo, about medicinal matter and deadly poisons*. Salamanca (Spain); 616 pp].

Lawlor, D. W. (2002). Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of Experimental Botany*, 53:773-787.

Levadoux, L. (1956). Les populations suavages et cultivées de *Vitis vinífera* L. *Annales de l'Amélioration des Plantes*, 1, 59-118. [The wild and cultivated populations of *Vitis vinífera*, L. *Annales de l'Amélioration des Plantes*, 1, 59-118.]

Lidon, F. C. and Henriques, F. S. (1991). Limiting step on photosynthesis of rice plants treated with varying copper levels. *Journal of Plant Physiology*, 138: 115-118.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids - pigments of photosynthetic biomembranes. *Methods in Enzymology*, 148:350-382.

Llugany, M., Lombini, A., Poschenrieder, C., Dinelli, E., Barceló, J. (2003). Different mechanisms account for enhanced copper resistance in *Silene armeria* ecotypes from mine spoil and serpentine sites. *Plant and Soil*, 251:55-63.

Logothetis, M. (1974). The contribution of the vine and wine to the Civilization of Greece and Eastern Mediterranean (in Greek with French summary). *In Epistemoniki Epetiris tis Geoponikis kai Dasologikis Sholis*, University of Thessaloniki ed.; Vol. 17, pp. 5-286.

Lovicu, G., Farci, M., Barchetta, G., Orrú, M., Pérez, M. A., Gómez, J. E., Ocete, R. (2009). Hábitats, estado sanitario y caracterización enológica de la vid silvestre en Cerdeña. *Enólogos*, 62:30-35. [Habitats, sanitary status and oenological characterization of wild vine in Sardinia. *Oenologists*, 62:30-35].

Maggioni, A. (1980). Iron-absorption by excised grapevine roots. *Vitis*, 19:105-112.

Maghradze, D., Ocete, M. E., Pérez, M. A., Chipashvili, R., Ocete, C. A., Ocete, R. (2009). El patrimonio vitícola de Georgia: el estado sanitario de sus poblaciones silvestres. In *Proceedings of XXXI Jornadas de Viticultura y Enología*, Tierra de Barros., Almendralejo, Badajoz, Spain; pp. 113-116. [The wine heritage of Georgia: the health status of its wild populations. In *Proceedings of XXXI Viticulture and Oenology Symposium*, Tierra de Barros., Almendralejo, Badajoz, Spain; pp. 113-116.]

Maghradze, D., Ocete, R., Garcia, J. L., Cantos, M. (2015). Micropropagation and in vitro germplasm conservation of Georgian wild grapevines. *Vitis*, 54:257-258.

Mangafa, M., Kotsakis, K. (1996). A new method for the identification of wild and cultivated charred grape seeds. *Journal of Archaeological Science*, 23:409-418.

Martínez de Toda, F. (1991). *Biología de la vid: fundamentos biológicos de la viticultura*; Mundi-Prensa. [*Vine Biology: Biological basis of viticulture*; Mundi-Prensa. Ed.].

McGovern, P. E. (2004). Wine and Eurasian grape: Archaeological and chemical perspectives on their origins. In *Proceedings of Actas do III Simpósio da Associaçao International de História e Civilizaçao da Vinha e do Vinho*, Funchal. Madeira. pp.291-307.

McGovern, P. E., Fleming, S. J., Katz, S. H. (1996). *The origins and ancient history of wine*; Overseas Publishers Association: Amsterdam.

Meléndez, E., Puras, P., Garcia, J. L., Cantos, M., Gómez-Rodríguez, J. A., Íñiguez, M., Rodríguez, Á., Valle, J. M., Arnold, C. Ocete, C. A., Ocete, R. (2016). Evolution of wild and feral vines from the Ega River gallery forest (Basque Country and Navarra, Spain) from 1995 to 2015. *Journal International Des Sciences de la Vigne et du Vin*, 50:65-75.

Mirlean, N., Roisenberg, A., Chies, J. O. (2007). Metal contamination of vineyard soils in wet subtropics (southern Brazil). *Environmental Pollution*, 149:10-17.

Negrul, A. M. (1938). Evolution of cultivated forms of grapes. *Comptes Rendues de l' Academie des Sciences de URSS*, 18:585-588.

Nikolic, M., Merkt, N. (2000). Effect of bicarbonate on uptake and translocation of 59Fe in two grapevine rootstocks differing in their resistance to Fe deficiency chlorosis. *Vitis*, 39:145-149.

Nikolic, M., Kastori, R. (2000). Effect of bicarbonate and Fe supply on Fe nutrition of grapevine. *Journal of Plant Nutrition*, 23, 1619-1627.

Ninkov, J., Papric, D., Sekulic, P., Zeremski-Skoric, T., Milic, S., Vasin, J., Kurjacki, I. (2012). Copper content of vineyard soils at Sremski Karlovci (Vojvodina Province, Serbia) as affected by the use of copper-based fungicides. *International Journal of Environmental Analytical Chemistry*, 92:592-600.

O.I.V. (2009). *Descriptor list for grape varieties and Vitis species*; A. Dendon: Paris (France).

Ocete, C. A., Martínez-Zapater, J. M., Ocete, R., Lara, M., Cantos, M., Arroyo, R., Morales, R., Iriarte-Chiapusso, M. J., Hidalgo, J., Valle, J. M., Rodríguez–Miranda, A., Armendáriz, I., Lovicu, G., Maghradze, D., Puig Pujol, A., Ibáñez, J. (2018). La vid silvestre euroasiática, un recurso fitogenético amenazado ligado a la historia de la humanidad. *Enoviticultura*, 50:4-21. [The Eurasian wild grapevine, a threatened plant genetic resource linked to the history of humanity].

Ocete, R. Gallardo, A. Pérez, M. A. Ocete, C. A., Lara, M., López, M. A. (2011). Usages traditionnels de la vigne sauvage en Espagne. *Territoires du vin* [en ligne], 03.2011: Les territoires du vin en Espagne, 22 février. [Traditional uses of the wild vine in Spain. *Wine territories* [online], 03.2011: The wine territories in Spain, February 22]. http://revuesshs.u-bourgogne.fr/territoiresduvin/document.php?id=872 ISSN 1760-5296.

Ocete, R., Arnold, C., Failla, O., Lovicu, G., Biagini, B., Imazio, S., Lara, M., Maghradze, D., López, M. A. (2011). Considerations on the European wild grapevine (*Vitis vinifera* L. ssp *sylvestris* (Gmelin) Hegi) and Phylloxera infestation. *Vitis*, 50:97-98.

Ocete, R., Cantos, M., López, M. A., Gallardo, A., Pérez, M. A., Troncoso, A., Lara, M., Failla, O., Ferragut, F. J., Liñán, J. (2007). *Caracterización y conservación del recurso fitogenético vid silvestre en Andalucía*. Consejería de Medio Ambiente, Junta de Andalucía: Sevilla. 410 pp. [*Characterization and conservation of the wild grapevine plant genetic resource in Andalusia*. Consejería de Medio Ambiente, Junta de Andalucía: Sevilla. 410p.].

Ocete, R., Fevereiro, P., Failla, O. (2015). Proposal for the wild grapevine (*Vitis vinifera* L. subsp *sylvestris* (Gmelin) Hegi) conservation in the European countries. *Vitis*, 54:281-282.

Ocete, R., López, M. A., Del Tío, R., Lara, M. (1999). Las poblaciones españolas de vid silvestre: Características de un recurso fitogenético a conservar; *Monografías INIA: Serie Agrícola. Madrid (Spain)*, Vol. 3, pp. 42. [Spanish populations of wild vines: Characteristics of a plant genetic resource to conserve; INIA *Monographs: Agricultural Series. Madrid (Spain)*, Vol. 3, pp. 42].

Ocete, R., López, M. A., Gallardo, A., Pérez, M. A, Rubio, I. M. (2004). Efecto de los daños de *Xylotrechus arvicola* (Olivier) (Coleoptera, Cerambycidae) sobre las características de los racimos de la variedad de vid Tempranillo en La Rioja. *Bol. San. Veg. de Plagas*, 30, 311-316. [Effect of damage of *Xylotrechus arvicola* (Olivier) (Coleoptera, Cerambycidae) on the characteristics of the bunches of the Tempranillo grape variety in La Rioja. *Bol. San. Veg. de Plagas*, 30, 311-316].

Ocete, R., Ocete, E., Ocete, C., Pérez, M. A., Rustioni, L., Failla, O., Chipashvili, R., Maghradze, D. (2012). Ecological and sanitary characteristics of the Eurasian wild grapevine (*Vitis vinifera* L. ssp *sylvestris* (Gmelin) Hegi) in Georgia (Caucasian region). *Plant Genetic Resour*ces, 10:155-162.

Pallas, P. S. (1799-1801) *Bemerkungen auf einer Reise in die Südlichen Statthalterschaften des Russischen Reichs* [*Remarks on a trip to the southern governorships of the Russian Empire*]. Ed. Gottfried Martin. Leipzig.

Pavloušek, P. (2013). Tolerance to Lime - Induced Chlorosis and Drought in Grapevine Rootstocks, Abiotic Stress. In *Plant Responses and Applications in Agriculture*, Vahdati, K., Leslie, C., Eds. IntechOpen; 10.5772/54793.

Puig-Pujol, A., Ferrando, N., Capdevila, F., Ocete, R., Revilla, E. (2016). Yeast biodiversity from *Vitis vinifera* L., subsp *sylvestris* (Gmelin) Hegi to face up the oenological consequences of climate change. In 3*9th World Congress of Vine and Wine*, Aurand, J. M., Ed.; Vol. 7.

Quer, J. (1784). *Continuación de la flora española o Historia de las plantas de España*; Joaquín Ibarra-Impresor de Cámara de SM: Madrid (Spain), [*Continuation of the Spanish flora or History of the plants of Spain*; Joaquín Ibarra-Impresor de Cámara de SM: Madrid (Spain)].

Rai, M. K., Kalia, R. K., Singh, R., Gangola, M. P., Dhawan, A. K. (2011). Developing stress tolerant plants through in vitro selection-An overview of the recent progress. *Environmental and Experimental Botany,* 71:89-98.

Riaz, S., Boursiquot, J. M., Dangl, G. S., Lacombe, T., Laucou, V., Tenscher, A. C., Walker, M. A. (2013). Identification of mildew resistance in wild and cultivated Central Asian grape germplasm. *BMC Plant Biology,* 13 (149) 21.

Rivera, D., Walker, M. J. (1989). A review of paleobotanical findings of early Vitis in the Mediterranean and on the origin of cultivated grapevines, with special reference to new pointers to prehistoric explotation in the Western Mediterranean. *Review of Palaeobotany & Palynology* 61(3-4):205-238.

Rodríguez-Miranda, A., Hidalgo, J., Arrimadas, J., Ocete, C. A., Duhart, F., Ocete, R., Iriarte-Chiapusso, M. J., Valle, J. M. (2016). El impacto antrópico sobre la vid silvestre en el territorio de Lapurdi (suroeste de Francia). *Munibe Ciencias Naturales. Natur zientziak,* 64:79-98. [The anthropic impact on the wild grapevine in the territory of Lapurdi (Southwest of France). *Munibe Natural Sciences*, 64: 79-98].

Romić, M., Romić, D., Dolanjski, D., Stričević, I. (2004). Heavy metal accumulation in topsoils from the wine-growing regions. Part 1. Factors which control retention. *Agriculturae Conspectus Scientificus,* 69:1-10.

Rout, G. R., Sahoo, S. (2007). In vitro selection and plant regeneration of copper-tolerant plants from leaf explants of *Nicotiana tabacum* L. cv. 'Xanthi'. *Plant Breeding,* 126:403-409.

Rustioni, L., Ciacciulli, A., Grossi, D., Brancadoro, L., Failla, O. (2016). Stem Xylem Characterization for Vitis Drought Tolerance. *Journal of Agricultural and Food Chemistry,* 64:5317-5323.

Santos, C., Fragoeiro, S., Phillips, A. (2005). Physiological response of grapevine cultivars and a rootstock to infection with Phaeoacremonium and Phaeomoniella isolates: an in vitro approach using plants and calluses. *Scientia Horticulturae,* 103:187-198.

Scali, M., Zifferero, A., Vignani, R. (2018). Distribution and Characterization of the *Vitis vinifera* L. subsp *sylvestris* in Southern Tuscany. *Recent Patents on Biotechnology,* 12:208-220.

Schumann, F. (1974). Berichte über die Verwendung der Wildrebe *Vitis vinífera* L. Var. Silvestris Gmelin. *Die Wein-Wissenschaft,* 26:212-218. [Reports on the use of the wild grape *Vitis vinífera* L. Var. Silvestris Gmelin. *Die Wein-Wissenschaft,* 26:212-218].

Serra, I., Strever, A., Myburgh, P. A., Deloire, A. (2014). Review: the interaction between rootstocks and cultivars (Vitis vinifera L.) to enhance drought tolerance in grapevine. *Australian Journal of Grape and Wine Research,* 20:1-14.

Skene, K. G. M., Barlass, M. (1988). Response to NaCl of grapevines regenerated from multiple-shoot cultures exhibiting mild salt tolerance in vitro. *American Journal of Enology and Viticulture,* 39:125-128.

Stummer, A. (1911). Zur urgeschichte der Rede und des Weinbaues. Mitteilungen der Anthropologischen. *Gesellschaft in Wien,* 41:283-296. [To the prehistory of speech and viticulture. Releases of the Anthropological. *Gesellschaft in Wien,* 41: 283-296].

Susaj, L. S., Jashari, F., (2014). Mature Leaf Features of Wild Grapevine: Populations grown in Three Different River Valleys of North Albania. *Albanian Journal of Agricultural Science,* (Special edition), 135-142.

Tagliavini, M., Rombolà, A. D. (2001). Iron deficiency and chlorosis in orchard and vineyard ecosystems. *European Journal of Agronomy,* 15:71-92.

Tangolar, S. G., Unlu, G., Tangolar, S., Dasgan, Y., Yilmaz, N. (2008). Use of in vitro method to evaluate some grapevine varieties for tolerance and susceptibility to sodium bicarbonate-induced chlorosis. *In Vitro Cellullar and Developmental Biology-Plant,* 44:233-237.

Terpó, A. (1969). A *Vitis silvestris* Magyar Középhegységi termöhelyi Viszonyainak visgàlata. *Botanikae Közlemények,* 56 (1):27-35. [*Vitis silvestris* in the Hungarian Central Mountains. *Botanikae Közlemények,* 56 (1):27-35].

Terral, J. F., Tabard, E., Bouby, L., Ivorra, S., Pastor, T., Figueiral, I., Picq, S., Chevance, J. B., Jung, C., Fabre, L., Tardy, C., Compan, M., Bacilieri, R., Lacombe, T., This, P. (2010). Evolution and history of grapevine (*Vitis vinifera*) under domestication: new morphometric perspectives to understand seed domestication syndrome and reveal origins of ancient European cultivars. *Annals of Botany*, 105:443-455.

Torres-Vila, L. M., Mosquera, J. L. (2004). Caracterización de las pepitas de vid de una ofrenda funeraria romana bajo-imperial (s. IV-Vth) de Los Bodegones (Mérida). *Excavaciones arqueológicas*, Memoria 2004, 7:455-465. [Characterization of the grape seeds of a low-imperial Roman funeral offering (IV-Vth) of Los Bodegones (Mérida). *Archaeological excavations*. Memory of 2004, 7:455-465.

Toselli, M., Baldi, E., Marcolini, G., Malaguti, D., Quartieri, M., Sorrenti, G., Marangoni, B. (2009). Response of potted grapevines to increasing soil copper concentration. *Australian Journal of Grape and Wine Research*, 15:85-92.

Troncoso, A., Matte, C., Cantos, M., Lavee, S. (1999). Evaluation of salt tolerance of in vitro-grown grapevine rootstock varieties. *Vitis*, 38:55-60.

Troncoso, A., Villegas, A., Mazuelos, C., Cantos, M. (1990). Growth and mineral composition of grapevine rootstocks cultured in vitro with different levels of ammonium nitrate. In *Plant Nutrition - Physiology and Applications,* Beusichem, M.L.V., Ed.; Vol. 41, pp. 653-654.

Vallecillo, C. G., Vega, I. (1995).Conservando parientes silvestres de las plantas cultivadas. *Ecosistemas*, 14:55-59. [Keeping wild relatives of cultivated plants. *Ecosistemas*, 14:55-59].

Varanini, Z., Maggioni, A. (1982). Iron reduction and uptake by grapevine roots. *Journal of Plant Nutrition*, 5:521-529.

Vassilev, A., Lidon, F. C., Matos, M. D., Ramalho, J. C., Yordanov, I. (2002). Photosynthetic performance and content of some nutrients in cadmium- and copper-treated barley plants. *Journal of Plant Nutrition*, 25:2343-2360.

Vavilov, N. I. (1926). Cemtry proiskhozhdenia kulturnikh rastenii (The centres of origin for cultivated plants). (in Russian). In *Proceedings of Trudi poprikladnoi botanike, genetike i selektsii*. (Proceedings of Applied Botany, Genetics and Breeding); pp. 133-137.

Vivier, V. A., Preotius I. S. (2002). Genetically tailored grapevines for the wine industry. *Trends Biotechnology*, 20:472-488.

Von Caemmerer, S., Farquhar, G. D. (1981). Some relationships between the biochemistry of photosynthesis and the gas-exchange of leaves. *Planta*, 153:376-387.

Zancan, S., Suglia, I., La Rocca, N., Ghisi, R. (2008). Effects of UV-B radiation on antioxidant parameters of iron-deficient barley plants. *Environmental and Experimental Botany*, 63:71-79.

Zdunic, G., Maul, E., Hancevic, K., Leko, M., Butorac, L., Mucalo, A., Radic, T., Simon, S.; Budic-Leto, I., Mihaljevic, M. Z., Maletić, E. (2017). Genetic Diversity of Wild

Grapevine *Vitis vinifera* L. subsp *sylvestris* (Gmel.) Hegi in the Eastern Adriatic Region. *American Journal of Enology and Viticulture*, 68:252-257.

Zimmermann, J. (1958). Die Bedeutung der wildrebe (*Vitis silvestris* Gmel.) in Jugoslavien für Forschung und Weinbau. *Wein– Wiss*, 8:79-87. [The importance of wild grape (*Vitis silvestris* Gmel.) In Yugoslavia for research and viticulture *Wein–Wiss,* 8:79-87].

Zohary, D., Spiegelroy, P. (1975). Beginnings of fruit growing in old world. *Science*, 187:319-327.

In: Vitis: Biology and Species
Editors: António M. Jordão et al.
ISBN: 978-1-53618-308-5

Chapter 5

ATLANTIC ISLANDS OF AZORES - A BRIEF REVIEW OF VITICULTURE AND GRAPE VARIETIES

Ana Romão[*]
Regional Laboratory of Oenology, Directorate of Agriculture Services,
Regional Secretariat of Agriculture and Forestry, Cafuinhas,
Pico Island, Azores, Portugal

ABSTRACT

Vines were introduced in the Azores Islands at the beginning of their settlement. They adjusted to the environment in very small connected plots surrounded by low retaining stone walls. However, their establishment was difficult, requiring constant and persistent hard work. The wine produced by then was sold by experts following the commercial circuits of the islands and later on exported. Transport by sea allowed profitable sales of wine even in small quantities. The plagues that devastated the vines in the middle of the 19th century (*Oidium* and *Philloxera*) forced the substitution of European vines with American ones. American vines like cv. Isabella led to the production of Foxy Smell Wine known in the region by “Vinho de Cheiro.” The lack of good quality of this product (already forbidden by the European Legislation) put at risk the growing of these vines and even if it does not go extinct it will worsen the financial difficulties of the growers. Today, the rhythm of changing the vines by European varieties translates in a growing visibility of the Azorean Wines, having contributed to the fact the classification by UNESCO of the Landscape of the Pico Island Vineyard Culture in 2004, as well as the promotion of Madalena do Pico as a Wine City in 2017. For the future, we predict an enormous evolution in vine growing in Azores and particularly in Pico Island, with the European incentives for the reconversion of the vines and the growing hope of progressively increasing the quality of the wines produced.

---

[*] Corresponding Author’s Email: ana.cc.romao@azores.gov.pt.

Keywords: Azores Islands, climatic conditions, European vines, grape varieties, vines training system

1. INTRODUCTION

The Azores Archipelago is formed by nine islands of volcanic origin and is part of the central crest of the North Atlantic. It is approximately 1500 km west from Europe's mainland coast and about 3900 km from the North America coast. It is located between 36º 55' and 39º 43' Latitude N and 24º 46' and 31º 26' Longitude W (Figure 1). The islands spread in a NW/SE direction and are divided in three groups: Eastern (Santa Maria and São Miguel), Central (Terceira, Graciosa, São Jorge, Pico and Faial), and Western (Flores and Corvo). The Archipelago has a total area of 2333 Km^2 and the distance between the most extreme islands (Santa Maria to the east and Corvo to the west) is about 600 km. This archipelago is one of the two autonomic regions of Portugal located in Atlantic Ocean.

The introduction of vine growing in the Azores archipelago goes back to the beginning of its settlement. The chronicler Gaspar Frutuoso (1522-1591) reported the abundance that the culture assumed in several places of the islands and the wine transactions that took place. Although, the precise date of this introduction is not known, Gaspar Frutuoso in XVI century, described that this culture was not only perfectly established but also occupied an important area, particularly on the islands of Terceira, São Jorge, Pico, Graciosa and São Miguel.

https://azo-cdn.azureedge.net/sites/default/files/inline-images/Handling-Map_0.jpg.

Figure 1. Geographical location of the Azores islands.

The crop has expanded to most of the islands, mainly due to climatic affinities, occupying mainly areas close to the sea and poorly developed soils, areas that presented better conditions for the development of the crop.

It is admitted that it was the Franciscan friars who introduced the cultivation of vines in these islands. The varieties then introduced came from the mainland, Madeira and other Mediterranean areas (Medeiros, 1994). Historical references to vine growing in the Azores are generally poor in the description of the varieties that were introduced here, as well as in their origin. Some bibliographical references indicate that the grape varieties were introduced in Azores from Madeira and Cyprus islands (Rebello, 1885; Silva, 1950), and also from the Portuguese mainland (Rebello, 1885; Medeiros, 1994). However, it is not known in most cases what source of information the authors rely on, and it is difficult to assess its reliability.

The first known reference to grape varieties appears at the end of the 16th century, indicating that in Terceira island there would be “grapevines and grapes from all over the grapevine, Verdelho, Mourisco, Açaria....” The most important and widespread Azorean grape variety, Verdelho is already mentioned on this date (Frutuoso, 1522-1591). In the period before the phytosanitary crises (that occurred in the middle of 19h century, around 1853) that devastated the vineyards in the Azores, the most extensive reference to the vine population in these islands is due to António Gyrão, Viscount of Vilarinho de São Romão (São Romão, 1822). This author presents a list of white and red grape varieties cultivated on the islands of São Miguel, Faial and Pico, indicating as varieties cultivated on São Miguel, such as ‘Boal,’ ‘Dona Branca,’ ‘Galego,’ ‘Malvasia,’ ‘Moscatel,’ ‘Tália,’ ‘Teta de Cabra’ and ‘Verdelho’ for white varieties and ‘Sobrainho’ and ‘Negra Mole’ for red grape varieties. For the islands of Pico and Faial the varieties indicated are ‘Alicante,’ ‘Boal,’ ‘Galego,’ ‘Verdelho,’ ‘Verdelho Valente’ or ‘Terrantez do Monte’ for the white varieties and ‘Bastardo’ and ‘Tinta’ for the red varieties. In addition, the intercalation of the vine in the mosaic of the cultivated species was done in an original way, well adjusted to the natural conditions, and without interfering with the area of expansion of the cereals and the crops of yield. The crisis in the 19th century with the appearance of powdery mildew at first and phylloxera and downy mildew later led to the decadence of the crop and the introduction of direct producers, with only the cultivation of traditional European varieties remaining significant on the islands of Pico, Terceira and Graciosa,

The wine produced in Azores became famous and was widely exported, particularly the fortified wine produced on the island of Pico, to Northern Europe and even to Russia. After the Bolshevik revolution in 1917, bottles of Pico Verdelho wine were found stored in the cellars of the former czars of Russia. However, today the ‘Verdelho’ wine, noble in character, a direct descendant of the ancient Verdelho, kept its race in small redoubts of the islands of Pico (Lajidos), Terceira (Biscoitos) and Graciosa (Figure 2). These spaces contain a treasure trove of experiences acquired and perpetuated over the centuries, by heritage bequeathed to subsequent generations and wisely preserved.

Figure 2. Traditional vineyards landscape in Pico island (Lajidos - Criação Velha).

Nowadays, the recovery of the vineyards in the Azores and particularly in the regions of Denomination of Origin (Pico, Graciosa and Terceira - Biscoitos region), already translated into more abundant and higher quality productions, which again travel the trails of internationalization, is equivalent to the rediscovery of a tradition. The reconversion of the vineyard has allowed a significant recovery of the traditional 'Verdelho' wine, having also successfully introduced new varieties from European origin and new training systems on the vines.

2. HISTORY OF VINE AND WINE IN THE AZORES

Even before the Bronze Age vine growing was already established in some regions like Greece, Egypt and the Aegean islands. Once introduced in Rome, it arrived in Europe along the expansion of the Roman Empire. Regarding the first Azorean strains, opinions diverge or even clash, thus it may not be stated with precision its provenance or when they were introduced in the region. About the introduction of the vine in Azores islands, Macedo (1981) considers: "... it's stated that the first vine bases came from the island of Cyprus to the islands of S. Jorge and Pico in 1470…," however Lima (1943), addressing the same question about the Pico island, writes: "It is said that the first vine bases came from the Madeira island." Similar opinion was shared also by Rebello (1885).

There are several references to this culture, as well as some of its productions, in the different islands of the Azores in the 16th century (Frutuoso, 1522-1591). Thus, when describing Santa Maria island, the author refers the vine growth along almost the entire coast, and regarding its production he tells us: "All these vines will produce every year one hundred barrels of wine (1 barrel = 480 liters), of the best grapes in the islands, ..., which will give us a lot of wine because there is no bushel of vine that does not provide a barrel of wine or more." About S. Miguel island, the same author comments several times on this culture implemented all over the island, referring specifically the port of Vale de Cabaças: “... valley and fajã of flat land and at sea level, surrounded by rock and slopes of many moios (1 moio = 60 bushels; 1 bushel = 968 m^2) of soil and biscoito (soil consisting fundamentally of unbundled volcanic slag) with stones that were used for vineyards.” Also about the wine production in this island, he writes: “In a year of good news, this island gave almost two thousand barrels of wine, seven hundred in the city, as many others in the village of Alagoa, four hundred in Ribeira Grande, and the rest in Nordeste and Povoação, and throughout the island. Now, in a good year, it produces five thousand barrels.” Like these islands, the author also left us important notes, regarding the other islands in the archipelago, namely in Terceira island. However, for Graciosa island, we find only one note, which confines the vineyard to the neighborhood of Praia village. About the S. Jorge island, the area extending from Manadas to Ribeira do Nabo, is highlighted as: “... land of biscoito that provides more wine than bread, ...” and some fajãs: “... produces a lot of bread and wine, ...” and “... now provides many wines.”

Regarding the Pico island, there are several references to its vineyards and their production. For example, in Vila das Lages village the mentioned authors described that “There are numerous vineyards in this parish that are growing immensely,” and also: “... many barrels of wine, once they’re produced in the same parish of Vila de S. Roque, more than seven hundred every year;.” Finally, for Faial island, we quote a passage referring to the port of Castelo Branco that summarizes the situation of the island’s viticulture around this time: “... where some caravels and boats arrive, and, having wine, as there are some promising vineyards, we can load them; but the cause of not having vineyards in the island, considering the fertile soil for the purpose, is that the residents don’t grow them and those early experiments were destroyed by some rabbits, cattle and got stolen.”

Being Azores islands a region with volcanic origin, and still having, during the first centuries of settlement, many manifestations of this nature, it is easy to deduce that there was no shortage of places, where viticulture could be established. Until the middle of the 19th century, the varieties cultivated in the region were European, deserving one of them, the ‘Verdelho,’ the consensus of historians as the most referenced and with greater expression.

Wine production in the Azores has been growing over the years, despite the problem of the crop and counter crop (Pereira, 1987). To get a comprehensive view on how viticulture in the region was developing in the beginning of the 18th century, we should take a look at Cordeiro (1981) referring to the production of some quality wine in several islands such as Santa Maria, S. Miguel, Faial and Terceira, where the productions sometimes were not enough for each island. However, in general the excellent wine came mainly from Pico island but also, in a small quantity, from Graciosa, and from S. Jorge islands, where the production was abundant enough to supply both the Portuguese ships and foreign ships.

At the beginning, in the Graciosa island the vineyard occupied, almost exclusively, the land of “Biscoito” region, and it was intended for self-consumption in the island. Then, since the middle of the 18th century until around 1840, it appears occupying the ploughed land and becoming one of the largest cultures in the island, although its production is very variable and precarious. Nevertheless, Graciosa’s vineyards produce more than any other island, because they were almost all planted in land that was previously ploughed. The wine of Graciosa was labeled as poor and everything leads to believe that its quality has even decreased, due to the fact that most of it was exported as brandy, which led the winemakers to neglect both the state of the grapes at the time of harvesting and the wine itself. The reasons that prevented the wine exportation are easily deduced. Thus, on the one hand, it would suffer disadvantageously competition from the best quality wines from Pico island and the Portuguese mainland. Instead, considering the low alcohol concentration, it was liable to be lost when transported over long distances and in addition, the transport of brandy was facilitated by the reduction in volume (Goulart, 1991).

Concerning to S. Jorge island, if in some years there was the need to import wine for domestic consumption, it is also true that the following years there was a surplus to export. Thus, viticulture has always assumed an important role in the economy of S. Jorge island. The production of the island reached a considerable level and its exportation started quite early. In some years the production of wine was such that it allowed exportation to England and Brazil, and also to S. Miguel and Madeira islands (Pereira, 1987).

In the first half of the 16th century, Pico was exporting wine. In 1670, the Chamber of Horta was authorized by the Prince Regent to send a ship with wine to Brazil every year, but finding this insufficient, in 1735, the farmers asked for an extension of this trade (Goulart, 1991). Undoubtedly this island, which produced the greatest quantities of wine, and due to its excellent quality, gained fame far beyond its borders. Sousa (2004) reported the exportation of white wines from Pico amounting to twelve thousand barrels. As for the main destinations of this product, Lima (1943) reported that the exportation was mainly to England, in smaller quantities to Germany, Russia and Brazil, and in a very small scale to the Portuguese mainland (Rebello, 1885).

When Azorean viticulture was in a pleasant situation, in the middle of the 19th century, *Oidium tuckeri* appeared, coming from America, which impetuously attacked the European vineyards, leading to decrease vertiginously the wine production. At this time, the American vine of the species *Vitis labrusca* arrived to the Azores, called the “uva de cheiro,” “The Isabel vine” or “Smell Wine,” and was introduced in the Azores islands between 1853 and 1854. In this time “Isabella” grape variety was introduced in Azores islands and it was found that this new grape variety was resistant to oidium, hence the interest shown by the winegrowers and therefore the creation of a wine commission, aiming to develop this new path of viticulture in the Azores.-However, the introduction of this new grape variety did not stop with oidium. Thus, in the last quarter of the 19th century, when it started to be successfully destroyed using sulphur, a new calamity of entomological origin appeared, the phylloxera, resulting from *Phyloxera vastatris*. The appearance of this insect took place through varieties from America, imported with the intention of replacing the European ones which were very sensitive to oidium. Phylloxera was the final stroke for European grape varieties, which were no longer in the best conditions.

The winegrowers tried to overcome their precarious situation, looking more and more into direct producers, which were less sensitive to diseases, easier to cultivate and more productive. Nevertheless, during the next decades the vines area and in general the wine sector reduced in the different Azores islands, although not completely extinct. As a result of all this situation, from the late 80's it became necessary to relaunch this culture in order to restore the markets that once existed and at the same time defend the rural populations deeply connected with the wine activity. Therefore, main goals and priorities were defined and translated into the following measures: investment aid programs, creation of demarcated areas and also the creation of the Regional Wine Commission of the Azores (CVR Azores).

Thus, the investment aids have resulted in significant increases on the restructured vineyard area, which until now, has allowed, first of all, to re-launch the culture in the islands with the greatest tradition, i.e., in Terceira (Biscoitos), Graciosa and Pico islands.

One of the main structural problems of this sector in this region is the small size of the plots (the average area per vineyard holding is approximately 0,3 ha) and their dispersion, associated to a traditional system of conduction called "currais," where the vines are conducted in the ground between stone walls, which requires high labor, not allowing the mechanization of some cultivation techniques (Figure 3).

Figure 3. Traditional vine landscape ("Currais") in Pico island.

Table 1. Production area before investment made and after the investments made in the reconversion of abandoned vineyards between 2014 and 2017

Production area before 2014 (ha)				
D.O.				I.G. Azores
Total	Pico	Biscoitos	Graciosa	
154	130	15	9	84
Production area in 2019 (ha) after investments (2104-2017)				
D.O.				I.G. Azores
Total	Pico	Biscoitos	Graciosa	
750,2	711,88	24,02	14,3	264,82

Source: CVR Azores.

Table 2. Evolution of certificated wine produced in Azores islands and for the different denominations of origin (D.O.) and geographical indication (I.G.) between 2004 and 2019 (source: CVR Azores)

Certificated wine produced in Azores islands (Liters)				
Harvest year	I.G. Açores	D.O. Pico	D.O. Biscoitos	D.O. Graciosa
2004	217850	11300	0	10000
2005	128920	8190	0	2100
2006	152190	10540	0	0
2007	181075	12800	0	5500
2008	172050	880	0	3500
2009	237165	2850	0	8000
2010	63750	0	0	3500
2011	200930	3900	0	2500
2012	60480	20000	4050	1000
2013	68960	46100	4050	2500
2014	106040	25350	0	0
2015	201846	74837	3500	3850
2016	40235	39550	2400	1200
2017	45796	37590	0	1100
2018	87600	153937,75	0	4500
2019	190621	162217,75	3900	4500

With the Decree-Law no. 17/94, of 25th January, which approved the Statute of Wine Areas for the Autonomous Region of the Azores, three production areas were established, suitable for the production of Quality Wines. Thus, the denominations of “Biscoitos” and “Pico” were established for the Quality Fortified Wines Produced in a Specified Region (VLQPRD) and “Graciosa” for the Quality Wines Produced in a Specified Region

(VQPRD). As a result of the approval of these demarcated regions, the Regional Wine Commission of the Azores was created.

The wine production in Azores has been increasing in the last years, which has allowed to update and improve the certification practices of this typical product. The CVR Azores is responsible for the certification and only products under these conditions are able to bear the word Azores on their labels, considering that this is a geographical indication (I.G.). Besides the word Azores (I.G.), there are other designations (DO - Denomination of Origin) that can also be used, as “Pico,” “Graciosa” and “Biscoitos,” once these are identified areas for certification purposes.

In terms of processing and marketing units, there are currently several cooperative wineries on each of the islands. Thus, in each island (Pico, Graciosa and Terceira) there is one of these type of wineries, but also a few private winemakers to produce red and white wines, including in S. Miguel and Faial islands.

Still, considering the priorities given to the sector we should state that there are a vast area to restructure in the Autonomous Region of the Azores. We can see in Tables 1 and 2 how the vineyard area in the different demarcated regions has increased as well as the volume of certificated wine produced in the last years, mainly due to the investment made in the reconversion of abandoned vineyards.

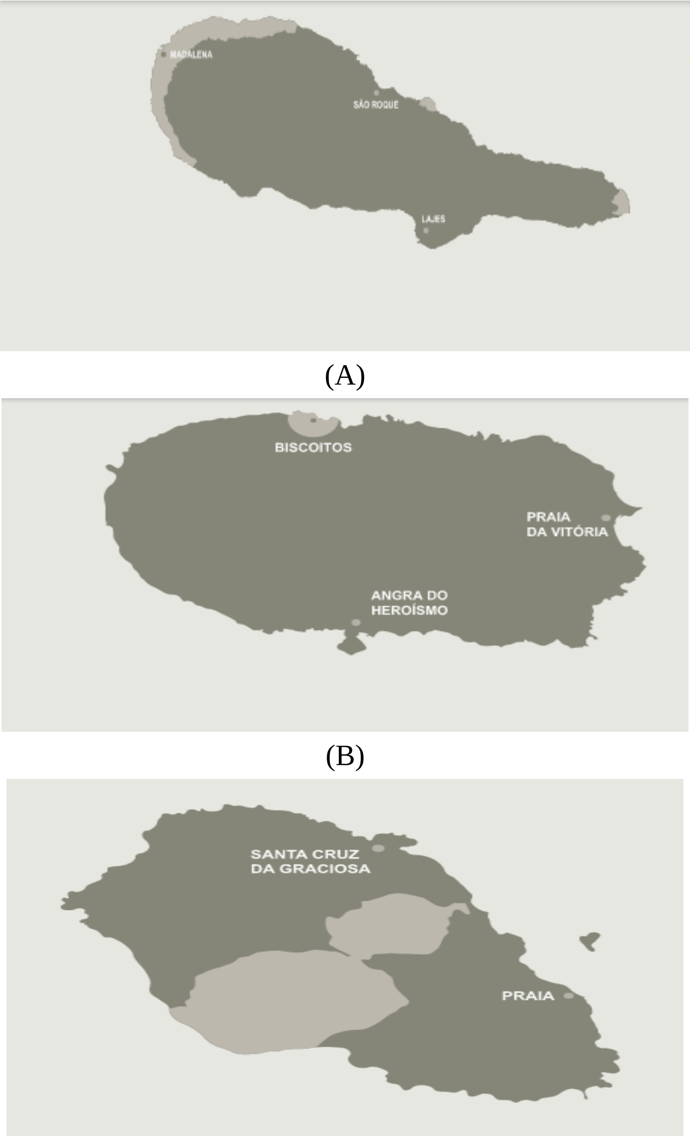
Thereby, a number of outlined development strategies for this sector are being put into practice, implemented on different islands and which essentially resume the process of restructuring, strengthening experimentation, publicizing and vulgarizing the experimentation carried out, professional training at different levels, as well as promoting and supporting associations, based on improving and modernizing the technical, human and material resources of producer organizations. In 2019, an ordinance was published updating the rules for the implementation of vineyards and wine production as well as the indication of authorized and recommended grape varieties for the production of I.G. and D.O. wine.

3. EDAFO-CLIMATIC CHARACTERIZATION AND VINEYARD TRAINING SYSTEM

Due to its location, the Azores have a climate with typically maritime characteristics, which translates into mild temperatures, with a small temperature range, high rainfall and high relative air humidity. The average annual temperature is around 17.4ºC. According to the Köppen classification, the Azores climate falls in the category of warm temperate climates (group C), once it presents summer and winter and the average temperature of the coldest month is below 18ºC. The temperatures are mild, with the annual average at sea level being between 17 and 18º C. February, with an average of 12ºC, is the coldest month and during summer (July-September) the average temperature is around 21ºC. The average daily temperature range is only 5ºC. However, given the special distribution of the islands, the archipelago's climate can be classified (from East to West) from Csb to Cfb, that is, temperate rainy climate with dry summer to temperate rainy climate with wet seasons, not exceeding the average temperature of the warmest month at 22ºC (Azevedo, 2008). The drop in temperature as a function of altitude is -0.9ºC for every 100 meters, until the dew point temperature is reached, which is on average close to 400 meters of altitude. From then on, the temperature decreases less abruptly, about -0.6ºC for every 100 meters, due to the yield of energy to the atmosphere by the condensation process (Azevedo, 2008).

Considering the geographic location of the islands in terms of global atmospheric and oceanic circulation and also the influence of the mass water, the Azores archipelago's climate is generally characterized by mild temperatures, high levels of air humidity, low rates of sunshine, regular and abundant rainfall and a vigorous wind regime. The four seasons of the year are recognizable, with winters being rainy but not very harsh and the summers mild. Rainfall occurs practically all year round, being January the rainiest month and July the driest. The rainfall is of the order of 1000 mm at sea level, varying with the altitude according to a gradient of 25% for each 100 m.

Figure 4. Traditional vine training systems in Pico and Terceira ("Biscoitos "region) islands.



(A)

(B)

(C)

Source: https://www.azoreanwines.com/.

Figure 5. Location of the different wine denominations of origin - Pico (A); Terceira - Biscoitos; (B) and Graciosa (C) islands.

Regarding the soils and their capacity of use, this is a region with a volcanic origin and with relatively recent formation, with part of its soils underdeveloped. The most represented classes are the andosols (saturated, unsaturated and ferruginous), the thin Indian soils (incipient, saturated and unsaturated), the lithosols and the regosols. In some islands, such as Pico, a substantial part of its soils is still incipient and therefore the main classes represented are lithosols, lithosols and andicos slender soils (University of the Azores, 1986).

The cultivation method, against the roughness of the volcanic terrain, almost with no vegetal soil, is made in "currais," which protects the vines from oceanic winds and creates conditions for the microclimate existing in these areas that favors the maturation of the grapes. The vine is conducted in the ground to protect it from wind and raised at the time of maturation to avoid the scalding of the cluster by contact with the black stone (Figure 4).

The DO Pico region (Figure 5A) is located in the municipality of Madalena, the parish of the same name and in Candelária, Criação Velha and Bandeiras, in areas with 100 m of altitude or lower. The vines are located in the municipality of São Roque, the parish of Santa Luzia and part of the parish of Prainha, place of the Bay of Canas, also in areas of altitude equal or lower than 100m; In the municipality of Lajes, the parish of Piedade, places of Engrade and Manhenha, in areas of altitude equal or lower than 100 m. In this specific location we can find non wet lithographic soils and lithosols, on a consolidated substrate of basalt or similar rocks, corresponding to recent lavas, in association with rocky outcrops, sometimes with disseminated rocky material and a consolidated lava mantle at the surface (Afonso, 2000).

In Terceira island (Figure 5B), the D.O. Biscoitos region (Figure 5B) is located in areas with 100 m or less in the parish of Biscoitos, in the municipality of Praia da Vitória. The soils are lithosol and non-humid lithosols, on a consolidated substrate of basalt or similar rocks and traquitos, generally corresponding to recent lavas, often associated with rocky outcrops and sometimes with widespread rocky material (Afonso, 2000). The D.O. of "Graciosa" island (Figure 5C) is spreader in the municipality of Santa Cruz, the parish with the same name and in Guadalupe, Praia and Luz, in areas with 150 m or less in altitude, where the soils are brownish andic, normal and not very thick, and regolic soils derived from basaltic rocks or pyroclastic materials based on basaltic rock at shallow depth (Afonso, 2000).

4. INDIGENOUS GRAPE VARIETIES FROM THE AZORES ISLANDS

The occurrence of oidium in the 19th century led to profound changes in the region's vine population and the gradual decline of European varieties in every islands, resulting in its total disappearance in São Miguel island. European varieties were replaced on a large scale with American varieties and, specially with 'Isabella' (*Vitis labrusca* L.).

During exhaustive prospecting work carried out by Mestre et al., (2016), it was possible to conclude that currently the oldest cultivars in the region is practically composed by 3 white varieties: "Verdelho," "Arinto dos Açores" and "Terrantez do Pico." The "Verdelho" variety differs ampelographically and genetically from the "Gouveio," a variety often referred to in the north of the Portugal mainland as "Verdelho" (Eiras-Dias et al., 2006; Veloso et al., 2010). Similarly, despite the phonetic similarities with the Spanish "Verdejo" and the Italian “Verdecchio,” in the case of the former, the microsatellite markers prove to be two distinct varieties, despite the morphological similarities (Eiras-Dias et al., 2006), while in the latter the ampelographic differences are unequivocal. This variety is included in the Catalogue of vine varieties cultivated in France, and its cultivation is also mentioned in Australia, South Africa and California. In the Madeira island is usually presented as the region from which the initial vegetative material was originated. What we can state with absolute certain is that the grape variety ‘Verdelho’ that exists in the Azores is the same that we can find in Madeira island, confirmed through molecular analysis (Lopes et al., 1999) and no synonymy known in the Portuguese mainland has been detected (Veloso et al., 2010).

Nowadays the variety “Arinto dos Açores” can be found in Pico island, where it constitutes the main part of the island’s vine production. In Terceira island, is known by the name “Terrantez” and in Graciosa island it is often called by both names. Until 2012, the official name of this variety was “Terrantez da Terceira,” having been changed to “Arinto dos Açores” by Ordinance n. 380/2012.

The name “Arinto” seems to be quite recent and is not mentioned in any older writings, nor in the list presented by São Romão (1822), or in the brief description of the Azorean grape varieties, prepared by Ernesto Rebello (1895). The name “Arinto” appears, however, in 1889 in a report of the Phylloxeric Services as a variety existing in S. Jorge island (Barros, 1892). Regardless, this variety seems to be an ancient knowledge entity in the region (Silva, 1950), so we can assume that it has existed for quite some time, but possibly designated by a different name.

According to molecular analysis, this variety is remarkably different from the “Arinto” variety cultivated in the Portuguese mainland, as well as some other varieties equally designated, such as “Arinto do Interior” or “Arinto do Douro.” Until now, this variety has not been found in mainland Portugal either (Veloso et al., 2010). The “Terrantez do Pico” variety presents another difficulty in the confusion resulting from the name “Terrantez” also being used in other islands (Terceira and Graciosa) as a synonym of “Arinto dos Açores.” This variety it is different from the variety “Terrantez” cultivated on the mainland and from the variety known by the same name in the island of Madeira, the latter corresponding to the variety “Folgasão.” In addition, until now, it has also not been identified in the rest of the Portuguese territory.

The development of the DNA analysis application for variety identification has become an indispensable tool for varietal discrimination. Since 1994, numerous studies have been carried out on the vine based on the inheritance of nuclear microsatellites (nSSR), highly polymorphic markers, to clarify the origin of several varieties (Sefc et al., 2009). Microsatellites are composed by small blocks of base pairs, which are repeated in variable numbers, generating polymorphism. The growing number of tools and molecular analyses available have also made possible to study the genetic relationships between several cultivated genotypes, particularly when used in large samples of cultivars, concluding that many of these were in fact derived from spontaneous hybridizations of other cultivars still existing (Zapater et al., 2013). The first reported case of clarification on the origin of a variety based in the new genetic tools was the well-known variety Cabernet Sauvignon, which proved to be a spontaneous hybrid between Cabernet Franc and Sauvignon Blanc (Bowers et al., 1997). Since then, many authors have proven the existence of hybridization in the origin of an increasing number of grape cultivars varieties (Cipriani et al., 1997; Ibanez et al., 2012; Lacombe et al., 2012).

In Portugal, the database of microsatellite markers of the 313 vine varieties was published in 2010, authorizing the indication for cultivation, using six nuclear microsatellite loci. The information obtained allowed a breakdown of all varieties studied, resulting in the final identification of 244 distinct genotypes (Veloso et al., 2010). Based on the published information, a database was created on the varieties cultivated in Portugal, which will be referred to as BDC, to which the published molecular profiles for the Spanish (http://sivvem.monbyte.com/) and French (http://plantgrape.plantnet-project.org/en/) varieties were added. The microsatellites used were VVMD5, VVMD7, VVMD27, Zag62, VrZag79 and VVS2, recommended by the OIV and present in all sources used.

The publication of these data and their analysis has made possible to observe a relevant component concerning the traditional Azorean grape varieties. Thus, both "Terrantez do Pico" and "Arinto dos Açores" shared at least one allele with the "Verdelho" variety at each loci of the microsatellites analyzed. The kinship between two varieties will be easier to find when two cultivars share at least one allele in each loci, being even a prerequisite to demonstrate the existence of a filial relationship. However, in order to demonstrate this relationship, the sharing of the alleles should be a matter of progeny and not chance (Vouillamoz et al., 2006).

Although, sharing the same geographical area, the morphological similarities between the varieties and the fact that the cultivation of these two varieties is yet to be found in mainland territory, has led to the strong belief that they result from a marriage between the "Verdelho" variety and other unknown varieties. On the other hand, even today the old vines of Pico, Terceira and Graciosa islands are characterized by the cultivation of mixed varieties in the vineyard. Therefore, it is easy to imagine that also in old times all the varieties that were historically referred to as part of the Azorean vine were cultivated in a similar way. While occupying the same space, with strong proximity between plants of different varieties, the existence of accidental or intentional crossings are quite plausible. As such, the existence of grape variety hybridization in Azorean vineyards as a foreseeable origin of some of the local varieties could, and should, be considered as a working hypothesis to seek further progress in the study of the respective origin. At this point it matters to characterize the traditional varieties mentioned above, namely "Verdelho," "Arinto dos Açores" ("Terrantez da Terceira"), and "Terrantez do Pico."

Regarding "Verdelho" the first parish priest in the island, the Franciscan Friar Gigante, had then the revelation that the land looked just like Sicily, and had the first strains of

Verdecchio planted 'on free standing,' coming from Madeira." This is how Catarina Carvalho (2004) refers to the introduction of the vine and the "Verdelho" variety in Pico. Morphologically, "Verdelho" variety it is known to having young green leaves with coppery areas, with a glabrous lower page (hairless) (Figure 6A) and a slightly reddish buds. The adult leaf is orbicular, underneath, slightly irregular, rather bubbly, with a low density of prostrate hairs on the underside. The petiolar sinus is closed, with a U-shaped base. The upper lateral sinuses are V-shaped open and the teeth are convex. The main veins are slightly reddened around the petiolar point and the petiole is reddish. The bunch is small and whispered and the berry is slightly elliptical. It is an early sprouting variety in average season and with a very early ripening. It easily produces two bunches per cane and its vigor is medium and the size semi-erect.

The most important variety in the archipelago of Azores is "Arinto dos Açores" ("Terrantez" from Terceira). This variety is known as "Arinto dos Açores" in Pico and "Terrantez" in Terceira. In Graciosa sometimes is called "Arinto" and sometimes "Terrantez" (Figure 6B). The hypothesis bounded by some farmers, who consider it to be known in S. Jorge island by "Verdelho," and hence justifying its antiquity and therefore the real "Verdelho." Given the available data, it seems likely that this variety must have had a later introduction, although its origin is not to be found. The molecular characterization, already mentioned, between 314 grape varieties authorized for winemaking propose in Portugal, shows that it is a different variety from the others grown in the country. This variety is also named "Terrantez da Terceira." This name was determined to avoid confusion between this variety and the "Arinto" cultivated in the Portuguese mainland. On the other hand, the geographical name is justified as well to avoid confusion with the "Terrantez" variety cultivated on the Portuguese mainland. Morphologically it is characterized by the young end branch with medium density of prostrate hairs. The young leaf is green with coppery areas, and has the lower middle density of prostrate hairs. Slightly striated red bulb and green buds, without anthocyanin pigmentation. The adult leaf is orbicular, trilobed, slightly irregular, medium bubbly, with the lower medium density of prostrate hairs. The petiolar sinus is slightly open, with a V-shaped base, and the upper lateral sinuses are V-shaped. The teeth are straight and the main veins are reddish near the petiolar point. The bunch is small to medium and the berry is small and elliptical. The budburst of this variety is late, hence its culture closer to the coast, in Graciosa island. It produces, on average, 2 to 3 clusters per launch. Its vigor is low and its posture semi-erect. (Eiras-Dias et al., 2006).

Between the three traditional varieties cultivated in Pico island ("Verdelho," "Arinto dos Açores" and "Terrantez do Pico"), this is the most resistant to adverse weather conditions (strong winds or sea fog or heavy rainfall), showing the greater production capacity allied to an oenological quality similar to cv. "Verdelho." All these reasons have led more and more winegrowers in Pico to replace cvs. "Verdelho" and "Terrantez" plants for cv. "Arinto dos Açores," showing a great tendency to increase the planted area of this variety compared to the others. “Terrantez do Pico” is a Pico variety with very restricted expansion (Figure 6C). This grape variety is exclusively from Pico island and it does not exist on the other islands. The use of the geographical name is justified to avoid confusion with the “Terrantez” variety grown in the Portuguese mainland. The molecular characterization, already mentioned, shows that it is a different variety from all the others cultivated in Portugal.

Morphologically it is characterized for having a young green leaf with coppery areas and a glabrous bottom page. The shoot is striped red and the buds are slightly reddish. The adult leaf is pentagonal, trilobed and irregular with high bubbles. The lower page has low density of prostrate hairs. The petiolar sinus is closed, with a U-shaped base. The upper lateral sinuses are open V-shaped. The teeth are convex and the main veins are reddish from the petiolar point to the first branch. In addition, the petiole is reddish. The bunch is small and very whispered, and the berry is rounded. The branch is dark brown to reddish. It can easily produce two bunches per cane. The vigor is low and the vegetative growth is semi-erect. It is a variety that is very sensitive to cryptogamic diseases (mainly powdery and mildew), that are very common in the Azores region, as a consequence of high relative humidity, mild temperatures and frequent rainfall that is a constant throughout the vine’s vegetative cycle.

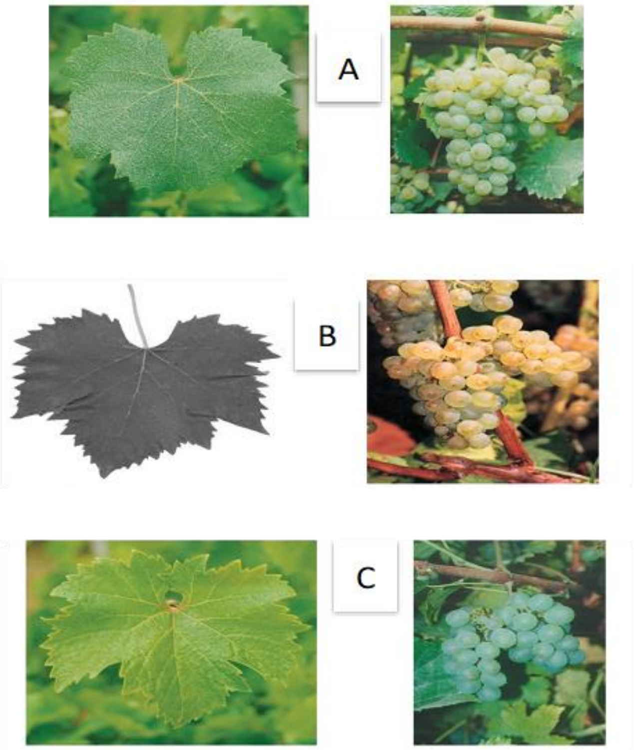


Figure 6. Leaves and bunches of “Verdelho” (A), “Arinto dos Açores” (B) and “Terrantez do Pico” (C) grape varieties.

CONCLUSION

Vine growing in the Azores has experienced a positive evolution, especially since 2014, when vineyards conversion had its greatest increment, mainly by support given to producers to return to native varieties vineyards. At the end of 2019, several governmental programs, providing the chance of recovering and reconverting vineyards in all the islands of the Azores, which could increase wine production for the coming years. Under this new program other islands like Santa Maria and Faial will be considered for viticulture and wine production, and where edafo-climatic characteristics as well as distinct soil will allow the production of different wines from the other D. O. regions.

Recently a few studies have been conducted concerning indigenous grape varieties, namely at the level of clones of grape varieties, such as “Arinto dos Açores,” “Verdelho” and “Terrantez do Pico.” In addition, also the yeasts population from these varieties has been study to contribute to explaining the uniqueness of wines produced in the regions of DO “Pico,” “Biscoitos” (Terceira) and Graciosa islands. However, there is still a long path away, until the recently placed vineyard starts to produce, and then, there will be an increase in opportunities to differentiate oenological products. Traditionally, the wines produced in the Azores are white wines and liqueur wines. Sparkling wines appear on the market but still have small production, nevertheless due to the curiosity and creativity of the winemakers, we will start seeing in the future other types of wines, which will bring new challenges for the winemaking activity of the Azores islands.

REFERENCES

Afonso, J. (2000). Azores: A region to discover. *Wine Magazine*, (118):55-60.

Azevedo, E. M. V. B. (2008). *The climate of the Azores. Master in Nature Management and Conservation 2007-2008*. Climate, Meteorology and Global Change Centre of the University of the Azores.

Barros, A. C. (1892). Inspection services to vineyards in the southern and northern agronomic circumscriptions in 1889. *General Directorate of Agriculture*, National Press, 93 pp.

Bowers, J. E., Meredith, C. P. (1997). The parentage of a classic wine grape, Cabernet Sauvignon. *Nature Genetics*, 16:84-87.

Carvalho, C. (2004). The heritage vineyards. *Expresso, Única Magazine*, 7:58-64.

Cipriani, G., Spadotto, A., Jurman, I., Di Gaspero, G., Crespan, M., Meneghetti, S., Frare, E., Vignani, R., Cresti, M., Morgante, M., Pezzotti, M., Pe, E., Policriti, A., Testolin, R. (2010). The SSR-based molecular profile of 1005 grapevine (*Vitis vinifera* L.) accessions uncovers new synonymy and parentages, and reveals a large admixture amongst varieties of different geographic origin. *Theoretical and Applied Genetics,* 121(8):1569-85.

Cordeiro, A. (1981). *Insular history of the islands to Portugal in the western ocean*. Regional Secretariat of Education and Culture, Angra do Heroísmo.

Eiras-Dias, J. E., Paulos, V., Mestre, S., Martins, J. T., Goulart, I. (2006). The grapevine varieties of Azores region, *Ciência e Técnica Vitivinícola,* 21(2):99-112.

Frutuoso, G. (1522-1591). *Saudades da Terra* (6 vols), Cultural Institute of Ponta Delgada, Ponta Delgada Available in https://sites.google.com/site/saudadesterra/.

Goulart, I. M. (1991). Contribution to the Study of Viticulture on Pico Island. *Final report of a degree in Agricultural Engineering,* University of the Azores. Angra do Heroísmo.

Ibanez, J., Teresa de Andres, M., Zinelabidine, L. H., Cabezas, J. A., Gaforio, L., Munoz, G., Cabello, F., Martínez-Zapater, J. M. (2013). Study of the kinship of vine varieties using DNA markers In: *Cultural heritage of the vine and wine*, pp 275-281, UAM Ediciones.

Lacombe, T., Boursiquot, J. M., Laucou, V., Vecchi-Staraz, M., Peros, P. (2012). Large-scale parentage analysis in an extended set of grapevine cultivars (*Vitis vinifera* L.). *Theoretical and Applied Genetics,* 126(2): 401-414.

Lima, M., (1943). *Annals of Horta Municipality,* Famalicão.

Lopes, M. S., Sefc, K. M., Eiras Dias, E., Steinkellner, H., Machado, M. L., Machado, A. C., 1999. The use of microsatellites for germoplasm management in a Portuguese grapevine collection. *Theoretical and Applied Genetics*, 99:733-739.

Macedo, A. L. S. (1981). *History of the four islands that make up the district of Horta, RAA*, Regional Secretariat of Education and Culture.

Medeiros, C. A. (1994). Contribution to the study of vineyards and wine in the Azores. *Finisterra,* 29:199-229.

Mestre, S. (2016). Traditional grapevine varieties from the Azores: notes on their origin. *Ciência e Técnica Vitivinícola,* 31(2):63-72.

Pereira, A. S. (1987). *The island of S. Jorge (XV-XVIII centuries).* Contribution to his study. Ponta Delgada. University of the Azores.

São Romão, V. V. (1822). *Theoric and practical treaty on vineyard agriculture: the extraction of must, goodness, and conservation of wines, and the distillation of brandies,* Lisboa, National Press, 239 pp.

Silva, M. R. (1950). The Island of Pico from a wine-growing point of view. *Bulletin of the Regulatory Commission for Cereals of the Azores Archipelago,* 14:45-46.

Rebello, E. (1885). *Azorean notes, archive of the Azores, Volum VII,* Ponta Delgada, 74-75 pp.

Regional Secretariat of Agriculture and Forestry (2007). *Rural development programme for the autonomous region of the Azores 2007-2013. Edition 2* - Version of November 18.

Sousa, P. S. (2004). For a history of the vine and wine of the Azores. *Separata of the Historical Bulletin of Terceira Island*. Volume LXII.

Sefc, K. M., Pejic, I., Maletic, E., Thomas, M. R., Lefort, F. (2009). Microsatellite markers for grapevine: tools for cultivar identification & pedigree reconstruction In: *Grapevine Molecular Physiology & Biotechnology*, K. A. Roubelakis - Angelakis(ed.), pp. 565-596.

University of the Azores, (1986). *Soil Charter of Pico Island*.

Veloso, M., Almandanin, M. C., Baleiras-Couto, M., Pereira, H. S., Carneiro L. C., Fevereiro, P., Eiras-Dias, J. (2010). Microsatellite database of grapevine (*Vitis vinífera* L.) cultivars for wine production in Portugal. *Ciência e Técnica Vitivinícola*, 25(2):53-61.

Vouillamoz, J. F., Grando, M. S. (2006). Genealogy of wine grape cultivars: ‘Pinot’ is related to Syrah. *Heredity,* 97:102-110.

Zapater, J. M., Lijavetzky, D., Fernández, L., Santana, J. C., Ibanez, J. (2013). The history written in the grapevine genome, 213-231, In: *Patrimonio cultural de la vid y el vino*, UAM Ediciones, Universidad Autónoma de Madrid, Madrid.

Websites

http://sivvem.monbyte.com/.
http://plantgrape.plantnet-project.org/en/.
https://i1.wp.com/funchalnoticias.net/wpcontent/uploads/2016/10/acoresmapa.jpg?ssl=1.
https://www.azoreanwines.com/.

In: Vitis: Biology and Species
Editors: António M. Jordão et al.

ISBN: 978-1-53618-308-5

Chapter 6

THE THREE DIFFERENT WINEGROWING ZONES IN BRAZIL ACCORDING TO CLIMATE CONDITIONS AND VINE MANAGEMENTS

Giuliano Elias Pereira[*]
Brazilian Agricultural Research Corporation, Embrapa Grape & Wine,
Bento Gonçalves, RS, Brazil

ABSTRACT

Brazil is the only country worldwide with three kinds of viticulture, producing grapes and wines. Two key factors play an important rule to distinguish these zones: different climate conditions and vine managements. The first winegrowing condition is the traditional viticulture, practiced in temperate and subtropical climates for over a hundred years in Southern and Southeastern Brazil, where wineries prune and harvest once a year, as in most of the world, in both Hemispheres. The second kind of viticulture is in Northeastern Brazil, in a tropical semi-arid climate, where wineries have been elaborating tropical wines since 1985, pruning and harvesting the same vine twice a year. By scheduling plots and vineyards, they are able to prune and harvest every single day of the year, programming production according to market demand, winemaking capacity and available tanks. Wine typicality varies due to intra-annual climate variability, resulting in very different wines, depending on harvest date. Finally, a new third winegrowing zone began in 2002 in the Southeastern and Northeastern regions of Brazil, between 800-1,100 m of altitude, where wineries prune twice and harvest once per year, producing only winter wines. In this area, vine management with the use of fitoregulators is the key to allow two vegetative cycles (development and production). All products of these winegrowing zones (white and red still and sparkling wines) have specific qualities and typicalities, each with its own characteristics.

Keywords: chemical and sensorial composition, grapes, typicality, *Vitis labrusca*,*Vitis vinifera* L.,wines

1. INTRODUCTION

Wines have been produced for decades, even centuries ago in many countries worldwide, in different climate/soil conditions, with specifical vine managements by winegrowers and enologists. Vine is originated from the East Coast of the Black Sea, in the Transcaucasia region located at Asia Minor, in a temperate climate zone (Mullins et al., 1992). But actually vines are being planted in many other different climate conditions, such as subtropical, tropical semi-arid and also in altitudes around 1,000 meters above sea level, which will be detailed in this chapter.

The traditional vitiviniculture started commercially several centuries ago in the called "Old World," mainly in Europe, including France, Italy, Portugal, Spain and Germany. Few decades ago, other countries started wine production, and actually are playing an important role in the wine market. These countries are known as taking part of the called "New World." The main countries are China, USA, Argentina, Chile, South Africa, Australia and New Zealand (OIV, 2019). These traditional countries from "Old" and "New" Worlds are producing wines in the north hemisphere (between 30-50º north latitude) and south hemisphere (between 22-50º south latitude) (Figure 1).

In 2018, the most important countries that presented the largest vineyard areas were Spain (969,000 hectars), China (875,000 ha), France (793,000 ha), Italy (705,000 ha), Turkey (448,000 ha), USA (439,000 ha), Argentina (218,000 ha) and Chile (212,000 ha), including all products, such as wine, grape juice, table grapes and raisins. Brazil has actually around 80,000 ha of vineyards, also for all uses (except raisins). In relation to the major grape producers, China is located on the top (11.7 million tonnes), followed by Italy (8.6), USA (6.9), Spain (6.9), France (6.2), Turkey (3.9), India (2.9) and Argentina (2.7 million tonnes). Brazil produced in 2018 around 1.6 million tonnes of grapes for all uses (Mello, 2018).

All countries together produced 292 million hL of wines in 2018, representing an increasing of 17%, as compared to 2017 (OIV, 2019). The highest wine production was observed in Italy (55 million hL), followed by France (49), Spain (44), USA (24), Argentina (15), Chile (13), Australia (13) and Germany (10 million hL) (OIV, 2019). Brazil produced around 2.8 million hL of wines in 2018, from grapes of the species *Vitis labrusca* and *Vitis vinifera* L. (Mello, 2018). In relation to the global wine consumption, 246 million hL were consumed by all countries in 2018. Some countries presented an increasing on wine consumption in 2018, as compared to 2014, such as USA, Canada, Portugal, Spain, Italy, Russia, China, India, South Africa and Australia (Figure 2) (OIV, 2019). Other countries presented a stability between 2014 and 2018, such as France, Germany, United Kingdom, New Zealand and Brazil. In contrast, Chile, Argentina, and some countries located in the East of Europe presented a decrease of wine coonsumption from 2014 to 2018.

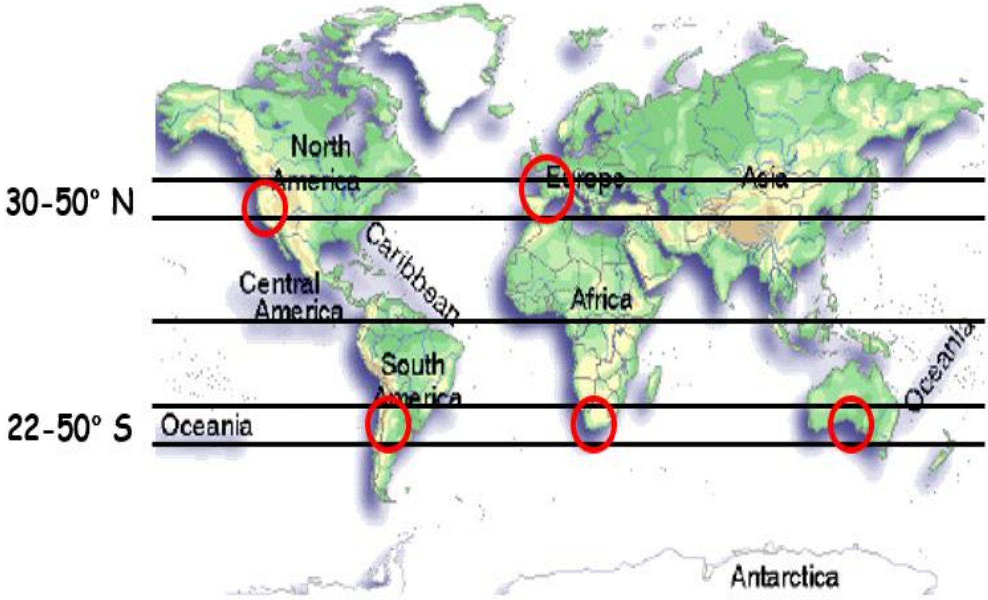


Figure 1. Different world winegrowing regions.
Circules in northern and southern hemispheres show traditional zones for grapes production in US, Europe, South America, South Africa, Australia and New Zealand (Pereira, G.E.).

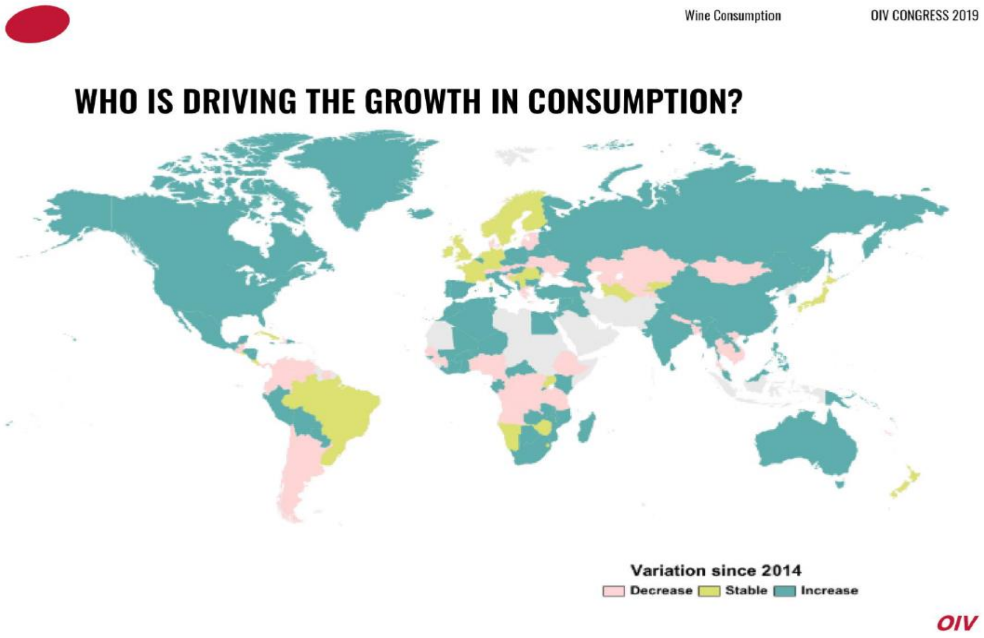


Figure 2. Global map showing countries increasing, stable or decreasing wine consumption in 2018, in relation to 2014 (OIV, 2019).

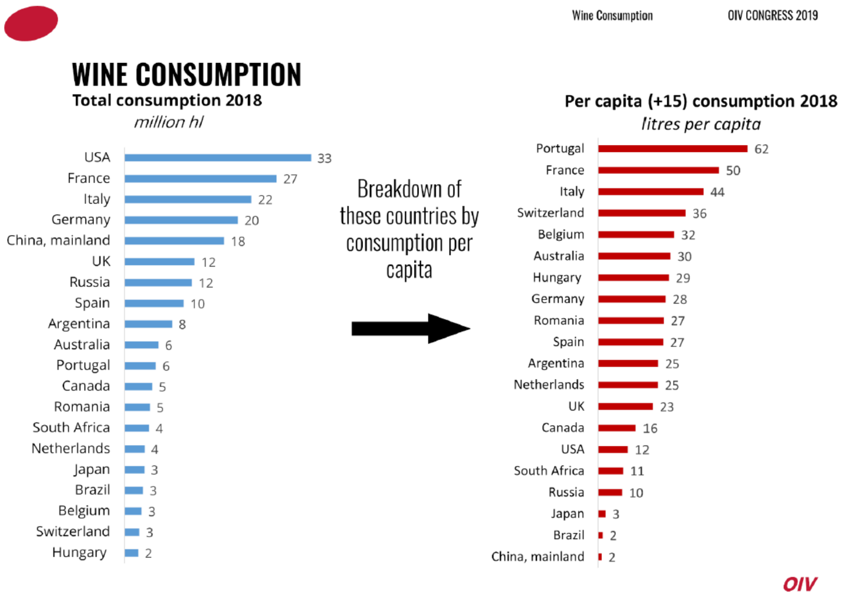


Figure 3. Wine consumption per country (left) and per capita (right) in 2018 (OIV, 2019).

In 2018, the countries which consumed the highest amounts of wines were USA (33 million hL), France (27), Italy (22), Germany (20), China (18), UK (12), Russia (12) and Spain (10) (Figure 3) (OIV, 2019). Brazil consumed around 1.3 million hL of wines from *Vitis vinifera* L. in 2018, being 85% from imported wines and 15% of national products (Mello, 2018). Including wines from *Vitis labrusca*, the country consommed around 3 million hL of wines. According to the per capita consumption, in 2018 Portugal was located on the top of the list, with 62 liters, followed by France (50), Italy (44), Switzerland (36), Belgium (32), Australia (30), Hungary (29) and Germany (28) (Figure 3) (OIV, 2019). In Brazil, the per capita wine consumption in 2018 was around 2 liters.

Brazil is producing and consuming low quantities as compared to the traditional wine countries around the world. However, there is a very interesting situation happening in the brazilian vitiviniculture, that we can not find elsewhere. There are actually three very different winegrowing zones, according to the climate conditions and vine managements. This topic will be the main objective of this chapter.

2. THE THREE WINEGROWING REGIONS IN BRAZIL

Brazil, oficially called of Federative Republic of Brazil, is a large country, presenting more than 8.5 million of square kilometers, and over 210 million people (IBGE, 2020). It is the largest country of both South (where it is located) and Latin America, the fifth-largest country by area and the sixth most populous country of the world. The capital is Brasilia (situated in the Federal District) and the most populous city is São Paulo, with more than

12 million habitants. The country is composed by 26 States more the Federal District, with 5,570 municipalities, situated in five geographical regions: North, where is located Amazonia, North-East, South-East, Center-West and South (IBGE, 2020).

Except in the North region, where there is not yet vineyards installed, because weather conditions are very warm and wet, and vines could present huge problems with fungal diseases, vines are being planted and commercialy explored in all other four regions in Brazil. There are vineyards for different uses, including wine grapes (from *Vitis labrusca* and/or *Vitis vinifera* L.), grape juices and table grapes. Different climate conditions and soil types are found in Brazil, as shown in the Figure 4.

In Brazil are produced actually 280 million liters of all wines per year (Mello, 2018). From these, around 85% (238 million liters) of the wines are elaborated with *Vitis labrusca* varieties, such as 'Isabella', 'Ives' (or Folha de Figo or Bordô), and other hybrids. These wines are nationally called table or common wines, and are all exclusively consummed in Brazil. On the other hand, the called fine wines, elaborated with *Vitis vinifera* L. varieties, are responsible by 15% of the wines (42 million liters).

Actually in Brazil, three different winegrowing zones are commercially explored, and wines are being produced with different qualities and typicalities (Figure 5). The first one is located in the South and South-East regions, where "traditional wines" have been produced since one hundred years ago. The second one is located in the North-East region, where tropical vitiviniculture is practiced to produce "tropical wines" commercially since 1985. The thirth one is the most recent zone, where the called "winter wines" are being produced in the South-East, North-East and Center West regions of Brazil, since 2004. Brazil is the only country around the world where these three winegrowing regions are possible, and information about these zones will be are detailled in this chapter. The two factors allowing these possibilities with a lot of variabilities are due to the climate conditions and vine managements.

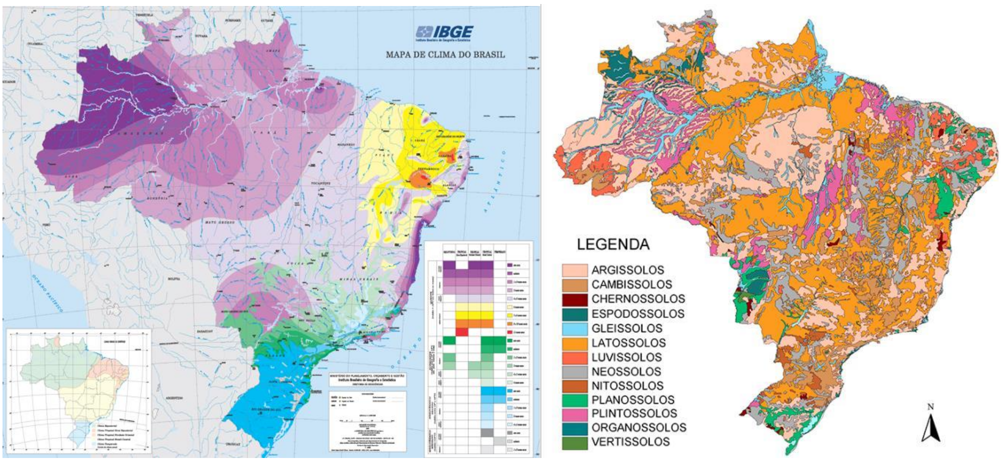


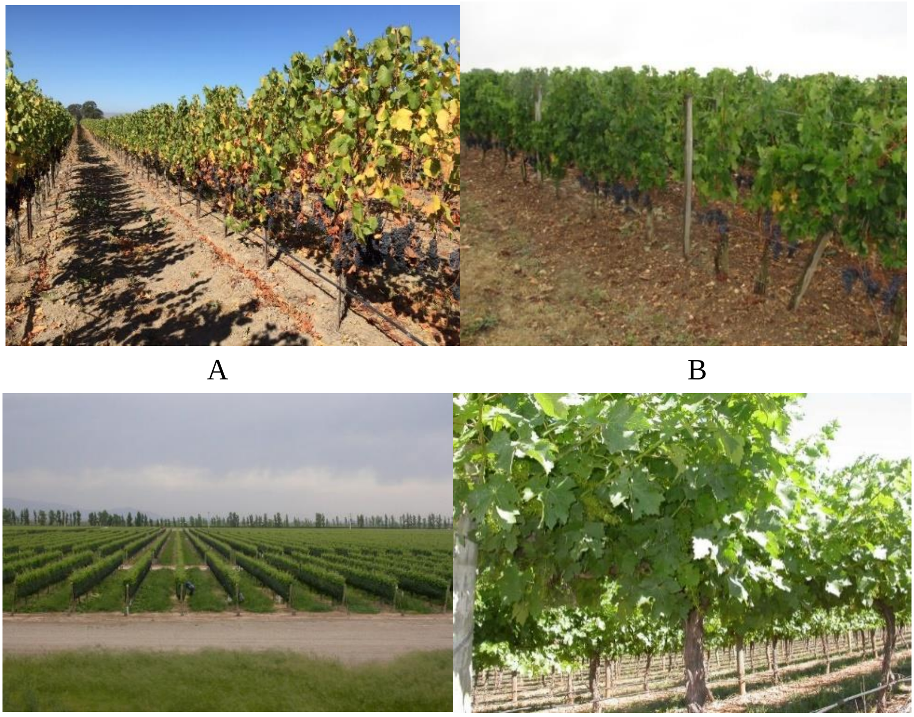
Figure 4. Climatic (left) and soil (right) variability in Brazil (IBGE, 2020; Embrapa, 2020).

Figure 5. The three different winegrowing zones in Brazil.
1: traditional wines, produced in the humid temperate and sub-tropical climates; 2: tropical wines, in a tropical-semi-arid climate; 3: winter wines, in the sub-tropical of altitude and tropical of altitude climates. North (Norte), Center-West (Centro-Oeste), North-East (Nordeste), South-East (Sudeste) and South (Sul) regions (IBGE, 2020, adapted by Pereira).

2.1. The First Winegrowing Zone: Traditional Wines

Traditional wines are produced in countries located in the “Old World” and “New World,” in both northern and southern hemispheres. In countries from the North Hemisphere, such as United States of America, Canada, Portugal, Spain, France, Italy, Germany, China and others, vines are pruned in January-February-March, and grape harvests are carried out between August to October, depending of the climatic conditions and grape varieties, if they are early (Chardonnay, Pinot Noir, Riesling, Sauvignon Blanc, and others), mid-season (Cabernet Franc, Malbec, Merlot, Syrah, Tempranillo, and others) or late (Cabernet Sauvignon, Grenache, Sangiovese, Zinfandel, and others). In countries located in the southern hemispheres, such as Chile, Argentina, Uruguay, Brazil (south and south-east regions), South Africa, Australia and New Zealand, pruning season is August-September, and harvest dates are between December to April, also depending of the same factors already mentioned (climates and varieties).

Figure 6 shows examples of vineyards in the northern hemisphere, in the USA (Figure 8A) and France (Figure 8B), close to harvest date, and also in the southern hemisphere at fruit set, in Argentina (Figure 8C) and Australia (Figure 8D). The climates of these winegrowing zones, for all countries in both hemispheres, are mainly temperate and humid subtropical (south-east of Brazil). The vine management for all of these winegrowing regions, in both northern and southern hemispheres, among other practices, is characterized by one pruning of vines and one harvest of grapes per year. This is mandatory, mainly due to the climate conditions.



A B

C D

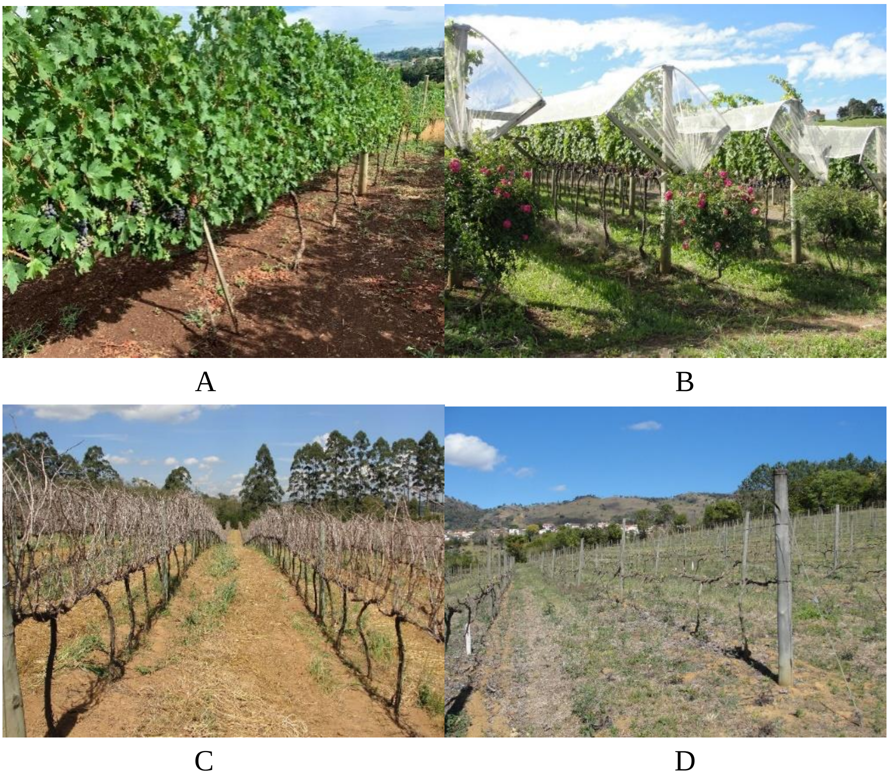
Figure 6. Vineyards in different phenological stages and winegrowing regions.
Few days before harvest of Cabernet Sauvignon in Napa Valley, California, USA, on October 2018.(A); few days before harvest of Merlot in Pomerol, France, on September 2011 (B); Malbec at fruit set in Mendoza-Argentina, on November 2007 (C); and also at fruit set of Syrah in Adelaide-Australia, on November 2008 (D). Pictures: Giuliano Elias Pereira.

In Brazil, the traditional wines, with one pruning of vines and one harvest of grapes per year, are produced in two regions, in the South and South-East. The most important and traditional winegrowing region is located in the South, and the vitiviniculture started for more than one century ago. Italians came to Brazil as immigrants, and planted first vines around 1875, and then adapted varieties to the weather conditions (Souza, 1996). The predominant climate is humid temperate, and vitiviniculture is practiced in all three States, in Paraná, Santa Catarina and Rio Grande do Sul (Wrege et al., 2012). The Serra Gaúcha (Gaucha Mountain), the most famous and traditional winegrowing region in Brazil, is located in the Rio Grande do Sul State, composed by 26 municipalities (Wrege et al., 2012). The annual average temperature of the region is around 17ºC. In the same way, in the South-East region of Brazil, the Sates of Minas Gerais and São Paulo have produced traditional wines for decades ago, at the same characteristic as found in the South of Brazil, but in different climate conditions and also soil types. These two States (Minas Gerais and São Paulo) are located in a humid sub-tropical climate, which annual average temperature is around 18ºC, and vines present the same behavior as well as found in the South, with one pruning and one harvest per year. All of these regions are producing traditional wines, in the South and South-East States of Brazil, are located between 50 and 1200 m of elevation above sea level. In these conditions, viticulture follows the natural season and the weather, as well as for all countries cited before, in the winegrowing zones located in the northern and southern Hemispheres. Describing the cycle, vines are pruned between winter and spring, then temperatures increase, vines get flowering period (blooming), some weeks later grapes start ripening (veraison), and then they are harvested between summer and autumn. After harvest, leaves rippen, dry and drop. Vines are naturally deciduous plants in these climate conditions (Carbonneau et al., 2015). In all countries and winegrowing zones around the world, the effect of climate, soil and human activity, with different vine managements and wine protocols, is called the "*terroir*" effect (Carbonneau et al., 2015). This definition is used mainly in the "Old World" to add value in many products, such as wines, cheeses, meats, vegetables, fruits, milks and other products.

Figure 7 shown different vineyards from traditional vitiviniculture in different States from Brazil, at different phenological stages. The main products elaborated in the South and South-East regions of Brazil are table/common wines, from *Vitis labrusca*, followed by sparkling wines from Muscat varieties (*Vitis vinifera* L.), such as Giallo Muscat, Bianco Muscat and others, and then red wines from *Vitis vinifera* L. (Nicolli et al., 2015).

In the South region of Brazil, in Rio Grande do Sul State, Embrapa Grape & Wine started a project to develop a Geographical Indication-GI for still and sparkling wines from *Vitis vinifera* L. varieties at the end of the 90's decade (Tonietto, 1993). In 2002, it was conceded by Brazilian Gouvernment (National Institute of Industrial Property-INPI) the first GI of Brazil, called Vale dos Vinhedos. At this moment, more than 20 varieties were used by wineries were allowed to produce and use the label of GI. In 2012, the region became an Apellation of Origin-AO Vale dos Vinhedos (Vineyards Valley) (Tonietto, 2003; 2008; Tonietto and Falcade, 2013).

The Association of the Wineries (Aprovale) is the owner of the label and the varieties allowed for AO Vale dos Vinhedos are Merlot for red wines (possible blend with Cabernet Sauvignon, Cabernet Franc and Tannat), Chardonnay for white wines (possible blend with Riesling Italic), and Pinot Noir and Chardonnay (possible blend with Riesling Italic) for sparkling wines (Tonietto et al., 2013). After these two recognition by INPI, the wine sector in the region changed completely, firstly with a sectorial organization, followed by an improvement of the wine quality. The consequence was a strong rise of the enoturism, demand for the products and, actually, an increase of the reputation, prices and sales of the wines with AO. Actually, Vale dos Vinhedos is an important winegrowing region of Brazil, located at the most important winegrowing region of the country - Serra Gaúcha (Figure 8).


A B

C D

Figure 7. Different vineyards located in several Brazilian winegrowing regions.
Vineyards of Cabernet Sauvignon after veraison in the South of Brazil, in Bento Gonçalves, at Rio Grande do Sul State, on January 2020 (A); vineyard of Cabernet Sauvignon close to veraison in São Joaquim, at Santa Catarina State, on December 2016 (B);vineyard of Cabernet Franc few days before pruning, in São Roque, at São Paulo State, on August 2012 (C); vineyard of Chardonnay few days after pruning in Caldas, Minas Gerais State, on August 2012 (D). Pictures: Giuliano Elias Pereira.

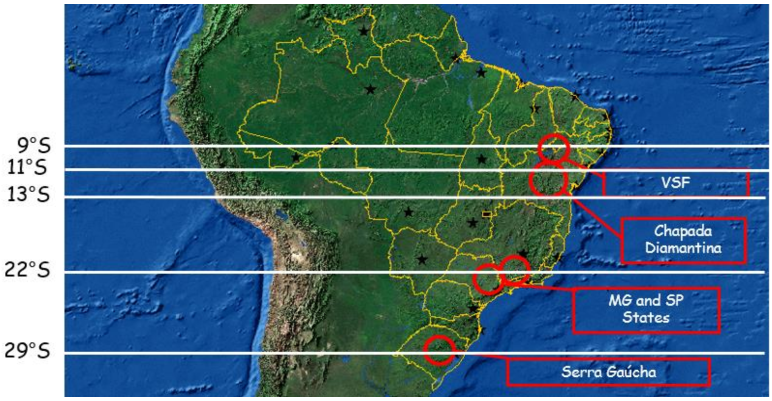


Figure 8. The three winegrowing zones in Brazil.
“Traditional wines” are produced at Serra Gaúcha (29º South Latitude), Minas Gerais-MG and São Paulo States (22º South Latitude); “tropical wines” are produced in the São Francisco Valley-VSF, north-eastern of Brazil (9º South Latitude); “winter wines” are produced recently in Minas Gerais-MG and São Paulo-SP States (22º South Latitude) and in the Chapada Diamantina (11º and 13º South Latitudes), in high altitudes (Pereira et al., 2018).

2.2. The Second Winegrowing Zone: Tropical Wines

The second winegrowing region in Brazil is quite new, and has started in the middle of the 1980's decade. Precisely, the first commercial wines were sold in 1986 (Pereira et al., 2011). The region is located in the north-east and tropical wines are produced in the Vale do São Francisco (São Francisco Valley), in a tropical semi-arid climate, where annual average temperature is 26.5ºC (Figure 8, 9º South Hemisphere). The main characteristic of the region is that winegrowers can make two prunings and two harvests per year from the same vine (Tonietto and Pereira, 2011; Pereira et al., 2016). But scaling different plots, they prune vines every week and harvest grapes every week during the year (Tonietto and Pereira, 2012; Pereira et al., 2018). Figure 9 shows an example of the plot management which wineries use to get prunings and harvests in all weeks/all days throughout the year. For example, in the plot 1, they prune vines in January and harvest grapes in May. Then, they give an "artificial dormance" during 20-30 days, and they prune again in June, which harvest of this plot is made in October. The same is planned for all plots of the wineries, and they have always vines being pruned and grapes being harvested for winemaking. For table grapes is the same, products are being harvested every week/every day during the year, for seed or seedless varieties.

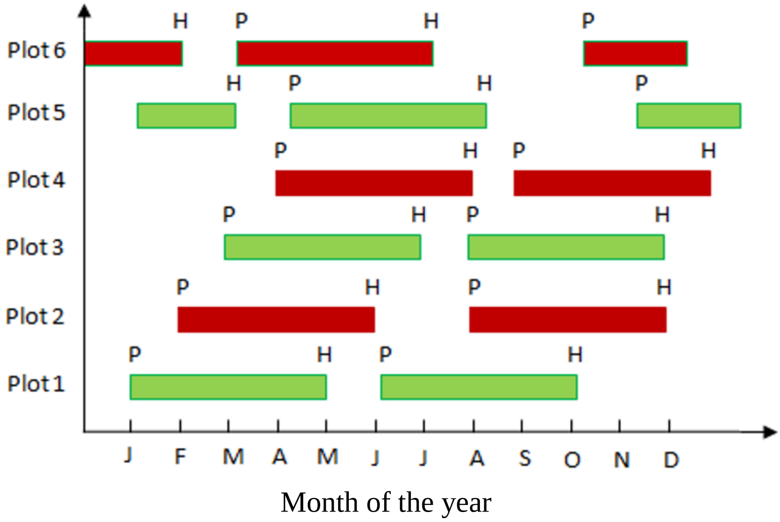


Figure 9. Plot management of the vineyards in the tropical semi-arid climate conditions of the north-eastern of Brazil.
P: pruning period; H: harvest period.

There are three main factor allowing wineries to decide when make prunings and harvests: high temperatures during the year (annual average of 26.5ºC), high solar radiation (more than 3,000 MJ m^{-2} $year^{-1}$) and water availability for irrigation from São Francisco river (Tonietto and Pereira, 2011; Tonietto et al., 2012; 2014). The vine management is very precisely, and wineries decide when prune or harvest according to the market, physical structure of the wineries for winemaking and also depending on potential wine quality and typicality. Figure 10 shows vineyards in different plots. It can be seen on the top the São Francisco river, from where water is used for irrigation. There are vineyards at different phenological stage, at fruit set, at veraison and after pruning. Among the vineyards there is the typical vegatation of the region, called caatinga.

Figure 10. Vines in different plots of vineyards, at different phenological stage.
Picture: Vitivinícola Santa Maria/Global Wines.

The season for the vines in the region is very different as compared to traditional zones, as described before. Starting a new cycle in the region, the producers increase the irrigation level for 100% of the crop coefficient, 2-3 days before pruning, after vines are pruned, then hydrogen cyanamide (Dormex®) is applied on the buds to homogeneize budburst (Figure 11). Blooming happens around 30-40 days after pruning, depending of the grape variety. Veraison starts around 70-80 days after pruning, and then harvest occurs between 95-135 days after pruning, depending of the cycle of each cultivar. After harvest, producers decrease irrigation for 5-10% of the crop coeficient (they can not suspend completely irrigation because vines die), and they promote an "artificial dormance" to vines. After 30-40 days of "artificial dormance," they prune vines, with leaves. Even vines being deciduous plant, they do not lose leaves in that condition, they are a little bit dry, yellowish/brown, but do not fall. This is a sumary of the tropical semi-aridi condition of Brazil, where tropical wines, grapes juices and table grapes are being produced since 35 years ago. It is possible to produce also tropical wines in other countries of the northern hemisphere, such as in India, Thailand, Myanmar and Venezuela (Pereira et al., 2018).

The region has around 13,000 ha of table grapes (*Vitis vinifera* L), with seed and seddless varieties, producing grapes for export and also for intern market in Brazil. The region has around 400 ha of labrusca varieties (Early Isabel, BRS Magna, BRS Violeta, and BRS Cora, that are Brazilian hybrids and *Vitis labrusca* created by Embrapa Grape & Wine) for grape juices and table wine production.

Figure 11. Application of hydrogen cyanamide (Dormex®) on the buds to homogeneize budburst (A and B); and vines in pergola (C) and spalier (D) trellys systems starting a new cycle, 10 days after the plant regulator application.
Pictures: Giuliano Elias Pereira.

Also in the Vale do São Francisco, there are 400 ha of *Vitis vinifera* L. varieties being used to produce around 4 million liters of wines per year. Sparkling wines are responsible for 65% of the production, then red wines with 24% and white wines responbible by 1% of total production (Pereira et al., 2018). Vineyards are conducted on pergola and spalier trellys systems, depending of the objective (Figure 12). Normally sparkling wines are produced with grapes from pergola system, which yield reaches 35-40 ton hectar^{-1} per vintage, which plot can produce twice, that means around 60-70 ton ha^{-1} year^{-1}. The main varieties for sweet sparklings are Italia and Canelli Muscat (muscats), elaborated by the Asti method. The producers also use some varieties for white and rosé sparklings, elaborated by Charmat method, such as Chenin Blanc, Sauvignon Blanc, Verdejo (or Verdelho), Viognier, Syrah, Grenache and Tempranillo. For red wines, the main grape varieties are Syrah, Tempranillo, Touriga Nacional, Alicante Bouschet, Cabernet Sauvignon, Ruby Cabernet, Merlot and Tannat, used mostly for young wines, and a bit for aged wines.

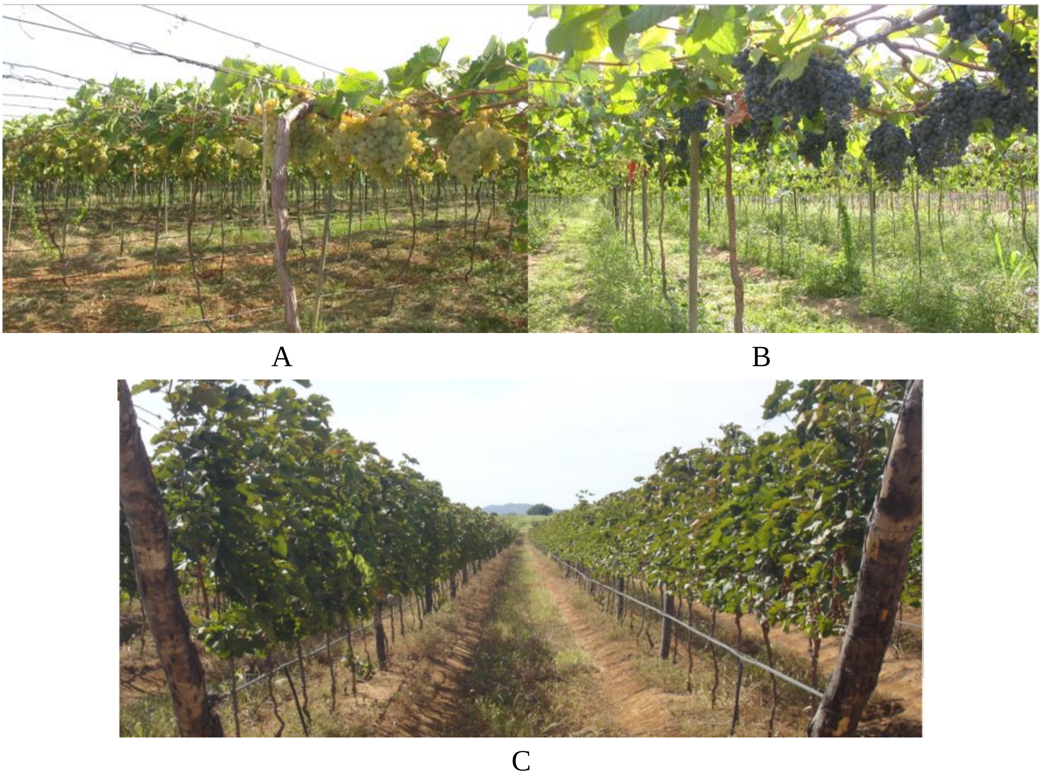


Figure 12. Vineyards producing grapes for sparkling wines in pergola (A and B) and in spalier systems for red wines (C) in the Vale do São Francisco.
Pictures: Giuliano Elias Pereira.

The composition and quality of the grapes, wines and grape juices vary according to the harvest date and the month which grapes were harvested (Padilha et al., 2016; 2019; Oliveira et al., 2019a). Normaly red wines elaborated between May and September present high color, aroma complexity and ripen tannins, and can be stable for years and decades. On the other hand, wines elaborated with grapes harvested between October and March present low color, aroma and unrippen tannins, due to the highest temperatures, and the vegetative cycle is shorter (Pereira et al., 2018; Oliveira et al., 2018; 2019b). For sparkling and white still wines, also there is an influence of the date on wine stability, but less as compared to red wines, because these products are consommed young, few months after bottling.

2.3. The Third Winegrowing Zone: Winter Wines

The third winegrowing zone in Brazil is very new, has started in 2002, after trials developped by Company of Farming Research of Minas Gerais (EPAMIG), in Minas Gerais State, south-eastern of Brazil (Regina et al., 2010). The main characteristic of this winegrowing condition is that vines are submited to double pruning, being the first one called a formation pruning and the second one the production pruning (Dias et al., 2017). As described before, in the southern hemisphere vines are normally pruned in August and harvested between December and April, according to the varieties. In this case for "winter wines," vines are pruned in August (end of winter), then they remove all clusters from the vines in October-November (end of spring), and plants end the cycle on January (middle/end of summer), presenting brown canes. Then, vines are pruned again on January-February, as it happens in the North Hemisphere, but with green/yellowish leaves. Soon after hydrogen cyanamide (Dormex®) is applied, and the harvest is performed between June and August, depending of the grape varieties.

In this kind of management, the harvest occurs in the winter, and Brazil is the only country around the world where grapes are harvested in this season, because temperatures can range between 0°C (in the morning, sometimes with frost) to 25°C (in the afternoon). This is the same season and region where the farmers harvest coffee with high quality. This new viticulture is being commercially exploited in Minas Gerais and São Paulo States, in the South-East region of Brazil, since 2005. In that regions there are around 350 hectars of vines planted, which the main varieties are Syrah, Cabernet Sauvignon and Cabernet Franc for reds, and Sauvignon Blanc and Chardonnay for whites. Wines are available in the market since 2008. Figure 13 shows pictures of this new winegrowing region, in Minas Gerais and São Paulo States.

In the same context producing winter wines, there is a specifical region in north-eastern Brazil, in Bahia State, at Chapada Diamantina, where there are around 50 hectars of vineyards, which were planted in 2011, after the success and potential of the "winter wines" observed in Minas Gerais and São Paulo States. Even being located in the north-east of Brazil, where climate is normally tropical semi-arid, warm and dry as described before, this region is different because it is located in high altitudes, around 1,000 m above sea level. The effect of the elevation at low latitudes (they are at 11º and 13º South Latitudes) is that the annual average temperature is around 19ºC, very fresh as compared to the tropical semi-arid climate (26,5°C) (Oliveira et al., 2019c; 2019d). The temperatures in the harvest season range from 6-8°C in the morning to 25°C in the afternoon.

The commercial wines will be available in the market only in 2021, using the same grape varieties used in Minas Gerais and São Paulo States, to produce whites, reds and sparkling wines (Figure 14). In the center-western region of Brazil, there are also some vineyards being introduced and winter wines are being produced in Goiás and Distrito Federal, close to the capital Brasilia, using the same grape varieties mentioned for the order states, and they will be also available in the market only in 2021.

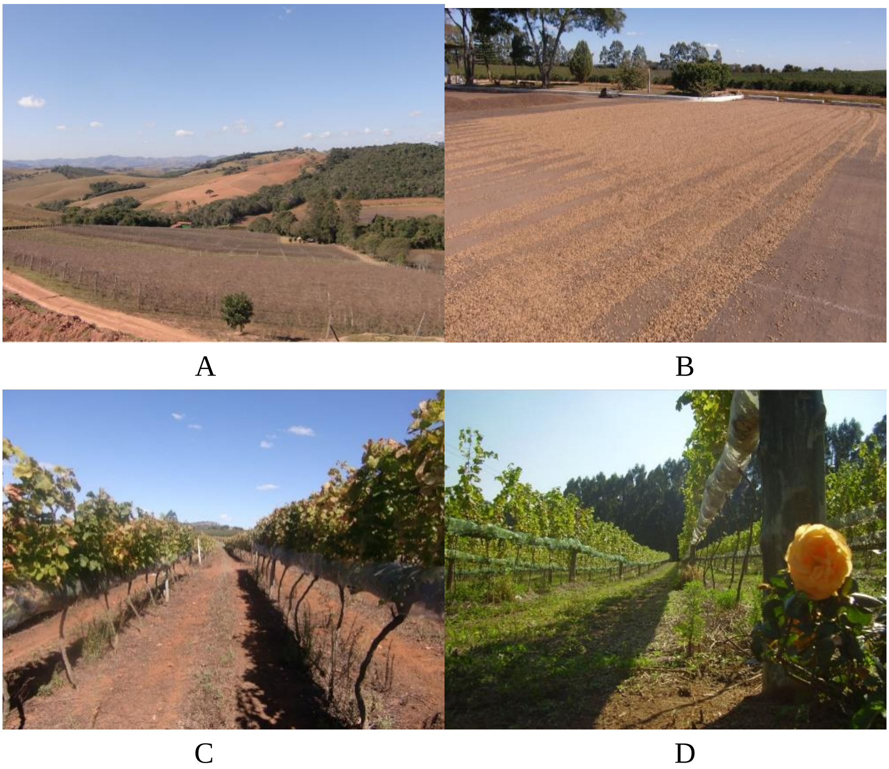


Figure 13. New winegrowing zones located in the South-East region of Brazil, where wineries are producing the winter wines.
Typical landscape of the region, in Minas Gerais, in July 2016 (A); coffee harvested and being dried on the ground, in July 2016 (B); vineyard of Syrah close to harvest, on July 2016, in Minas Gerais State (C); vineyard of Sauvignon Blanc close to harvest, on July 2016, in São Paulo State (D). Pictures: Giuliano Elias Pereira.

Scientific researches were carried out showing a comparison of the differences between grapes and wines compounds elaborated from the tropical semi-arid (tropical wines) and tropical of elevation (winter wines) climates, in the north-east of Brazil (Oliveira et al., 2019c and 2019d). Results show that Syrah grapes harvested from Chapada Diamantina (high altitudes) presented higher amounts of some phenolic compounds as compared to Syrah grapes harvested from São Francisco Valley (low altitudes).

Brazilian winegrowing regions are being organized by the GI in many different regions (Tonietto and Falcade, 2018). Figure 15 shows the Brazilian map with AO and GI already registered (in purple) and being implemented (in green), in the south and north-east regions. Wineries producing the winter wines from the south-east and north-east regions are also interested to get the GI for their products, and a project will be started soon. The producers of the "winter wines" created an Association of all wineries (Asprovin), and they are starting the requests to demand and get in the future the GI for these wines.

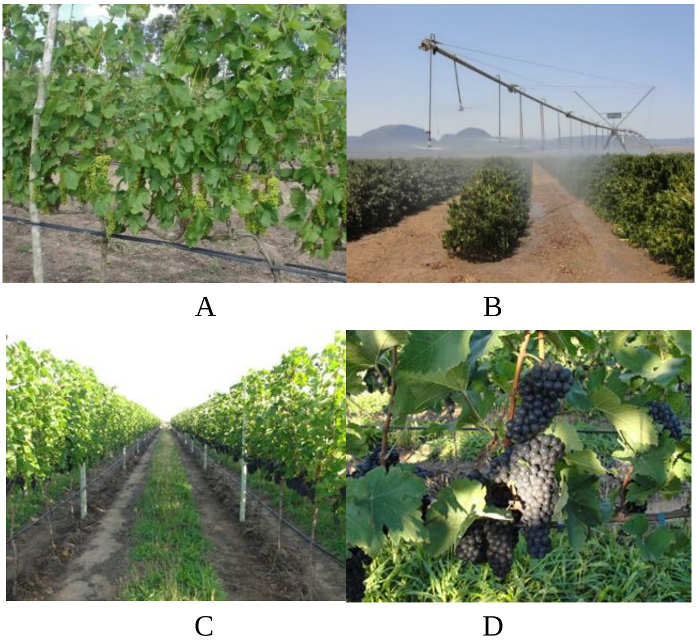


Figure 14. New winegrowing zones located in the north-eastern region of Brazil, where vines are also producing winter wines. Vineyard of Syrah at pea size in Morro do Chapéu, at Chapada Diamantina-Bahia State, on May 2015 (A); typical production of coffee in Mucugê, irrigated by central pivot, also at Chapada Diamantina-Bahia State, on July 2017 (B); vineyard of Cabernet Sauvignon close to harvest in Mucugê, on July 2017 (C); grape clusters of Cabernet Sauvignon in Mucugê, on July 2017 (D). Pictures: Giuliano Elias Pereira.

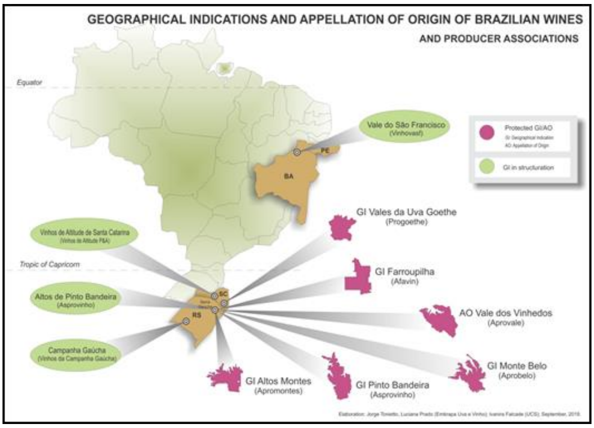


Figure 15. Map of the Brazilian Geographical Indications obtained and being implemented by wineries in the southern and north-eastern regions of Brazil. (Tonietto and Falcade, 2018).

CONCLUSION

Brazil is the only country around the world where it is possible to find three different winegrowing conditions, according to different climate conditions and vine managements.

The first one is the same which happens in the most part of the main countries producing wines, in both northern and southern hemispheres, where vines are pruned and grapes are harvested just once per year, producing traditional wines. The called “traditional wines” are being produced since more than one century ago in Brazil, located in the humid temperate and humid sub-tropical climates, in the southern and south-eastern regions of the country.

The second type of winegrowing zone started in 1985, in a tropical semi-arid climate of north-eastern Brazil, where vines are pruned and grapes are harvested twice a year, producing the called “tropical wines.” By scaling the plots of vineyards, it is possible to harvest grapes and make wines every day of the year. The third Brazilian winegrowing region started in 2002, in a sub-tropical of elevation and tropical of elevation climates, both close to 1,000 m above sea level, where vines are pruned twice a year, one for formation and other for production, and harvest date is in the winter, where wineries are producing the called “winter wines.” These wines are being produced in the south-east and north-east regions of Brazil, and soon will be also produced in the center-west region. Thus, wines from Brazil are presenting different qualities and typicalities, at different prices, from these three winegrowing zones. They are being produced in different natural conditions of climates and soils, with specifical managements by viticulturists and enologists, valuing the different Brazilian *terroirs*. The “traditional wines” from Brazil are already known in the national and international markets, because some products are even exported. They present specifical qualities and very good products are available for consummers, among young and aged wines. The “tropical wines” are new but already available in the brazilian market, with good products. The wines from São Francisco Valley are in the great majority young and fresh, elaborated with grapes from several harvests throughout the year.

The “winter wines” are just in the beginning, few products in the market, but soon they will be also in the national and international markets, with high quality and typicality. In my personal opinion, the “winter wines” will change the brazilian market, because they will have in the future a big success. These wines will be the most expensive products from Brazil, because they will be purchased also by american, european and asian markets, and once we have competition, the prices will increase a lot.

REFERENCES

Carbonneau, A., Deloire, A., Torregrosa, L., Pellegrino, A., Metay, A., Ojeda, H., Lebon, E., Abbal, P. (2015). *Traité de la Vigne: Physiologie, Terroir, Culture.* Paris: Dunod, 592 p.

Dias, F.A.N.,Mota, R.V., Souza, C.R., Pimentel, R.M.A., Souza, L.C., Souza, A.L., Regina, M.A. (2017). Rootstock on vine performance and wine quality of ‘Syrah’ under double pruning. *Scientia Agricola*, 74(2):134-141.

Empresa Brasileira de Pesquisa Agropecuária-Embrapa [*Brazilian Agricultural Research Corporation-Embrapa*]. https://www.embrapa.br/en/international.

International Organization of Vine and Wine-OIV (2019). www.oiv.int.

Instituto Brasileiro de Geografia e Estatística-IBGE [*Brazilian Institute of Geography and Statistics-IBGE*], 2020. www.ibge.gov.br.

Mello, L.M.R. (2018). *Vitivinicultura brasileira: panorama* [*Brazilian vitiviniculture: overview*]. Embrapa, 2019. https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/1113215/1/ComunicadoTecnico210.pdf.

Mullins, M.G., Bouquet,A., Williams, L.E. (1992). *Biology of the Grapevine*. Cambridge University Press, Cambridge, United Kingdom. 239p.

Nicolli, K.P., Welke, J.E., Closs, M., Caramão, E.B., Costa, G., Manfroi, V., Zini, C.A. (2015). Characterization of the volatile profile of Brazilian moscatel sparkling wines through solid phase microextraction and gas chromatography. *Journal of Brazilian Chemistry Society*, 26(7):1411-1430.

Oliveira, J.B., Faria, D.L., Duarte, D.F., Egipto, R., Laureano, O., Castro, R., Pereira, G.E., Ricardo-da-Silva, J.M. (2018). Effect of the harvest season on phenolic composition and oenological parameters of grapes and wines cv. ‘Touriga Nacional’ (*Vitis vinifera* L.) produced under tropical semi-arid climate, in the State of Pernambuco, Brazil. *Ciência e Técnica Vitivinícola*, 33:145-166.

Oliveira, J.B., Egipto, R., Laureano, O., Castro, R., Pereira, G.E., Ricardo-da-Silva, J.M. (2019a). Chemical characteristics of grapes cv. Syrah (*Vitis vinifera* L.) grown in the tropical semiarid region of Brazil (Pernambuco state): Influence of rootstock and harvest season. *Journal of the Science of Food and Agriculture*, 99:5050-2063.

Oliveira, J.B., Egipto, R., Laureano, O., Castro, R., Pereira, G.E., Ricardo-da-Silva, J.M. (2019b). Chemical composition and sensory profile of Syrah wines from semiarid tropical Brazil - Rootstock and harvest season effects. *LWT-Food Science and Technology*, 114:108415.

Oliveira, J.B., Egipto, R., Laureano, O., Castro, R., Pereira, G.E., Ricardo-da-Silva, J.M. (2019c). Climate effects on physicochemical composition of Syrah grapes at low and high altitude sites from tropical grown regions of Brazil. *Food Research International*, 121:870-879.

Oliveira, J.B., Egipto, R., Laureano, O., Castro, R., Pereira, G. E., Ricardo-da-Silva, J.M. (2019d). Chemical and sensorial characterization of tropical Syrah wines produced at different altitudes in Northeast of Brazil. *South African Journal of Enology and Viticulture*, 40:3101.

Padilha, C.V.S., Biasoto, A.C.T., Correa, L.C., Lima, M.S., Pereira, G.E. (2016). Phenolic compounds profile and antioxidant activity of commercial tropical red wines (*Vitis vinifera* L.) from São Francisco Valley, Brazil. *Journal of Food Biochemistry*, 1-9.

Padilha, C.V.S., Lima, M.S., Toaldo, I.M., Pereira, G.E., Bordignon-Luiz, M.T. (2019). Effects of successive harvesting in the same year on quality and bioactive compounds of grapes and juices in semi-arid tropical viticulture. *Food Chemistry*, 301:125170.

Pereira, G.E. (2011). Autour des vins tropicaux au huitième parallèle de l'Hemisphère Sud, Nord-Est du Brésil [Around tropical wines at the eighth parallel of the Southern Hemisphere, Northeastern Brazil]. In: Perard, J., Perrot, M. (Org.). *Des hommes et du vin: Le vin, patrimoine et marqueur d'identité culturelle*. 1ed. Dijon: Centre Georges Chevrier, 1:29-46.

Pereira, G.E., Padilha, C., Marques, A.T.B., Canuto, K.M., Mendes, A., Souza, J.F. (2016). Le poids des consommateurs sur l'evolution des vins : l'exemple de la Vallée du Sao Francisco [The influence of consumers on the evolution of wines: the example of the Sao Francisco Valley], Brésil. In: Perard, J. et Perrot, M. (Org.). *Vin et civilisation, les étapes de l'humanisation. Centre Georges Chevrier*, Dijon, 301-310.

Pereira, G.E., Guerra, C.C., Amorim, F.M., Nascimento, A.M.S., Souza, J.F., Lima, L. L.A., Lima, M.S., Padilha, C., Protas, J.F.S., Zanus, M.C., Tonietto, J. (2018). Vinhos tropicais do semiárido do Brasil [Tropical wines from the semiarid region of Brazil]. *Territoires du Vin*, 9:1-13.

Regina, M.A., Carmo, E.L., Fonseca, A.R., Purgatto, E., Shiga, T.M., Lajolo, F.M., Ribeiro, A.P., Mota, R.V. (2010). Influência da altitude na qualidade das uvas "Chardonnay" e "Pinot Noir" em Minas Gerais [Influence of altitude on the quality of "Chardonnay" and "Pinot Noir" grapes in Minas Gerais]. *Revista Brasileira de Fruticultura*, 32(1):143-150.

Souza, J.S.I. (1996). Uvas para o Brasil [Grapes for Brazil]. *FEALQ*. Piracicaba, SP. 791p.

Tonietto, J., Falcade, I. (2018). Indicações Geográficas de Vinhos do Brasil. A estruturação e a valorização da produção nos territórios do vinho [Geographical Indications of Wines from Brazil. The structuring and enhancement of production in the wine territories]. *Territoires du Vin*, 9:1-6.

Tonietto, J. (1993). *O conceito de denominação de origem: uma opção para o desenvolvimento do setor vitivinícola brasileiro* [*The concept of designation of origin: an option for the development of the Brazilian wine sector*]. Bento Gonçalves: EMBRAPA. 20p.

Tonietto, J., Falcade, I. (2003). Vinhos regionais: regulamentação no Brasil [Regional wines: regulations in Brazil]. *X Congresso Brasileiro de Viticultura e Enologia*. Bento Gonçalves.

Tonietto, J: (2003). Vinhos brasileiros de 4° geração: o Brasil na era das indicações geográficas [4th generation Brazilian wines: Brazil in the era of geographical indications]. Bento Gonçalves: Embrapa – Uva e Vinho, *Comunicado Técnico*, 45.

Tonietto, J. (2008). Geographical indicators for grapes. *Acta Horticulturae*, 785:467-476.

Tonietto, J., Zanus, M.C., Falcade, I., Guerra, C.C.(2013). *O Regulamento de uso da denominação de origem Vale dos Vinhedos: vinhos finos tranquilos e espumantes* [*The*

Regulation for the use of the Vale dos Vinhedos appellation of origin: fine, still and sparkling wines]. Bento Gonçalves: Embrapa Uva e Vinho, Documentos 84, 35p.

Tonietto, J., Pereira, G.E. (2012). A concept for the viticulture of “tropical wines.” In: *Proceedings of the IXth International Terroir Congress*, 34-37.

Tonietto, J., Pereira, G.E. (2011). The development of the viticulture for a high quality tropical wine production in the world. In: *International Symposium of the Group of International Experts of Vitivinicultural Systems for Cooperation*, 17, Asti. Proceedings. Le Progrès Agricole et Viticole, 25-28.

Tonietto, J., Mandelli, F., Zanus, M.C., Guerra, C.C., Pereira, G.E. (2012). O clima vitícola das regiões produtoras de uvas para vinhos finos do BRASIL [The viticultural climate of the grapes producing regions for fine wines in BRAZIL]. In: Tonietto, Jorge; Sotés Ruiz, Vicente; Gómez-Miguel, Vicente D. (Org.). *Clima, zonificación y tipicidad del vino em regiones vitivinícolas iberoamericanas*. 1ª ed., Madri: Cyted, 2012, 111-145.

Tonietto, J., Ruiz, V. S., Zanus, M. C., Montes, C., Uliarte, E. M., Bruno, L. A., Climaco, P., Pena, A., Guerra, C. C., Catania, C. D., Kohlberg, E. J., Pereira, G. E., Ricardo-Da-Silva, J. M., Ragout, J. V., Navarro, L. V., Laureano, O., Castro, R., Monte, R. F., Monte, S. A., Gomez-Miguel, V., Carbonneau, A (2014). The effect of viticultural climate on red and white wine typicity. Characterization in Ibero-American grapegrowing regions. *Journal International des Sciences de la Vigne et du Vin*, 1:19-23.

Wrege, M.S., Steinmetz, S., Reisser Junior, C., Almeida, I.R. (2012). *Atlas climático da região sul do Brasil. Estados do Paraná* [*Climatic atlas of southern Brazil*], Santa Catarina e Rio Grande do Sul. Embrapa: Brasília, 334p.

In: Vitis: Biology and Species
Editors: António M. Jordão et al.
ISBN: 978-1-53618-308-5

Chapter 7

GRAPEVINES DISEASES: DESCRIPTIONS AND CONTROL

Carine Rusin[1,*], Marcus André Kurtz Almança[2], Cacilda Márcia Duarte Rios Farias[1], Aline José Maia[1] and Renato Vasconcelos Botelho[1]
[1]Department of Agronomy, State University of Mid-Western of Paraná – UNICENTRO, Guarapuava, Paraná State, Brazil
[2] Federal Institute of Education, Science and Technology of Rio Grande do Sul State/Campus Bento Gonçalves, Bento Gonçalves, Rio Grande do Sul State, Brazil

ABSTRACT

The cultivation of grapevine spreads throughout practically the entire world areas. Several species and cultivars are produced for fresh consumption and for industrialization. Due to the diverse climatic conditions to which they are subjected, countless diseases can occur with different severity in the vineyards, becoming one of the main obstacles to the world grape production. These diseases can limit the development of plants and fruits, causing qualitative and quantitative losses in the production of grapes, wines, juices and derivatives, consequently increasing costs and making the production impossible. Vine diseases can be caused by fungi, bacteria, viruses and nematodes, affecting roots, trunk, leaves and bunches. Among these diseases, the fungal ones represent the majority and occur in all producing regions, being the main fungal diseases, mildew, powdery mildew, anthracnose, scoriosis, gray rot, ripe grape rot and vine decline. Vine decline may also be associated with the presence of nematodes and bacterial diseases such as bacterial canker. Bacteria are also responsible for Pierce's disease and 'fireblight' reported in vineyards in many countries. With symptoms easily confused with those of other diseases, viruses can affect the vines latently, semi-latently and in general with the onset of symptoms that may

---

[*] Corresponding Author's Email: carine.rusin@gmail.com.

vary in intensity according to vineyard conditions. In this chapter, we will address the major vine pathogens that occur worldwide, the damage caused by them, as well as their control measures.

Keywords: bacteria, fungi, fungicides, pathogens, phytossanity, viruses

1. INTRODUCTION

All crops can show decreases in production, caused by natural phenomena, which results in a favorable environment for the development of diseases (Mello, 2011). The grapevine is cultivated in the practically the entire world area, being then exposed to different climatic conditions, conducive to the appearance of numerous diseases with different severities in the vineyards, becoming one of the main obstacles to world grape production. Diseases can compromise the root and/or vascular system, leaves and fruits causing qualitative and quantitative losses in the production of grapes, wines, juices and derivatives.

2. DISEASES OF ROOTS AND TRUNK

Grapevine diseases of roots and trunk are important due to the loses caused by plant death and decrease of production, as well as expenses with renovation of vineyards. Fungi that cause the decline and death of vines represent a problem for world viticulture, being in some cases compared to the problem caused by Phylloxera, an insect native to North America that devastated French vineyards around 1863 (Smart and Fontaine, 2014). The main diseases and genera of fungi associated with diseases of wood and roots are: Eutypa dieback or Eutypiose (*Eutypa lata*), Petri disease or Young vine decline (*Phaeoacremonium* spp., *Phaeomoniella chlamydospora* e *Cadophora luteo-olivaceae*), Esca (*Phaeoacremonium* spp., *Phaeomoniella chlamydospora*, *Fomitiporia mediterranea* and *F. australiensis*), Black dead arm or Botryosphaeria canker (Botryospheriaceous fungi), Phomopsis decline (*Phomopsis viticola*), black foot (*Campylocarpon* spp., *Dactylonectria* spp., *Ilyonectria* spp.) and fusariosis (*Fusarium oxysporum* f.sp. *herbemontis*) (Mugnai et al., 1999; Gatica et al., 2001; Halleen et al., 2004; Halleen et al., 2006; Mondello et al., 2018).

The symptoms of decline associated with these diseases have occurred in several wine regions of the world. However, in some countries, these symptoms have also been reported and associated with the presence of soil insects such as the pearl-of-the-earth (*Eurhizococcus brasiliensis*) and Phylloxera (*Daktulosphaira vitifoliae*) (Botton and

Walker, 2009; Dambrós et al., 2016) and related to the interaction of these insects with soil fungi such as *Fusarium* spp. (Edwards et al., 2007; Idris and Arabi, 2014).

Regarding the occurrence of GTD (Grapevine Trunk Diseases) in vineyards, there is a tendency for symptoms to appear more frequently (around 20% of plants) from 10 years of age on in the vineyard and a more evident incidence until 15-20 years. On the other hand, there is a tendency for losses not to be noticed until the age of 5.

In Europe and in general, it is estimated that there are significant areas that are affected by GTD. In Italy the incidence ranged from 8 to 19%, in Spain around 10% and in France close to 13%. In France, losses were estimated at around 1 billion of Euros and 10 to 15% of potential production. In California (USA) a loss of at least US$260 million per year were reported (De La Fuente et al., 2016). These increasing in the incidence and losses by GTD are caused by some strategies used (or not) in the nurseries and vineyards, as lack of effective fungicides to control, changes with intensive cultural practices in vineyards and, lack of pruning wound protection alternatives and, poor low-quality propagation material (Gramaje et al., 2018).

2.1. Fusariosis

The main species of occurrence in wine regions is *Fusarium oxysporum* (Grigoletti Junior, 1993; Omer et al., 1999; Castillo-Pando et al., 2001). In addition to this species, Idris and Arabi (2014) observed that *Fusarium solani* was able to infect vine roots. These two species are also associated with the occurrence of Phylloxera in the roots, as observed by several autors (Granett et al., 1998; Idris and Arabi, 2014).

External symptoms in plants are yellowing and withering of the leaves. Smaller sprouts and marginal necrosis are also observed on the leaves. The bunches may also wither, but remain attached to the canes. In less severe infections, the symptoms are observed in a cane, part of the plant or in the whole plant. Internal symptoms are characterized by the darkening of the xylem, which may extend from the roots to the trunk. The death of the plant can occur after one or more vegetative cycles. Any injuries on the roots or base of the trunk can serve as a gateway for this fungus (Sonego et al., 2005).

One of the main alternatives for control is to avoid injury to the root system and to avoid cultivation in flooded soils (Sonego et al., 2005). Regarding rootstocks, Andrade et al. (1993) reported that ‘SO4’ was susceptible and ‘Paulsen 1103’ showed resistance to *F. oxysporum* f.sp. *herbemontis*. Between American cultivars planted grown in its own roots, cv. 'Isabella' (*Vitis labrusca*) showed resistance. Omer et al. (1999) observed a resistance reaction in the rootstocks ‘3309 C’, ‘420 A’, ‘5 C’ and ‘Freedom’, and a susceptibility reaction in the ‘Carignane’, ‘AXR # 1’ and ‘110 R’. When the disease is already installed in the vineyard, it is recommended to eradicate the infected plants, removing as much of the roots as possible and burning them, avoiding damage to the roots and disinfecting tools after use (Garrido et al., 2004).

2.2. Black Foot Disease of Grapevine

The disease known as “Black foot” is one of the most important of the Grapevine trunk diseases. Historically, it was linked to species of the genus *Cylindrocarpon,* the two most important species being *Cylindrocarpon destructans* and *C. liriodendri*. From the 2000s, phylogeny studies reclassified fungi of the genus *Cylindrocarpon* and these currently belong to the genera *Campylocarpon*, *Cylindrocladiella*, *Dactylonectria*, *Ilyonectria*, *Neonectria* and *Thelonectria* (Halleen et al., 2004; Carlucci et al., 2017; Aigoun-Mouhous et al., 2019). This disease is considered one of the most important of viticulture, affecting mainly young nurseries and vineyards worldwide (Alaniz et al., 2007). Fungal species associated with blackfoot infect vine plants by crossing the root tissue and colonizing the root ends, causing depressed necrotic lesions and a reduction in organ biomass (Halleen et al., 2003). The removal of the bark reveals an intense black discoloration and necrosis of the woody tissue developed from the base of the rootstock. Cutting roots and trunks transversely, partial or complete internal darkening of the xylem is seen (Figure 1A).

It is also possible to observe the presence of dark gum in the lesions, a result of the production of tilosis by the plant in defense against to the fungus attack. Reddish-brown color can still be seen in the internal tissues (Figure 1B and 1C). There is the presence of roots in the shape of "J” (Figure 1D) associated with the presence of these fungi, a consequence of planting seedlings with deficient roots. Other symptoms includes loss of vigor, shortening of internodes, formation of sparse and minute foliage with leaves containing chlorotic internal lesions and necrosis, often leading to plant death (Scheck et al., 1998; Gubler and Petit, 2013). Because it is a soil-dwelling fungus, managing the species is difficult. In this sense, it is important that management strategies are used from seedling production to planting in the field. Control measures should always be used preventively, since curative methods have not been successful. The focus should be on reducing stress conditions for the plant, as noted by Dambrós et al. (2016). According to these authors with the use of ridges, there is a significant reduction of plants with symptoms of decline.

The use of chemical fungicides is still an alternative in development and presents variable results, with many vine-producing countries not having registered products. Several authors (Rego et al., 2006; Halleen et al., 2007; Alaniz et al., 2011) reported results of efficient control at field level by the use of tebuconazole, carbendazim + flusilazole, cyprodinil + fludioxonil, imazalil and captan.

Both in nurseries and when planting in the field, a possible strategy to be employed is the use of biocontrol agents such as *Trichoderma* spp. In South Africa, Fourie et al. (2001)

observed that rootstocks inoculated with *Trichoderma harzianum* had a lower incidence of *Cylindrocarpon* spp, with results similar to the fungicides. In addition to the use of chemical and biological methods in nurseries, a possibility of control measure is the use of heat treatment with hot water. This is a highly recommended practice for reducing the inoculum of these fungi in propagation materials, and the immersion at 50 ºC for 30 minutes has shown efficiency (Gramaje et al., 2010).

2.3. Esca Complex and Petri Disease

Esca is a disease complex, caused by different species of *Phaeoacremonium*, *Phaeomoniella chlamydospora*, *Cadophora luteo-olivaceae* and the species of basiodiomycetes *Fomitiporia*, *Phellinus*, *Stereum*, *Inocutis*, *Inonotus* and *Fomitiporella*. In general, the typical syntoms of Esca complex disease are inner necrosis in grapevine wood tissues and external symptoms known as “tiger-striped” leaves (Figure 1E) or black measles on the berries. Internally, it is also observed wood discoloration, vascular infections and white decays and, externally, a generaland progressive decline with stunted development, chlorosis (Figure 1F) and apoplexy (Figure 1G). Initially, this disease can cause loss of productivity and eventually death of the vine (Mugnai et al., 1999; Graniti et al., 2000; Cloete et al., 2016; Fischer and Ashnaei, 2019).

Currently, Grapevine leaf stripe disease (GLSD) is considered the most important and widespread disease within the esca complex. Other diseases that are discussed within esca include brown wood streaking, Petri disease, white rot and esca proper. In old vineyards is common see symptoms of GLSD and esca proper co-existing (Surico, 2009).

As explained by Mondello et al. (2018), this disease complex and its occurrence are related to the age of the vine plants, symptoms observed and fungi involved. In young plants, from 1 to 7 years old, normally is observed symptoms of black spotting and dark wood streaking (Figures 1H and 1F), characteristic of Petri disease. Around 8 years old, the tipycal simptoms are those related to GLSD, called “tiger- striped” on leaves. After 8 year common symptoms are soft and spongy rotted wood (Figure 1J) associated to White rot disease, brown wood discoloration and apoplexy. The observed rates of decline/mortality of plants are not always in accordance with the incidence and intensity of external symptoms (Andreini et al., 2014).

Associated with the occurrence of internal and external symptoms to the Esca complex in vine plants, another important characteristic of these diseases is that in many situations the fungi, such as *P. chlamydospora* and *Phaeoacremonium* spp., are isolated from asymptomatic plants. In this case, it is important to consider that these fungi have a latency phase within the plant, demonstrating that there is a variable period of time between the stage of infection and the expression of symptoms (Di Marco and Osti, 2009; Bruez et al., 2014; Elena et al., 2018). This variation in the time between infection and the onset of symptoms can possibly be explained by the variation in the environmental factors associated with each plant and vineyard (Di Marco and Osti, 2009). The stress caused by water deficiency can increase the severity of internal symptoms, as observed by Fischer and Kassemeyer (2012) in plants inoculated with *P. chlamydospora*.

The entry of these fungi basically occurs in the nursery or in vineyards in practices that cause injuries to the plants (Bertsch et al., 2013). Eskalen et al. (2007) found that the wounds were susceptible to *P. chlamydospora* and *Phaeoacremonium aleophilum* for at least 4 months.

The inoculum of these fungi, mainly due to the formation of pycnids, can be present in the tissue of the infected plants and remain in the rest of pruning. This inoculum associated with times of rain and relative high humidity are released into the air and can infect new wounds. Furthermore, the inoculum of these fungi can be transported to new areas through contaminated vegetative material (Bertsch et al., 2013; Gramaje et al., 2018).

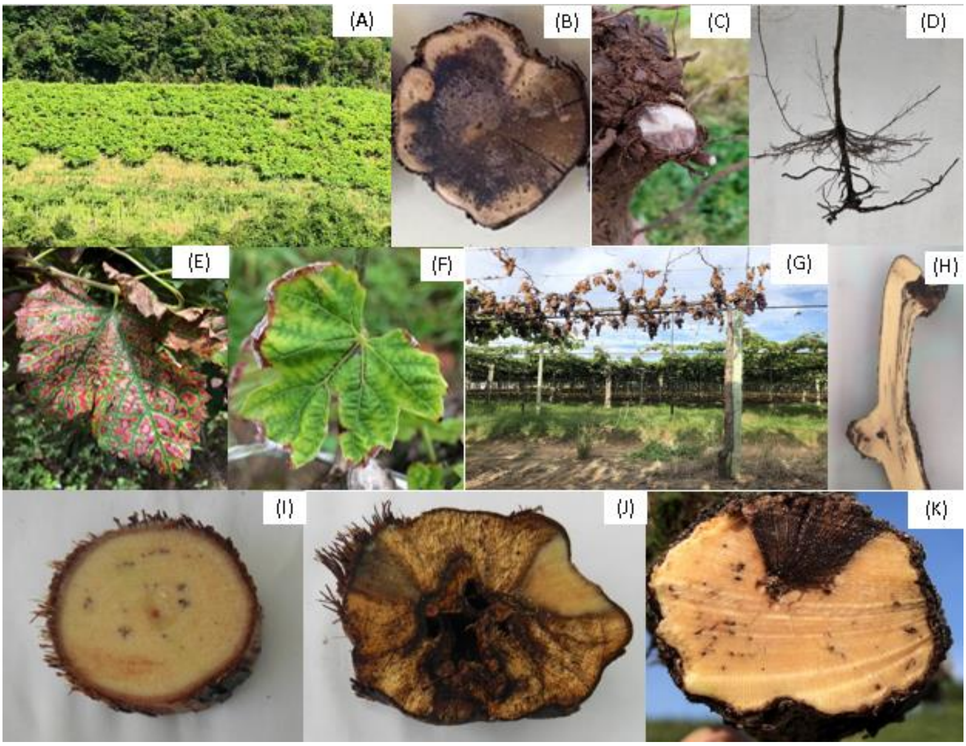


Figure 1. Vineyard with areas without plants due to the decline of vines (A), internal darkening caused by wood fungi (B) and (C), “J” shaped roots due to black-foot disease (D), esca symptoms “tiger-striped” leaves (E), chlorosis and necrosis in the leaf margin caused by esca (F), apoplexy (G), dark wood streaking (H), black spotting (I), soft and spongy rotted wood (J) and browning of the wood commonly in a “V” shape (K). Photos: Marcus A. K. Almança.

Considered by several authors within the Esca complex, possibly being the beginning, it is Petri's disease. This disease is known as the decline of young vine plants and its occurrence is associated with nurseries and young plants up to approximately 7 years of age (Crous and Gams, 2000; Bertsch et al., 2013; Gramaje et al., 2018; Mondello et al., 2018). The fungal species associated with Petri disease are: *P. chlamydospora*, at least 29 species of *Phaeoacremonium*, *Pleurostoma richardsiae* and 6 species of *Cadophora* (Gramaje et al., 2018). The typical internal symptoms of Petri disease are black spotting and dark wood streaking in the xylem vessels, with exudation of dark gum when these vessels are cut across. The external symptoms observed are chlorosis and necrosis in the leaves and decreased sprouting.

The control of fungi associated with the Esca complex and Petri disease is still a major challenge in viticulture, however, in a broad way, some measures can be taken, such as the reduction of stresses, protection of wounds caused to plants, the elimination of contaminated vegetative materials and disinfestation of tools, equipment and supplies used in the production of seedlings and also vineyard management.

The protection of wounds is done with the use of chemical fungicides and biological control agents. For chemical control, some fungicides have demonstrated the possibility of use in protecting and/or reducing symptoms such as carbendazim, methyl thiophanate, pyraclostrobin, diphenoconazole, tebuconazole and fosetyl-Al. However, in many countries these products are not registered for use in control of these diseases (Mondello et al., 2018). In biological and alternative control, products based on *Trichoderma* and *Bacillus* species, chitosan, garlic extract and seaweed extract have been used in the protection of injuries (Mutawilla et al., 2011; Gramaje et al., 2018; Mondello et al., 2018).

2.4. Eutypiosis

Eutypiosis, caused by Diatrypaceous fungus *Eutypa lata* (Pers.) Tul. & C. Tul (= *E. armeniacae* Hansf. & M.V. Carter), is present in many wine-growing areas worldwide. The fungus has a wide range of hosts including approximately 80 species, distributed in at least 27 families (Garrido and Sonego, 2004). The consequences of eutypiosis include loss of productivity, reduced vineyard longevity, increased costs for handling and renewing the vineyard. In 1991, the winegrowers in California (USA) lost an estimated value of more than 260 million dollars due to eutypiosis (Siebert, 2001).

Eutypiosis is known as one of the most destructive diseases of vine woody tissues, associated with the decline of the vine. When it infects the plant, external symptoms occur that are very similar to the other wood fungi, being easily confused with the fungi that cause descending rot. It presents a slow development, manifesting in the green organs when half of the trunk section is destroyed, about 2 or 3 years after the infection. Therefore, when the symptoms appear, it is already in an advanced stage. The severity of symptoms increases from year to year. The death of the attacked arm occurs 3 to 5 years after the first symptoms appear.

The disease spreads through surviving ascospores in periteciums that grew on dead wood. *E. lata* periteciums reach maturity in early spring, and ascospores are spread with raindrops (> 1 mm) and wind, being able to be transported for distances that can reach 100 km. Ascospores take 11 to 12 hours to germinate at an optimum temperature of 20 to 25ºC, and can remain viable for up to 2 months. Germination occurs within the conducting vessels, usually 2 mm or less from the wound surface (Garrido and Sonego, 2004). The infected plants present deformation and discoloration in the new canes, during the beginning of the growth, and the shortening of the internodes. The young leaves are smaller than normal, the edges are curved and chlorotic, margins are torn and sometimes with necrotic areas larger with age, causing an early defoliation. There may also be an excess of sprouting of affected canes and low fruit set. In the infected cane, several necroses are observed and internally, the browning of the affected wood occurs.

If prevention and control measures are not adopted, the symptoms evolve until the death of the plant, spreading the pathogen to other plants in the vineyard. Control is based on using healthy material, not pruning immediately after precipitation, removing diseased material from the vineyard, disinfecting pruning tools and applying fungicides right after pruning. The choice of the cultivar to be implanted is also important, as some cultivars have greater tolerance. According to Cadort et al. (2019), the cultivars Cabernet Sauvignon and Ugni Blanc are more susceptible to the disease than cv Merlot.

The use of double-pruning can reduce the incidence of the disease in the sprouts of the vine cane (Weber et al., 2007). In this management, the producer can perform a pruning in late autumn, before the period of the beginning of the rains, even with the presence of leaves on the vine. Pruning must be carried out high, from 30 to 45 cm beyond the point where the final pruning will be carried out. As a result, any Eutypa infections that may occur in these wounds will be completely removed during the final pruning, which can be performed in a late period, providing faster healing.

In a study carried out by Sosnowski et al. (2008), carbendazim was the most effective in reducing the colonization of *E. lata* pruning wounds in the field. Other fungicides, including fluazinam, pyrimethanil and pyraclostrobin, and the use of physical barriers, such as acrylic paint (with or without fungicides) also protected wounds from *E. lata* infection. Protection of wounds with fungicides can be effective when applied up to six days after infection and can protect wounds up to 14 days after application (Ayres et al., 2017).

The use of biological control agents to protect pruning wounds has been tested. Ferreira et al. (1991) used *Bacillus subtilis*, combined or not with mineral oil and benomyl, obtaining significant reductions in infection by *E. lata,* in relation to untreated plants. Kotze et al. (2009), also obtained protection from injuries with the use of *Trichoderma* and *Bacillus subtilis*.

2.5. Descending Rot

Caused by *Botryospheriaceous* fungi, which penetrate the vines by pruning injuries or other injuries produced on the plants. Several species of fungi of the Botryosphaeriaceae family are found worldwide, being endophytic, parasitic and saprophytic from numerous annual and perennial plants, usually associated with stress conditions (Urbez-Torres, 2011; Slippers et al., 2013). The fungi of this family are ecologically diverse, but commonly associated with leaf spots, rotting of fruits and roots, decline or death of plants.

Among the species, *Botryosphaeria stevensii* (Diplodia mutila) was the first to be associated with descending death in Tokay, Hungary (Lehoczky, 1974). Subsequently, several Botryosphaeriaceae species were related to the descending rot of the vine, among them, those belonging to the genera *Botryosphaeria*, *Diplodia*, *Dothiorella*, *Fusicoccum*, *Guignardia*, *Neofusicoccum*, *Lasiodiplodia* and *Phaeobotryosphaeria* (Amponsah et al., 2012).

The spores of these pathogens remain in the air, and the spread is favored in periods of rain when temperatures are around 10ºC. The spread can also occur during sprinkler irrigation (Úrbez-Torres et al., 2010) and in some cases the spread happens without the presence of rain, suggesting that other environmental factors are involved in this process. The optimum germination temperature of these fungi is between 23 and 26ºC (Úrbez-Torres et al., 2010; Van Niekerk et al., 2010).

Fungi penetrate through pruning wounds, being easily isolated in areas such as grafting and canes of the plant. This disease is characterized by a set of symptoms. The attacked plant may show delayed sprouting, reduction of vegetative growth and production, shortening of internodes, overgrowth, wilting of canes, smaller than normal leaves, deformed, reddish, chlorotic and with small necrosis on the margins, irregular fruiting. The attack can even cause plant death. Symptoms can progress slowly or cause the plants to die quickly (stroke). Internally, when the plants are infected by *Botryosphaeriaceae* fungi, the browning of the wood is observed, commonly in the form of a “V” (Figure 1K) (Larignon and Dubos, 2001). Some management measures can help to reduce this disease in the vineyard, such as the use of healthy seedlings, disinfestation of tools and the elimination of pruned canes. Protecting of pruning wounds with fungicides and biological agents are also effective against this disease.

3. LEAF DISEASES

The Leaf diseases interfere directly and indirectly in grapes production, by decreasing production due to the smaller leaf area available for assimilation of photosynthesized and production losses due to fungi attack during flowering. During the 19th century, pathogens such as the causal agents of powdery mildew (*Uncinula necator*) and and mildew

(*Plasmopara viticola*) were transported to Europe from North America, probably with cuttings from American cultivars used to replant French vineyards destroyed by phylloxera and have become the main risk factors for viticulture (Fischer et al., 2004; Gessler et al., 2011). In addition to downy mildew and powdery mildew, foliar diseases such as anthracnose, scoriosis, leaf blight and, more recently, the presence of grape rust in several viticultural areas in the world, cause losses similar to those described above, and it is necessary to know the symptoms and control method for reducing losses and maintaining the quality of fruits and derivatives.

3.1. Powdery Mildew

Also known as peronospora, mufa or mold, the disease is caused by the oomycete *Plasmopara viticola* (Berk. & Curtis) Berlese & De Toni. The severity of the disease increases mainly in regions of tropical viticulture, as conditions of high relative humidity (above 95%) and temperature between 18 and 25 ºC are favorable for the development of the pathogen. The presence of free water on the surface of plant tissues, whether from rain, dew or gutting, for a minimum period of 2 hours is essential for infection to occur. The infection occurs by stomata and pedicels (Garrido and Sonego, 2003).

Mildew can affect all structures of the aerial part of the plant, such as leaves, inflorescences and fruits (Elsharkawy et al., 2018). In favorable climatic conditions and lack of control measures or of high severity, it can drastically reduce production. This can occur due to the reduction of biomass accumulation in the affected plants, which can be determined by the presence of necrotic spots on the leaves, decreasing the interception of light and/or reducing the photosynthetic efficiency of the rest of the green leaf area (Moriondo et al., 2005). Direct losses of production can also occur when the pathogen affects the bunch, especially at the time of flowering, and can reduce up to 100% the production of the affected vineyard (Gessler et al., 2011).

The symptoms observed on the top leaves are yellow spots, translucent against the sun, called 'oil spots' (Figure 2A). Underside of the leaves, in high relative humidity, the white sporulation of the fungus appears (Figure 2B), then necrosis begins in the affected area (Garrido and Sonego, 2003). With leaf necrosis, the leaves may fall, causing the early defoliation of the vineyard in very severe attacks.

The pathogen also attacks the inflorescences, which when infected, the rachis darken, with the possibility of sporulation of the fungus, followed by the drying and falling of the flower buds. In the most developed berries, the fungus penetrates through the pedicels and develops inside, making them dark, hard, with a depressed surface, causing them to fall (Garrido and Sonego, 2003; Barbosa et al., 2010). To avoid major damage, the control of mildew must be done preventively, carrying out practices that reduce the humidity conditions in the vegetation of the vines such as: use of adequate spacing, avoid installing the vineyard in lowland areas, carry out defoliation. Techniques such as the use of plastic cover can reduce the incidence of downy mildew in areas with excessive rainfall in critical periods (Chavarria and Santos, 2009). In general, *Vitis labrusca* cultivars exhibit a generally higher degree of resistance than *V. vinifera* (Atak et al., 2017).

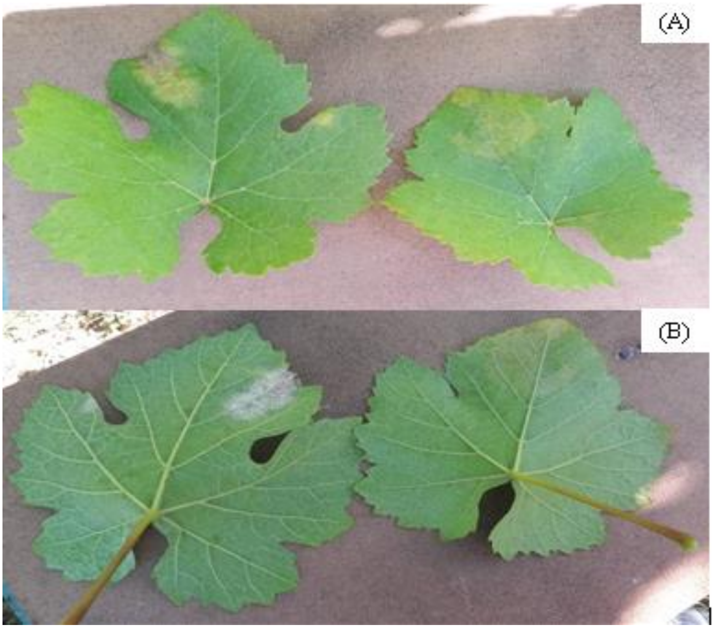


Figure 2. Symptom on the top of the leaf called "oil stain" (A), on the underside of the leaf, presence of characteristic white mold (B).
Photos: Marcus A. K. Almança.

Several multisite fungicides, such as mancozeb, folpet, dithianon, captan, cuprics, metalaxyl-M, cymoxanil, benalaxyl strobirulins and several inhibitors of cellulose biosynthesis and rRNA polymerization are indicated today for the control of downy mildew (Garrido and Sonego, 2003; Cavalcanti et al., 2019). Other products based on plant extracts and substances based on phosphorous acid and phosphonates (phosphites) were tested to control downy mildew, demonstrating efficiency and can be used by producers who choose not to use chemical fungicides (Speiser et al., 2000).

3.2. Oidium

Disease present in all wine-growing areas worldwide, caused by the pathogen *Erysiphe necator* Schwein (syn. *Uncinula necator*), a mandatory parasitic fungus, that is, it needs the live host for its growth and reproduction, and can infect all green organs of the plant.

Environmental conditions limit germination and development of the pathogen. The ideal conditions are mild temperatures (20ºC - 27ºC) and low relative humidity. Relative air humidity above 90% is unfavorable to the disease, as the conidia germinate abnormally in contact with water. Moreover, the disease develops well in low light conditions (Boso et al., 2018).

The symptoms are easily identifiable, the fungus forms a white or slightly gray powdery mold on shoots, leaves and berries (Figure 3A). Small attacked berries and flowers dry and fall. In early infections, the berries crack, exposing the seeds, and may be the gateway for other pathogens in the bunch, such as *Botrytis cinerea* (Boso et al., 2018).

The use of resistant cultivars prevents large losses due to the presence of powdery mildew. Some vine species, such as *V. riparia*, *V. rupestris*, *V. labrusca* and *V. rotundifolia*, are considered resistant to powdery mildew, but almost all varieties of *V. vinifera* are susceptible, with a difference in susceptibility according to the cultivar (Atak et al., 2017; Boso et al., 2018).

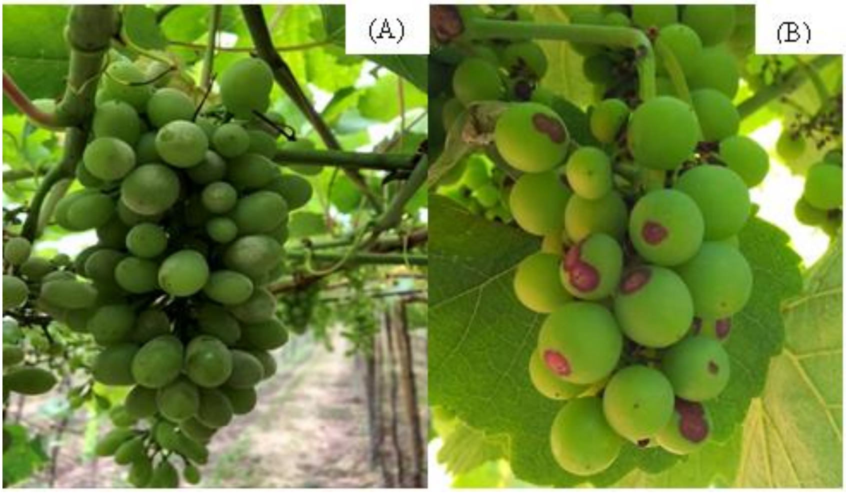


Figure 3. Presence of powdery mildew (*Uncinula necator*) (A) and symptoms of anthracnose (B) in berries.
Photos: Marcus A. K. Almança.

The monitoring of the vineyard must be constant, to start the treatments in the appearance of the first signs of the disease. Among the products, those based on sulfur are efficient, relatively inexpensive and with permission for organic production (Garrido and Sonego, 2003). In addition, biological control using mycoparasitic fungi, such as *Ampelomyces quisqualis* Ces., or the use of disease resistance inducers in plant tissues have constituted new methodologies for controlling powdery mildew (Colcol and Baudoin, 2016). Pascale et al. (2017) reported that applications of *Trichoderma harzianum* and *T. atroviride* suppressed the development of powdery mildew. In addition, Abdu Allah et al. (2017) also concluded that the extracts of azadiractin (neem extract), jojoba oil and *Reynoutria sachalinensis* had a positive effect on the control of powdery mildew.

3.3. Anthracnose

Anthracnose is a disease of European origin, caused by *Sphaceloma ampilina* de Bary, (syn. *Gloeosporium ampelophagum* (Pass.) Sacc, perfect stage *Elsinoe ampelina* (de Bary) Shear); it is also known as bird's eye. The favorable climatic conditions for the development of the fungus are cold winds and high relative humidity, with rain during the spring; free water on susceptible tissues for at least 12 hours and temperatures between 2 and 30°C, with an optimal temperature of 24-26°C (Thind et al., 2004).

European vine cultivars (*V. vinifera*) are susceptible to anthracnose, while some American species, such as *V. labrusca* and *V. aestivales* are more tolerant to this disease (Kono et al., 2009). The fungus attacks all the green organs of the plant, and the typical symptom is characterized by circular leaf spots, with black margins and round or irregular edges. When infection occurs during the flowering period, the inflorescence shows darkening and fall of the flower buds. In the berries, necrotic round spots of gray color in the center and black on the edges are observed (Figure 3B), which is why they are commonly called “bird's eye.”

The control of anthracnose must be initiated at the time of pruning, with the destruction of diseased canes and with winter chemical treatment with lime sulfur mixture, in order to eliminate or reduce the initial inoculum. Spraying with fungicides should be carried out from the beginning of the sprouting, since at that time the high humidity and the tender tissues favor the infection, and must be continuous until the compacting of the bunches. The recommended fungicides are dithianon, imibenconazole, diphenoconazole and methyl thiophanate (Naves et al., 2006). For organic systems, in addition to the lime sulfer, treatments based on plant extract can help control the disease, Botelho et al. (2009) tested the effect garlic extract on the *in vitro* mycelial growth of *Elsinoë ampelina* and obtaining a reduction of the pathogen.

3.4. Scoriosis

Grapevine scoriosis has for many years been known as 'dead-arm': However, in 1976, researchers demonstrated that 'dead-arm' and scoriosis were two distinct diseases that often occurred simultaneously. The fungus *Eutypa lata* was then identified as the cause of the 'dead-arm', and the fungus *Phomopsis viticola Sacc* (sin Fusicoccum viticola Reddick), causal agent of scoriosis (Erincik et al., 2001). The disease is widespread in several regions of the world, causing losses ranging from 10 to 40% of production (Garrido et al., 2006).

Scoriosis can affect most of the vine, including canes, leaves, rachis, flowers, tendrils and bunches (Erincik et al., 2001). The main way of spreading the pathogen is through rain, and temperature between 23 and 25°C with at least 4 h of humidity are necessary for the infection to occur. The lesions on the branches and leaves appear three to four weeks after infection, while on the berries and on the peduncle they appear one to three weeks before harvest. The main symptoms are on young leaves, including small punctuated chlorotic spots (Figure 4A), evolving to necrotic spots; on the shoots, superficial dark brown abrasion appear, which may involve the entire basal portion of the canes, or in the form of longitudinally elongated, dark and superficial lesions. The choice of the planting location is essential to avoid the disease, and places with direct sunlight, good soil drainage and good air circulation should be chosen. Performing cultural practices that reduce humidity, increase air circulation and light penetration such as green pruning are important for reducing infection. During the dormancy period, remove infected canes with pruning to reduce the inoculum (Garrido et al., 2006).

Figure 4. Symptom of scoriosis (A) and rust pustules (B) in grapevine leaves.
Photos: Marcus A. K. Almança and Renato V. Botelho.

3.5. Rust

Grapevine rust, a disease caused by the fungus *Phakopsora euvitis* Ono, can be quite destructive, mainly due to its great potential for dissemination, when it finds favorable environmental conditions for its development and is not quickly controlled. This disease occurs mainly in tropical and subtropical regions, and can also appear in temperate regions.

The pathogen is a mandatory parasite, in the leaves of the grapevine it presents yellowish-colored uredinic pustules on the underside and necrotic lesions on the upper part (Amorim et al., 2016). It is an end-of-cycle disease, preferentially infecting mature leaves, which, in severe attacks of the fungus, cause lesions to senescence and early defoliation (Figure 4B), leading to decreased productivity, reduced fruit quality and plant vigor in the next cycle (Ono, 2000). The rust pustules are formed five to six days after inoculation and the temperature for germination of the uredospores varies from 8 to 32 ºC (Bayer and Costa, 2006). For infection to occur, a minimum period of humidity (free surface water) of 6 hours is required at a temperature range of 16 to 30 ºC, with a greater infection being reported when the leaf moisture period is longer than 12 hours (Angelotti et al., 2013).

For the control of this disease, several protective measures must be adopted, such as the use of resistant varieties and spraying with fungicides, in order to avoid primary pathogenic infection (Primiano et al., 2017). Preliminary records have shown that American cultivars such as Niagara and Isabella and several rootstocks are more susceptible than European cultivars (*V. vinifera*) (Bayer and Costa, 2006). For the chemical control of grapevine rust, specific spraying is not normally necessary, since the disease generally occurs concomitantly with other leaf diseases important to the crop, such as downy mildew, powdery mildew and ripe grape rot, and the treatments used for these diseases also control rust (Buffara et al., 2013). Demethylation-inhibiting fungicides (IDM, "triazoles"), external quinone inhibitors (IQe, "strobilurins"), dithiocarbamate and copper are the most effective in controlling rust (Buffara et al., 2013; Angelotti et al., 2014).

3.6. *Mycosphaerella* Leaf Spot

The leaf spot, also known as isariopsis, is a disease that occurs at the end of the vegetative cycle, therefore it does not attract the attention of producers, but when treatments are not carried out after harvest, this disease can cause premature leaf fall, causing weakening of the plant and deficiency in the maturation of the branches and, consequently, bad sprouting in the next cycle (Naves et al., 2005). This disease is caused by *Mycosphaerella personata* (anamorph Pseudocercospora vitis = synonym of *Isariopsis clavispora*) and has a great importance in American cultivars, especially in warmer regions, where the disease evolves rapidly (Maia et al., 2003; Naves et al., 2005; Garrido and Gava, 2014). The symptoms manifest mainly in the leaves. In the leaf blade, well-defined patches appear, up to 2 cm in diameter, with an irregular outline and initially brown-reddish color, which later become dark. They have a yellowish or light green halo clearly visible and, on the opposite side of the leaf, in the corresponding tissue, a brown color is observed, which are the reproductive structures of the fungus. These structures develop on both the top and the bottom of the leaf. As the disease progresses, the spots may coalesce. The disease develops in conditions of high temperature and humidity (Maia et al., 2003; Lenz et al., 2009; Garrido and Gava, 2014). Therefore, treatment is essential and must be carried out when the first symptoms appear. Chemicals based on mancozeb, methyl thiophanate, diphenoconzole, tebuconazole and dithianon can be used (Maia et al., 2003; Naves et al., 2005).

4. ROTTEN IN BUNCHES

The incidence of bunch rot has increased significantly in the world vineyards, causing loss of yield and grape quality, both from table production and in the production of processing grapes. In addition to the depreciation of the fruit, these diseases can affect the characteristics of products from grapes. Compounds found in bunch rot affected grapes and wine are typically described as having mushroom, earthy odors and include geosmin, 2-methylisoborneol, 1-octen-3-ol, 2-octen-1-ol, fenchol, and fenchone (Steel et al., 2013). In sensory analysis, Lopez Pinar et al. (2017) observed that the bunch rot predominantly induced an increase in the intensities of peach-like/fruity, floral and liquor-like/toasty aroma notes, influenced the aroma profiles of the varieties Riesling, Red Riesling and Gewürztraminer.

The infection of the bunches can occur from the spring, in the flowering, until the moment of ripening of the fruits. The main rot of the bunches are the black rot (*Guignardia bidwellii),* botrytis bunch rot (*Botrytis cinerea*), ripe rot (*Colletotrichum* spp.) and bitter rot (*Greeneria uvicola*).

The gray rot in bunches of vines, also known as Botrytis bunch rot or gray mold, is caused by the fungus *Botryotinia fuckeliana* (from Bary) Whetzel, sexual form of *Botrytis cinerea* Pers.Fr.. Present in practically in vineyards of the whole world (Thomidis et al., 2016), this fungus has a saprophytic behavior and survives in the soil, in cultural remains, also in mummified fruits of the previous crop. In the spring period the spores are produced infecting leaves and bunches, mainly in the bunches bases and pedicels (Holz et al., 2003; Carisse, 2016). The symptoms can occur before and during flowering, affecting the flower organs that are adhered to the inflorescence, causing them to fall. In the berries, in the maturation period, the symptoms begin with spots, of lilac coloration and circular in the pellicle. Under favorable conditions, the fungus develops inside the berry developing the appearance of gray mold, which evolves into the darkening and rotting of the berry (Figure 5). The infection progresses from the diseased berries to the neighboring berries and can take the whole bunch (Bettiga and Gubler, 2013).

Rotten grape rot, caused by the fungus *Glomerella cingulata* (Stonemam) Spauld & Schrenk, perfect or sexual phase of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. The imperfect or asexual phase, focuses on ripe grapes or in the process of ripening. The fungus can survive saprophytically from one year to the next on crop remains, dead pedicels and mummified fruits remaining on the plants, serving as a source of inoculum for the next cycle.

The main symptom of this disease is the rotting of ripe fruits. Initially, red-brown spots appear on the berries, which subsequently reach the whole fruit, darkening it. Under conditions of high humidity, the structures of the fungus appear in the form of dark gray dots, from which a pink mass exudes, which are the conidia. This rosy mass also serves to differentiate it from bitter rot (Sonego and Garrido, 2003).

Figure 5. Botritis rot in grapevines cv. Sangiovese.
Photo: Renato V. Botelho

Bitter rot, caused by *Greeneria uvicola*, when it affects the stem, prevents the free flow of sap to the berries. These become wrinkled and mummified and fall easily. The direct attack of the fungus on the berries causes them to acquire, at first, a reddish-brown color, while altering their conformation. It is possible to observe later, black punctuations constituted by structures typical of the fungus (acervulus). The remaining berries that remain in the bunch, wilt, become black, hard and dry mummies (Sonego and Garrido, 2003).

The predisposing conditions for the occurrence of bunch rot in general are mainly high temperatures and high humidity. In a trial carried out by Schenato et al. (2008), it was observed that the combination that most favored the infection was the 24°C and 24 h wetting. In the case of *Glomerella cingulata*, excess of nitrogen also favors infection and fungus development (Sonego and Garrido, 2003). Regardless of the type of rot, cultural management is important to improve treatment conditions and decrease the incidence of disease, such as: removal and destruction of mummified fruits; elimination of pruned canes; balanced fertilization and canopy aeration. Canopy management with defoliation can help reduce disease. Leaf removal carried out before veraison during the phenological stages of full bloom, buckshot berries, or pea-sized berries should reduce Botrytis bunch rot (Würz et al., 2020).

Chemical treatment should be started at the end of flowering, and repeat spraying to protect the berries at all stages of development (Sonego and Garrido, 2003). Among fungicides, systemic ones are usually effective due to the ability to penetrate the tissue and eradicate latent infections. However they should not be used intensively in order to avoid the appearance of fungal isolates resistant to the product. Rotation with contact fungicides is recommended practice. Several chemicals are registered for control with good efficiency, as an example the application of pyraclostrobin in flowering and veraison reduces the severity of ripe rot and bitter rot in the harvest (Samuelian et al., 2014). The use of biological control is a viable option in the management of rot. Several biological control agents are available, such as *Aureobasidium pullulans* that showed potential to suppress bitter rot of grapes (Rathnayake et al., 2018) and *Candida sake* in the control of Botrytis rot (Garrido et al., 2017).

5. DISEASES CAUSED BY BACTERIA

The susceptibility of grapevines to bacterial diseases can compromise the quantity and quality of fruits in the producing regions. Thus, it is necessary to have knowledge about the symptoms that the plant presents when infected and the favorable conditions that allow the disease to progress.

5.1. Crown Gall

Crown gall is a serious and common disease in producing regions worldwide, especially in cultivars of *Vitis vinifera*. The disease weakens vines and causes significant economic losses in vineyards and nurseries (Johnson et al., 2016; Yepes, 2019). The disease is caused by the bacterium *Agrobacterium tumefasciens* (E.F. Smith & Touwnsend) Conn., Family Rhizobiaceae. It is a gram-negative, rod-shaped, aerobic bacterium and has a polar flagellum. *A. tumefasciens* has three biovars, and predominant in grapevine is the biovar 3, which came to be called *Agrobacterium vitis* (Lima, 2019).

The formation of gall is the typical symptom of the disease, which can arise in roots and in the collar of the plant. The galls can be of different size and shape, reaching several centimeters in diameter. As the bacteria is adapted to live in the vascular system and in the parenchyma of vine plants, it promotes tissue disorganization, leaving them with variable texture, spongy or hardened. The galls, when young, have a light and smooth surface and when older, they become rough and dark (Tolba and Zaki, 2011).

A. vitis has a large number of hosts, infecting about 600 species of dicotyledonous plants. As it persists systemically in vine plants without symptoms, it can be efficiently disseminated to distant geographic areas through international trade in propagating material (Kuzmanović et al., 2018). Dissemination over short distances can occur through soil water, rain splashes and scissors used in the growth and pruning of plants, as the pathogen can be found in all parts of the vine, including internodes, tips of sprouts and leaves (Johnson et al., 2016; Orel et al., 2017). The penetration of bacteria in the plant can occur through injuries caused during pruninn or other cultural pactices, natural openings and injuries caused by low temperature (Lima, 2009). For the control of the disease, it is necessary to make sure that the propagating material is certified and wound-free mainly on the roots and collar of the plant. As a cultural practice, crop rotation, elimination of plants with symptoms, may be partially effective in controlling the disease (Lima, 2009).

5.2. Bacterial Canker

The causative agent of bacterial cancer in grapevine is *Xanthomonas campestris* pv. *viticola*, a bacterium that produces rounded, convex, shiny and smooth-edged colonies. The bacterium is gram-negative, does not produce pigments and has a polar flagellum. The ideal conditions for the development of the disease are temperatures of 25 to 30°C and the high relative humidity of the air (Chand et al., 1994). The symptom of the disease can occur in the leaves, branches, inflorescence, stems and berries. The symptom on the leaves begins with small lesions (1 to 2 mm in diameter) with or without a yellowish halo that coalesce to form large necrotic areas. In the branches, petioles and stems, necrosis occurs in the epidermis, forming open cancers. The stem also has a dark color and necrosis in the epidermis. The berries have dark, rounded lesions with more prominent edges and the berries are uneven in size and color (Nascimento and Mariano, 2004). In plants within the same vineyard, the spread of the bacteria is favored by the occurrence of heavy rains associated with strong winds. Strong winds can cause injury to the leaf blade, making it easier for the bacteria to enter the plant. The dissemination between plants can also occur through splashes of rain or irrigation water, by pruning shears and agricultural implements (Almança et al., 2015).

5.3. Pierce's Disease

The causative agent of the disease is the bacterium *Xylella fastidiosa* Wells et al., an aerobic gram-negative bacilliform shape (Wells et al., 1987). The bacterium survives and multiplies in the xylem vessels. The spread of the bacteria is efficiently by vectors that comprise sharpshooters belonging to the subfamily Cicadellinea and Cercopoidea. The form of transmission is the non-circulating propagative mode during insect feeding, that is, there is no latency period between acquisition and inoculation (Esteves, 2019).

On the vine, the symptoms of Pierce's disease vary, depending on the cultivar and the climatic conditions. They appear as the sudden dryness of a large part of the leaf, turning brown to necrotic and the periphery of the tissues becoming yellowish with reddish streaks (Naves et. al., 2012). In infected plants, fruit production can occur, however, the bunches wither or become mummified. Severely affected plants can die in one to two years or stay alive for five years or more, depending on the species, cultivar, age of the plant and local climatic conditions (Eppo, 2016). For the control of this disease it is recommended to use resistant cultivars, adoption of management and sanitary measures, in addition to control of insect vectors. As the introduction of the pathogen can occur from neighboring areas or from other hosts, the removal of diseased parts partially contributes to solving the problem (Marques and Garrido, 2018).

6. VIROTIC DISEASES

The grapevine can be affected by numerous virures economically important, showing characteristic symptoms that may vary in intensity and depending on the cultivar, viral diseases can become latent, difficult to be diagnosticated and controlled (Kuhn and Farjado, 2004).

6.1. Grapevine Leafroll Disease (GLD)

It is an important disease that occurs in all grape-producing regions of the world, and normally is latent in rootstocks. However, the disease causes serious damage and can affect not only the quantity but also the quality of the fruits and decrease the longevity of the plants (Radaelli et al., 2009). The Grapevine Leafroll disease is caused by a complex of ten viruses, of the Closteroviridae family (Grapevine leafroll-associated virus, GLRav) and each virus can occur in isolation. Winding viruses are pathogens restricted to the genus *Vitis*.and the most prevalent disease-associated viruses among the 10 are: GLRa V-1, GLRa V-2 and GLRa V-3 (Amorim et al., 2016). The virus is transmitted efficiently by scale insects from the family *Pseudococcidae*, Coccidae, aphids, or by vegetative propagation by cuttings, and is present worldwide, wherever vines are grown (Sforza et al., 2003; Thompson et al., 2019).

The symptoms of the plants that contain the virus may vary according to the time of year, soil fertility, strain of the virus and with the cultivar. Symptoms are usually easily recognized in sensitive cultivars at the end of the crop cycle, before the leaves fall, although the symptoms also appear before flowering (Kuhm and Farjado, 2004). The characteristic symptom of the disease is the curling of the leaf edges downwards (Figure 6). In red wine cultivars (*Vitis vinifera*), the limbus takes on a red color, with the tissue along the main veins remaining green (Naidu et. al., 2014). Symptoms usually appear from the base of the branches, evolving to the remaining leaves at the end, after the berries ripen. The plant reduces its yield and fruit quality and has reduced vigor. In infected white grape vines, the limbus takes on a slight yellow color. Regardless of the cultivars, the infected leaf tissue is rough and thicker in consistency (Kuhm and Farjado, 2004).

The control of this virus can be through the use of seedlings and/or healthy propagating material (Lima and Fajardo, 2012) and the monitoring of scale insects and aphids should also be carried out in order to reduce the spread of the virus (Tsai et al., 2010).

6.2. Grapevine Rugose Wood Complex Disease

It is a disease that causes changes in the wood, caused by a complex of four viruses, being Corky bark (Grapevine virus B. GVB), Kober stem grooving (Grapevine virus A, GVA), Rupestris stem pitting (Rupestris stem pitting-associated virus, RSPaV) and LN33 stem grooving (unidentified virus). These viruses can occur in isolation and the last three causing changes in the stem pitting type of wood and the first causing corky bark as the main symptom (Martelli, 2014).

The preventive management of the grapevine rugose wood compleax disease includes mainly the use of healthy propagation materials, as many cultivars can present the virus in latency (Lima, 2009). Hu et al. (2018) carried out a research with the objective of evaluating the efficiency of 15 and 20 μg mL^{-1} of ribavirin to eliminate the virus associated with Ruspestris stem pitting (GRSPaV) in 'Kyoho' grapevines grown *in vitro*, and found that chemotherapy can be effective in viral eradication.

Figure 6. Grapevine Leafroll disease in cv. Pinotage.
Photos: Marcus A.K. Almança.

6.3. Grapevine Degeneration

The causal agent of grapevine degenerationt is the Grapevine fanleaf virus (GFLV), a virus of the genus Nepovirus and of the family *Secoviridae*, that reduces vines yield causing great losses to the wine grower (Martelli et al., 2014). The disease affects all organs of the vine. In the leaves the characteristic symptoms are: deformations with abnormal distribution of the veins; petiole angle too open or closed; leaf asymmetry and size reduction, in addition to stains of different shapes. It is also common to occur in the leaves of translucent stains of different shapes, usually observed in the spring. Another symptom is the yellow-gold color in the leaves, caused by a specific GFLV strain that induces yellow mosaic or only yellowing of the tissue is observed along the main vein, which can extend to the secondary ones. In the canes, internodes are short, with bifurcations, flattening and double nodes, proliferation of buds and weak and delayed sprouting. In the bunches, the number and size of the berries are smaller and remain green. Diseased plants are generally less developed (Kuhn and Fajardo, 2004). GFLV is transmitted specifically from plant to plant by *Xiphinema* spp., an ectoparasitic nematode that inhabits the soil and feeds on growing root tips (Hewitt et al., 1958; Wyss, 2000).

The strategies to control a GFLV in vineyards aim to eradicate or reduce *Xiphinema* spp. populations; such as, the removal of root debris and the realization of a prolonged soil fallow (ten years); rotation of cultures from two to four years, with plants not hosted on the nematode and; use of rootstocks resistant to the vector. Areas where only viruses occur, without a vector, can be cleaned more easily by removing infected plants and planting healthy vines (Castro and Moreira, 2012; Lima and Fajardo, 2012).

6.4. Grapevine Fleck Disease

This disease is caused by the Grapevine fleck virus (GFkV) and is present in several wine-growing areas around the world (Martelli et al., 2014). GFkV is latent in *Vitis vinifera*, but induces specific leaf symptoms in the *Vitis rupestris* indicator, in which phloem cells cause highly characteristic cytopathic characteristics, the affected plants are less developed and in the leaves, they have irregular, translucent and chlorotic spots, following the position of the veins (Kuhn and Fajardo, 2004). This virus is spread only through infected propagation material, therefore, thermotherapy in conjunction with micropropagation is a simple and fast method for virus elimination (GFkV) (Bota et al., 2014).

7. NEMATODES

7.1. Gall Nematodes

The genus *Meloidogyne*, a gall nematode, is considered one of the most important causes of damage to grapevines. The most important species of this genus for the vine are

M. incognita (Kofoide & White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood, *M. hapla* Chitwood and *M. ethiopica* Whitehead, the first four being widely distributed in most regions where the vine is grown (Lordello and Lordello, 2003).

The characteristic symptoms of the nematode attack of the vine galls occur in the roots where thickening called galls is observed. Little infected plants have small galls on the absorbent roots, while highly infected ones may have a reduction in the root system with large and elongated galls and, consequently, restriction of water absorption, nutrients and plant growth which has low vigor, symptoms of nutritional deficiency and reduced production.

Some practices can prevent infestation in the vineyard such as prevent flooding from infested areas; avoid reinfestation of the area by infested machinery, agricultural implements and irrigation water; plant brown hemp (*Crotalaria juncea*) or velvet bean (*Mucuna pruriens*) to reduce the nematode population (Dias-Arieira et al., 2010). Some rootstocks are resistant to this pathogen such as 'Salt Creek', 'Kober 5BB', 'SO4', 'Harmony' and 'Solferino' (Somavilla et al., 2012).

7.2. Dagger Nematodes

Four species of dagger nematodes are found to be associtated with vine roots: *Xiphinema americanum*, *X. index*, *X. brasiliensis* and *X. krugi*. Among these species, only the symptoms induced by *X. index* have been studied in detail. These ectaparasites, in addition to causing direct damage to the vine, have their importance related to the fact that some species of this genus are vectors of important viruses, as is the case of *X. index*, which can transmit the vine degeneration virus (Grapevine fanleaf virus, GFLV) (Demangeat et al., 2010). The attacked vines have roots with terminal thickening, curvature, necrosis and a decrease in the number of roots. Extensive necroses in the main roots result in the lateral proliferation of other roots, in an effect similar to witches' broom. The presence of *X. americanum* has also been related to the reduction of the root system of the grapevine (Campos et al., 2003).

Among the strategies for the control of this nematode, biological control can be effective. Arbuscular mycorrhizal fungi can reduce the damage caused by plant nematodes and, consequently, represent alternative or complementary biological protection (Pozo and Azcon-Aguilar, 2007; Schouteden et al., 2015). Another alternative is the use of solarization, which is the covering of the soil with transparent plastic film, during the period of greatest solar radiation. The effects of solarization, in reducing nematode populations in the soil, were verified for the genera *Meloidogyne*, *Xiphinema* and *Pratylenchus* (Campos et al., 2003).

CONCLUSION

Pathogens associated with grapevines are a problem that entails many costs as well as important losses for wine growers. Therefore, integrated measures for the management of grapevine diseases are essential for the success of the activity from the social and economic point of view. In addition, considering the concern with the environmental impact, measures that increase the sustainability of the production system must be recommended, leading to the search for new disease control technologies, such as the use of biological control, resistant cultivars and rootstocks, among others.

REFERENCES

Alaniz, S., Abad-Campos, P., García-Jiménez, J., Armengol, J. (2011). Evaluation of fungicides to control *Cylindrocarpon liriodendri* and *Cylindrocarpon macrodidymum* in vitro, and their effect during the rooting phase in the grapevine propagation process. *Crop Protection* 30:489-484.

Alaniz, S., León, M., Vicent, A., García-Jiménez, J., Abad-Campos, P., Armengol, J. (2007). Characterization of *Cylindrocarpon* species associated with black foot disease of grapevine in Spain. *Plant Disease*, 91(9):1187-1193.

Amorim, L., Spósito, M. B., Kuniyuki, H. (2016). Doenças da videira. In: Amorim, L., Rezende, J. A. M., Bergamin Filho, A., Camargo, L. E. A. (5ed) Manual de fitopatologia, vol.2. *Doenças de Plantas Cultivadas*. Agronomica Ceres, 810p.

Amponsah, N. T., Jones, E., Ridgway, H.., Jaspers, M. V. (2012). Susceptibility of grapevine tissues to *Neofusicoccum luteum* conidial infection. *Plant Pathology*, 61(4):719-729.

Andrade, E. R., Schuck, E., Bo, M. (1993). Avaliação da Resistencia de *Vitis* spp a *Fusarium oxysporum* f. sp. *herbemontis* em condições controladas. *Pesquisa Agropecuária Brasileira*, 28(11):1287-1290.

Andreini, L., Cardelli, R., Bartolini, S., Scalabrelli, G., Viti, R. (2014). Esca symptoms appearance in *Vitis vinifera* L.: influence of climate, pedo-climatic conditions and rootstock/cultivar combination. *Vitis*, 53:1, 33-38.

Angelotti, F., Regina, C., Buffara, S., Vieira, R. A., Vida, J. B. (2014) Protective, curative and eradicative activities of fungicides against grapevine rust. *Ciência Rural*, 44:1367-1370.

Angelotti, F., Scapin, C. R., Tessmann, D. J., Vida, J. B., Canteri, M. G. (2013). The effect of temperature, leaf wetness and light on development of grapevine rust. *Australasian Plant Pathology*, 43:9-13.

Atak, A., Akkurt, M., Polat, Z., Celik, H., Kahraman, K. A., Akgül, D. S., Eibach, R. (2017). Susceptibility to downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*) of different Vitis cultivars and genotypes. *Ciência e Técnica Vitivinícola*, 32:1, 23-32.

Ayres, M., Billones-Baaijens, R., Savocchia, S., Scott, E., Sosnowski, M. (2017). Critical timing for application of pruning wound protectants for control of grapevine trunk diseases. *Wine & Viticulture Journal*, 32:1-38.

Barbosa, M. A. G., Castro, J. M. C., Terao, D., de Lima, M. F., Batista, D. C., Tavares, S. C. C. H. (2010). *Cultivo da Videira* [*Vine cultivation*]. Embrapa Semiárido, Sistemas de Produção 1. 2° edição. Versão eletrônica, 2010. In: http://www.cpatsa.embrapa.br:8080/sistema_producao/spuva/doencas.htm.

Bayer, T. M., Costa, I. F. D. D. (2006). Ocorrência de *Phakopsora euvitis* Ono em Santa Maria [Occurrence of Phakopsora euvitis Ono in Santa Maria], Rio Grande do Sul. *Ciência Rural*, 36(4):1307-1308.

Bertsch C., Ramirez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Fontaine, F. (2013). Grapevine trunk diseases: complex and still poorly understood. *Plant Pathology*, 62:243-265.

Bettiga, L., Gubler, W. (2013) Bunch rots. In: *Grape Pest Management*. Bettiga, L. (Ed.). University of California Division of Agriculture and Natural Resources, Oakland, p. 93-103.

Boso, S., Gago, P., Santiago, J. L., Martínez, M. C. (2018). Variation in sensitivity of different grapevine genotypes to *Erysiphe necator* growing under unfavourable climatic conditions. *South African Journal of Enology and Viticulture*, 39(1):100-105.

Bota, J., Cretazzo, E., Montero, R., Rosselló, J., Cifre, J. (2014). Grapevine fleck virus (GFkV) elimination in a selected clone of *Vitis vinifera* L. cv. Manto negro and its effects on photosynthesis. *OENO One*, 48:11-19.

Botelho, R. V., Maia, A. J., Pires, E. J. P., Terra, M. M. (2009). Efeito do extrato de alho na quebra de dormência de gemas de videiras e no controle *in vitro* do agente causal da antracnose [Effect of garlic extract on breaking dormancy of grape buds and in vitro control of the causal agent of anthracnose] (*Elsinoe ampelina* Shear). *Revista Brasileira de Fruticultura*, 31(1):96-102.

Botton, M., Walker, M. A. (2009). Grape phylloxera in Brazil. *Acta Horticulturae*, 816:39-40.

Bruez, E., Vallance, J., Gerbore, J., Lecomte, P., Costa, J. P., Rey, P. (2014). Analyses of the temporal dynamics of fungal communities colonizing the healthy wood tissues of esca leaf-symptomatic and asymptomatic vines. *PloS One*, 9:15.

Buffara, C. R. S., Angelotti, F., Tessmann, D. J., de Souza, C. D., Vida, J. B. (2013). Atividade de fosfito de potássio na pré e pós-infecção de *Phakopsora euvitis* em folhas de videira [Potassium phosphite activity in the pre and post-infection of Phakopsora euvitis in grape leaves]. *Embrapa Semiárido*-Artigo em periódico indexado (ALICE).

Campos, V. P., Maximiniano, C., Ferreira, E. A. (2003). Uva para processamento: fitossanidade [Grape for processing: plant health]. Bento Gonçalves: *Embrapa Uva e Vinho*, Brasília: EMBRAPA, (Frutas do Brasil).

Carisse, O. (2016). Epidemiology and Aerobiology of Botrytis spp. In: *Botrytis–the Fungus, the Pathogen and its Management in Agricultural Systems*. Springer, Cham. p. 127-148.

Carlucci, A., Lops, F., Mostert, L., Halleen, F., Raimondo, M. L. (2017). Occurrence fungi causing black foot on young grapevines and nursery rootstock plants in Italy. *Phytopathologia Mediterranea*, 56(1):10-39.

Castillo-Pando, M., Somers, A., Green, C. D., Priest, M., Sriskanthades, M. (2001). Fungi associated with dieback of Semillon grapevines in the Hunter Valley of New South Wales. *Australasian Plant Pathology*, 30:1, 59-63.

Cavalcanti, F., Cassuba, K., Fioravanço, J. (2019). *Produtos com menor teor de cobre para o controle do míldio em viticultura* [*Products with lower copper content to control mildew in viticulture*]. Embrapa Uva e Vinho-Circular Técnica (INFOTECA-E).

Chand, R., Singh, P. N., Singh, D., Singh, R. (1994) Copper and streptomycin resistance in *Xanthomonas campestris* pv. *viticola*. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz* 101:487-491.

Chang, C. J., Garnier, M., Zreik, L., Rossetti, V., Bové, J. M. (1993). Culture and serological detection of the xylem-limited bacterium causing citrus variegated chlorosis and its identification as a strain of *Xylella fastidiosa*. *Current Microbiology* 27:137-142.

Chavarria, G., Santos, H. P. (2009). Manejo de videiras sob cultivo protegido [Grapevine management under protected cultivation]. *Ciência Rural*, 39:1917-1924.

Cloete, M., Fischer, M., Du Plessis, I. L., Mostert, L., Halleen, F. A. (2016). New species of *Phellinus sensu* stricto associated with esca on grapevine in South Africa. *Mycological Progress*, 15:3-25.

Colcol, J. F., Baudoin, A. B. (2016). Sensitivity of *Erysiphe necator* and *Plasmopara viticola* in Virginia to QoI fungicides, boscalid, quinoxyfen, thiophanate methyl, and mefenoxam. *Plant Disease*, 100:337-344.

Crous, P. W., Gams, W. (2000). *Phaeomoniella chlamydospora* gen. et comb. nov., a causal organism of Petri grapevine decline and esca. *Phytopathologia Mediterranea*, 39(1):112-118.

Daly, A. M., Hennessy, C. R., Schultz, G. C. (2005). New host record for the grapevine leaf rust fungus, *Phakopsora euvitis. Australasian Plant Pathology*, 34(3):415-416.

De La Fuente, M., Fontaine, F., Gramaje, D., Armengol, J., Smart R., Corio-Costet, M. F. (2016). *Grapevine Trunk Diseases. A Review*. International Organization of Vine and Wine (OIV), 24 p.

Demangeat, G., Komar, V., Van-Ghelder, C., Voisin, R., Lemaire, O., Esmenjaud, D., Fuchs, M. (2010). Transmission competency of single female *Xiphinema index* lines for Grapevine fanleafvirus. *Phytopathology* 100:384-389.

Di Marco, S., Osti, F. (2009) Effect of biostimulant sprays on *Phaeomoniella chlamydospora* and esca proper infected vines under greenhouse and field conditions. *Phytopathologia Mediterranea*, 48:47-58.

Dias-Arieira, C. R., Molina, R. D. O., Costa, A. T. (2010). Nematóides causadores de doenças em frutíferas [Disease-causing nematodes in fruit trees]. *Revista agro@mbiente on-line* 2:46-56.

Edwards, J., Pascoe, I. G., Salib, S. (2007). Impairment of grapevine xylem function by *Phaeomoniella chlamydospora* infection is due to more than physical blockage of vessels with 'goo'. *Phytopathologia Mediterrane*a, 46(1): 87-90.

Elena, G., Bruez, E., Rey, P., Luque, J. (2018). Microbiota of grapevine woody tissues with or without esca-foliar symptoms in northeast Spain. *Phytopathologia Mediterranea* 57:425-438.

El-Sharkawy, L. H. H. A., Abo-El-Wafa, T. S. A., Ibrahim, S. A. A. (2018). Biological control agents improve the productivity and induce the resistance against downy mildew of grapevine. *Journal of Plant Pathology* 100(1):33-42.

Eppo (European and Mediterranean Plant Protection Organization). 2016. Diagnostics *Xylella fastidiosa*. Bulletim OEPP/EPPO *Bulletin*, 2:1-38.

Erincik, O., Madden, L. V., Ferree, D. C., Ellis, M. A. (2001). Effect of growth stage on susceptibility of grape berry and rachis tissues to infection by *Phomopsis viticola*. *Plant Disease*, 85:517-520.

Eskalen, A., Feliciano, A. J., Gubler, W. D. (2007). Susceptibility of grapevine wounds and symptom development in response to infection by *Phaeoacremonium aleophilum* and *Phaeomoniella chlamydospora. Plant Disease* 91:1100-1104.

Esteves, M. B. (2019). *Eficiência de transmissão de estirpes de Xylella fastidiosa subsp. pauca por cigarrinhas e o papel da adesina XadA2 na formação do biofilme no inseto vetor* [*Transmission efficiency of Xylella fastidiosa subsp. leafhopper and the role of XadA2 adhesin in the formation of biofilm in insect vector*] (Doctoral dissertation, Universidade de São Paulo).

Ferreira, J. H. S., Matthee, F. N., Thomas, A. C. (1991). Biological control of *Eutypa lata* on grapevine by an antagonistic strain of *Bacillus subtilis*. *Phytopathology*, 81:283-287.

Figueiredo, G. M. D., Mota, R. V. D., Souza, C. R. D., Peregrino, I., Fernandes, F. D. P., Rhegina, M. A. (2020). Late defoliation of ‘Chardonnay’ grapevine in subtropical highland climate. *Bragantia*, 1-13.

Fischer, B. M., Salakhutdinov, I., Akkurt, M., Eibach, R., Edwards, K. J., Töpfer, R., Zyprian, E. M. (2004). Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. *Theoretical and Applied Genetics*, 108(3):501-515.

Fischer, M., Kassemeyer, H. H. (2012). Water regime and its possible impact on expression of esca symptoms in *Vitis vinifera:* growth characters and symptoms in the greenhouse after artificial infection with *Phaeomoniella chlamydospora*. *Vitis,* 51:129-135.

Fischer, M., Peighami-Ashnaei, S. (2019). Grapevine, esca complex, and environment: The disease triangle. *Phytopathologia Mediterranea, 58:*1, 17-37.

Fourie, P. H., Halleen, F., Van der Vyver, J., Schreuder, W. (2001). Effect of *Trichoderma* treatments on the occurrence of decline pathogens in the roots and rootstocks of nursery grapevines. *Phytopathologia Mediterranea,* S:473-478.

Garrido, C. C., Usall, J., Torres, R., Teixidó, N. (2017). Effective control of Botrytis bunch rot in commercial vineyards by large-scale application of *Candida* sake CPA-1. *BioControl*, 62:2, 161-173.

Garrido, L. D. R., Sônego, O. R. (2004). *Eutipiose: epidemiologia, sintomatologia e controle* [*Eutypiosis: epidemiology, symptomatology and control*]. Embrapa Uva e Vinho, Bento Gonçalves, Brasil, Circular 53.

Garrido, L. D. R., Sônego, O. R., Fochesato, M., Naves, R. D. L. (2006). *Escoriose da videira: sintomatologia, epidemiologia e controle* [*Vine scoriosis: symptomatology, epidemiology and control*]. Bento Gonçalves, Brasil, Embrapa Uva e Vinho. Circular Técnica 70.

Garrido, L. R., Sônego, O. R. (2003). *Uvas viníferas para processamento em regiões de clima temperado* [*Wine grapes for processing in temperate regions*]. Porto Alegre, Brasil. EMBRAPA, 10-16.

Garrido, L. R., Sônego, O. R., Gomes, V. N. (2004). Fungos associados com o declínio e morte de videiras no Estado do Rio Grande do Sul [Fungi associated with the decline and death of grapevines in the State of Rio Grande do Sul]. *Fitopatologia Brasileira* 29:322-324.

Garrido, L. R., Gava, R. (2014). *Manual de doenças fúngicas da videira* [*Handbook of fungal diseases of the vine*]. Bento Gonçalves, Brasil, Embrapa Uva e Vinho, 101p.

Gatica, M., Césari, C., Magnin, S., Dupont, J. (2001). *Phaeoacremonium* species and *Phaeomoniella chlamydospora* in vines showing "hoja de malvón" and young vine decline symptoms in Argentina. *Phytopathologia Mediterranea,* S:317-324.

Gessler, C., Pertot, I., Perazzolli, M. (2011). *Plasmopara viticola:* a review of knowledge on downy mildew of grapevine and effective disease management. *Phytopathologia Mediterranea*, 50(1):3-44.

Gramaje, D., Úrbez-Torres, J. R., Sosnowski, M. R. (2018). Managing grapevine trunk diseases with respect to etiology and epidemiology: current strategies and future prospects. *Plant Disease*, 102(1):12-39.

Granett, J., Omer, A. D., Pessereau, P., Walker, M. A. (1998). Fungal infections of grapevine roots in phylloxera-infested vineyards. *Vitis*, 37(1):39-42.

Graniti, A., Surico, G., Mugnai, L. (2000). Esca of grapevine: a disease complex or a complex of diseases?. *Phytopathologia Mediterranea*, 39(1):16-20.

Grigoletti Júnior, A.(1993).gr *Fusariose da videira: resistência de cultivares, sintomas e controle* [*Vine fusariosis: cultivar resistance, symptoms and control*]. Bento Gonçalves, Brasil, Embrapa Uva e Vinho, Circular 18.

Gubler, W. D., Petit, E. (2013). Black Foot Diseases. In: Bettiga, L. J. (Ed.). *Grape pest management*. 3rd ed. Oakland, University of California-Division of Agriculture and Natural Resources. p.90-92.

Halleen, F., Fourie, P. H., Crous, P. W. (2007). Control of black foot disease in grapevine nurseries. *Plant Pathology,* 56: 637-645.

Halleen, F., Crous, R. W., Petrin, O. (2003). Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. *Australasian Plant Pathology*, 32(1):47-52.

Halleen, F., Fourie, P. H., Crous, P. W. (2006). A review of black foot disease of grapevine. *Phytopathologia Mediterranea*, 45:55-67.

Halleen, F., Schroers, H. J., Groenewald, J. Z., Crous, P. W. (2004). Novels species of *Cylindrocarpon* (*Neonectria*) and *Campylocarpon* gen. nov. associated with black-foot disease of grapevines (*Vitis* spp). *Studies in Mycology,* 50: 431-455.

Hewitt, W. B., Raski, D. J., Goheen, A. C., (1958). Nematode vector of soil-borne fanleaf virus of grapevines. *Phytopathology* 48:586-595.

Holz, G., Gütschow, M., Coertze, S., Calitz, F. J. (2003). Occurrence of *Botrytis cinerea* and subsequente disease expression at different positions on leaves and bunches of grape. *Plant Disease*, 87(4):351-358.

Idris, I., Arabi, M. I. E. (2014). The relationship between grape phylloxera and Fusarium root infection. *Advances in Horticultural Science,* 14-19.

Johnson, K. L., Cronin, H., Reid, C. L., Burr, T. J. (2016). Distribution of *Agrobacterium vitis* in grapevines and its relevance to pathogen elimination. *Plant disease*, 100:791-796.

Kono, A., Nakaune, R., Yamada, M., Nakano, M., Mitani, N., Ueno, T. (2009). Effect of culture conditions on conidia formation by *Elsinoë ampelina*, the causal organism of grapevine anthracnose. *Plant Disease*, 93(5):481-484.

Kotze, C., Van Niekerk, J. M., Halleen, F., Fourie, P. H. (2009). Identifying potential biocontrol agents for grapevine pruning wound protection against trunk pathogen infection. 6th International Workshop on Grapevine Trunk Diseases. Anais. Florence, Italy: *Phytopathologia Mediterranea*.184.

Kuhn, G. B., Fajardo, T. V. M. (2004). *Importância da origem do material de propagação na qualidade da muda de videira* [*Importance of the origin of propagating material in the quality of the vine seedling*]. Bento Gonçalves, Brasil Embrapa Uva e Vinho. 8p. (Circular Técnica 50).

Kuzmanović, N., Puławska, J., Hao, L., Burr, T. J. (2018). Ecologia de *Agrobacterium vitis* e manejo da doença em vinhedos [Agrobacterium vitis ecology and disease management in vineyards]. In *Agrobacterium Biology,* 15-53.

Larignon, P., Dubos, B. (2001). The villainy of black dead arm. *Wines Vines*, 82: 86-89.

Lehoczky, J. (1974). Black dead-arm disease of grapevine caused by *Botryosphaeria stevensii* infection. *Acta Phytopathologica Academiae Scientiarum Hungaricae,* 9 (3/4):319-327.

Lenz, G., Costa, I. D. D., Balardin, R. S., Marques, L. N., Arrué, A., Stefanelo, M. S., Zemolin, C. R. (2009). Elaboração e validação de escala diagramática para quantificação da mancha de isariopsis da videira [Elaboration and validation of a diagrammatic scale to quantify the isariopsis stain of the vine]. *Ciência Rural*, 39(8):2301-2308.

Lima, M. F. (2009). *Detecção e controle de viroses em videiras* [*Detection and control of viruses in vines*]. Circular Técnica 90.

Lima, M. F., Fajardo, T. M. V. (2012). Doenças causadas por vírus [Diseases caused by viruses]. In: Lima, M. F., Moreira, F. R. B. (ed.) *Uva de Mesa: Fitossanidade*. 2 ed. Brasília: Embrapa, p.43-58 (*Frutas do Brasil*, 14).

Lopez Pinar, A., Rauhut, D., Ruehl, E., Buettner, A. (2017). Effects of bunch rot (*Botrytis cinerea*) and powdery mildew (*Erysiphe necator*) fungal diseases on wine aroma. *Frontiers in Chemistry,* 5:20.

Lordello, R. R. A.; Lordello, A. I. L. (2003). Doenças e nematoides [Diseases and nematodes] in: POMER, C. V. *Uva: tecnologia de produção, pós-colheita, mercado*. Porto Alegre: Cinco Continentes 568-596.

Maia, J. D. G., Naves, R. L., Garrido, L. R., Sônego, O. R., Kuhn, G. B. (2003). Doenças e seus controles [Doenças e seus controles]. In: Kuhn, G. B., Melo, G. W., Nachtigal, J. C., Maia, J. D. G., Protas, J. D. S., Mello, L. M. R., Naves, R. D. L. *Cultivo da videira Niágara Rosada em regiões tropicais do Brasil*. Bento Gonçalves, Brasil, Embrapa Uva e Vinho.

Marques, A. D. A., Garrido, L. D. R. (2018). *Xylella fastidiosa subsp. fastidiosa (Xanthomonadales: Xanthomonadaceae).* Embrapa Recursos Genéticos e Biotecnologia-Capítulo em livro científico *(*ALICE*)*.

Martelli, G. P. (2014). Directory of viruses and diseases similar to the vine and its agents. *Journal of Plant Pathology*, 96:1-136.

Mello, L. M. R. (2011) *Viticultura Brasileira: Panorama 2010.* Comunicado Técnico 111. Embrapa, Bento Gonçalves, RS.

Mondello, V., Songy, A., Battiston, E., Pinto, C., Coppin, C., Trotel-Aziz, P., Fontaine, F. (2018). Grapevine trunk diseases: a review of fifteen years of trials for their control with chemicals and biocontrol agents. *Plant Disease*, 102(7):1189-1217.

Moriondo, M., Orlandini, S., Giuntoli, A., Bindi, M. (2005). The effect of downy and powdery mildew on grapevine (*Vitis vinifera* L.) leaf gas exchange. *Journal of Phytopathology*, 153(6):350-357.

Mugnai, L., Graniti, A., Surico, G. (1999). Esca (black measles) and brown wood-streaking: two old and elusive diseases of grapevines. *Plant Disease*, 83(5): 404-418.

Naidu, R., Rowhani, A., Fuchs, M., Golino, D., Martelli, G. P. (2014). Grapevine leafroll: A complex viral disease that affects a high-value fruit crop. *Plant Disease* 98:1172-1185.

Nascimento, A. R. P., Mariano, R. D. L. R. (2004). Cancro bacteriano da videira: etiologia, epidemiologia e medidas de controle [Bacterial cancer of the vine: etiology, epidemiology and control measures]. *Ciência Rural*, 34:301-307.

Naves, R. D. L., Garrido, L. D. R., Sônego, O. R., Fochesato, M. (2006). *Antracnose da videira: sintomatologia, epidemiologia e controle* [*Vine anthracnose: symptomatology, epidemiology and control*]. Bento Gonçalves, Brasil, Embrapa Uva e Vinho-Circular Técnica 69.

Naves, R. L., Garrido, L. R., Sônego, O. R., Kuhn, G. B. (2005). Doenças e seu controle. In: Guerra, C. C., Hickel, E. R., Kuhn, G. B., Nachtigal, J. C., Maia, J. D. G., Fráguas, J. C., Botton, M. *Sistema de produção de uvas rústicas para processamento em regiões tropicais do Brasil* [*System of production of rustic grapes for processing in tropical regions of Brazil*]. Bento Goncalves, Brasil, Embrapa Uva e Vinho. Available in: https://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Uva/UvasRusticasParaProcessamento/doencas.htm.

Naves, R. D. L., Garrido, L. D. R., Fajardo, T. V. M. (2012). *Doenças da videira causadas por fungos, vírus e bactérias* [*Vine diseases caused by fungi, viruses and bacteria*]. Bento Gonçalves, Brasil, Embrapa Uva e Vinho. 41p.

Omer, A. D., Granett, J., Wakeman, R. J. (1999) Pathogenicity of *Fusarium oxysporum* on different Vitis rootstocks. *Journal of Phytopathology*, 147(7-8):433-436.

Ono, Y. (2000) Taxonomy of the *Phakopsora ampelopsidis* species complex on vitaceous hosts in Asia including a new species, *P. euvitis*, *Mycologia*, 92(1): 154-173.

Orel, D. C., Reid, C. L., Fuchs, M., Burr, T. J. (2017). Identifying environmental sources of *Agrobacterium vitis* in vineyards and wild grapevines. *American Journal of Enology and Viticulture* 68:213-217.

Pascale, A., Vinale, F., Manganiello, G., Nigro, M., Lanzuise, S., Ruocco, M., Lorito, M. (2017). *Trichoderma* and its secondary metabolites improve yield and quality of grapes. *Crop Protection*, 92:176-181.

Pearson, R. C., Goheen, A. C. (1988). *Compendium of Grape Diseases*. St Paul: APS Press, 93 p.

Pozo, M. J., Azcon-Aguilar, C. (2007). Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology*, 10:393-398

Primiano, I. V., Loehrer, M., Amorim, L., Schaffrath, U. (2017) Asian grapevine leaf rust caused by *Phakopsora euvitis*: an important disease in Brazil. *Plant Pathology*, 66(5):691-701.

Radaelli, P., Fajardo, T. V., Nickel, O., Eiras, M., Pio-Ribeiro, G. (2009). Variabilidade do gene da proteína capsidial de três espécies virais que infectam videiras no Brasil [Variability of the capsid protein gene from three viral species that infect grapevines in Brazil]. *Tropical Plant Pathology*, 34(5):297-305.

Rathnayake, R. M. S. P., Savocchia, S., Schmidtke, L. M., Steel, C. C. (2018). Characterisation of *Aureobasidium pullulans* isolates from *Vitis vinifera* and potential biocontrol activity for the management of bitter rot of grapes. *European Journal of Plant Pathology*, 151(3):593-611.

Rego, C., Farropas, L., Nascimento, T., Cabral, A., Oliveira, H. (2006). Black foot of grapevine: sensitivity of *Cylindrocarpon destructans* to fungicides. *Phytopathologia Mediterranea*, 45:93-100.

Samuelian, S. K., Greer, L. A., Savocchia, S., Steel, C. C. (2014). Application of Cabrio (ai pyraclostrobin) at flowering and veraison reduces the severity of bitter rot (*Greeneria uvicola*) and ripe rot (*Colletotrichum acutatum*) of grapes. *Australian Journal of Grape and Wine Research*, 20(2):292-298.

Scheck, H. J., Vasquez, S. J., Gubler, W. D., Fogle, D. (1998). First report of three *Phaeoacremonium* spp. causing young grapevine decline in California. *Plant Disease*, 82(5):590.

Schenato, P., Minozzo, D., Nardin, R. D., Garrido, L. D. R., Fialho, F. (2008). *Influência da temperatura e do período de molhamento na infecção de bagas de uva por Glomerella cingulata* [*Influence of temperature and wetting period on the infection of grape berries by Glomerella cingulata*]. Congresso Brasileiro de Viticultura e Enologia. Bento Gonçalves, Brasil, Embrapa Uva e Vinho.

Schouteden, N., De Waele, D., Panis, B., Vos, C. M. (2015). Arbuscular mycorrhizal fungi for the biocontrol of plant-parasitic nematodes: a review of the mechanisms involved. *Frontiers in Microbiology*, 6:1280.

Sforza, R., Boudon-Padieu, E., Greif, C. (2003). Novas espécies de cochonilhas que vetorizam os vírus-1 e-3 associados ao leafroll de videira [New species of mealybugs that vectorize virus-1 and-3 associated with vine leafroll] (GLRaV-1 e-3). *Revista Brasileira de Patologia Vegetal*, 109:975-981.

Siebert, J. B. (2001). Eutypa: The economic toll on vineyards. *Wines & Vines*, 50-56.

Slippers, B., Boissin, E., Phillips, A. J. L., Groenewald, J. Z., Lombard, L., Wingfield, M. J., Postma, A., Burgess, T., Crous, P. W. (2013). Phylogenetic lineages in the Botryosphaeriales: a systematic and evolutionary framework. *Studies in Mycology*, 76:31-49.

Smart, R. E., Fontaine, F. (2014) Grapevine trunk diseases in 2014. How comparable to phylloxera in 1881?, 9th International Workshop on Grapevine Trunk Diseases, 2014. *Phytopathologia Mediterranea,* 591. Adelaide, Australia.

Somavilla, L., Gomes, C. B., Quecini, V. M. (2012). Registro da ocorrência de *Meloidogyne incognita* no porta-enxerto 'IAC 766-Campinas' no estado de Pernambuco e reação de porta-enxertos e de cultivares copa de videira a *Meloidogyne* spp. [Record of the occurrence of Meloidogyne incognita in the 'IAC 766-Campinas' rootstock in the state of Pernambuco and reaction of rootstocks and vine crown cultivars to Meloidogyne spp.] *Revista Brasileira de Fruticultura*, 34:750-756.

Speiser, B., Berner, A., Häseli, A. L., Tamm, L. (2000). Control of downy mildew of grapevine with potassium phosphonate: effectivity and phosphonate residues in wine. *Biological Agriculture & Horticulture*, 17(4):305-312.

Steel, C. C., Blackman, J. W., Schmidtke, L. M. (2013). Grapevine bunch rots: impacts on wine composition, quality, and potential procedures for the removal of wine faults. *Journal of Agricultural and Food Chemistry*, 61(22):5189-206.

Surico, G. (2009). Towards a redefinition of the diseases ithin the esca complex of grapevine. *Phytopathologia Mediterranea*, 48:5-10.

Thind, T. S., Arora, J. K., Mohan, C., Raj, P. (2004). Epidemiology of powdery mildew, downy mildew and anthracnose diseases of grapevine. In: *Diseases of Fruits and Vegetables Volume I* (pp. 621-638). Springer, Dordrecht.

Thomidis, T., Ziozou, E., Koundouras, S., Karagiannidis, C., Navrozidis, I., Nikolaoub, N. (2016). Effects of nitrogen and irrigation on the quality of grapes and the susceptibility to *Botrytis* bunch rot. *Scientia Horticulturae*, 212:60-68.

Thompson, B. D., Dahan, J., Lee, J., Martin, R. R., Karasev, A. V. (2019). A novel genetic variant of Grapevine leafroll-associated virus-3 (GLRaV-3) from Idaho grapevines. *Plant Disease,* 103:509-518.

Tolba, I. H., Zaki, M. F. (2011). Characterization of *Agrobacterium vitis* isolates obtained from galled grapevine plants in Egypt. *Annals of Agricultural Sciences*, 56:113-119.

Tsai, C. W., Rowhani, A., Golino, D. A., Daane, K. M., Almeida, R. P. (2010). Mealybug transmission of grapevine leafroll viruses: an analysis of virus–vector specificity. *Phytopathology*, 100:830-834.

Úrbez-Torres, J. R. (2001). The status of Botryosphaeriaceae species infecting grapevines. *Phytopathologia Mediterranea*, 50(4):5-45.

Van Niekerk, J. M., Calitz, F. J., Halleen, F., Fourie, P. H. (2010). Temporal spore dispersal patterns of grapevine trunk pathogens in South Africa. *European Journal of Plant Pathology*, 127(3):375-390.

Weber, E. A., Trouillas, F. P., Gubler, W. D. (2007). Double pruning of grapevines: a cultural practice to reduce infections by *Eutypa lata. American Journal of Enology and Viticulture*, 58:1, 61-66.

Würz, D. A., Rufato, L., Bogo, A., Allebrandt, R., Bem, B. P., Marcon Filho, J. L., Bonin, B. F. (2020). Effects of leaf removal on grape cluster architecture and control of Botrytis bunch rot in Sauvignon Blanc grapevines in Southern Brazil. *Crop Protection*, 131:105-170.

Wyss, U., (2000). *Xiphinema index*, Maintenance and feeding in monoxenic cultures. In: Maramorosch, K., Mahmood, F. (Eds.), *Maintenance of Human, Animal and Plant Pathogen Vectors*. Science Res. Assoc., Chicago, 251-281.

Yepes, L. M., Burr, T., Reid, C., Fuchs, M. (2019). Elimination of the Crown Gall Pathogen, Agrobacterium vitis, from Systemically Infected Grapevines by Tissue Culture. *American Journal of Enology and Viticulture* 70:243-248.

In: Vitis: Biology and Species
Editors: António M. Jordão et al.
ISBN: 978-1-53618-308-5

Chapter 8

DOWNY MILDEW-GRAPEVINE INTERACTION

***Petra Štambuk*[1,2], *Ivana Tomaz*[1,*], *Iva Šikuten*[1,2],**
***Zvjezdana Marković*[1], *Edi Maletić*[1,2], *Darko Preiner*[1,2]**
***and Jasminka Karoglan Kontić*[1,2]**
[1]Department of Viticulture and Enology, University of Zagreb,
Faculty of Agriculture, Zagreb, Croatia
[2]Centre of Excellence for Biodiversity and Molecular Plant Breeding,
University of Zagreb, Faculty of Agriculture, Zagreb, Croatia

ABSTRACT

Downy mildew is one of the most destructive diseases affecting viticulture, caused by *Plasmopara viticola* Berl. and De Toni, an obligate biotrophic oomycete whose life cycle is mostly maintained in any grapevine green tissue. Infection of grapevine begins in spring with dispersed sporangium produced from oospores and lasts until the end of summer. The most common way for controlling this disease is by repeated fungicide applications which cause the development of resistant strains, residual toxicity, and pathogen pressure. Moreover, it is harmful to the environment and human health, but also economically expensive. In the absence of treatments, and with favourable weather conditions, downy mildew can devastate the crop in one season causing a serious economic loss. Unlike the innate disease tolerance present in several wild American and Asian *Vitis* species, there are different levels of susceptibility among cultivated *Vitis vinifera* L. varieties. In the era of "omics" approaches, it became possible to detailly investigate the early host response mechanism and metabolomic background of resistant or partially resistant varieties. Phenolic compounds are used as biomarkers of resistance due to their higher concentration in resistant or tolerant genotypes compared to susceptible ones upon the downy mildew infection. With the introduction of deep sequencing, resistance proteins can be identified and their resistance gene analogues which is of utterly importance for using non-*vinifera*

---

[*] Corresponding Author's Email: itomaz@agr.hr.

germplasm in breeding programs. Thus, the aim of this review is to congregate the most contemporary knowledge about the downy mildew biology and the complete background of grapevine's subsequent response.

Keywords: defence mechanism, omics studies, *Plasmopara viticola*, *Vitis vinifera*

1. INTRODUCTION

Fast recognition of host signals and early activation of infection mechanisms in *Plasmopara viticola* are decisive for successful infestation of the cultivated grapevine (*Vitis vinifera* L.). Virulent pathogen strains can spread rapidly, colonising the tissue of susceptible grapevine cultivars within hours by forming primary haustoria and intercellular mycelia (Gomez-Zeledon and Spring, 2018). The development of new resistant varieties to the oomycete *Plasmopara viticola* is a promising strategy to combat downy mildew, one of the major diseases threatening the cultivated grapevine (Vezzulli et al., 2019). Very detailed and scrutinized analyses of grapevine–downy interaction, which are nowadays available and approachable, enable a deeper understanding of this antagonism, including the host defence mechanism as well as the pathogen parasitism. Upon downy mildew infection, the groups of compounds included in grapevine's secondary metabolism, namely polyphenolics, volatile organic compounds and lipids play an important role in defence mechanism (Algarra Alarcon et al., 2015; Chitarrini et al., 2017; Negrel et al., 2018). Prior to and early after infection with *P. viticola*, protein changes in grapevine lead to the discrimination of resistant and susceptible cultivars. This approach highlights the involvement of reactive oxygen species-signalling events that restrict fungal growth. Lipid associated signalling, with the involvement of jasmonic acid, play a major role in the establishment of the defence mechanism in a resistant cultivar. Many proteins involved in photosynthesis and primary energy metabolism are repressed during infection (Figueiredo et al., 2017). The genetic basis of wild *Vitis* and *Muscadinia* genotypes are encouraging, since resistance genes to downy mildew have been identified among them, which are in turn applied in breeding programmes (Töpfer et al., 2011).

1.1. Classification and History of *Plasmopara viticola* Spreading

Plasmopara viticola is a microorganism which causes the downy mildew disease of grapevine, one of the most destructive diseases in all grape-growing areas characterized by the temperate climate and frequent rain during spring and summer. It was first noticed and collected in 1834 in the north-eastern part of the USA by Schweinitz, who referred it to *Botrytis cana* Link. To date, several recategorizations have ensued. In 1848, Berkley and

Curtis re-classified it as the new species of *Botrytis viticola*. During this period (from 1845 to 1849) *Phytophthora infestans* severely infected areas in the west and south of Ireland, causing the potato late blight and consequently the Great Famine. Having scrutinized the life cycle of the potato late blight fungus, De Bary described the asexual and the sexual stages the grape pathogen in 1863 and placed in the genus *Peronospora*. Farlow published full botanical description of this microorganism and attributed it to *Peronospora viticola* in 1876. In 1886, several clear differences were found by Schröder between a few species of the *Peronospora* genus which resulted in the separation of this genus into *Peronospora* and *Plasmopara*. Finally, using Schröder's classification system, Berlese and de Toni renamed this microorganism *Plasmopara viticola* (Gessler et al., 2011).

Downy mildew is caused by the obligate biotrophic oomycete *Plasmopara viticola*. Oomycetes are fungal-like members of the kingdom Chromista and are closely related to heterokont algae forming a distinct group different from true fungi, green algae or plants (Rumbolz et al., 2002). They were once classified as fungi because of their filamentous growth, production of spores and feeding on decaying matter. However, discovering that its cell walls contain cellulose instead of chitin, and its cell nuclei are diploid, not haploid like those of fungi it belongs to the class of oomycetes. Belonging to the infrakingdom or phylum heterokonta it is closely related to marine algae (such as kelps and diatoms) (Keller, 2015). As an obligate biotroph, this pathogene obtains nutrients from living cells of hosts in order to complete its life cycle (Guerreiro et al., 2016), unlike necrothrophic microorganisms which thrive on dead or senescing host tissues (e.g., *Botrytis cinerea*) (Glazebrook, 2005). *Plasmopara* morphology is rather simple since it consists of vegetative intercellular mycelium from which grow sporangiophores with monopodial branching, mostly at right angles, with terminal branches (sterigmata) that are mostly trichotomous (Gessler et al., 2011).

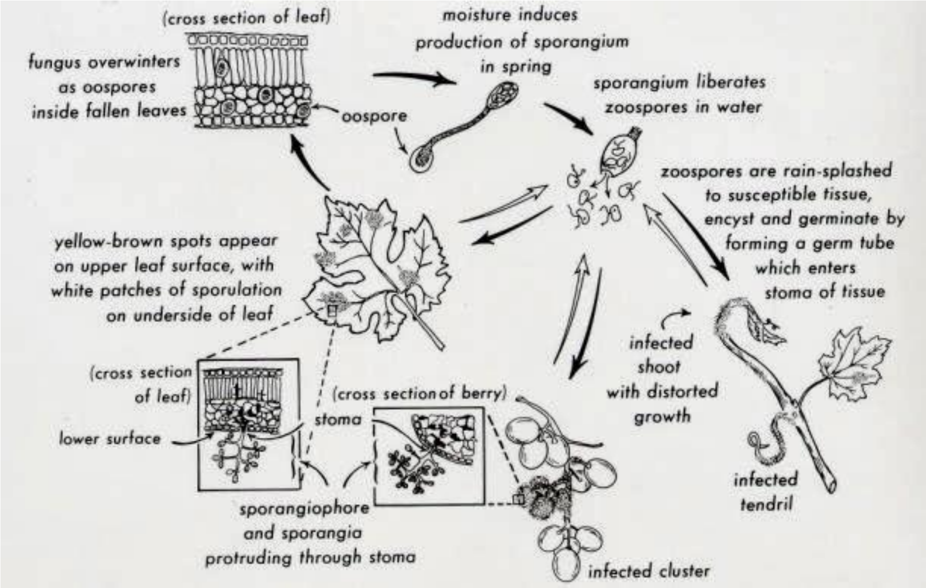
Striking years for the European viticulture were 1845, 1863 and 1878, when powdery mildew (*Erysiphe necator*), phylloxera (*Daktulosphaira vitifoliae*) and downy mildew were introduced, respectively. Wild *Vitis* species of North America are endemic hosts of these pathogens and their co-evolution made them resistant to these pathogens. The development and spreading of *Plasmopara viticola* during the second half of the 19th century was excruciating for the viticultural production in Europe since the European grapevine, *Vitis vinifera*, is generally very susceptible to the downy mildew disease. In 1878, the symptoms of this disease were firstly noticed in the French vineyards as far as the European continent is concerned. As already mentioned, two other epidemics caused severe losses before the downy mildew introduction. Willing to replant destroyed vineyards throughout Europe with resistant American cuttings, viticulturists imported them together with the third dangerous disease unintentionally. Since then, scientists from all over the world have been seeking for the effective disease management, especially in the form of chemical protection and through the breeding programmes which are priority toward the sustainable viticultural production (Töpfer et al., 2011). *Plasmopara viticola* presents a high evolutionary potential as several isolates are able to break down plant resistance of interspecific hybrids (Guerreiro et al., 2016). Not only that downy mildew has a great economic impact on the grapevine, but also on other crops, e.g., *Pseudoperonospora humuli* on hop (*Humulus lupulus*) and *Peronospora tabacina* on tobacco (*Nicotiana* spp.) (Voglmayr, 2008).

2. THE LIFE CYCLE OF *PLASMOPARA VITICOLA*

The life cycle of *Plasmopara viticola* consists of two main stages – the sexual overwintering phase in which primary inoculum is produced and the asexual multiplication cycles over the period of grapevine vegetative growth. Thorough understanding of its life process is essential in relation to the pathology and epidemiology, and for the development of better control of disease (Burruano 2000). In other words, the downy mildew disease cycle possesses both the oospores and the zoospores (asexual spores), which cause primary and secondary infections, respectively (Figure 1). Thus, it is a polycyclic disease with both forms responsible for its proliferation (Judelson, 2009).

2.1. Oosporogenesis

A form in which *Plasmopara viticola* overwinters is called oospore which is placed within fallen decaying leaves and other host tissues on vineyards' ground or buries in the soil. Oospores are the sexual stage of the pathogen the main source of inoculum for primary downy mildew infections in the next season (Rossi et al., 2008). They are produced from the fertilization of oogonia by antheridia, which are female and male gametangium, respectively. This thick-walled sexual form develops from a fertilized oosphere which is the female reproductive cell formed in the oogonium. There is one oosphere in each oogonium. The young gametangia contain many small haploid gametic nuclei which are products of gametangial meiosis in this diploid oomycete. The formation of gametangia and oospores is at the highest level during the autumn. The fertilization begins when a single antheridial nucleus passes through a fertilization tube into the oosphere and comes near to single oosphere nucleus. The remaining oosphere nuclei migrate to the periplasmic space, surrounded by a thick fibrillar oogonium wall. In the oosphere, single haploid antheridial and oogonial nuclei fuse producing a thick-walled diploid oospore while periplasmic nuclei degenerate.



https://enoviti-hanumangirl.blogspot.com/2014/02/downy-mildew.html.

Figure 1. *Plasmopara viticola* life cycle.

As a heterothallic organism with two mating types, P1 and P2, the oogonia of *Plasmopara viticola* can only be fertilized by antheridia from another colony ensuring that self-fertilization is impossible. It was noticed by conducting a series of laboratory studies in which there was no chance to obtain oospores when single-sporangiophore isolates of the oomycete were inoculated onto untreated grape leaves (Wong et al., 2001). With the availability of molecular markers, it became possible to affirm that each oospore is derived from a recombination event involving two *P. viticola* genotypes. During the winter months, oospores are dormant and will not germinate before January even if exposed to favourable conditions (the optimum temperature being 20°C and relative humidity reaching more than 95%). Simultaneously, the oospore maturation takes place which is an important process because only mature oospore will rapidly germinate (within 24 h) and form sporangia once exposed to a water film (Burruano, 2000; Gessler et al., 2011).

The climate is one of the crucial factors affecting the severity of primary infection, with rainfall and temperature being the most relevant and considered. Based on a 30-year observation by Rouzet and Jacquin (2003), it was concluded that the more it rains in autumn (October and November), the more serious are the infections in the following summer. Likewise, spring rainfall in April and May is positively correlated with the severity of primary foci. March and April are the months involved in the severity of primary infections: the more it rains (rainfall greater than 5 mm within 24 h) and the warmer it is (maximum daily temperature over 13°C), the more serious are the infections. The number of mature oospores appears to decrease through the spring, and this decrease is accelerated by warm dry periods. In some years, the germination stops probably due to dryness in spring, as only long dry periods can stop oospore development (Rouzet and Jacquin, 2003).

Over half a century ago, it was suggested that primary infections do not occur unless the minimum atmospheric temperature is about 10°C, at least 10 mm of rain have fallen during the previous two days and the shoot length is about 10 cm (the corresponding leaf surface being about 6 to 8 cm^2) (Gessler et al., 2011) which is still a trustworthy forecasting model. These adequate climate conditions are usually met during the spring period, when they activate oospores in fallen leaves and other decayed grapevine tissues. Once activated, oospore germinate and form a germ tube terminating in a macrosporangium. Within this structure, the self-motile biflagellate zoospores are formed. They are released and dispersed onto grapevine tissues by rain or they are airborne. In the presence of water drop or water film on the abaxial leaf side, the zoospores can encyst near stomata and produce germ tube which penetrates through functional stomata (Armijo et al., 2016; Buonassisi et al., 2017). While the oospores induce most primary infections of the grapevine in Europe (Gobbin et al., 2005), South Africa (Koopman et al., 2007) and the north-eastern part of the United States (Kennelly et al., 2007), some secondary infections may also occur depending on the climate conditions. However, in Western Australia oosporogenesis is rare because of an uneven distribution of the two mating types (Killigrew and Sivasithamparam, 2005). Since downy mildew was introduced into this area, only in the late 1990s (McKirdy et al., 1999), this absence of oospores may be caused by a bottle-neck situation, due to the presence of only a few genotypes (Gessler et al., 2011). This emphasises the significance of both mating types presence in many regions of the world where oospores are an important inoculum along with zoospores for the pathogen’s evolution and genetic improvement.

Thich-walled oospores are the culmination of sexual reproduction within the class Oomycotina. Their winter-survival dominion is accomplished by forming the oogonial cytoplasm, which becomes rich in lipids, proteins, and b-linked glucans after fertilization. The ooplast is a phosphate-rich vacuolar body, which takes about half of the oospore volume and largely defines its architecture. Not only that the oospores are important because in that form *Plasmopara viticola* ensures its survival in inhospitable environments (such as freezing or dry conditions and microbial degradation), but that is also the source of genetic variation (Judelson, 2009). Maturation of the oospores involves establishing a thick multi-layered wall accompanied with ribosomes and cytochromes disappearing, which indicates that the metabolism is very reduced. The combination of very low metabolism, a thick wall, and a lipid-rich cytoplasmic matrix makes oospores extremely effective resting structures specialized for surviving harsh environmental conditions (Judelson, 2009).

2.2. Asexual Reproduction

There are two main phases of the asexual reproduction in the oomycetes: sporangiogenesis, which is the formation of multinucleate sporangia, and zoosporogenesis, which is the formation of uninucleate, molatile biflagellate zoospores. These asexual zoospores are the main dispersive agents for oomycete and the most important means of initiating host infection. When asexual reproduction is induced, the mycelia produce sporangia on the tips of branched sporangiophores. The mature sporangia may detach and function as dispersive propagules. The multinucleate sporangia germinate indirectly by forming biflagellate zoospores. These flagella make the zoospores motile which is an important feature because of improving the organism's chances to find a suitable source of nutrients. The zoospores encyst when they reach a potential host or other nutrient sources. During encystment, the zoospores detach the two flagella. Typically, within half an hour the zoospores germinate forming the germ tube and attempt to penetrate through the host stoma. Zoospore germination and germ tube growth return the organism to the vegetative state before sexual reproduction is initiated by the end of a growing season (Hardham, 2009).

Sporangiogenesis, involves the differentiation of hyphal tips to form sporangia through the production of a sporangiophore on whose distal end sporangia subsequently develop. The sporangia eventually detach and function as dispersive propagules. Formation of the basal septum corresponds with the accomplishment of the sporangium final size. In the *Peronosporales*, the sporangia are globose to lemon shaped. The wall of sporangium is thick and impermeable to molecules in the external solution. These characteristics are important for mechanisms involved in a subsequent release of the motile zoospores. The sporangial apex often differentiates as a specialized papilla that ruptures on zoospore discharge, which is driven by hydrostatic pressure that builds up within the sporangium. Cleavage of mature sporangia that releases uninucleate zoospores is the process of zoosporogenesis (Hardham, 2009).

The matured sporangium is capable to release zoospores that infect plant tissue in the presence of water. Zoospores have two flagella that are unequal in length and surface morphology, and that feature put them in the phylum Heterokonta. The shorter flagellum moves the zoospore forward, while the longer cause them to turn. Zoospores rotate as they swim and mostly move straight forward with suddenly changing directions. Flagella emerge the zoospore ventral surface, that faces the potential host. After reaching the appropriate host cell (the guard cells on the lower surface of the leaf), the zoospore become immotile and start to encyst. The process of encystment is rapid during which the flagella are detached while spores change its shape to spherical and become adhesive (Hardham, 2009). The zoospores of *Plasmopara viticola*, which is a biotrophic foliar pathogen, target stomatal complexes and the hyphae enter the leaf via stomatal pores (Gindro et al., 2003). The components of encysted zoospores, actin and microtubules, are required for its further germination (Riemann et al., 2002). Zoospores are not enveloped by a cell wall, and their plasma membrane is covered by an extracellular carbohydrate layer (glycocalyx) that is rich in glucosyl and mannosyl residues. Because of this feature, zoospores cannot induce hydrostatic pressure. Thus, they actively pump out excessive water by a contractile vacuole in order to maintain cell size and integrity (Hardham, 2009).

After being inoculated onto the abaxial surface of a leaf, zoospores germinate to form a germ tube which grows through a stoma. Once inside host tissue, a substomatal vesicle starts to develop which gives rise to the intercellular mycelium. The mycelium is composed of many haustoria whose main task is to penetrate the cell walls of the spongy mesophyll leaf layer. The spreading of mycelium is restricted by the leaf veins, which appeared impermeable for hyphae due to the dense package of cells in the vascular tissue. For the first four to five days after the infection, the downy mildew disease is latent, but it develops progressively inside the leaf tissue. The first visible sign of the infection appears on the adaxial leaf surface in the form of the typical yellow-brownish oil-spot lesions.

If the condition of inoculation is supported with high humidity, hyphae grow into the substomatal cavity and give rise to sporangiophores emerging from the stoma. One stoma can bear several multinucleate sporangiophores but usually, only one matures, while the other degenerate after 6-8 hours. The sporangiophore elongation emerges while several nuclear divisions occur. After the elongation, the first lateral branch develops on a sporangiophore and nuclei migrate into the branch. There are branches of the first, second and third order. On the branch, tips are the sporangial initials which are anucleate and spherical. As soon as enlargement is complete the round or elongate nuclei migrate through the sterigmata into the sporangium, until a basal septum is formed. Mature sporangia are drop-shaped (Burruano, 2000; Rumbolz et al., 2002).

Short and repeated disease cycles are characteristics of the asexual part of the life cycle of the pathogen. Under favourable conditions (temperature of 20 ±2°C with a relative humidity of nearly 100%) the mycelium can sporulate three days after the infection. In other words, the intercellular mycelium grows out of stomatal cells forming hyphae that branch in a species-typical manner. A branched hypha is called sporangiophore which bears developing or mature sporangia at its distal end (Rumbolz et al., 2002).

2.2.1. Inside the Host Cell

In the oomycetes, as in fungal phytopathogens, the invading hyphae synthesize and secrete enzymes that degrade plant cells wall components such as pectin, cellulose, and xyloglucans. They also secrete proteins that protect the pathogen against plant defence molecules. Such counter-defence proteins include glucanase and protease inhibitors (Hardham, 2009).

As an obligate biotroph, *Plasmopara viticola* does not invade its host with the aim to destroy it but to establish a stable relationship with living host cells. Thus, disruption of the host is minimized because it is important to maintain the integrity of the host cells. The haustoria form in the mesophyll cells. During its development, the grapevine cell wall is locally degraded, and a thin penetration hypha grows through the wall to invaginate the plant plasma membrane as it enters and continues to expand within the cell. From the hyphae arise haustoria, which are nutrient-absorbing outgrowths. After creating an interaction with a host, a pathogen makes rapid changes in the organization of the host cell. These initial responses are associated with the basal host defence response. Successful colonization of the grapevine by *Plasmopara viticola* culminates in sporulation. Within a few days (usually not more than seven) asexual sporangia form on the plant surface during a growing season. In nearly autumn conditions, sexual spores (oospores) form inside infected fallen leaves and berries while mycelium overwinters in dormant twigs. Then, the pathogen life cycle begins again (Hardham, 2009).

3. CHANGES OF GRAPEVINE UPON DOWNY MILDEW INFECTION

3.1. Structural and Histological Features of Resistant and Susceptible Varieties

Very soon after being infected with *P. viticola*, the inside tissue of grapevine leaves goes through changes and the manner of these changes depends on the grapevine – *P. viticola* interaction which can be incompatible or compatible. The susceptibility is defined as a compatible plant–pathogen interactions, which is characterised by the great symptoms' manifestations in the host, a well as the capability of the pathogenic agent to finalise and propagate its infection cycle (Kranz, 2003). In this regard, noble *V. vinifera* varieties are compatible with *P. viticola*, while incompatible interactions are usually ascribed to wild *Vitis* species and interspecific hybrids. Molecular mechanisms and genetic regulation are responsible for these possible types of interaction. In resistant genotypes, the first changes occurring at the chemical level include, the accumulation of reactive oxygen species (ROS), the increase of phenolic antimicrobial compounds, as well as the action pathogenesis related (PR) proteins and increased peroxidase activity. Subsequently, morphological alternations in cells, including cell wall thickening, callose deposition in stomata, necrosis and activation of hypersensitive response take place (Nascimento-Gavioli et al., 2019).

Brazilian interspecific *V. labrusca* variety 'Ives' (syn. 'Bordô') is a very interesting resistance source for breeding purposes, which makes it appropriate to employ as a model variety for understanding the underlying mechanisms responsible for resistance (Nascimento-Gavioli et al., 2019). Moreover, 'Ives' possesses dense hydrophobic trichomes on its abaxial leaf sides. Trichomes act as a physical barrier against *P. viticola* infection since in their presence the water droplets remain spherical and are unable to reach and are unable to reach leaf surface. Thus, the adherence of water droplet to the abaxial leaf surface is reduced while *P. viticola* requires free water to infect. Eventually, the presence of hydrophobic trichomes increases the host resistance (Kortekamp and Zyprian, 1999).

It has been shown that stomata release amino acids, pectinases, and isoflavones when open. Thus, they behave as navigators during zoospores encystment. Moreover, stoma's cavity is directly connected to mesophyll, which makes a perfect entrance for a zoospore's germ tube to penetrate inside a leaf tissue. However, in some cases, germ tubes seem to be disoriented while penetrating stomata, but could colonise the mesophyll of a resistant cultivar, which indicates that the mechanism that blocks the pathogen development occurs after the host colonisation (Nascimento-Gavioli et al., 2019). Histological changes of variety 'Ives' leaf tissue upon inoculation include compact mesophyll cells, while cells of spongy parenchyma are arranged in 3-5 with large intercellular spaces. An increase of protoplasm granules and starch in cells of palisade and spongy parenchyma is observed, together with an accumulation of phenolic compounds. The observed pathogen hyphae are damaged in this case. This can be ascribed to the presence of small and compact mesophyll cells which do not leave many intercellular spaces needed for mycelium growth. Thus, pathogen growth is interrupted and limited. Moreover, densely grouped leaf tissues cells can establish structural barriers at the host level, preventing further pathogen development in the leaf mesophyll (Yin et al., 2017). The significant reduction of hyphae growth and the high frequency of lesions with arrested pathogen development in the initial colonisation phase are features associated with resistance responses (Nascimento-Gavioli et al., 2019).

Necrosis is a typical response of *P. viticola* infection in resistant and partially resistant grapevines. While in totally resistant genotypes the hypersensitive response is detected just hours after inoculation, in partially resistant ones occurs some days after inoculation (Liu et al., 2015). The black spots observed at the inoculation site in cv. 'Ives,' and the complete block of the pathogen development, suggest that hypersensitive response occurs as a resistance mechanism to *P. viticola* (Bellin et al., 2009).

Typical basal resistance mechanisms include the high density of trichomes on the abaxial leaf surface, a more compact mesophyll, and changes in cell density in the spongy parenchyma. Complementary to these mechanisms are induced post-infection defences, such as the triggering of hypersensitive response, once necrosis formation is observed at the site of infection (Díez-Navajas et al., 2008).

In a susceptible variety, the pathogen is capable to reach the substomatal cavity and to develop sporangiophores from the stomata, usually four to five days post inoculation. The complete cell wall rupture and mesophyll cells disorganisation caused by the colonisation of the pathogen are observed in cv. 'Cabernet Sauvignon'. Without trichomes on this variety's abaxial leaf surface, the zoospores of the pathogen were already observed at six hours post inoculation around the stomata. At 12 hours post inoculation, from one to four zoospores per stomata were observed, emitting and penetrating the germ tubes in the substomatal cavity. Finally, mature sporangia carrying mature zoospores were detected from four days post inoculation, which correlates with the macroscopic symptoms of the disease, appearing as white cotton-like cover (Nascimento-Gavioli et al., 2019). The leaf veins containing sclerenchyma that act as a natural borderline are the only limitation for hyphae expansion. The mycelium colonises the intercellular space of a susceptible genotype until four days post inoculation and then the sporangiophores development begins in the substomatal cavities, followed by the appearance of the first symptoms. This abundance of hyphae in mesophyll triggers the sporulation, which is regulated by the photoperiod (Rumbolz et al., 2002; Unger et al., 2007; Liu et al., 2015).

3.2. Phenotyping

The first step of providing differences among the *Vitaceae* family in sense of susceptibility to *Plasmopara viticola* is the phenotyping approach. Although this kind of experiments usually requires patience, precision, focusing and are time-consuming; they are informative, selective and useful for further detailed analyses. To date, the genetic background of numerous crop plants is well-known and investigated while phenotypic characterization is mostly neglected or poorly described.

Over the past decades, a lot of effort has been put in breeding and obtaining resistant cultivars (Töpfer et al., 2011). Since leaves are the pioneers in providing the first visual symptoms of the downy mildew disease, a phenotyping method on leaf discs that are inoculated and maintained in controlled conditions has been widely applied among plant pathologists, breeders and geneticists who are willing to obtain differences between genotypes in regard to their downy mildew susceptibility. Moreover, this phenotyping tool characterizes resistance sources in the *Vitis* genus regarding *P. viticola*. The main materials for conducting the leaf disc bioassay are healthy, untreated and unstressed grapevine leaves and suspension of *P. viticola* sporangia.

Grapevine genotypes of interest need to be grown indoors (e.g., greenhouse, phytotron or climate chamber) under controlled conditions (temperature at 24 ± 2°C, photoperiod 16/8 h, relative humidity 70%) without application of fungicides against *P. viticola*. For that purpose, green of hardwood cuttings can be used instead of more expensive grafted cuttings since this trial mostly takes place during one vegetative period. Collected dormant hardwood cutting can be stored in plastic bags at 4°C until required. After preparing and cutting them to be around 20 to 30 cm long containing five to six nodes, the basal bud needs to be eliminated since from the basal node the root system develops. It is recommended to treat that basal part of cutting with IBA (indol-3-butiric acid) powder for 30 seconds or to soak it overnight in an aqueous solution containing IBA. Two to three months after planting, from successfully rooted cuttings, shoots will be developed carrying 10 to 12 leaves. Young leaves (ideally fourth to sixth from the shoot apex) are the most susceptible to *P. viticola*, thus they are appropriate for the leaf disc bioassay. For the older leaves, ontogenic resistance takes place.

From detached leaves, leaf discs (from 1 to 2,5 cm of diameter) are excised with a cork borer and placed onto wet filter paper or water agar (from 0,8 to 1% agar) in Petri dishes with the abaxial side up. Suspension can be prepared either with a pure sporangia culture (single sporangium inoculum) or with mixed sporulation taken from the untreated vineyard. Both ways have their advantages: while a pure culture provides more uniform results, a mixed one more confidently describes the real virulence of *P. viticola* taken from a specific site. Suspension concentration needs to be adjusted from $2,5 \times 10^4$ to 1×10^5 sporangia mL^{-1} and this is usually done by the Neubauer counting chamber under the microscope. Suspension can be sprayed or pipetted onto leaf discs abaxial sides. Spraying is faster and provides equally dispersed droplets over the entire disc surface.

Closed and sealed with parafilm, Petri dishes need to be placed in a climate chamber for incubation period in the dark that lasts 24 h and subsequent photoperiod of 16/8 h (light/dark) for seven days at 21°C. It is possible to notice the first symptoms (sporulation) after four days on susceptible varieties. However, on some varieties, even more than seven days will pass until visual changes of infection while resistant ones develop only necrotic spots with no signs of sporulation (Bellin et al., 2009; Buonassisi et al., 2017; Gomez-Zeledon et al., 2017; Nascimento-Gavioli et al., 2019). Using early bioassays on leaves ensures a satisfactory prediction of disease severity on bunches in the field (Calonnec et al., 2013).

Different types and regimes of light affect *Plasmopara viticola* sporulation. White light irradiation causes abnormal differentiation of external hyphae. More precisely, in that case, the hyphae (without bearing sporangia) are either spatial organized in clusters, unbranched and shortened or extremely elongated, with a reduced diameter compared with standard sporangiophores. Continuous blue light, as well as continuous white light, lead to a strong reduction of sporangia, while continuous irradiation with red or far red light has not an inhibitory effect. Finally, the dark phases at the end or in the middle of the incubation lead to a significant increase in the number of sporangia (Rumbolz et al., 2002).

Gomez-Zeledon et al., (2017) proposed a comprehensive phenotyping method that includes classifying the reaction of the pathogen (sporulation) together with the reaction of the plant (necrosis). This idea came from completely different plant responses among the *Vitaceae* family and cognition that there are many different *P. viticola* strains even within a single vineyard during the same period. Genetic heterogeneity of this oomycete provides isolates with different virulence to different *Vitis* genotypes. In order to obtain more precise and consistent plant reactions in phenotyping experiments, it is necessary to use genetically homogeneous strains of the pathogen. Since plant and pathogen make specific reactions when they interact, two separate scales have been designed. Especially when utilizing a broader range of pathogen isolates, sporulation and necrosis on leaves should be treated as independent features. Thus, for leaf disc bioassay the assessment of the sporulation and the necrosis level is defined using different symbols. As for the pathogen aggressiveness, this scale uses letters from A, which indicates that very strong sporulation is developed, to E meaning that sporulation is absent. Complementary to sporulation scale, the resistance reaction of the plant is categorized in four classes that rank necrosis (a hypersensitive reaction) from strong (+++) to absent ( ) (Gomez-Zeledon et al., 2017).

Out of more than 150 characteristics marked as descriptors for grape varieties and *Vitis* species prescribed by the International Organisation of Vine and Wine (OIV, 2001), three descriptors are related to the downy mildew infection. Descriptors 452 and 452-1 are made to be used on leaves in the field and as leaf disc bioassay, respectively, while descriptor 453 assess disease development on clusters in the field. The former two descriptors provide five classes all of which relate to a particular stage of disease development. This scale uses odd numbers from 1 (meaning that a genotype is completely susceptible; sporulation is spread and dense) to 9 (meaning that a genotype is completely resistant; sporulation is absent while necrotic spots can be developed) (Figure 2). Disease severity can also be visually assessed the percentage of the abaxial leaf surface are covered by sporulation, while disease incidence can be calculated as the percentage of leaves showing sporulation (EPPO, 2001).

According to the descriptor 453, clusters should be assessed at two periods: about three weeks after the onset of flowering and before vérasion of berries. Strongly affected clusters are marked with scores 1 to 3. Score 5 means that 20 – 30% of clusters are attacked, while scores from 7 to 9 mean that cluster are either sparsely attacked or completely healthy (OIV, 2001). Moreover, for quantifying sporulation on leaf discs computer vision methods for image analysis are routinely used. ImageJ software (Peressotti et al., 2011) requires a single image for every leaf disc that is being phenotyped, which is a time-consuming step if there are thousands of leaf discs to phenotype. Another method (Khiook et al., 2013) requires images of leaf discs taken on a photographic reproduction bench under artificial lightning. Finally, Divilov et al. (2017) developed a computational pipeline dedicated to estimating the percentage area of sporulation on leaf discs whose images can be taken from a smartphone camera. The same method can be used to quantify the percentage of leaf trichome area (Divilov et al., 2017).

Figure 2. Sporulation on a leaf disc of 'Cabernet Sauvignon' (OIV class 1) (left) and necrotic spots on a leaf disc of *Vitis riparia* (OIV class 9) (right).

Similar to the OIV descriptor 452-1 that describes the downy mildew development on leaf discs in controlled (laboratory) conditions, Buonassisi et al., (2018) proposed a bioassay applicable on grapevine inflorescences as a complementary method to the leaf discs' one. Therefore, the evaluation of a cultivar susceptibility/resistance will be more proper and the final effect on yield/wine will be more predictable. This method suggests using detached inflorescences from plants at the E-L (Eichhorn-Lorenz) 17 phenological stage grown in the untreated field or under controlled conditions (e.g., phytotron). The E-L 17 stage, which corresponds to flower button formation, proves to be the most robust and suitable stage for laboratory evaluations. Inflorescences should be infected by soaking in *P. viticola* suspension (1 x 10^4 sporangia mL^{-1}) for two hours to allow spore adhesion and germination. Next step is placing inflorescences in sterile boxes with peduncles inserted in 1% agar covered with sterile wet filter paper. The incubation period should be carried out at 21°C in dark conditions for 48 h in a growth chamber with a photoperiod of 16/8 h (light/dark) for 7 days afterwards. Finally, the visual disease symptoms should be described by two parameters: Dis, distribution of *P. viticola* on the total length of the inflorescence, and Sd, sporulation density of *P. viticola* considering every single spot (Buonassisi et al., 2018).

Method for producing oospores in the laboratory conditions suggests the coinoculation of abaxial sides of leaf discs with droplets containing sporangia. The incubation period lasts for 5 days at room temperature (20-22°C) with 12 h photoperiod to facilitate disease development with ensuing transfer to a 12°C growth chamber for 14 days with 12 h photoperiod. Subsequently, leaf discs are cleared with 95% ethanol to assist in detecting oospores by light microscopy (magnification ×100) (Wong et al., 2001).

3.3. Description of Different Omics Studies

Grapevine, and horticultural cultures in general, are hard to study compared to herbaceous crops mainly due to their long life-cycle, and the requirement for large cultivation space. However, the invention and improvement of analytical instruments, such as next generation DNA sequencer (NGS) and mass spectrometer (MS), have enabled enviable progress even in this field. Development of NGS has made whole genome sequencing of horticultural crops possible. As a result of this technology, grapevine genome was sequenced in 2007, which put grapevine on the first place among fruit trees and on the fourth place among plants, after Arabidopsis, rice and poplar, whose whole genome has been sequenced (Jaillon et al., 2007). Describing the whole genome of an organism means to precisely depict a full haploid set of chromosomes with its gene set, which is the subject of genomics. Generally, comprehensive studies are called omics and their development and popularity have soared in the last two decades bringing an enormous contribution to plant science (Shiratake and Suzuki, 2016).

Apart from genomics, comprehensive analysis targeting transcriptome, proteome, metabolome, ionome and phenome is called transcriptomics, proteomics, metabolomics, ionomics and phenomics, respectively. Even advanced studies called multi-omics or integrated-omics represent the combination and integration of several omics performed on a single sample or material (Shiratake and Suzuki, 2016).

3.4. Metabolomics

Upon downy mildew infection, the metabolomic profile of grapevine tissue changes and affects both the primary and secondary metabolism. As far as primary metabolism is concerned, pathogen infection causes a lack of sugar and alters carbohydrate partitioning between different plant organs. Thus, a dramatic alternation of carbohydrate metabolism is in correlation with *P. viticola* development in leaves. A reduction of photosynthesis enzymes indicates a source-to-sink transition in infected leaf tissue. At the early stages in the incompatible interaction (*P. viticola* and resistant *Vitis amurensis*) repression of photosynthesis and fatty acid synthesis is observed (Li et al., 2015).

A few striking metabolomic changes have been described to date: the biosynthesis of large amounts of stilbene phytoalexins in both compatible and incompatible interactions (Malacarne et al., 2011; Ali et al., 2012; Chitarrini et al., 2017), the biosynthesis of volatile organic compounds (VOCs) (Algarra Alarcon et al., 2015; Chalal et al., 2015) and the biosynthesis of lipid compounds (Negrel et al., 2018). *Plasmopara viticola* is able to infect green tissues and establish biotrophism widely across the *Vitis* genus. Unlike the European *V. vinifera*, some accessions in North American wild species have evolved host resistance. The later can activate defence responses upon pathogen infection, which culminate in localised necrosis, resulting into lower rates of sporangia release compared to susceptible varieties (Bellin et al., 2009; Polesani et al., 2010).

3.4.1. Phenolic Compounds

One of the partially resistant grapevine cultivars is ‘Bianca’, a hybrid between ‘Bouvier’ and the resistant cultivar ‘Villard Blanc,’ the donor of the *Rpv3* locus responsible for resistance phenotype. This locus controls the ability to initiate a localized hypersensitive response (HR) soon after the infection. Close to infection sites, HR limits the development of biotrophic pathogens, restricting their endophytic growth. Other early inducible responses include pathogen cell wall degrading and releasing of reactive oxygen species. These early responses are controlled by interactions between pathogen virulence gene products and host receptors. In order to activate them, multiple signalling pathways occur firstly because plant defence responses require energy and activation of signalling molecules, namely carbohydrates, organic acids, amines, amino acids, and lipids supplied by primary metabolism. Secondary metabolites are also important for expressing plant defence, which is often related to specific functions such as toxicity against pathogens or acting as signal molecules after stress. Stress-related metabolites are known as phytoalexins and their class of stilbenes that provide active compounds with antifungal activity against *Plasmopara viticola*. Different *Vitis* species exhibit specific pattern and accumulation of stilbenes upon the pathogen infection. Resistant varieties produce stilbenes faster and in higher concentrations in comparison to susceptible ones. *Muscadinia rotundifolia* defence response to *P. viticola* is mediated by stilbene accumulation induced by abscisic acid and salicylic acid phytohormones (Wang et al., 2018). It is also possible to achieve a downy mildew resistance in the absence of stilbene accumulation (Bellin et al., 2009; Di Gaspero et al., 2012; Rojas et al., 2014).

An experiment conducted on artificially inoculated leaf discs of ‘Bianca’ showed changes in metabolomic profile at different time points after the infection. At the very early stage of the pathogen invasion, mostly volatile compounds were found which were probably produced in mesophyll air spaces. Both sugars and amino acids were lower at the beginning of infection while organic acids were higher than controls. An interesting modulation of amino acid proline was noticed, which can classify this molecule as a biomarker of resistance, together with benzaldehyde. Moreover, they are components of salicylic acid-mediated resistance, contributing to cell death. Among unsaturated fatty acids, ceramide started accumulating very early as an essential signalling molecule in the activation of defence-related plant programmed cell death likewise. Generally, this primary metabolism serves as an energy supplier and provides the precursors of secondary metabolites, building blocks of pathogenesis-related (PR) proteins and components of defence signalling cascade (Chitarrini et al., 2017).

On the other hand, secondary metabolites were more affected during the later stages after the infection, or more particularly phenolic compounds, such as phenylpropanoids and flavonoids (Chitarrini et al., 2017). These compounds were used for distinguishing the resistant cultivar ‘Regent’ from the susceptible cultivar ‘Trincadeira’ (Ali et al., 2012). *Trans*-resveratrol is considered as a biomarker of resistance against pathogens since it is a precursor of phytoalexins, which are toxic to pathogens. In the resistant cultivar ‘Bianca’, a higher concentration of *trans*-resveratrol was accumulated soon after the infection while the subsequent accumulation of resveratrol derivates such as *trans*-*ε*-viniferin, *trans*- and *cis*-piceid, isorhapontin, ampelopsin H + vaticanol C-isomer, *α*-viniferin and pallidol (Chitarrini et al., 2017). To simplify, the defence mechanism that includes phenolic compounds starts with the synthesis of resveratrol and progresses with the synthesis of its dimers and oligomers. A similar pattern was observed in a segregating population of ‘Merzling’ x ‘Teroldego’ where higher content of oligomers did not correlate with the percentage of sporulation upon the infection. At the same time, the monomers *trans*-resveratrol and *trans*-piceid were highly accumulated in sensitive genotypes with dense sporulation (Malacarne et al., 2011).

3.4.2. Volatile Organic Compounds

Resistance to downy mildew disease can be enhanced by the application of an elicitor, such as sulfated laminarin (PS3), which induces the production of volatile organic compounds (VOCs). These compounds are well-known for their resistance role against pathogens (Chalal et al., 2015). Elicitors are compounds perceived by plants as danger signals that trigger cascades of signaling events leading to plant defence expression (Garcia-Brugger et al., 2006). Different biochemical classes contain elicitors, including lipids, proteins and carbohydrates. Cell walls of algae and microorganisms are composed of oligosaccharides (elicitors) referred to as microbial-associated molecular patterns (MAMPs) that are recognized by matching plant receptors (Jones and Dangl, 2006). A natural laminarin can be extracted from the brown algae *Laminaria digitata*. This oligosaccharide elicits defence and induces resistance in different plant species (Aziz et al., 2003). By the chemical sulfation of natural laminarin, a sulfated laminarin (PS3) is obtained. Generally, sulfated laminarin is a much more effective inducer of resistance than the native compound and in grapevine, it elicits the expression of defence genes, callose deposition, phytoalexin (resveratrol and derivates) production, and induced resistance against *Plasmopara viticola* (Trouvelot et al., 2008).

Not only phytoalexin resveratrol and its derivates can be functional grapevine defence metabolites, but also volatile organic compounds triggered by sulfated laminarin. This class includes terpenes, green-leaf volatiles, and benzenoids. Together with phenolic compounds, volatile compounds contribute to the plant’s secondary metabolism. They are emitted from above and belowground plant organs and can act as repellents of herbivore insects or attractants to their enemies. As airborne defence signals, they can initiate defence reactions against pathogens in remote plants (Kishimoto et al., 2006; Yi et al., 2009).

Upon grapevine treatment with sulfated laminarin and subsequent inoculation with *Plasmopara viticola* in a greenhouse under controlled conditions, an interesting fluctuation of volatile organic compounds emission was observed (Chalal et al., 2015). At the end of the monitoring period (four days post treatment), the monoterpene emission rates were higher in the samples treated with sulfated laminarin, while inoculation with *Plasmopara viticola* did not affect the monoterpene emission rates. Their emission is closely related to photosynthetic activity, which is typical for leaves without storage pools. A similar trend was noticed for the sesquiterpene emission. In the control samples, the methyl salicylate emission increased following inoculation. Among the identified terpenes, *trans*-β-ocimene and (*E,E*)-α-farnesene were the most abundant mono- and sesquiterpenes, respectively, produced by plants in response to sulfated laminarin. These compounds are also known to be products that are commonly emitted during plant-pathogen interactions, supporting the concept that elicitors can mimic pathogen attack.

It appears that the induction of volatile organic compounds is a common feature of elicitor treatment, including treatment with oligosaccharides. A positive correlation was found between methyl salicylate production and the infection rate in both the control and treated plants suggesting that this compound act as a disease marker. Similarly, some phytoalexins can accumulate as a response to pathogen infection, but do not allow its restriction. On the other hand, the plants with a lower infection rate at the end of experiment correspond to those that emitted more terpenes prior to or at the beginning of the grape-pathogen interaction. Therefore, an early terpene production, regardless of whether it is constitutive or elicited, may play a role in grape defence. Finally, Chalal et al. (2015) concluded that sesquiterpenes, particularly (*E,E*)-*α*-farnesene, represent a specific response to sulfated laminarin. Thus, they are considered as biomarkers of the grapevine response to sulfated laminarin treatment. The whole point of the induced resistance is to boost a plant natural immune system instead of using pesticides which directly destroy pathogens or insects.

Examples of genotypes whose resistance to *Plasmopara viticola* is based on both constitutive factors (structural barriers, hairy and water repellent leaf surfaces, and phytoanticipins) and inducible defence mechanisms (localized cell death, production of reactive oxygen species, synthesis of phytoalexins and pathogenesis-related proteins) are grapevine rootstocks SO4 and Kober 5BB (hybrids of *Vitis berlandieri* and *Vitis riparia*). On *in vitro* grown plants of these resistant hybrids together with the susceptible cultivar 'Pinot noir', Algarra Alarcon et al. (2015) screened the emission of volatile organic compounds at different time points after inoculation with *Plasmopara viticola*. Sporulation appeared on the abaxial leaf surface of 'Pinot noir' seven days after inoculation. Moreover, on the tenth day after inoculation, sporulation was visible on the adaxial leaf surface, petioles and stems. While SO4 showed only slight sporulation on the abaxial leaf surface and small necrotic spots, Kober 5BB showed only diffuse necrotic spots with no sporulation at all on the tenth day post inoculation, demonstrating the successful defence response.

Necrotic spots are one of the earliest phenotypic differences that are noticed between susceptible and resistant genotypes, and they are attributed to a hypersensitivity reaction causing programmed cell death at the infection site and associated with reductions in pathogens performance and symptom development (Bellin et al., 2009). The slight sporulation observed on the resistant hybrid SO4 could be explained by growing in the high humidity conditions, which favour the development of downy mildew sporangia on *in vitro*-grown resistant grapevines (Dai et al., 1995). The emission of sesquiterpenes was grater from resistant genotypes (SO4 and Kober 5BB) in comparison with the susceptible genotype ('Pinot noir'). At one time point, *Plasmopara viticola*-inoculated plants of Kober 5BB showed the highest emission of sesquiterpenes. Only from *Plasmopara viticola*-inoculated plants of the SO4 genotype emission of monoterpenes was detected, demonstrating that their production was activated in response to the infection. Although these compounds could be synthesized either by the grapevine or the pathogen, their emission was high in plants with sparse sporulation, suggesting that they are mainly produced by plant cells. In brief, volatile organic compounds are implicated in plant defence responses against pathogens, thus they play a role in the resistance against downy mildew by direct toxicity or by inducing grapevine resistance (Algarra-Alarcon et al., 2015).

For analysing volatile organic compounds emission, a Proton Transfer Reaction-Time of Flight-Mass Spectrometer (PTR-TOF-MS) (Algarra Alarcon et al., 2015) and a Proton Transfer Reaction-Quadrupole-Mass Spectrometer (PTR-Q-MS) prove to be powerful tools (Chalal et al., 2015).

3.4.3. Lipid Markers

A high-resolution mass spectrometry-based non-targeted metabolomic approach can be used to characterise metabolites differently accumulated in grapevine leaves and in *P. viticola* (Negrel et al., 2018). Unlike other experiments whose aim is to examine the metabolomic profile of infected grapevine tissue, Negrel et al. (2018) studied the metabolome of *P. viticola,* which cannot be obtained in pure culture due to its obligatory biotrophic way of life. Determination of these lipid compounds, namely ceramides, derivates of arachidonic and eicosapentaenoic acid, which are not found in healthy grapevine tissues, favours to efficiently monitor the infection progress of downy mildew, long before the appearance of any visible symptom of the disease. Moreover, this method is applicable for monitoring the downy mildew development in both susceptible and resistant grape varieties, with different patterns of lipid accumulation in each group. While the infection process is quickly stopped in a resistant genotype (*Vitis riparia*) presumably as the result of plant defence reaction and rapid lipid accumulation, a susceptible variety (‘Syrah’) accumulates considerably more lipids compared to a partially resistant (‘Bianca’) or resistant one (*Vitis riparia*) only during the later stages after being infected (72 hours and 6 days post-inoculation) (Negrel et al., 2018). By comparison of image analysis-based quantification of sporulation area on leaf discs and quantification of lipid markers of *P. viticola* (mostly arachidonic and eicosapentaenoic acid), a highly positive correlation was observed. Thus, these lipids can be considered as *Plasmopara*-specific, in the context of grapevine-downy mildew interaction. Although pre-symptomatic detection of downy mildew can be achieved using chlorophyll fluorescence imaging (Cséfalvay et al., 2009), this approach is not quantitative. Abovementioned *P. viticola*-lipid markers are characterised by ultra-high-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS)-based method that allows a sensitive, continuous and quantitative monitoring of downy mildew progression inside infected grapevine tissue (Negrel et al., 2018).

3.4.4. Infection Strategies – Jasmonic/Salicylic Acid

Based on the lifecycle and infection strategies, pathogenic microorganisms can be classified as necrotrophic, biotrophic or hemibiotrophic. After being attacked, plants activate their defence mechanism that is tightly regulated by hormone-mediated signalling pathways, mainly jasmonic acid and salicylic acid. It is generally assumed that inducible defence against leaf-chewing insects and necrotrophic microbes (e.g., *Botrytis cinerea*) is mediated by jasmonic acid and ethylene-dependent signaling, whereas salicylic acid is activated when a pathogen is a biotroph or hemibiotroph (e.g., *Plasmopara viticola*, *Erysiphe necator*, *Agrobacterium vitis*, *Xylella fastidiosa*, viruses) and systemic acquired resistance is established (Glazebrook, 2005). Moreover, it is believed that plants infected by biotrophic pathogen supress jasmonic acid-mediated responses. However, these generalities are disputed in grapevine as jasmonic acid signalling has been involved in resistance against biotrophs (Guerreiro et al., 2016).

On the first hours after *Plasmopara viticola* inoculation, both salicylic acid and jasmonic acid signalling pathways are simultaneously activated and act synergistically, but an antagonistic mechanism between the two pathways may be present at later time-points (Guerreiro et al., 2016). After that, reactive oxygen species (ROS) starts to increase following a localized programmed cell death in the infected green tissue. In that way, grapevine destroys its own infected cells together with the pathogen since the pathogen access to nutrients and water was limited (Glazebrook, 2005). This occurrence is realistic only for resistant cultivars due to their ability to recognize and respond swiftly to the pathogen invasion.

Generally, plants employ sophisticated defence mechanisms in order to prevent diseases caused by pathogenic microorganisms. To gain a better understanding of the infection process, it is essential to understand both sides: the attacking strategy of the pathogen along with the defence reaction of the host plant (Gómez-Zeledón et al., 2017). Three types of plant–pathogen interactions are known to date. The first one is a basal immune response called Microbe Associated Molecular Patterns (MAMP) or Pathogen Associated Molecular Patterns (PAMP)-triggered immunity (PTI) which is activated after recognition of non-adapted pathogens. This represents the first line of defence, and it is a feature which is prevailing in all plants of the same species facing a potentially pathogenic organism. It is activated by the recognition of conserved molecules (or patterns). Plant cells possess Specific Pattern Recognition Receptors (PRRs) which are placed on their plasma membranes. These plant receptors perceive the presence of pathogen- and host-derived molecules released during infection within the host tissue apoplast (the plant intracellular space).

The effector-triggered susceptibility (ETS) is the second type of interaction. In this case, certain microorganisms are able to overcome the basal plant response due to their secretion of virulence factors (proteins called effectors) which inhibit PTI, thus the disease continues to develop. A host immune system is suppressed and manipulated to the pathogen's benefit, e.g., by promoting nutrient leakage. Consequently, plants evolved the effector-triggered immunity (ETI) where a plant genotype can identify pathogen effectors through disease-resistance proteins (R), which are plant receptors (Armijo et al., 2016; Buonassisi et al., 2017). Jones and Dangl (2006) proposed the 'zigzag' model which describes a strong selective pressure on both the pathogen and the host plant. While the pathogen struggles to avoid ETI by diversifying its own effectors or by obtaining new ones, the plant, in turn, evolves new receptors so that ETI is triggered again. If an effector is recognized by a resistant protein, it is considered as an avirulence factor (AVR). This interaction leads to the activation of hypersensitive response (HR) meaning that the pathogen is avirulent to the plant (Armijo et al., 2016; Buonassisi et al., 2017). The metabolomic changes that happen in grapevines' leaves upon downy mildew infection are presented in the Table 1.

3.5. Proteomics

Proteomics is the comprehensive study of proteins in an organism, organ, tissue or cell. Nowadays mass spectrophotometry is used for proteins identification and quantification in almost all proteomics. To identify proteins by mass spectrophotometry, a protein sequence database is required. In comparative proteomics 2D-PAGE and iTRAQ are used in order to determine differences in protein amounts among different samples. Post-translational modifications of proteins, such as acetylation, glycosylation and phosphorylation, can be monitored in proteomics (Shiratake and Suzuki, 2016). A few studies regarding proteomics of grapevine as a response to downy mildew have been conducted (Palmieri et al., 2012; Nascimento-Gavioli et al., 2017). Their aim is to identify proteins related to defence response, including pathogenesis related (PR) proteins and proteins involved in signal transduction of defence response. These types of analyses usually show induction of PR-like protein and nucleotide-binding-site-leucine-rich repeat (NBS-LRR) proteins, which detect pathogens (Shiratake and Suzuki, 2016). Downy mildew infection causes a strong and rapid induction of pathogenesis-related proteins and enzymes required for the synthesis of phenylpropanoid-derived compounds (Negrel et al., 2018).

Generally, in the grapevine genotype-downy mildew pathosystem that mainly relies on genetic resistance, it is useful to know the whole subsequent mechanism in detail. Many quantitative trait loci (QTL) have been identified among or sourced from wild *Vitis* and *Muscadinia* species (Merdinoglu et al., 2003; Fischer et al., 2004; Bellin et al., 2009). Grapevine defence systems rely on the resistance genes (R-genes). Nucleotide binding (NB) and leucine rich repeat (LRR) domains are parts of a major class of resistant genes and are arranged in clusters in plat genomes. Nonetheless, it is not easy to find the connection between the defence-related genes and the biochemical pathways they trigger since the proteins implicated in these reactions are also included in many different signalling pathways. However, during the last decade, scientists' expertise in plant–pathogens interactions have improved by virtue of proteomic analysis. These interactions are crucial in deciphering grapevine defence response and signalling cascades, together with clarifying which pathogenicity factors and pathogen effectors are included in these reactions (Nascimento-Gavioli et al., 2017).

Resistance can be either constitutive or induced. Induced resistance is characterised by a change in the immune response to a pathogen attack that is triggered by a pathogen attack. Thus, this defence mechanism is active only when the infection is present (Colova-Tsolova et al., 2009). Different pathogenesis-related proteins can accumulate in leaves and berries in either constitutive or induced manner. However, it is not known to what extent they contribute to disease resistance or stress reduction. In some cases, although pathogenesis-related proteins are detected during the pathogen invasion, a host is not completely resistant, meaning that these proteins require supplementary more specific proteins (Gomès and Coutos-Thévenot, 2009). There are two major pathways that are included in down-regulation of the proteome of infected plants: the asparagine biosynthesis and glyoxylate cycle and fatty acid degradation pathway. The amino acid asparagine proves to be ideal nitrogen storage and transport compound due to its high N/C ratio. Thus, it has an important biological role in plants acting as a nitrogen recycler during abiotic and biotic stresses since it transports nitrogen from source to sink (Gaufichon et al., 2010).

Unlike induced resistance, constitutive resistance involves morphological or biochemical entities present in a host before a pathogen attack, as a part of host basal immune system (Colova-Tsolova et al., 2009). Such a pathogenesis-related protein is a thaumatin-like protein (PR-5), which is detected in mock inoculated samples (Wielgoss and Kortekamp, 2006). The same protein is detected in both resistant and susceptible variety after being inoculated with downy mildew as a part of constitutive resistance. Another protein (PR-10) also found as a constitutive part of resistance improved its expression upon inoculation. However, two chitinases (PR-3 and PR-4) are constitutively expressed in resistant cultivar without additional induction (Kortekamp, 2006).

Conducting research on completely susceptible and resistant grapevines containing pyramided Rpv1 and Rpv3 loci, which are responsible for defence response to P. viticola, Nascimento-Gavioli et al. (2017) concluded that major proteomic alternation takes place 48 and 96 hours after infection. Identified proteins are mainly included in functional categories of redox and energy metabolism. L-ascorbate degradation pathway is the major altered pathway which up-regulates antioxidant metabolism in response to apoplastic oxidative burst caused by infection.

Table 1. Metabolomic changes in grapevines' leaves upon downy mildew infection

<table>
<tr><th rowspan="2">Genotype</th><th colspan="6">Hours (hpi) or days (dpi) post inoculation</th><th rowspan="2">Reference</th></tr>
<tr><th>6 hpi</th><th>12 hpi</th><th>24 hpi</th><th>48 hpi</th><th>96 hpi</th><th>6 dpi</th></tr>
<tr><td rowspan="5">Bianca</td><td rowspan="5"></td><td colspan="3">Sugars, organic acids, amino acids</td><td rowspan="4">Phenypropanoids, flavonols, stilbenes, stilbenoids (<i>trans</i>- and <i>cis</i>-piceid, isorhapontin, ampelopsin H + vaticanol C-like isomer, α-viniferin, pallidol)</td><td rowspan="5"></td><td rowspan="5">Chitarrini et al., (2017)</td></tr>
<tr><td><i>Trans</i>-resveratrol</td><td colspan="2">Proline</td></tr>
<tr><td rowspan="3"></td><td>Ceramide</td><td><i>Trans</i>-ε-viniferin</td></tr>
<tr><td rowspan="2"></td><td>Phenylalanine</td></tr>
<tr><td colspan="2">Benzaldehyde</td></tr>
<tr><td>F1 population of Merzling × Teroldego</td><td></td><td></td><td></td><td></td><td></td><td><i>Trans</i>-resveratrol, trans-piceid, trans-pterostilbene, (+)-E-ε-viniferin, aviniferin, pallidol, ampelopsin D, quadrangularin A, Z- and E- ω-viniferin, Z- and E-miyabenol C, isohopeaphenol, ampelopsin H and vaticanol-C-like isomer</td><td>Malacarne et al., (2011)</td></tr>
<tr><td>Regent</td><td></td><td colspan="2">12-oxophytodienoic acid, jasmonic acid, (+)-7-iso-jasmonoyl-L-isoleucine, salicylic acid</td><td></td><td></td><td></td><td>Guerreiro et al., (2016)</td></tr>
<tr><td rowspan="4">Trincadeira</td><td>Glucose, fructose, glutamic acid, succinic acid, ascorbic acid</td><td>Starch</td><td rowspan="2"></td><td rowspan="4"></td><td rowspan="4"></td><td rowspan="4"></td><td rowspan="4">Nascimento et al., (2019)</td></tr>
<tr><td colspan="2">Hydrogen peroxide</td></tr>
<tr><td colspan="3">Lipid peroxidation</td></tr>
<tr><td>Fatty acyls</td><td>Fatty acyls</td><td>Flavonoids, phenylpropanoids</td></tr>
<tr><td>SO4</td><td></td><td></td><td></td><td></td><td>Monoterpenes, sesquiterpenes</td><td></td><td rowspan="2">Algarra Alarcon et al., (2015)</td></tr>
<tr><td>Kober 5BB</td><td></td><td></td><td></td><td></td><td>Sesquiterpenes</td><td></td></tr>
<tr><td>Marselan</td><td></td><td></td><td></td><td></td><td>Monoterpenes (<i>trans</i>-β-ocimene), sesquiterpenes ((<i>E</i>,<i>E</i>)-α-farnesene)</td><td></td><td>Chalal et al., (2015)</td></tr>
<tr><td>Syrah, Bianca, <i>V. riparia</i></td><td></td><td></td><td colspan="4">Ceramides, derivates of arachedonic and eicosapentaenoic acid</td><td>Negrel et al., (2018)</td></tr>
</table>

It is possible to discriminate genotypes according to their proteome profile, either constitutively or in response to the pathogen, according to Figueiredo et al., (2017) who have compared the grapevine cultivars ‘Regent’ (resistant) and ‘Trincadeira’ (susceptible). At each time point, (0, 6, 12 and 24 hours post inoculation) of analysis accumulation of mainly specific proteins is determined. Prior to inoculation, higher accumulation of proteins involved in primary metabolism and biosynthetic machinery is found in ‘Regent’, which proves its constitutive resistance, hence resulting in more stress-resistant plants. However, at 12 hours post infection photosynthesis and carbohydrate-related proteins are down-accumulated in ‘Regent’. This can be explained by inducing defence mechanisms in order to protect the photosynthetic system. Reactive oxygen species (ROS) are important in plant resistance since they strengthen host cell walls via cross-linking of glycoproteins, lipid peroxidation and activation of ROS-signalling networks leading to the establishment of a resistance response. In addition, they are related to other plant signalling molecules, particularly with salicylic acid (SA) and nitric oxide (NO). After inoculation with *P. viticola*, production of hydrogen peroxide is quicker and stronger in the resistant genotype. Despite the ROS burst in the resistant genotype, the total antioxidant capacity is not significantly different in comparison with susceptible variety. A key factor in incompatible interactions and cell death responses is the secondary production of ROS. By maintaining a low abundance of ROS scavenging proteins and not increasing the total antioxidant capacity, ‘Regent’ is probably promoting the secondary production of ROS following pathogen recognition. At 12 hours post inoculation, a plastid lipid associated protein is found in high levels in ‘Regent.’ That protein belongs to the group termed fibrillins, which are the basis for producing lipid droplets that contain free fatty acids, carotenoids, phytols, quinones, and other lipophilic compounds. Their level is correlated with the level of jasmonic acid in stressed plants. Jasmonic acid is a signalling molecule that induced the resistance response in ‘Regent’ (Figueiredo et al., 2017).

One of the key processes in early plant defence signalling is enhanced lipid peroxidation and production of a vast array of oxylipins. Lipid peroxidation is often linked to cell apoptosis, necrosis and programmed cell death, and to the synthesis of jasmonic acid. After inoculation with *P. viticola*, lipid peroxidase increased in resistant and susceptible variety. However, its product is much higher in the resistant one. By comparing resistant and susceptible genotypes, an increased number of infection responsive proteins is mainly found at 24 hours post inoculation (Figueiredo et al., 2017), while earlier changes can be found at the transcriptional level (6 hours post inoculation) in both types of genotypes (Figueiredo et al., 2012).

Palmieri et al. (2012) used the microorganism *Trichoderma harzanium* T39 (T39) that induces plant-mediated resistance and reduces the severity of downy mildew in susceptible grapevines. Treatments with this filamentous fungus have been found to activate grapevine resistance to downy mildew without negative effects on grapevine growth. T39 induced resistance is mediated by direct modulation of defence-related genes and by their enhanced expression after pathogen inoculation (Perazzolli et al., 2008; 2011). While T39 acts antagonistically to zoospores germination, it leads to pronounced accumulation of callose and high amounts of reactive oxygen species (ROS) in stomata guard cells at early stages upon inoculation (Palmieri et al., 2012).

Concerning grapevines affected by *P. viticola* and untreated with T39, grapevine proteome response reveals weak pathogen recognition coupled with an ineffective attempt to activate a resistance response early after infection. NBS-R proteins are responsible for microbial recognition and signal transduction. Although their increasing in abundance is possible, it has not corresponded to an effective activation of resistance response in grapevine. This is a part of pathogen defence suppression strategy. Suppression of endogenous signalling pathways by pathogenic effectors is probably required to establish compatible interactions and is followed by metabolic reprogramming associated with compatibility. Suppression of defence responses at early stages post infection is related to the repression of grapevine proteins involved in signal transduction processes (e.g., an RAF-like MAP3Ks involved in ET-mediated signalling and the homologue of resistance-inducing protein PBS1). In addition, proteins associated with hormone metabolism and hormone signalling (e.g., a 1-aminocyclopropane-1-carboxilate synthase, a lipoxygenase, an RRM-containing protein, polyubiquitin 10, and a PKN/PRK1 effector-like domain protein) are less abundant after *P. viticola* inoculation. A decreasing can be found among two NADPH-oxidases, a peroxiredoxin 2B and peroxiredoxin Q (PrxQ), which is also found at the oil spot stage (Polesani et al., 2008; Milli et al., 2012; Palmieri et al., 2012).

Moreover, a decreased abundance of proteins associated with defence, responses to abiotic stress, secondary metabolism and transport are found in early phases upon inoculation with *P. viticola*. Proteins related to transport, voltage-dependent anion channel (VDAC) proteins, are involved in the formation of permeability transition pores, which can contribute to cell shrinkage during the hypersensitive response. Since obligate biotrophic oomycetes require living host cells to complete their infection cycle, the decrease in abundance of VDAC could be attributed to *P. viticola* attempt to keep the host alive. Finally, the main decrease in abundance induced by *P. viticola* inoculation involves proteins related to photosynthesis (PSII D2 proteins, PSI subunit F and D1, ferredoxin-NADP-oxidoreductase 1 and 2), pentose phosphate cycle (aldolase, GAP, FBPase, phosphoglycerate kinase, and PRK) and photorespiration (glycine decarboxylase P-protein 1 and 2) (Palmieri et al., 2012).

Treatment of susceptible grapevine variety with T39 directly affects proteins associated with signal transduction, response to stresses, response to stimuli and energy metabolism. Increasing abundance takes place among the receptor-recognition proteins, a probable LRR-kinase, a receptor-like protein kinase, and three NBS-R proteins. Among the stress related proteins, T39 increases the abundance of a member of the non-specific lipid transfer proteins (nsLTP) family. Several LTPs possesses antimicrobial properties. LTPs isoforms are specifically involved in plant responses since one nsLTP isoform increases in abundance after T39 treatment, while another nsLTP isoform increases in abundance upon *P. viticola* inoculation of T39-treated plants. Regarding hormone metabolism and signalling, a marker for the jasmonic acid pathway displays a decrease in abundance upon T39 treatment (Palmieri et al., 2012).

In case when *Trichoderma*-treated plants are challenged with a pathogen, defence gene expression and protective enzyme activity are enhanced compared to inoculated control plants. Proteins affected by *P. viticola* in T39-treated plants are mainly associated with response to stress, photosynthesis, redox signalling, and energy metabolism. Proteins associated with photosynthesis and energy metabolism mostly increased in abundance in T39-treated plants in response to *P. viticola*, highlighting a specific reaction of plants treated with the resistance inducer. Defence-related reinforcement of cell walls is evidenced by callose deposition around stomata upon inoculation. An increased abundance of the protein associated with signal transduction, which has been associated with resistance to *Peronospora parasitica* and *Pseuomonas syringae* in *Arabidopsis,* suggest the existence of a common protein that may be required for multiple resistance protein signal cascades (Warren et al., 1998; Palmieri et al., 2012).

The concentration of oxidising species induced upon *P. viticola* inoculation is kept under control by an array of enzymes (a glutathione reductase, a copper/zinc superoxide dismutase, a glutaredoxin) in order to avoid biological damage. *Trichoderma*–plant cross talk is dynamic and regulation of jasmonic acid/ethylene and salicylic acid pathways may be essential for an efficient defence mechanism. Thus, in the *Trichoderma*–grapevine interaction, cooperation between hormone pathways could help the plant to minimise energy costs and to create a flexible signalling network to adjust defence response to invaders (Hermosa et al., 2012; Palmieri et al., 2012).

3.6. Genomics

The plant–pathogen interaction is a complex system since it involves the interference between two genomes, that of the host and of the pathogen. Not only that terms ‘susceptible’ or ‘resistant’ to *P. viticola* is important in these interactions, but also is ‘specificity.’ It covers both the extent to which a pathogen is limited to a specific host and reverse: extent to which the plant is or not susceptible to a specific pathogen. The gene-for-gene hypothesis is established in classical genetics work, which explains that a single resistance gene in plant recognises only those pathogen biotypes that have a corresponding avirulence gene (Colova-Tsolova et al., 2009).

Grapevine is the first fruit crop whose genome is completely sequenced (Jaillon et al., 2007). The first draft genome sequence of *P. viticola* is provided as well (Dussert et al., 2016). Comparison of genome sequences among different cultivars is an effective approach to identify genes for cultivar-specific traits (Shiratake and Suzuki, 2016). During recent years, numerous genetic loci in grapevine have been described that are linked with a specific phenotype, which is of high value for marker-assisted selection (MAS) in grapevine breeding. To date, a total of 27 Quantitative Trait Loci (QTLs) have been found and described among *Vitis* germplasm. This database is available within the *Vitis* International Variety Catalogue (VIVC) (Maul and Topfer, 2015) and proves to be systemic in the naming of loci (Hausmann et al., 2019). The major loci that carries resistant genes have their origins in *Muscadinia rotundifolia* (Merdinoglu et al., 2003), *Vitis riparia* (Marguerit et al., 2009; Moreira et al., 2011), *Vitis amurensis* (Blasi et al., 2011; Venuti et al., 2013), *Vitis cinerea* (Ochssner et al., 2016) and *Vitis rupestris* (Divilov et al., 2018).

The resistance mechanism starts with gene-for-gene recognition, thus providing signal cascades and ends with defence response. In that sense, locus *Rpv3* is of specific interest since within its genomic region a hot spot of NBS-LRR genes is identified. QTL mapping shows that downy mildew resistance is associated with *Rpv3-3* haplotype and stilbenoid induction. Genes belonging to the NBS-LRR superfamily are detected in the regions underlaying QTLs with disease resistance parameters (Vezzulli et al., 2019), in agreement with the first studied Rpv3 resistance haplotype encoding NB-LRR and LRR-kinase receptors (Di Gaspero and Foria 2015). According to the Effector-Triggered Immunity model, resistant gene products sense the pathogen effectors and activate signal transduction pathways (Cui et al., 2015).

In grapevine, *Rpv-3* dependent resistance follows this model of gene-for-gene interaction. The resistance haplotype is revealed to be necessary and sufficient to trigger a hypersensitive response (HR) leading to the cell death in the proximity of sites infected by *P. viticola* (Bellin et al., 2009). Although most polyphenols do not have a known QTL associated, Vezzulli et al., (2019) have identified some a few mQTLs which reveal polyphenols with a central role in *P. viticola*–grapevine interaction. In particular, this analysis allowed the identification of mQTLs associated with 17 different stilbenoid-related parameters, therefore representing a thorough characterisation of stilbenoid regulation upon *P. viticola* infection on leaves. To date, many loci responsible for downy mildew resistance have been identified among interspecific hybrids, wild *Vitis*, and *Muscadinia* species, some of which are presented in the Table 2.

Table 2. Loci of resistance to the downy mildew disease

Genotype	Rpv1	Rpv2	Rpv3	Rpv3-1 299-279	Rpv3-2 null-297	Rpv3-3 null-271	Rpv3 321-312	Rpv3 361-299	Rpv3 299-314	Rpv3 null-287	Rpv10	Rpv12	Rpv14	Reference
Artaban	Rpv1		Rpv3											Schneider et al., (2019)
Bianca				Rpv3-1 299-279										Bellin et al., (2009)
Börner													Rpv14	Ochssner et al., (2016)
Bronner						Rpv3-3 null-271					Rpv10			Zini et al., (2019)
Chancellor							Rpv3 321-312			Rpv3 null-287				
Couderc 13									Rpv3 299-314					
Esther				Rpv3-1 299-279										
Fanny								Rpv3 361-299						
Floreal	Rpv1		Rpv3											Schneider et al., (2019)
GM 6495-3						Rpv3-3 null-271					Rpv10			Zini et al., (2019)
Katharina				Rpv3-1 299-279	Rpv3-2 null-297									
Kunleany												Rpv12		
Merzling						Rpv3-3 null-271								Vezzulli et al., (2019)
Munson					Rpv3-2 null-297									Di Gaspero et al., (2012)
Muscadinia rotundifolia	Rpv1	Rpv2												Merdinoglu et al., (2003) Wiedemann-Merdinoglu et al., (2006)
Muscat Bleu				Rpv3-1 299-279			Rpv3 321-312							Zini et al., (2019)

Genotype	Rpv1	Rpv2	Rpv3	Rpv3-1 299-279	Rpv3-2 null-297	Rpv3-3 null-271	Rpv3 321-312	Rpv3 361-299	Rpv3 299-314	Rpv3 null-287	Rpv10	Rpv12	Rpv14	Reference
Nero				Rpv3-1 299-279										
Noah						Rpv3-3 null-271	Rpv3 321-312							Di Gaspero et al., (2012)
Palatina				Rpv3-1 299-279										Zini et al., (2019)
Petra												Rpv12		
Philipp				Rpv3-1 299-279										
Pölöskei Muskotaly				Rpv3-1 299-279										
Regent			Rpv3	Rpv3-1 299-279										Welter et al., (2007); Van Heerden et al., (2014)
Seibel 4614				Rpv3-1 299-279										Zini et al., (2019)
Severnyi											Rpv10			
Seyval					Rpv3-2 null-297	Rpv3-3 null-271								
Seyve Villard 12375				Rpv3-1 299-279				Rpv3 361-299						
Solaris											Rpv10			Schwander et al., (2012)
Sophie				Rpv3-1 299-279										Zini et al., (2019)
V. amurensis											Rpv10	Rpv12		Schwander et al., (2012) Venuti et al., (2013)
Vidoc	Rpv1		Rpv3											Schneider et al., (2019)
Villard Blanc				Rpv3-1 299-279				Rpv3 361-299						Zyprian et al., (2005)
Voltis	Rpv1		Rpv3											Schneider et al., (2019)
VRH30-82-1-42	Rpv1													Zini et al., (2019)
Zarya Severa												Rpv12		

CONCLUSION

In this chapter, the importance of omics studies is described and highlighted as they are used for detailed metabolic pathways upon downy mildew infection in grapevines. Knowing the secondary metabolic pathway, which is specific for each plant species and very often for each variety, is of primary value for improving the plant's natural immunity. In this way, it is also possible to distinguish between less and more susceptible grapevine varieties, which is a decisive feature in breeding programmes. Implementation of uniform scales and worldwide genetic databases, such as OIV descriptors and VIVC table of loci for resistance, make scientific efforts systemic, traceable and comparable. The exponential development of high-throughput technologies reveals genes, metabolites, metabolic pathways and physiological processes included in defence mechanism against downy mildew disease in grapevines. Further tendencies lie in the field of enhancing the activity of compounds and pathways responsible for the grapevines' immunity system in an environmentally friendly manner that will reduce the use of chemical protection.

REFERENCES

Algarra-Alarcon, A., Lazazzara, V., Cappellin, L., Bianchedi, P. L., Schuhmacher, R., Wohlfahrt, G., Pertot, I., Biasioli, F., Perazzolli, M. (2015). Emission of volatile sesquiterpenes and monoterpenes in grapevine genotypes following *Plasmopara viticola* inoculation in vitro. *Journal of Mass Spectrometry,* 50 (8):1013-1022. doi:10.1002/jms.3615.

Ali, K., Maltese, F., Figueiredo, A., Rex, M., Fortes, A. M., Zyprian, E., Pais, M. S., Verpoorte, R., Choi, Y. H. (2012). Alterations in grapevine leaf metabolism upon inoculation with Plasmopara viticola in different time-points. *Plant Science,* 191-192:100-7. doi: 10.1016/j.plantsci.2012.04.014.

Armijo, G., Schlechter, R., Agurto, M., Muñoz, D., Nuñez, C., Arce-Johnson, P. (2016). Grapevine pathogenic microorganisms: understanding infection strategies and host response scenarios. *Frontiers in Plant Science,* 7:382. doi: 10.3389/fpls.2016.00382.

Aziz, A., Poinssot, B., Daire, X., Adrian, M., Bezier, A., Lambert, B., Joubert, J. M., Pugin A. (2003). Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. *Molecular Plant-Microbe Interactions,* 16 (12):1118-1128. doi: 10.1094/mpmi.2003.16.12.1118.

Bellin, D., Peressotti, E., Merdinoglu, D., Wiedemann-Merdinoglu, S., Adam-Blondon, A. F., Cipriani, G., Morgante, M., Testolin, R., Di Gaspero, G. (2009). Resistance to *Plasmopara viticola* in grapevine 'Bianca' is controlled by a major dominant gene causing localised necrosis at the infection site. *Theoretical and Applied Genetics,* 120 (1):163-176. doi: 10.1007/s00122-009-1167-2.

Blasi, P., Blanc, S., Wiedemann-Merdinoglu, S., Prado, E., Ruhl, E. H., Mestre, P., Merdinoglu, D. (2011). Construction of a reference linkage map of *Vitis amurensis* and genetic mapping of *Rpv8*, a locus conferring resistance to grapevine downy mildew. *Theoretical and Applied Genetics,* 123 (1):43-53. doi: 10.1007/s00122-011-1565-0.

Buonassisi, D., Cappellin, L., Dolzani, C., Velasco, R., Peressotti, E., Vezzulli, S. (2018). Development of a novel phenotyping method to assess downy mildew symptoms on grapevine inflorescences. *Scientia Horticulturae,* 236:79-89. doi: 10.1016/j.scienta. 2018.03.023.

Buonassisi, D., Colombo, M., Migliaro, D., Dolzani, C., Peressotti, E., Mizzotti, C., Velasco, R., Masiero, S., Perazzolli, M., Vezzulli, S. (2017). Breeding for grapevine downy mildew resistance: a review of “omics” approaches. *Euphytica,* 213 (5):103. doi: 10.1007/s10681-017-1882-8.

Burruano, S. (2000). The life-cycle of *Plasmopara viticola,* cause of downy mildew of vine. *Mycologist,* 14 (4):179-182. doi:https://doi.org/10.1016/S0269-915X(00)80040-3.

Calonnec, A., Wiedemann-Merdinoglu, S., Deliere, L., Cartolaro, P., Schneider, C., Delmotte, F. (2013). The reliability of leaf bioassays for predicting disease resistance on fruit: a case study on grapevine resistance to downy and powdery mildew. *Plant Pathology,* 62 (3):533-544. doi: 10.1111/j.1365-3059.2012.02667.x.

Chalal, M., Winkler, J. B., Gourrat, K., Trouvelot, S., Adrian, M., Schnitzler, J.P., Jamois, F., Daire, X. (2015). Sesquiterpene volatile organic compounds (VOCs) are markers of elicitation by sulfated laminarine in grapevine. *Frontiers in Plant Science,* 6:9. doi: 10.3389/fpls.2015.00350.

Chitarrini, G., Soini, E., Riccadonna, S., Franceschi, P., Zulini, L., Masuero, D., Vecchione, A., Stefanini, M., Di Gaspero, G., Mattivi, F., Vrhovsek, U. (2017). Identification of biomarkers for defense response to *Plasmopara viticola* in a resistant grape variety. *Frontiers in Plant Science,* 8:11. doi: 10.3389/fpls.2017. 01524.

Colova-Tsolova, V., Perl, S., Krastanova, A., Samuelian, S., Atanassov A. (2009). Progress in genetic engineering of grapevine for disease & stress tolerance. In *Grapevine Molecular Physiology & Biotechnology,* edited by Kalliopi A. Roubelakis-Angelakis, 509-533. Dordrecht: Springer Netherlands.

Cséfalvay, L., Di Gaspero, G., Matouš, K., Bellin, D., Ruperti, B., Olejníčkov, J. (2009). Pre-symptomatic detection of Plasmopara viticola infection in grapevine leaves using chlorophyll fluorescence imaging. *European Journal of Plant Pathology,* 125 (2):291-302. doi: 10.1007/s10658-009-9482-7.

Cui, H. T., Tsuda, K., Parker, J. E. (2015). Effector-triggered immunity: from pathogen perception to robust defense. *Annual Review of Plant Biology,* 66:487-511. doi: 10. 1146/annurev-arplant-050213-040012.

Dai, G. H., Andary, C., Mondolotcosson, L., Boubals, D. (1995). Involvement of phenolic-compounds in the resistance of grapevine callus to downy mildew (*Plasmopara viticola*). *European Journal of Plant Pathology,* 101 (5):541-547. doi: 10.1007/bf01874479.

Díez-Navajas, A. M., Wiedemann-Merdinoglu, S., Greif, C., Merdinoglu, D. (2008). Nonhost versus host resistance to the grapevine downy mildew Plasmopara viticola, studied at the tissue level. *Phytopathology,* 98:776-780. doi: 10.1094/phyto-98-7-0776.

Di Gaspero, G., Copetti, D., Coleman, C., Castellarin, S. D., Eibach, R., Kozma, P., Lacombe, T., Gambetta, G., Zvyagin, A., Cindric, P., Kovacs, L., Morgante, M., Testolin R. (2012). Selective sweep at the *Rpv3* locus during grapevine breeding for downy mildew resistance. *Theoretical and Applied Genetics,* 124 (2):277-286. doi: 10.1007/s00122-011-1703-8.

Di Gaspero, G., Foria, S. (2015). Molecular grapevine breeding techniques. In *Grapevine Breeding Programs for the Wine Industry,* edited by Andrew Reynolds, 23-37. Oxford: Woodhead Publishing.

Divilov, K., Wiesner-Hanks, T., Barba, P., Cadle-Davidson, L., Reisch B. I. (2017). Computer Vision for High-Throughput Quantitative Phenotyping: A Case Study of Grapevine Downy Mildew Sporulation and Leaf Trichomes. *Phytopathology,* 107 (12):1549-1555. doi: 10.1094/phyto-04-17-0137-r.

Divilov, K., Barba, P., Cadle-Davidson, L., Reisch, B. I. (2018). Single and multiple phenotype QTL analyses of downy mildew resistance in interspecific grapevines. *Theoretical and Applied Genetics,* 131 (5):1133-1143. doi: 10.1007/s00122-018-3065-y.

Dussert, Y., Gouzy, J., Richart-Cervera, S., Mazet, I. D., Deliere, L., Couture, C., Legrand, L., Piron, M. C., Mestre, P., Delmotte, F. (2016). Draft Genome Sequence of Plasmopara viticola, the Grapevine Downy Mildew Pathogen. *Microbiology Resource Announcements,* 4 (5):2. doi: 10.1128/genomeA.00987-16.

Figueiredo, A., Monteiro, F., Fortes, A. M., Bonow-Rex, M., Zyprian, E., Sousa, L., Pais, M.S. (2012). Cultivar-specific kinetics of gene induction during downy mildew early infection in grapevine. *Functional & Integrative Genomics*, 12 (2):379-386. doi: 10.1007/s10142-012-0261-8.

Figueiredo, A., Martins, J., Sebastiana, M., Guerreiro, A., Silva, A., Matos, A. R., Monteiro, F., Pais, M. S., Roepstorff, P., Coelho, A. V. (2017). Specific adjustments in grapevine leaf proteome discriminating resistant and susceptible grapevine genotypes to *Plasmopara viticola*. *Journal of Proteomics,* 152:48-57. doi: 10.1016/j.jprot.2016.10.012.

Fischer, B. M., Salakhutdinov, I., Akkurt, M., Eibach, R., Edwards, K. J., Topfer, R., Zyprian, E. M. (2004). Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. *Theoretical and Applied Genetics,* 108 (3):501-515. doi: 10.1007/s00122-003-1445-3.

Garcia-Brugger, A., Lamotte, O., Vandelle, E., Bourque, S., Lecourieux, D., Poinssot, B., Wendehenne, D., Pugin, A. (2006). Early signaling events induced by elicitors of plant defenses. *Molecular Plant-Microbe Interactions,* 19 (7):711-724. doi: 10.1094/mpmi-19-0711.

Gaufichon, L., Reisdorf-Cren, M. J. Rothstein, S., Chardon, F., Suzuki, A. (2010). Biological functions of asparagine synthetase in plants. *Plant Science,* 179 (3):141-153. doi: https://doi.org/10.1016/j.plantsci.2010.04.010.

Gessler, C., Pertot, I., Perazzolli, M. (2011). *Plasmopara viticola*: a review of knowledge on downy mildew of grapevine and effective disease management. *Phytopathologia Mediterranea,* 50 (1):3-44. doi: 10.14601/Phytopathol_Mediterr-9360.

Gindro, K., Pezet, R., Viret, O. (2003). Histological study of the responses of two *Vitis vinifera* cultivars (resistant and susceptible) to *Plasmopara viticola* infections. *Plant Physiology and Biochemistry,* 41 (9):846-853. doi: 10.1016/s0981-9428(03)00124-4.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology,* 43:205-227. doi: 10.1146/annurev.phyto.43.040204.135923.

Gobbin, D., Jermini, M., Loskill, B., Pertot, I., Raynal, M., Gessler, C. (2005). Importance of secondary inoculum of Plasmopara viticola to epidemics of grapevine downy mildew. *Plant Pathology,* 54 (4):522-534. doi: 10.1111/j.1365-3059.2005. 01208.x.

Gomès, E., Coutos-Thévenot, P. (2009). Molecular Aspects of Grapevine-Pathogenic Fungi Interactions. In *Grapevine molecular physiology and biotechnology*: Second Edition, 407-428. doi: 10.1007/978-90-481-2305-6_15.

Gomez-Zeledon, J., Kaiser, M., Spring, O. (2017). Exploring host-pathogen combinations for compatible and incompatible reactions in grapevine downy mildew. *European Journal of Plant Pathology,* 149 (1):1-10. doi: 0.1007/s10658-017-1156-2.

Gomez-Zeledon, J., Spring, O. (2018). Up-regulated RxLR effector genes of *Plasmopara viticola* in synchronized host-free stages and infected leaves of hosts with different susceptibility. *Fungal Biology,* 122 (12):1125-1133. doi: 10.1016/j.funbio.2018.07.006.

Guerreiro, A., Figueiredo, J., Silva, M. S., Figueiredo, A. (2016). Linking jasmonic acid to grapevine resistance against the biotrophic oomycete *Plasmopara viticola*. *Frontiers in Plant Science,* 7:7. doi: 10.3389/fpls.2016.00565.

Hardham, A. (2009). The Asexual Life Cycle. In *Oomycete Genetics and Genomics: Diversity, Interactions, and Research Tools*, 93-119. doi: 10.1002/9780470475898.ch5.

Hausmann, L., Maul, E., Ganesch, A., Toepfer, R. (2019). Overview of genetic loci for traits in grapevine and their integration into the VIVC database. *Acta Horticulturae*, 221-226. doi: 10.17660/ActaHortic.2019.1248.32.

Hermosa, R., Viterbo, A., Chet, I., Monte, E. (2012). Plant-beneficial effects of Trichoderma and of its genes. *Microbiology-Sgm,* 158:17-25. doi: 10.1099/mic.0.052274-0.

Jaillon, O., Aury, J. M., Noel, B., Policriti, A., Clepet, C., Casagrande, A., Choisne, N., Aubourg, S., Vitulo, N., Jubin, C., Vezzi, A., Legeai, F., Hugueney, P., Dasilva, C., Horner, D., Mica, E., Jublot, D., Poulain, J., Bruyere, C., Billault, A., Segurens, B., Gouyvenoux, M., Ugarte, E., Cattonaro, F., Anthouard, V., Vico, V., Del Fabbro, C., Alaux, M., Di Gaspero, G., Dumas, V., Felice, N., Paillard, S., Juman, I., Moroldo, M., Scalabrin, S., Canaguier, A., Le Clainche, I., Malacrida, G., Durand, E., Pesole, G., Laucou, V., Chatelet, P., Merdinoglu, D., Delledonne, M., Pezzotti, M., Lecharny, A., Scarpelli, C., Artiguenave, F., Pe, M. E., Valle, G., Morgante, M., Caboche, M., Adam-Blondon, A. F., Weissenbach, J., Quetier, F., Wincker, P. (2007). French-Italian public consortium for grapevine genome characterization. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature,* 449 (7161):463-U5. doi: 10.1038/nature06148.

Jones, J. D. G., Dangl, J. L. (2006). The plant immune system. *Nature,* 444 (7117):323-329. doi: 10.1038/nature05286.

Judelson, H. S. (2009). Sexual Reproduction in Oomycetes: Biology, Diversity, and Contributions to Fitness. In *Oomycete Genetics and Genomics: Diversity, Interactions, and Research Tools,* 121-138. doi: 10.1002/9780470475898.ch6.

Keller, M. (2015). *The Science of Grapevines: Anatomy and Physiology*: Academic Press.

Kennelly, M. M., Gadoury, D. M., Wilcox, W. F., Magarey, P. A., Seem, R. C. (2007). Primary infection, lesion productivity, and survival of sporangia in the grapevine downy mildew pathogen *Plasmopara viticola*. *Phytopathology,* 97 (4):512-522. doi: 10.1094/phyto-97-4-0512.

Khiook, I. L. K., Schneider, C., Heloir, M. C., Bois, B., Daire, X., Adrian, M., Trouvelot S., (2013). Image analysis methods for assessment of H2O2 production and *Plasmopara viticola* development in grapevine leaves: Application to the evaluation of resistance to downy mildew. *Journal of Microbiological Methods,* 95 (2):235-244. doi: 10.1016/j.mimet.2013.08.012.

Killigrew, B. X., Sivasithamparam K., (2005). Absence of oospores of downy mildew of grape caused by *Plasmopara viticola* as the source of primary inoculum in most Western Australian vineyards. *Plant Disease,* 89 (7):777-777. doi: 10.1094/pd-89-0777c.

Kishimoto, K., Matsui, K., Ozawa, R., Takabayashi, J. (2006). Analysis of defensive responses activated by volatile allo-ocimene treatment in Arabidopsis thaliana. *Phytochemistry,* 67 (14):1520-1529. doi: 10.1016/j.phytochem.2006.05.027.

Koopman, T., Linde, C. C., Fourie, P. H., McLeod, A. (2007). Population genetic structure of *Plasmopara viticola* in the Western Cape province of South Africa. *Molecular Plant Pathology,* 8 (6):723-736. doi: 10.1111/j.1364-3703.2007.00429.x.

Kortekamp, A. (2006). Expression analysis of defence-related genes in grapevine leaves after inoculation with a host and a non-host pathogen. *Plant Physiology and Biochemistry,* 44 (1):58-67. doi: 10.1016/j.plaphy.2006.01.008.

Kortekamp, A., Zyprian E. (1999). Leaf hairs as a basic protective barrier against downy mildew of grape. *Journal of Phytopathology-Phytopathologische Zeitschrift*, 147 (7-8):453-459. doi: 10.1046/j.1439-0434.1999.00409.x.

Kranz, J. (2003). *Comparative epidemiology of plant diseases*. doi: 10.1007/978-3-662-05261-7.

Li, X. L., Wu, J., Yin, L., Zhang, Y. L., Qu, J. J., Lu, J. (2015). Comparative transcriptome analysis reveals defense-related genes and pathways against downy mildew in *Vitis amurensis* grapevine. *Plant Physiology and Biochemistr* 95:1-14. doi: 10.1016/j.plaphy.2015.06.016.

Liu, R. Q., Wang, L., Zhu, J. L., Chen, T. T., Wang, Y. J., Xu, Y. (2015). Histological responses to downy mildew in resistant and susceptible grapevines. *Protoplasm* 252 (1):259-270. doi: 10.1007/s00709-014-0677-1.

Malacarne, G., Vrhovsek, U., Zulini, L., Cestaro, A., Stefanini, M., Mattivi, F., Delledonne, M., Velasco, R., Moser, C. (2011). Resistance to *Plasmopara viticola* in a grapevine segregating population is associated with stilbenoid accumulation and with specific host transcriptional responses. *Bmc Plant Biology,* 11:13. doi: 10.1186/1471-2229-11-114.

Marguerit, E., Boury, C., Manicki, A., Donnart, M., Butterlin, G., Nemorin, A., Wiedemann-Merdinoglu, S., Merdinoglu, D., Ollat, N., Decroocq, S. (2009). Genetic dissection of sex determinism, inflorescence morphology and downy mildew resistance in grapevine. *Theoretical and Applied Genetics,* 118 (7):1261-1278. doi: 10.1007/s00122-009-0979-4.

Maul, E., Topfer, R. (2015). Vitis International Variety Catalogue (VIVC): A cultivar database referenced by genetic profiles and morphology. *BIO Web of Conferences* 5, 01009. doi: 10.1051/bioconf/20150501009.

McKirdy, S. J., Riley, I. T., Cameron, I. J., Magarey, P. A. (1999). First report of grapevine downy mildew (*Plasmopara viticola*) in commercial viticulture in western Australia. *Plant Disease*, 83 (3):301-301. doi: 10.1094/pdis.1999.83.3.301d.

Merdinoglu, D., Wiedeman-Merdinoglu, S., Coste, P., Dumas, V., Haetty, S., Butterlin, G., Greif, C. (2003). Genetic analysis of downy mildew resistance derived from *Muscadinia rotundifolia*. *Acta Horticulturae,* 603:451-456. doi: 10.17660/ActaHortic.2003.603.57.

Milli, A., Cecconi, D., Bortesi, L., Persi, A., Rinalducci, S., Zamboni, A., Zoccatelli, G., Lovato, A., Zolla, L., Polverari, A. (2012). Proteomic analysis of the compatible interaction between *Vitis vinifera* and *Plasmopara viticola*. *Journal of Proteomics*, 75 (4):1284-1302. doi: 10.1016/j.jprot.2011.11.006.

Moreira, F. M., Madini, A., Marino, R., Zulini, L., Stefanini, M., Velasco, R., Kozma, P., Grando, M. S. (2011). Genetic linkage maps of two interspecific grape crosses (*Vitis* spp.) used to localize quantitative trait loci for downy mildew resistance. *Tree Genetics & Genomes*, 7 (1):153-167. doi: 10.1007/s11295-010-0322-x.

Nascimento-Gavioli, M. C. A., Agapito-Tenfen, S. Z., Nodari, R. O., Welter, L. J., Mora, F. D. S., Saifert, L., da Silva, A. L., Guerra, M. P. (2017). Proteome of *Plasmopara viticola*-infected *Vitis vinifera* provides insights into grapevine Rpv1/Rpv3 pyramided resistance to downy mildew. *Journal of Proteomics,* 151:264-274. doi: 10.1016/j.jprot. 2016.05.024.

Nascimento-Gavioli, M. C. A., Rockenbach, M. F., Welter, L., Guerra, M. P. (2019). Histopathological study of resistant (*Vitis labrusca* L.) and susceptible (*Vitis vinifera* L.) cultivars of grapevine to the infection by downy mildew. *The Journal of Horticultural Science and Biotechnology,* 1-11. doi: 10.1080/14620316.2019. 1685411.

Negrel, L., Halter, D., Wiedemann-Merdinoglu, S., Rustenholz, C., Merdinoglu, D., Hugueney, P., Baltenweck, R. (2018). Identification of Lipid Markers of *Plasmopara viticola* Infection in Grapevine Using a Non-targeted Metabolomic Approach. *Frontiers in Plant Science,* 9 (360). doi: 10.3389/fpls.2018.00360.

Ochssner, I., Hausmann, L., Topfer, R. (2016). Rpv14, a new genetic source for Plasmopara viticola resistance conferred by *Vitis cinerea*. *Vitis,* 55 (2):79-81. doi:10.5073/vitis.2016.55.79-81.

OIV (2009). Descriptor list for grape varieties and *Vitis* species, 2nd Edn. *Office International de la Vigne et du Vin*. Available online at: http://www.oiv.org.

OEPP/EPPO (2001). Guidelines for the efficacy evaluation of fungicides. OEPP/EPPO *Bulletin,* 31:313-317.

Palmieri, M. C., Perazzolli, M., Matafora, V., Moretto, M., Bachi, A., Pertot, I. (2012). Proteomic analysis of grapevine resistance induced by Trichoderma harzianum T39 reveals specific defence pathways activated against downy mildew. *Journal of Experimental Botany,* 63 (17):6237-6251. doi: 10.1093/jxb/ers279.

Perazzolli, M., Dagostin, S., Ferrari, A., Elad, Y., Pertot, I. (2008). Induction of systemic resistance against *Plasmopara viticola* in grapevine by *Trichoderma harzianum* T39 and benzothiadiazole. *Biological Control*, 47 (2):228-234. doi: 10.1016/j.biocontrol. 2008.08.008.

Perazzolli, M., Roatti, B., Bozza, E., Pertot, I. (2011). *Trichoderma harzianum* T39 induces resistance against downy mildew by priming for defense without costs for grapevine. *Biological Control*, 58 (1):74-82. doi: 10.1016/j.biocontrol.2011.04.006.

Peressotti, E., Duchene, E., Merdinoglu, D., Mestre, P. (2011). A semi-automatic non-destructive method to quantify grapevine downy mildew sporulation. *Journal of Microbiological Methods*, 84 (2):265-271. doi: 10.1016/j.mimet.2010.12.009.

Polesani, M., Desario, F., Ferrarini, A., Zamboni, A., Pezzotti, M., Kortekamp, A., Polverari, A. (2008). cDNA-AFLP analysis of plant and pathogen genes expressed in grapevine infected with *Plasmopara viticola*.” *Bmc Genomics*, 9:14. doi: 10.1186/1471-2164-9-142.

Polesani, M., Bortesi, L., Ferrarini, A., Zamboni, A., Fasoli, M., Zadra, C., Lovato, A., Pezzotti, M., Delledonne, M., Polverari, A. (2010). General and species-specific transcriptional responses to downy mildew infection in a susceptible (*Vitis vinifera*) and a resistant (*V. riparia*) grapevine species. *Bmc Genomics*, 11:16. doi: 0.1186/1471-2164-11-117.

Riemann, M., Buche, C., Kassemeyer, H. H., Nick. P. (2002). Cytoskeletal responses during early development of the downy mildew of grapevine (*Plasmopara viticola*). *Protoplasma*, 219 (1-2):13-22. doi: 10.1007/s007090200001.

Rojas, C. M., Senthil-Kumar, M., Tzin, V., Mysore, K. S. (2014). Regulation of primary plant metabolism during plant-pathogen interactions and its contribution to plant defense. *Frontiers in Plant Science*, 5:12. doi: 10.3389/fpls.2014.00017.

Rossi, V., Caffi, T., Bugiani, R., Spanna, F., Della Valle, D. (2008). Estimating the germination dynamics of Plasmopara viticola oospores using hydro-thermal time. *Plant Pathology,* 57 (2):216-226. doi: 10.1111/j.1365-3059.2007.01738.x.

Rouzet, J., Jacquin, D. (2003). Development of overwintering oospores of *Plasmopara viticola* and severity of primary foci in relation to climate*. *EPPO Bulletin,* 33 (3):437-442. doi: 10.1111/j.1365-2338.2003.00670.x.

Rumbolz, J., Wirtz, S., Kassemeyer, H. H., Guggenheim, R., Schafer, E., Buche, C. (2002). Sporulation of *Plasmopara viticola*: Differentiation and light regulation. *Plant Biology,* 4 (3):413-422. doi: 10.1055/s-2002-32342.

Schneider, C., Onimus, C., Prado, E., Dumas, V., Wiedemann-Merdinoglu, S., Dorne, M. A., Lacombe, M. C., Piron, M. C., Umar-Faruk, A., Duchêne, E., Mestre, P., Merdinoglu, D. (2019). INRA-ResDur: the French grapevine breeding programme for durable resistance to downy and powdery mildew. *Acta Horticulturae,* 1248: 207-214. doi: 10.17660/ActaHortic.2019.1248.30.

Schwander, F., Eibach, R., Fechter, I., Hausmann, L., Zyprian, E., Töpfer, R. (2012). Rpv10: a new locus from the Asian Vitis gene pool for pyramiding downy mildew resistance loci in grapevine. *Theoretical and Applied Genetics*, 124 (1):163-176. doi: 10.1007/s00122-011-1695-4.

Shiratake, K., Suzuki, M. (2016). Omics studies of citrus, grape and rosaceae fruit trees. *Breeding Science*, 66 (1):122-138. doi: 10.1270/jsbbs.66.122.

Töpfer, R., Hausmann, L., Harst, M., Maul, E., Zyprian, E. (2011). New horizons for grapevine breeding. In *Methods in Temperate Fruit Breeding*, edited by Henryk Flachowsky and Magda-Viola Hanke, 79-100. Ikenobe, Japan: Global Science Books, Ltd.

Trouvelot, S., Varnier, A. L., Allegre, M., Mercier, L., Baillieul, F., Arnould, C., Gianinazzi-Pearson, V., Klarzynski, O., Joubert, J. M., Pugin, A., Daire, X. (2008). A beta-1,3 glucan sulfate induces resistance in grapevine against Plasmopara viticola through priming of defense responses, including HR-like cell death. *Molecular Plant-Microbe Interactions*, 21 (2):232-243. doi: 10.1094/mpmi-21-2-0232.

Unger, S., Bueche, C., Boso, S., Kassemeyer, H. H. (2007). The course of colonization of two different vitis genotypes by Plasmopara viticola indicates compatible and incompatible host-pathogen interactions. *Phytopathology*, 97 (7):780-786. doi: 10. 1094/phyto-97-7-0780.

Venuti, S., Copetti, D., Foria, S., Falginella, L., Hoffmann, S., Bellin, D., Cindric, P., Kozma, P., Scalabrin, S., Morgante, M., Testolin, R., Di Gaspero, G. (2013). Historical introgression of the downy mildew resistance gene Rpv12 from the asian species *Vitis amurensis* into grapevine varieties. *Plos One*, 8 (4):7. doi: 10.1371/journal.pone. 0061228.

Van Heerden, C. J., Burger, P., Vermeulen, A., Prins, R. (2014). Detection of downy and powdery mildew resistance QTL in a 'Regent' × 'RedGlobe' population. *Euphytica*, 200:281-295. doi: 10.1007/s10681-014-1167-4.

Vezzulli, S., Malacarne, G., Masuero, D., Vecchione, A., Dolzani, C., Goremykin, V., Mehari, Z. H., Banchi, E., Velasco, R., Stefanini, M., Vrhovsek, U., Zulini, L., Franceschi, P., Moser C. (2019). The Rpv3-3 Haplotype and stilbenoid induction mediate downy mildew resistance in a grapevine interspecific population. *Frontiers in Plant Science*, 10:23. doi: 10.3389/fpls.2019.00234.

Voglmayr, H. (2008). Progress and challenges in systematics of downy mildews and white blister rusts: new insights from genes and morphology. In *The Downy Mildews - Genetics, Molecular Biology and Control*, edited by Aleš Lebeda, Peter T. N. Spencer-Phillips and B. M. Cooke, 3-18. Dordrecht: Springer Netherlands.

Wang, C. X., Wu, J., Zhang, Y. L., Lu, J. (2018). *Muscadinia rotundifolia* 'Noble' defense response to *Plasmopara viticola* inoculation by inducing phytohormone-mediated stilbene accumulation. *Protoplasma*, 255 (1):95-107. doi:10.1007/s00709-017-1118-8.

Warren, R. F., Henk, A., Mowery, P., Holub, E., Innes, R. W. (1998). A mutation within the leucine-rich repeat domain of the arabidopsis disease resistance gene RPS5 partially suppresses multiple bacterial and downy mildew resistance genes. *Plant Cell*, 10 (9):1439-1452. doi: 10.1105/tpc.10.9.1439.

Welter, L. J., Göktürk-Baydar, N., Akkurt, M., Maul E., Eibach, R., Töpfer R., et al., (2007). Genetic mapping and localization of quantitative trait loci affecting fungal disease resistance and leaf morphology in grapevine (*Vitis vinifera* L). *Molecular Breeding*, 20:359-374. doi: 10.1007/s11032-007-9097-7.

Wiedemann-Merdinoglu, S., Prado, E., Coste, P., Dumas, V., Butterlin, G., Bouquet, A., et al., (2006). Genetic analysis of resistance to downy mildew from Muscadinia rotundifolia. In *9th International Conference on Grape Genetics and Breeding* (Vol. 2, No. 06.07, p. 2006).

Wielgoss, A., Kortekamp, A. (2006). Comparison of PR1 expression in grapevine cultures after inoculation with a host-and a non-host pathogen. *Vitis - Journal of Grapevine Research*, 45.

Wong, F. P., Burr, H. N., Wilcox, W. F. (2001). Heterothallism in Plasmopara viticola. *Plant Pathology,* 50 (4):427-432. doi: 10.1046/j.1365-3059.2001.00573.x.

Yi, H. S., Heil, M., Adame-Alvarez, R. M., Ballhorn, D. J., Ryu C. M. (2009). Airborne induction and priming of plant defenses against a bacterial pathogen. *Plant Physiology,* 151 (4):2152-2161. doi: 10.1104/pp.109.144782.

Yin, X., Liu, R. Q., Su, H., Su, L., Guo, Y. R., Wang, Z. J., Du, W., Li, M. J., Zhang, X., Wang, Y. J., Liu, G. T., Xu, Y. (2017). Pathogen development and host responses to Plasmopara viticola in resistant and susceptible grapevines: an ultrastructural study. *Horticulture Research,* 4:10. doi: 10.1038/hortres.2017.33.

Zini, E., Dolzani, C., Stefanini, M., Gratl, V., Bettinelli, P., Nicolini, D., Betta, G., Dorigatti, C., Velasco, R., Letschka, T., Vezzulli, S. (2019). *R*-loci arrangement versus downy and powdery mildew resistance level: A *Vitis* hybrid survey. *International Journal of Molecular Sciences,* 20(14):3526. doi: 10.3390/ ijms20143526.

In: Vitis: Biology and Species
Editors: António M. Jordão et al.
ISBN: 978-1-53618-308-5

Chapter 9

CONTROL OF GRAPEVINE DISEASES IN AN 'OMIC' ERA

***Antonio Biasi*[1], *M. Mercedes Maldonado-González*[2] *and David Ruano-Rosa*[3,*]**
[1]Department of Postharvest Science, The Volcani Center, Rishon, Lezion, Israel
[2]Research Group in Engineering and Sustainable Agriculture,
Department of Engineering and Agricultural Sciences,
Environment Institute Natural Resources and Biodiversity,
University of León, León, Spain
[3]Woody and Horticultural Crops Unit (Agricultural Research Area),
Technological Agricultural Institute of Castilla y León, Valladolid, Spain

ABSTRACT

Grapevines are continually threatened by a number of diseases that impact negatively on the plant itself, affecting diverse plant compartments including roots, trunk, leaves and fruits with the consequent impact on wine production. Different strategies are proposed to address their control such as cultural, chemical, physical, biological or the combination of several of them. In the last few years, due to the technological advances, those strategies have begun to make use of omics technologies. Omics use to refer in a general way to different fields such as genomic, transcriptomic, proteomic, metabolomic and others. These high-throughput tools generate a large amount of data that allow to evaluate cellular responses to changes in the environment (biotic or abiotics factors). Their application in plant pathology are providing a major advance for a better understanding of pathogen interaction with the plant and/or the biological control agents, the effect of chemical treatments, etc. The present chapter provides an overview on the state of the art in the application of omics technologies as a tool to control grapevine diseases.

---

[*] Corresponding Author's Email: ruanodavid@gmail.com.

Keywords: disease control, genomics, metabolomics, plant pathogens, proteomics, transcriptomics

1. INTRODUCTION

Plant diseases have an enormous impact on agriculture not only quantitatively but also qualitatively and therefore, are responsible for substantial economic losses (Esker et al., 2012; Savary et al., 2019). In addition, some pathogens reduce the food security since they produce toxic substances with important consequences over human and animal health (Moretti et al., 2017). On the other hand, many times, plant diseases are difficult to control. These control methods are usually classified as regulatory, cultural, biological, physical, and chemical, being the last one the most common strategy to manage phytopathogens (Agrios, 2005). Even when the control usually is based on chemical products, there is a current trend focused in using environmental friendly strategies frequently based on an integrated management (Ruano-Rosa and Mercado-Blanco, 2015) and proof of this is the increase in scientific works addressing this subject. This has become one of the main challenges of modern agriculture since it is also necessary to reach levels of production that allow to supply for global food without forgetting reducing the use of phytochemical (Gómez-Lama and Mercado-Blanco, 2017).

The case of grapevine is special, given the presence of diseases that are difficult to control such as grapevine trunk diseases (GTDs) (Gramaje et al., 2018), or under resistance risk to other diseases, such as powdery and downy mildew (Merdinoglu et al., 2018). Since the principal diseases and their control strategies are defined in a previous Chapter of this book ("*Grapevines diseases: descriptions and control*" by Carine Rustin and collaborators), the present chapter will not be dedicated to describe them. To address all the diseases challenges affecting grapevines, numerous strategies have been proposed by plant pathologists. Nevertheless, in recent years, there are plenty of techniques that the researchers are applying to manage the new and old threats related to the control of phytopathogens. Between all of them we would like to highlight the omics methodologies maybe due to their transversal character, it means, they can be applied to all the control strategies focused to a better development of the strategy, to determine its success, its consequences, etc.

Since these techniques appeared in scene, the literature on this subject has been steadily increasing. For these reasons, the motivation to write this chapter is to provide a brief overview about the state of the art in the use of these novel technologies applied to the fight against grapevine diseases. Throughout this chapter, we would also like to awaken reader's interest by introducing these techniques in the general context of phytopathology discipline, following by a more specific revision of how omics have been integrated in the principal strategies to control pathogens in grapevines. Finally, we will make a brief reflection about future prospects.

2. OMICS AND THEIR IMPORTANCE IN DISEASE CONTROL

The suffix -omics refers to several, novel and comprehensive approaches used in biology for the study of genetic and/or molecular profiles of the organisms. These allow the study of the complex interactions between genes and molecules that affects the phenotype, and they can be useful tools for among other, screening and diagnosis of pathogens, understanding of the etiology and the control of diseases or unraveling the plant-microorganism interaction. Among the plethora of omic strategies, we could highlight the following ones: 1) meta/genomic, which addresses the study of the genes in a given organism (genome) or group of organisms (metagenome) without the necessity of being previously isolated; 2) meta/transcriptomic, which studies RNA molecules in, for instance, a microorganism (transcriptome) or from an environmental sample (metatranscriptome); 3) meta/proteomic, which addresses the large-scale analysis and characterization of the protein profile of a cell, tissue, microorganism, etc (proteome), or from a microbial community (metaproteome); or 4) meta/metabolomic, studying the metabolites in a specific biological sample (metabolomic) or from a group of organisms or environmental samples (meta-metabolomic) (Clark and Pazdernik, 2013; Ameen and Raza, 2017) (Figure 1).

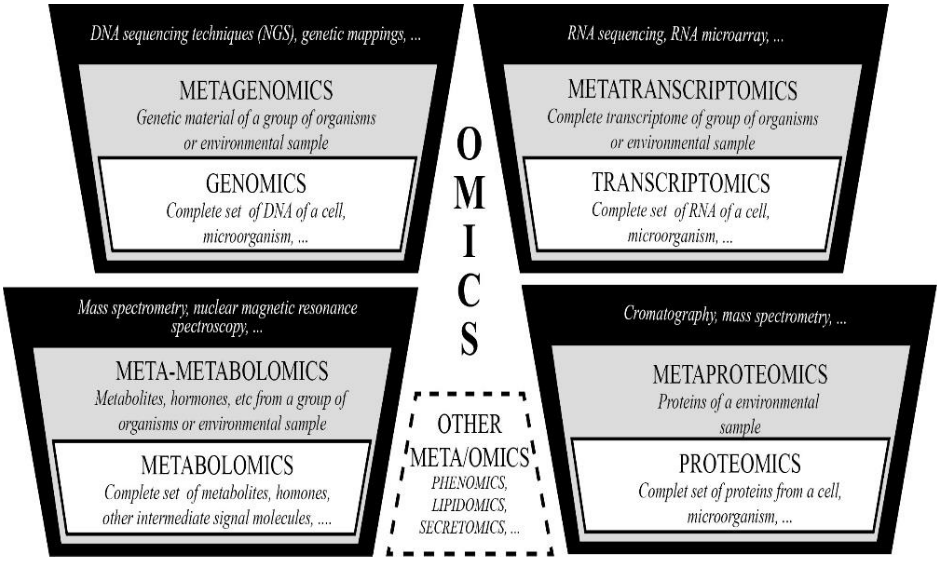


Figure 1. Overview of principal omics branches and related techniques to be applied in both single and combined way in plant pathology.

The wide number of omic tools available is playing an important role in scientific disciplines such as phytopathology (Van Emon, 2016). The continuous improvement in this area is resulting in a better study of genomes, transcriptomes, proteomes and metabolomes of plants and microorganisms. As consequence, we are improving our knowledge in plant-pathogen interaction, the life cycle of microorganisms, identification of new virulence factors and their host targets, or helping to develop newer and cost-effective control methods (Anandaral, 2016; Gomez-Casati et al., 2016). Plant modulates their omics profiles to face the disturbances of the surrounding natural area for their own benefit (Gupta et al., 2013). Currently, the trend is a multi-omic approach, since the achieved information allows progress in the understanding of more complex situations and, to that end, bioinformatics tools have been a key part (Bergna et al., 2019). In the following paragraphs, the most used omics in plant pathology are described in more details.

2.1. Genomics

If we understand the genome as a complete set of DNA from a given micro/macroorganism, the genomic is the area of the molecular biology focused on studying the genes, their relationship, functions and also the techniques involved. Genomic, across all their applications, is increasing since 90s of last century giving valuable information with application to several research areas (Jorrin et al., 2006). The improvement of technologies, such as Next Generation Sequencing (NGS) (Illumina/Solexa, Ion Torrent Personal Genome Machine, and Pacific Biosciences sequencing methods) has overturned the entire approach to genomic research. One of the reasons of this revolution, other than the technical development, is the decrease of the cost of the large-scale DNA sequencing, which have allowed a higher availability of these new tools to the scientific community, and these have resulted in an increase of the number of sequenced organisms. For instance, these improvements have facilitated the study of germoplasm of crop plants, giving important information about reservoirs of natural genetic events. The exploitation of this genetic variability is crucial to overcome problems associated with low genetic diversity of modern cultivars (Deshmukh et al., 2010). The advancement in NGS technologies have also permitted the availability of genes sequence data in the public domain, making possible the development of new markers-based models (Genic Molecular Markers, GMMs), with important consequences for plant breeding (Bhat et al., 2016; Crossa et al., 2016). Furthermore, genomics can be fruitfully used for understanding the plant disease-resistance mechanisms against any kind of pathogens (Nejat et al., 2016; Kankaala et al., 2019). The knowledge of pathogens' genomes, as well as the responses that occur in both the pathogen and the host during the infection, is opening up new methods for disease control in crops. The characterization of beneficial microorganisms genomes will bring to an increase of the yield and the reduction environmental hazards that may be associated with the current agronomic use of available pesticides (Anandaraj, 2016) (Figure 1).

2.2. Transcriptomics

All living beings transcribe their genes into RNA (protein coding mRNAs and non-coding small RNAs), and the so-called transcriptome includes the global analysis of those transcripts of a given cell, tissue or even organism (Anandaraj 2016). Transcriptomic is essential in functional genomics (Bhadauria et al. 2007) and can be addressed both from a qualitative and quantitative point of view, by identifying which transcripts are present and through analyzing how much is expressed for each transcript, respectively (Hasin et al., 2017). High-throughput methods used for transcriptomics include hybridization-based (microarray technology) and sequencing-based approaches (RNAseq) (Warren et al., 2007). One of the advantages offered by transcriptomic tools is the possibility of identify cell responses to biotic or abiotic stresses (Massa et al., 2013; Rasmussen et al., 2013; Sham et al., 2014; AbuQamar et al., 2016). By monitoring transcriptome changes is possible to determine the molecular basis of microorganism-plant interactions, relations between microorganisms and pathogenesis events (Bhadauria et al., 2007; Cairns et al., 2010; Gkarmiri et al., 2015) (Figure 1).

2.3. Proteomics

Proteins are a crucial part of any living organism and take part in all their physiological metabolic pathways (Anandaraj, 2016). The proteome, understood as the protein complement of genome (Pandey and Mann, 2000), can be studied on a large-scale by the proteomics. The techniques applied for investigating the proteome, allow the analysis of proteins in cells and tissues in different physiological situations and their characteristics (expression level, post-translational modification, etc.). This information gives us among others, an integrated view of disease and cellular processes and networks at the protein level. In the case of plant-pathogen interaction (Anandaraj, 2016), give us valuable information about pathogenicity or virulence factors, implication of proteins in adaptation process (González-Fernández et al., 2010) for a better understanding of the pathogen, other microorganisms and the hosts.

Advancements in proteomic techniques (MS, protein purification, post-translational modifications and 3D structure determination) offer new possibilities of characterization of unknown genes and the complex network of cell regulation. For instance, recent findings in plant disease control allowed to discover the overlap of the two categories of resistance mechanisms, innate and R-gene mediated (Popescu et al., 2007). The new model suggests the use of components of both innate and R-gene mediated responses, leading to the assumption that resistance and susceptibility are not just alternatives, but a continuum of plausible interactions ranging from complete resistance to extreme susceptibility (Anandaraj, 2012). Comparative proteomic analysis can be useful to discriminate the proteins involved in the pathogenesis pathway and to individuate the ones that are implicated in the defense mechanisms (González-Fernández et al., 2010; González-Fernández and Jorrin-Novo, 2012).

2.4. Metabolomics

Plants and microorganisms synthesize thousands of metabolites (both primary and secondary) that sometimes are exclusive, which gives them a special singularity. The production of these substances is usually as response to biotic (e.g., insects and pathogens) or abiotic (e.g., drought, temperature) factors (Kessle and Kalske, 2018) and, therefore, could be modulated, generating changes that are possible to register. Metabolomics concerns all the high-throughput techniques utilized for the identification and quantification of those metabolites with the aim of determine phenotypic variation as consequence of these *stimuli* (Cevallos-Cevallos et al., 2009). In other words, as defined Jorrín et al., (2006), it concerns ‘the understanding of the metabolic consequences of gen expression and protein activity.’

Metabolomic profiles are performed by the combination of several technologies, such as Nuclear Magnetic Resonance Spectroscopy, MS techniques, gas and liquid chromatography, capillary electrophoresis or by carbohydrates and metabolites microarrays. Changes of the measurement of some primary or secondary metabolites can be considered as indicators for instance of dysfunction or rearrangements in response to particular stress conditions such as biotic threats. This is a powerful way of unravel plant-pathogen interaction, attack methods, activation of defense mechanism (Castro-Moretti et al., 2020), and interaction between micro/macroorganisms (e.g., Biological control agent-phytopathogen). Consequently, they can represent useful biomarkers for different stresses (e.g., pathogen infections) (Martinelli et al., 2014). Considering the plethora of chemicals involved in stress responses and communication, it is very important to have a broad knowledge of the specific metabolic pathways. Additionally, another important challenge is the characterization of the enzymes and proteins involved in generating and regulating the metabolome, and the receptors and transporters involved on the transportation of the metabolites. However, the physiochemical complexity of the metabolome is disadvantageous since, unlike the genetic code, there is no genomic template for the direct identification and quantification of a metabolite (Argueso et al., 2019). In addition, the identification of metabolites in non-targeted approach with authentic reference compounds remains difficult. To overcome that, authentic standards, platforms and spectral databases are used for both chromatographic profile and mass spectrum, although they can be expensive or hardly commercialized.

3. OMICS IN PHYTOPATHOGEN CONTROL STRATEGIES OF GRAPEVINE

3.1. Biological Control in Grapevine and Omics

Traditionally, to surmount viticulture economic losses caused by plant pathogens, farmers had systematically applied synthetic chemical pesticides to crops. To overcome this dependency, avoid the negative impact of chemicals on both human/animal health and environment, and the comprehensive preoccupation of possible chemical residues in food and its derivatives, researchers have turned to innovative approaches betting on a sustainable disease management. However, not only scientists are concerned, chemical companies are also being implicated in this new approach developing novel eco-friendly products such as biological control agents (BCAs) (with different modes of action against phytopathogens) and botanical active ingredients. In this sense, Droby and Wisniewski (2018) effectively indicated that we are moving from an age of chemistry to an age of biology. Conversely, although there are accessible registered microbial biocontrol products, the belief on the part of the viticulturist, that these 'new' bioproducts are less effective than traditional pesticides (Gramaje and Di Marco, 2015) demonstrates that there still is a long way to go. Readers interested in knowing more about the mechanisms of action of these BCAs in a general way are encourage to read for instance several authors, such as, Massart et al.,· (2015) and Köhl et al., (2019).

Today, the trend in biological control is to consider them as part of microbial communities (plant microbiome) (Turner et al., 2013). Those, as gut microbiome in mammals, have been defined as the plant's second genome. Therefore, plant fitness is not only dependent of the plant itself but also for its associated microbiota, both co-evolving (Berendsen et al., 2012; Lemanceau et al., 2017) and resulting in a stable microbiome-organism which collectively form the so-called holobiont (Vandenkoornhuyse et al., 2015; Berg et al., 2017). Then, the role of both, plant and microbiome, is equally important in plant health, productivity and cultivar development. In general, the microbiota contributes to the plant on diverse levels, highlighting in this case the resistance against biotic stress factors (pathogen and parasite defence) (Vandenkoornhuyse et al., 2015). In viticulture, even vine characteristics may be influenced by the microbiota (Alaimo et al., 2018).

Therefore, an in-depth knowledge of the microbiota is vital to understand this crop and an important subject such as plant pathology, since diseases can be associated to a disequilibrium or swing in microbial populations. As consequence, the necessity of connecting plant microbiome and diseases and hence develops precision diagnostics and treatments is gaining in importance (Gilbert et al., 2016). On the other hand, to date, mostly fungi and bacteria have been exploited for biocontrol purposes in grapevine, and among them, some genera in particular leaving other possible candidates out of a more thorough study (Pertot et al., 2017). This is the case of genus *Lysobacter*, given its recent establishment (40 years), its biology and potential for biocontrol on plants needs to be addressed in comparison to other well-known bacteria such as *Bacillus* and *Pseudomonas* (Puopolo et al., 2018). Furthermore, the existence of an entire domain, Archaea, lately recognized as part of the plant microbiome (Müller et al., 2012) whose function and potential biocontrol traits are entirely unidentified and the occurrence of non-cultivable BCAs, open up the urgent necessity of a fast and reliable approach.

In this new scenario, an entire description of the microbial community in association with its host and/or the surrounding environment (ecosystem) is crucial, and the sole use of traditional culturing methods is out of question. Consequently, culture-independent molecular techniques such as DNA sequencing and meta/omics tools and, *in silico* analysis, are the new “heroes” facing the adventure of starting a new era in plant-microbe interactions. Thus, meta/genomics, meta/proteomics, meta/transcriptomics, meta/metabolomics, among other molecular approaches, alone or in combination, are currently allowing us to start shedding light over the role of these microbial communities in plant and ecosystem health as well as in its composition and dynamics.

Regarding to bacterial communities, two recent works revealed that the bacteriome is linked to diverse samples types (bulk soil, rhizosphere, roots, leaves, grapes and flowers). According to Zarraonaindia et al., (2015), the composition and structure of the bacterial microbiome depended significantly on the compartment under study. However, most of the organ-associated taxa rise from the soil, reflecting the extensive influence of biogeographic factors and vineyard management. Their studies revealed a possible function of the soil as a bacterial source for different grapevine organs. In this regard, good root colonizers were selected from soil by the grapevine, predominantly plant growth-promoting bacteria (PGPB). On the other hand, soil and root microbiota structure were correlated to soil pH and C:N ratio, while leaf- and grape-associated microbiota was related to soil carbon and presented interannual variation.

Marasco et al., (2018) focused their effort in analysis of the bacteriome and demonstrated that both root tissues and rhizosphere, were significantly influenced by the rootstock. Curiously, although different rootstocks recruited specific PGPB, these plant growth promoters displayed similar traits in all rootstocks. These are only two good examples about how important is to know all the different factors that modulate the microbial communities in a plant. In Martínez-Diz et al., (2019) an increase of knowledge of how grapevine modulates its fungal community and its relation with crop management and yield was studied. Thus, fungal communities associated with three soil-plant compartments (bulk soil, rhizosphere and endorhizosphere) were analyzed. This study revealed that whereas the spatial variation among the five vineyards under screening was low, the fungal communities were highly affected both in diversity and structure in different soil-plant compartments. Thus, the endorhizosphere compartment contrasted comparing to the others, showing an increase in the relative abundances of potential plant pathogens, endophytes and arbuscular mycorrhiza, and a decrease in wood, dung and undefined saprotrophs. Another remarkable result, not related with biocontrol, was that internal asymptomatic roots tissues were colonized by soilborne fungi known in relation to GTDs. Therefore, future experiments related with how, when and why endophytic and/or latent pathogens become virulent under specific conditions taking advantage of these new-omic tools, would be of interest in disease management.

Likewise, two studies characterized microbial population (bacteria and fungi) in grapevine. Thus, Berlanas et al., (2019) analyzed the microbial communities in five different rootstocks at two vineyards with identical soil properties over two growing seasons, and the influence of several factors (growing region, year, sampling date, grapevine genotype, and their interactions on microbiome diversity). Host genetic was the most important factor controlling the microbiome in the rhizosphere in mature vineyards. Remarkably, 140 Ru and 161-49C rootstocks presented the lowest number of black-foot pathogens and an increase of relative abundance of the genus *Bacillus*, a bacterial genus widely documented as biocontrol agents. On the other hand, Perazzolli et al., (2014) studied the effect of a BCA (*Lysobacter capsici* AZ78) on leaf bacterial and fungal populations in grapevine at three locations. The strain AZ78 is well known for its high efficacy against grapevine downy mildew caused by the oomycete *Plasmopara viticola*. The analysis of amplicons showed that the major factor affecting the richness and diversity of microbial populations was grapevine location, being slightly affected by AZ78. Therefore, studies like this gives us important information about source of markers applicable to a more effective selection of microorganisms useful in biological control.

If we look to transcriptomic approaches, some studies give also important information about source of markers applicable to a more effective selection of microorganisms useful in biological control. In this sense, Perazzolli et al., (2012) determined, through a global transcriptional study, how the biological control agent *Trichoderma harzianum* T39, also effective against *P. viticola*, induced partially the expression of genes related with resistant grapevine genotypes to downy mildew. Before pathogen inoculation, T39 activated microbial recognition mechanisms, while after *P. viticola* inoculation increased defense genes. Furthermore, transcriptomics may be used to compare possible differences in the mechanism of action of different BCAs. This is the case of the study exploring the mechanisms of mycoparasitism of several *Trichoderma* spp., two cosmopolitan opportunistic species, *Trichoderma atroviride* and *Trichoderma virens*, as well as one tropical ecologically restricted species, *Trichoderma reesei*, through *in vitro* confrontations against a grapevine pathogen, *Rhizoctonia solani* (Atanasova et al., 2013). All three *Trichoderma* spp. displayed completely different transcriptomic response: *T. atroviride* expressed genes involved in secondary metabolites synthesis (GH16 ß-glucanases, proteases and small secreted cysteine rich proteins); *T. virens* increased the expression of genes encoding for gliotoxin (antifungal activity) biosynthesis; and *T. reesei* expressed genes related with solute transport and the production of cellulases and hemicellulases.

A study focused in the impact of the soilborne pathogen *Armillaria mellea* and its biocontrol agent *T. atroviride* SC1 in the transcriptome of non-target soil microorganisms was carried out by Perazzolli et al., (2016). To do this, a small community was modeled by generating a basic soil microcosm by adding 11 microorganisms to the soil. As expected, the transcriptome varied when the established microorganism consortium was grown in the company of the new partners (BCA and pathogen). Interestingly, both the biological control agent and the pathogen were recognized by the soil microbial community, but in very different means. Meanwhile SC1 triggered early defense, *A. mellea* produced down-regulation of defense mechanism, meaning that the pathogen was perceived as a non-competitive intruder. This kind of recreation of small scenarios may become more complicated over time approaching as to a more realistic scene.

Another research related to transcriptomics found that several mixtures of two isolates of the oomycete and BCA *Pythium oligandrum* priming the plant producing a major mobilization in its defense reactions in response to *Phaeomoniella chlamydospora*, one of the most important causal agents of esca disease (GTD) (Yacoub et al., 2016). The researchers pointed that expression levels of 6 genes associated with several pathways (PR proteins, phenylpropanoid pathways, oxylipin and oxydo-reduction systems) were down-regulated in pre-*P. oligandrum* treated plants. Another stimulating study focused in microbial community composition as well as the main physiological functions of the xylem sap during bleeding period of 'Rosario Bianco' grapevine at three developmental stages was completed (Zheng et al., 2019). According to this studied, the transcriptional profile showed that the primary functions of the sap were implicated in plant growth promotion and pathogen resistance. In the case of microbial community its functions were also related with disease resistance in addition to microorganism growth. Palmieri et al., (2012) studied the proteome on *Trichoderma*-treated grapevine plants (induced systemic resistance) before and after the inoculation of the fungal pathogen *P. viticola*. *Trichoderma* treatment shifts the abundance of proteins, being most of them linked to signal transduction (stimulation of microbial recognition machinery) and response to stress and redox balance (defense activation). In this regard, an increase of reactive oxygen species (ROS) and callose at the inoculation sites was also observed.

A review based in *Trichoderma* spp. studies highlighted that is the great importance the role of the translatome, active mRNA population (Sharma et al., 2017). Since gene expression is dynamic in time and space, the variation in the rate of translation is specific for each protein and can be affected by the own nature of the mRNA and the external *environmental stimuli*, is crucial to study the translational status of the active mRNAs along with other omic data. Bona et al., (2019) developed the first study describing the proteome of the rhizospheric bacterial community in *V. vinifera* cv. Pinot Noir in Italy. In this research bacterial genera *Streptomyces*, *Bacillus*, *Bradyrhizobium*, *Burkholderia* and *Pseudomonas* were the most active in regards of protein expression and their major biological activity were linked to primary metabolic processes. Works like this open a new way of isolating and screening beneficial microorganisms since, while meta/genomic approaches shed light to the composition of the microbiome, addition of proteomic studies give information about its activity. For this reason, combination of different approaches is essential to achieve better results. Nevertheless, biological control is not only performed applying bacteria and/or fungi. Census of the viral population of diverse asymptomatic plant tissues (shoots, stem, leaves and petioles) or showing diverse disease symptoms by metagenomics were recently and extensively review by Roossinck et al., (2015). RNA and DNA analyzed showed the most prevalent species and novel virus not previously reported in a specific location and/or not previously identified in grapevine, as well as viruses associated with grapevine pathogens (e.g., *Botrytis cinerea*). Later, Fajardo et al., (2017) analyzed grapevine bark scrapings collected from experimental fields and vine collections to identify grapevine viruses in Brazil. In this sense, the identification of mycoviruses as responsible of alteration in the fungal pathogenicity (hypovirulence) has been known for some time. Probably, the best-known case is the chestnut blight caused by *Cryphonectria parasítica* (Anagnostakis, 1982). The expression of mRNAs of hypovirulent fungi infected with this kind of viruses using transcriptomic give us useful information about the mechanism implicated in the diminution of virulence (Lee et al., 2014). Similarly, Wang et al., (2018) performed comparative studies with *Botryosphaeria dothidea*. This fungus is an important pathogen for many woody crops such as apple, nuts, pear and also grapevine. In this research, *B. dothidea* strains infected with hypovirulent mycoviruse chrysovirus 1 (BdCV1) and non-hypovirulent mycoviruse partittivirus 1 (BdPV1) were compared to a transcriptomic level. The results highlighted that while the former presented high effect in the transcriptome and consequently on phenotype, the latter displayed a moderate effect. In addition to virulence, BdCV1 also showed a broader effect among others on conidiomata, mycelia or transmission capability (Hu et al., 2019). This information about the transcriptome improves our knowledge concerning the response to mycovirus infection and enhances the possibilities of applying it as efficient control strategy.

3.2. Chemical Control in Grapevines and Omics

Since the aforementioned trend to find a more respectful solution regarding to human health and environment started, reduction of chemical inputs has attracted the most attention. One point of concern about phytochemicals is how their application can affect the grapevine microbiome and break an equilibrium that may be necessary for controlling biotic threats. Pinto et al., (2014) unraveled the natural microbiome diversity (prokariotics and eukariotics) in grapevine leaves. They performed this study along the vegetative cycle but also analysed how the microbial biodiversity was modified before and after chemical treatments. One of the most important achievement was the evidence of a negative impact of phytochemicals in the microbial community, highlighting the breakup of the balance between species potentially pathogenics and those that have been described as beneficial for this crop. For instance, the presence of genus *Alternaria* increased after treatment, showing an opportunistic behaviour, when *Aureobasidium*, with recognized protectant effect was less abundant. On the other hand, Perazzolli et al., (2014) addressed the effect of penconazole, a systemic fungicide usually applied against *Erysiphe necator*, the causal agent of powdery mildew, with a broad effect against ascomycetes and basidiomycetes, on non-target microorganisms of grapevine leaves. They determined that richness and biodiversity of both, bacteria and fungi were affected minimally, evidencing that the microbiological equilibrium was not broken. Del Frari et al., (2019) studied the effect of common fungicides applied against downy and powdery mildew (copper-sulfur and systemics). They observed that these treatments not only altered the structure of the microbial communities but also the capability of some endophytes of colonizing the plant. However, the effect observed for an important GTD such as *P. chlamydospora* was scarce. Due to the important value of endophytic microorganisms and their stability as the front line of the struggle, the authors connected this alteration with the increase of phytopathological problems. Gobbi et al., (2020) applying NGS techniques and qPCRs, analyzed the evolution along the season of the leaves microbiome also treated with copper or the BCA *Lactobacillus plantarum* MW-1. While bacterial microbiome remained with low modifications along the sampling period, the composition of fungal community changed. This kind of studies can be applied to improve the use of phytochemicals, since they allow us to choose the best moment of application to increase the effectiveness of the treatment.

Another important concern about the use of phytochemicals is the development of resistances due to the consequences on the disease management. However, the improvement in omics tools is pushing the development and the registration of new more effective products. Cools and Hammond-Kosack (2013) performed a deep analysis on how omics can be used to improve fungicides efficiency, identifying for instance the impact on the development pipeline. They considered the genome sequencing of pathogens an opportunity for a better understanding of the resistance process, the mode of action and potential targets of phytochemical, and the collateral effects on other micro/macroorganisms. In the last years this subject has acquired substantial importance in diseases such as downy and powdery mildew due to a high fungicide pressure and a low alternation of phytochemical with different modes of action, and therefore affecting to their control managements. In this way, NGS have emerged as a great help to amplify the knowledge about the resistance to chemicals of downy mildew (Jones et al., 2014) and its evolution. In the case of *P. viticola*, Delmas et al., (2017) determined that this rapid evolution from susceptible to resistant involves soft selective sweeps, it means, several haplotypes rise in frequency. Dussert et al., (2019) through a high-quality genome of this pathogen, have determined the importance of this knowledge for a better understanding on how the environmental parameters guide its adaptation.

Another kind of chemical control is based on the use of substances with capacity to induce resistance in the plant. These substances can be from very different nature including, for instance, synthetic chemicals or biological inducers (e.g., some fungi, bacteria and algal extracts). However, these inducers, generically called elicitors usually need to be combined with other strategies because they are not totally effective by themselves (Walter et al., 2013). In the plant, the effect of these compounds is to trigger a complex net of reactions that result in defense actions. These are mediated by expression of genes that make possible the synthesis of proteins and/or defensive substances. It is in that point where transcriptomic and proteomic approaches can give us high valuable information. For instance, Lemaître-Guillier et al., (2017) performed a comparative proteomic study to improve the information about the lack of correlation between the mentioned defense actions and the induced resistance in the *P. viticola* - *V. vinifera* pathosystem and two β-1,3 glucans (PS3 and H13) with different activity as inducers. This study shows the possibility of using omics to discriminate between elicitors with the aim of choosing those that trigger induced resistance as a beneficial point in the control of phytopathogen. Likewise, the transcriptomic study of Gauthier et al., (2014) demonstrated the role of PS3 in induced resistance and the modulation of ROS dependent defenses while others such as Héloir et al., (2018) addressed the impact of this inducer from a metabolomics point of view.

3.3. How Omics Can Help in Grapevine Breeding

As is well known, traditional breeding programs have a common important problem due to their development is time consuming, especially when the target crop is a woody plant with long generation cycle such as grapevine (Töpfer et al., 2011). Usually, the first steps of selection are focused on resistance traits not only to abiotic factors but also to biotics such as diseases (Eibach and Töpfer, 2015). In this way, for a long time, breeders have focused their efforts on selecting genotypes resistant to important diseases, such as powdery and downy mildew and, Pierce's disease. However, since molecular techniques busted into breeding programs they have given an important impulse to this research area (e.g., with the whole sequencing of grapevine genome, identification of genes and their functions, genetic engineering, etc.) (Tracy, 2015). Readers interested in knowing more about the importance of new molecular technique in this scientific area are encouraged to read for instance "*Plant Breeding in the Omics Era*" (Ortíz Ríos, 2015). Nevertheless, due to these new genetic tools, consumers, producers, politicians and research community are seriously concerned about some aspect such as how genetic modification could have a negative consequence in the environment throughout, for example the horizontal gene transfer.

Hily et al., (2018) performed a metagenomics-based study to evaluate the possible impact of a genetically modified grapevine rootstock developed to confer resistance to one of the most important viral diseases affecting vineyards around the world, the Grapevine fanleaf virus (GFLP). They analyzed the structure and genetic diversity of this virus in experimental fields plants (genetically modified and no modified rootstocks) and their non-target microorganisms such as rhizospheric bacteria. As conclusion, they determined that the used rootstocks did not favor both the development of recombinant viruses and/or endophytes, and the modification of the microbial community. Studies like this can help us to develop more accurate molecular breeding programs aimed to workable solutions to difficult phytopathogenic problems and reassure the population about the risks associated of its use.

As mentioned above, among the alternatives to phytochemicals highlights the use of resistance varieties from breeding programs (Töpfer and Eibach, 2016). Thus, genetic resistance to pathogens can be also explored through a better knowledge of the transcriptome. In this sense, one of the most studied pathogens is *E. necator* since is a major pathogen affecting this crop. Among all, powdery mildew genomes available are quite well studied, and we can find omic studies such as genomics focused on resistance to phytochemicals (Jones et al., 2014) or transcriptomics to study the differential gene expression during morphogenesis of the pathogen (Wakeflield et al., 2014). They are particularly relevant comparative transcriptomic studies since they provide high information's to breeding programs. For instance, Amrine et al., (2015) performed a study focused on the characterization of the plant-pathogen interaction from a transcriptomic point of view and the improvement of the comprehension of resistant phenotypes understanding (e.g., by determining the up/down regulation of genes).

Another interesting example of transcriptomic applied to this pathosystem related to breeding is the work of Weldon et al., (2020). They took as a basis previous studies which linked transient quantitative resistance to powdery mildew with a period of cold stress. Evidently, low temperatures had an important influence over the biology of the pathogen, but also produce several metabolic changes in the plant that create a suboptimal environment for the establishment of the pathogen. This study identified a group of genes related with these changes and therefore opens up the possibility of developing new libraries of genes, which may be used for the improvement of breeding programs.

As cited previously for others control strategies, the combination of tools has important benefits. For this reason, we would like to highlight here the work of Figueiredo et al., (2008) combining transcriptomic and metabolomics approaches. The use of these techniques enabled to discriminate among resistant and susceptible grapevine to mildews (cultivars Regent and Trincadeira Preta, respectively). Both cultivars have the genetic information to trigger a resistance response. However, while ‘Regent’ grapevines active these responses more efficiently, in the susceptible cultivar Trincadeira Preta is slow. More recently, the same authors (Figueiredo et al., 2017) observed how the proteomic profile of grapevine leaves is different and identifiable between susceptible and resistance cultivars both before and after inoculation with the pathogen. They also shed light on the role of lipid associated events and ROS signaling in the interaction *P. viticola* - *V. vinifera.*

3.4. Others Omics Applications Associated to Grapevine Diseases

Another emerging trend related with omics is the characterization of the plant microbiota related with a disease. These characterizations may help, between others, to a better understanding of the microbial ecology in an infection process and in early diagnosis. As a consequence, we will able to improve our preventative measures. In this way, for instance Faist et al., (2016) characterized the endophytic microbial community of grapevine associated with the bacteria *Agrobacterium vitis*, causal agent of crow gall disease. Surprisingly, the pathogen only affected the microbiota of grafting unions where an increase of different bacteria was register. However, not only the microbial community identification is useful. A crucial aspect is to develop a proper disease diagnosis, overall when the disease is caused by a complex of phytopathogenic microorganisms as in GTDs. For example, the use of metatranscriptomics to monitor the virulence activities of various GTPs *in planta* was tested by Morales-Cruz et al., (2018). To do this, naturally infected samples from symptomatic fields were used. This method allowed a quantitative evaluation of species composition, in addition to the transcriptional profile of potential virulence factors throughout the genome (cell wall degradation, secondary metabolism and nutrient absorption) for all GTPs. Morales-Cruz and colleagues suggested that this approach may be interesting also for establishing gene expression patterns related with disease symptoms in different plant compartments testing different combinations of various pathogens under a specific environmental condition.

Hot-water treatment (HWT) is a control technique useful in nurseries against GTDs (Elena et al., 2015) due to the combination of temperature and time that allow a decrease in the disease incidence of phytopathogenic endophytes (Gramaje and Armengol, 2011; Ortíz-Ríos, 2015). Eichmeier et al., (2018) explored the dynamics of potentially active fungal communities in internal grapevine wood when grapevine plants were subjected to HWT. To do so, two plant zones (grafting area and the basal end of the rootstock) in two plants genotypes were analyzed at two different moments during the propagation process. Metatranscriptomic revealed that fungal diversity was due to plant genotypes as well as temporal variation. Besides, a high internal fungal diversity was found in treated vines demonstrating the impossibility of sterilizing inner wood of grapevine plants. Nevertheless, a decrease of fungal trunk disease pathogens was observed. In an integrated management program frame, these findings claim the use of this technique in combination with others such as application of BCAs.

Finally, agriculture practices (e.g., conventional, organic, integrated, etc.) have been also subject of study in relation with their deep impact on microbial diversity. These control strategies may alter the microbiome since they use different activities to control the pathogens. In this way, while some of them apply synthetic substances others use only natural products, microorganisms or combination of different methods. In these cases, molecular techniques are also useful to elucidate how the management of the vineyard influences in modifying the microbial endophytic community. Campisano et al.' (2014) addressed the differences between organic and integrated pest management (IPM) for two cultivars (Chardonnay and Merlot) and observed differences in bacterial communities. However, it was impossible to determine the way in which this modification was done, if as a direct consequence of the management or derived from a restructuring after fungal community modification. Pancher et al., (2012) observed that grapevines, under organic and integrated management, had similar fungal endophytic communities but with enough differences to distinguish between both managements. Similarly, Castañeda et al., (2018) analyzed the phyllosphere fungal diversity in vineyards cv. Carménère under organic and conventional management and determined that the diversity indexes did not change. However, the community structure showed sensibility to management strategy since the common species present in both showed different abundances. Another example, on this occasion exploring IPM (in the broad sense including control of weeds, pathogens and insects) in vineyard cv. Pinot Noir is the study of Novello et al., (2017). They analyzed samples with different origin (bulk soil and rhizosphere) in two different moments of sampling (flowering and early fruit) and their differences were more related with the origin of the sample than the moment of sampling. Additionally, they observed that soil could act as source of microorganisms for the rhizosphere. A brief resume of all omics applications here described can be found in Figure 2.

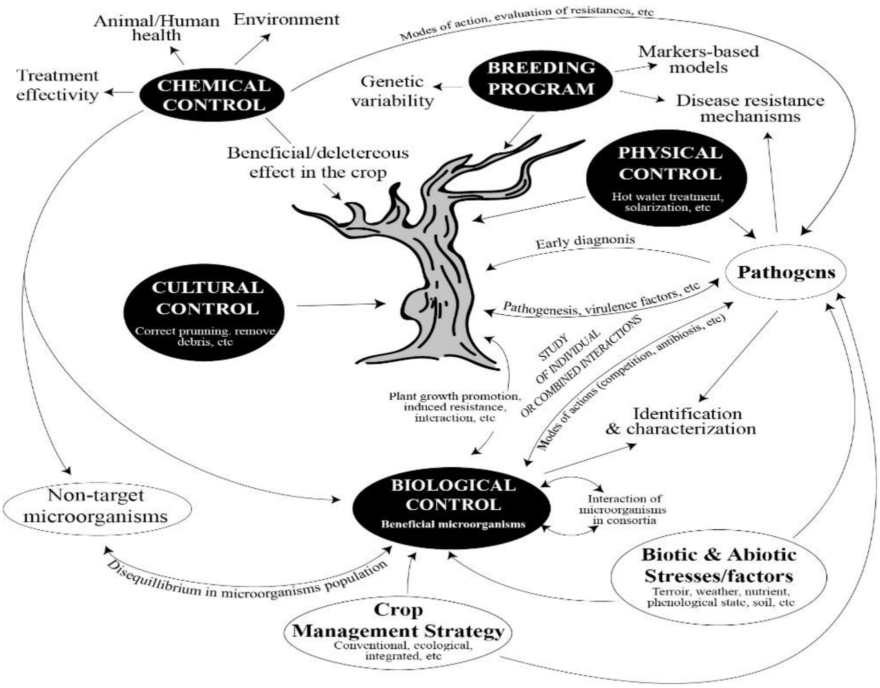


Figure 2. Overview of the different strategies in grapevine disease control for which omics sciences can be useful to face plant pathogens.

CONCLUSIONS AND FUTURE SCENARIO

Nowadays, we are witnessing to a constant advance of methodologies concerning the molecular biology, and this is closely linked to the continuous scientific and technological innovations that enabled to better understand the world around us. Regarding to the main subject of this chapter, omics in grapevine plant pathology, we have tried to describe several aspects in which the use of these new techniques allowed us to reinforce our knowledge. Among all of them, biological control is probably the science that benefited the most of these new sciences. Proof of our speculation is the plethora of applications that omics offer to select the biological control agents, to study microbial consortia, their combination with other techniques, the mechanisms of action, the effectiveness of treatment, etc. Therefore, these techniques open the door to more effective control alternatives.

Breeding programs have been ameliorated thanks to omics since, through their techniques, it has been possible to better understand the infection process, virulence factors, all of them important aspects for the identification of a good selection marker, which also led to a less time-consuming and more precise breeding programs. From our point of view, one of the most relevant aspect as consequence of the utilization of omics in grapevine disease control, and generally in plant pathology, concerns the possibility of monitoring hypothetical side effects deriving from the use of any control strategy, especially those from the chemical control and its impact over the environment and human/animal health.

Those applications represent only “*the tip of the iceberg*” of their entire potentiality, and many applications are constantly emerging. For the future, omics will bring to a more effective disease control and treatment design, with a consequent better comprehension of the diseases, by minimizing secondary undesirable effects, an improvement of our knowledge of pathogens and beneficial microorganisms, the identification of more effective markers for the development of cultivars with an enhanced disease resistance, early detection of emerging pathogens, etc. However, it is worth mentioning that these advances must go hand in hand with a considerable capability to deal with an enormous quantity of data, as consequence of assays using omics techniques, thus it becomes a priority the development and the optimization of suitable software tools to integrate data achieved from two or more different omics sciences.

REFERENCES

Abu Qamar, S. F., Moustafa, K., Tran, L. S. P. (2016). “Omics” and plant responses to *Botrytis cinerea*. *Frontiers in Plant Science,* 7(1658):1-8.

Agrios, G. N. (2005). “Control of Plant Diseases” In *Plant Pathology,* edited by George N. Agrios, 293-353. New York: Elsevier.

Alaimo, S., Marceca, G. P., Giugno, R. Ferro, A., Pulvirenti, A. (2018). Current knowledge and computational techniques for grapevine meta-omics analysis. *Frontiers in Plant Science,* 8: 241.

Ameen, A., Raza, S. (2017). Metaproteomics approaches and techniques: A review. *International Journal of Advances in Scientific Research,* 3(05):49-51.

Amrine, K. C., Blanco-Ulate, B., Riaz, S., Pap, D., Jones, L., Figueroa-Balderas, R., Walker, A., Cantu, D. (2015). Comparative transcriptomics of Central Asian *Vitis vinifera* accessions reveals distinct defense strategies against powdery mildew. *Horticulture Research,* 2(1):1-11.

Anagnostakis, S. L. (1982). Biological control of chestnut blight. *Science,* 215(4532): 466-471.

Anandaraj, M. (2016). The science of “omics” for plant pathologists. *Indian Phytopathology,* 69 (1):1-9.

Argueso, C. T., Assmann, S. M., Birnbaum, K. D., Chen, S., Dinneny, J. R., Doherty, C. J., Eveland, A .L., Friesner, J., Greenlee, V. R., Law, J. A., Marshall-Colón, A., Mason, G. A., O’Lexy, R., Peck, S. C., Schmitz, R. J., Song, L., Stern, D., Varagona, M. J., Walley, J. W., Williams, C. M. (2019). Directions for research and training in plant omics: Big questions and big data. *Plant Direct,* 3(4):1-16.

Atanasova, L., Le Crom, S., Gruber, S., Coulpier, F., Seidl-Seiboth, V., Kubicek, C. P., Druzhinina, I. S. (2013). Comparative transcriptomics reveals different strategies of *Trichoderma* mycoparasitism. *BMC Genomics* 14(1):1-15.

Berendsen, R. L., Pieterse, C. M. J., Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science,* 17 (8):478-486.

Berg, G., Köberl, M., Rybakova, D., Müller, H., Grosch, R., Smalla, K. (2017). Plant microbial diversity is suggested as the key to future biocontrol and health trends. *FEMS Microbiology Ecolog,* 93(5):fix050.

Bergna, A., Cernava, T., Zachow, C., Berg, G. (2019). Analysing seed endophytes for Biotechnology. In *Endophyte Biotechnology: potential for agriculture and Pharmacology,* edited by Alexander Schouten, 42-58. Cabi Biotechnology Series.

Berlanas, C., Berbegal, M., Elena, G., Laidani, M., Cibriain, J. F., Sagües, A., Gramaje, D. (2019). The fungal and bacterial rhizosphere microbiome associated with grapevine rootstock genotypes in mature and young vineyards. *Frontiers in Microbiology,* 10:1142.

Bhadauria, V., Popescu, L., Zhao, W. S., Peng, Y. L. (2007). Fungal transcriptomics. *Microbiological Research,* 162(4):285-298.

Bhat, J. A., Ali, S., Salgotra, R. K., Mir, Z. A., Dutta, S., Jadon, V., Tyagi, A., Mushtaq, M., Jain, N., Singh, P. K., Singh, G. P., Prabhu, K. V. (2016). Genomic selection in the era of Next Generation Sequencing for complex traits in plant breeding. *Frontiers in Genetic,s* 7: 221.

Bona, E., Massa, N., Novello, G., Boati, L., Cesaro, P., Todeschini, V., Magnelli, V., Manfredi, M., Marengo, E., Mignone, F., Berta, G., Lingua, G., Gamalero, E. (2019). Metaproteomic characterization of *Vitis vinifera* rhizosphere. *FEMS Microbiology Ecology,* 95(1):fiy204.

Cairns, T., Minuzzi, F., Bignell, E. (2010). The host-infecting fungal transcriptome. *FEMS Microbiology Letters,* 307(1):1-11.

Campisano, A., Antonielli, L., Pancher, M., Yousaf, S., Pindo, M., Pertot, I. (2014). Bacterial endophytic communities in the grapevine depend on pest management. *PloS one,* 9(11):e112763.

Castañeda, L. E., Miura, T., Sánchez, R., Barbosa, O. (2018). Effects of agricultural management on phyllosphere fungal diversity in vineyards and the association with adjacent native forests. *PeerJ,* 6:e5715.

Castro-Moretti, F., Gentzel, I., Mackey, A. (2020). Metabolomics as an emerging tool for the study of plant-pathogen interactions. *Metabolites,* 10:52.

Cevallos-Cevallos, J. M., Reyes-De-Corcuera, J. I., Etxeberria, E., Danyluk, M. D., Rodrick, G. A. (2009). Metabolomic analysis in food science: a review. *Trends in Food Science and Technology,* 20:557-566.

Clark, D. P., Pazdernik. N. J. (2013). “Proteomics: The Global Analysis of Proteins.” In *Molecular Biology*, edited by Clark David P., and Pazdernik Nanette J., 309-314. UK: Elsevier.

Cools, H. J., Hammond-Kosack, K. E. (2013). Genomics and fungicide research. *Molecular Plant Pathology,* 14(2):197-210.

Crossa, J., Jarquín, D., Franco, J., Pérez-Rodríguez, P., Burgueño, J., Saint-Pierre, C., Vikram, P., Sansaloni, C., Petroli, C., Akdemir, D., Sneller, C., Reynolds, M., Tattaris, M., Payne, T., Guzman, C., Peña, R. J., Wenzl, P., Singh, S. (2016). Genomic prediction of gene bank wheat landraces. *G3: Genes, Genomes, Genetics,* 6(7):1819-1834.

Del Frari, G., Gobbi, A., Aggerbeck, M. R., Oliveira, H., Hansen, L. H., Boavida Ferreira, R. (2019). Fungicides and the grapevine wood mycobiome: a case study on tracheomycotic ascomycete *Phaeomoniella chlamydospora* reveals potential for two novel control strategies. *Frontiers in Plant Science,* 10:1405.

Delmas, C. E. L., Dussert, Y., Delière, L., Couture, C., Mazet, I. D., Richart Cervera, S. R., Delmotte, F. (2017). Soft selective sweeps in fungicide resistance evolution: recurrent mutations without fitness costs in grapevine downy mildew. *Molecular Ecology,* 26:1936-1951.

Deshmukh, R., Singh, A., Jain, N., Anand, S., Gacche, R., Singh, A., Gaikwad, K., Sharma, T., Mohapatra, T., Singh, N. (2010). Identification of candidate genes for grain number in rice (*Oryza sativa* L.). *Functional and Integrative Genomics,* 10:339-347.

Droby, S., Wisniewski, M. (2018). The fruit microbiome: A new frontier for postharvest biocontrol and postharvest biology. *Postharvest Biology and Technology,* 140:107-112.

Dussert, Y., Mazet, I. D., Couture, C., Gouzy, J., Piron, M. C., Kuchly, C., Bouchez, O., Rispe, C., Mestre, P., Delmotte, F. (2019). A high-quality grapevine downy mildew genome assembly reveals rapidly evolving and lineage-specific putative host adaptation genes. *Genome Biology and Evolution,* 11(3):954-969.

Eibach, R., Töpfer, R. (2015). Traditional grapevine breeding techniques. In *Grapevine breeding programs for the wine industry,* edited by Andrew Reynolds, (Elsevier) p. 3-22.

Eichmeier, A., Pečenka, J., Peňázová, E., Baránek, M., Català-García, S., León, M., Armengol, J., Gramaje, D. (2018). High-throughput amplicon sequencing-based analysis of active fungal communities inhabiting grapevine after hot-water treatments reveals unexpectedly high fungal diversity. *Fungal Ecology,* 36:26-38.

Elena, G., Di Vela, V., Armengol, J., Luque, J. (2015). Viability of Botryosphaeriaceae species pathogenic to grapevine after hot water treatment. *Phytopathologia Mediterranea,* 54(2):325-334.

Esker, P. D., Savary, S., McRoberts, N. (2012). Crop loss analysis and global food supply: focusing now on required harvests. *CAB Reviews,* 7(52):1-14.

Faist, H., Keller, A., Hentschel, U., Deeken, R. (2016). Grapevine (*Vitis vinifera*) crown galls host distinct microbiota. *Applied and Environmental Microbiology,* 82(18):5542-5552.

Fajardo, T. V. M., Silva, F. N., Eiras, M., Nickel, O. (2017). High-throughput sequencing applied for the identification of viruses infecting grapevines in Brazil and genetic variability analysis. *Tropical Plant Pathology,* 42(4):250-260.

Figueiredo, A., Fortes, A. M., Ferreira, S., Sebastiana, M., Hae Choi, Y., Sousa, L., Acioli-Santos, B., Pessoa, F., Verpoorte, R., Pais, M. S. (2008). Transcriptional and metabolic profiling of grape (*Vitis vinifera* L.) leaves unravel possible innate resistance against pathogenic fungi. *Journal of Experimental Botany,* 59(12):3371-3381.

Figueiredo, A., Martins, J., Sebastiana, M., Guerreiro, A., Silva, A., Matos, A. R., Monteiro, F., Pais, M. S., Roepstorff, P., Coelho, A. V. (2017). Specific adjustments in grapevine leaf proteome discriminating resistant and susceptible grapevine genotypes to *Plasmopara viticola*. *Journal of Proteomics,* 152:48-57.

García-Pedrajas, M. D., Cañizares, M. C., Sarmiento-Villamil, J. L., Jacquat, A. G., Dambolena, J. S. (2019). Mycoviruses in biological control: from basic research to field implementation. *Phytopathology,* 109(11):1828-1829.

Gauthier, A., Trouvelot, S., Kelloniemi, J., Frettinger, P., Wendehenne, D., Daire, X., Joubert, J. M., Ferrarini, A., Delledonne, M., Flors, V., Poinssot, B. (2014). The sulfated laminarin triggers a stress transcriptome before priming the sa- and ros-dependent defenses during grapevine's induced resistance against Plasmopara viticola. *PloS one,* 9(2):e88145.

Gilbert, J. A., Quinn, R. A., Debelius, J., Xu, Z. Z., Morton, J., Garg, N. Jansson, J. K., Dorrestein, P. C., Knight, R. (2016). Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature,* 535:94-103.

Gkarmiri, K., Finlay, R. D., Alström, S., Thomas, E., Cubeta, M. A., Högberg, N. (2015). Transcriptomic changes in the plant pathogenic fungus *Rhizoctonia solani* AG-3 in response to the antagonistic bacteria *Serratia proteamaculans* and *Serratia plymuthica*. *BMC Genomics,* 16:630.

Gobbi, A., Kyrkou, I., Filippi, E., Ellegaard-Jensen, E., Hestbjerg Hansen, L. (2020). Seasonal epiphytic microbial dynamics on grapevine leaves under biocontrol and copper fungicide treatments. *Scientific Report,* 10:681.

Gomez-Casati, D. F., Pagani, M. A., Busi, M. V., Bhadauria, V. (2016). Omics approaches for the engineering of pathogen resistant plants. *Current Issues in Molecular Biology,* 19:89-98.

Gómez-Lama, C., Mercado-Blanco, J. (2017). "Ómicas, microbiomas vegetales y control biológico de enfermedades [Omics, plant microbiomes and biological control of diseases]." In *Control biológico de enfermedades vegetales*, edited by Antonieta De Cal and Paloma Melgarejo, 49-57. Valencia: Phytoma-España.

Gonzalez-Fernandez, R., Jorrin-Novo, J. V. (2012). Contribution of proteomics to the study of plant pathogenic fungi. *Journal of Proteome Research,* 11(1):13-16.

González-Fernández, R., Prats, E. Jorrín-Novo, J. V. (2010). Proteomics of plant pathogenic fungi. *BioMed Research International,* 2010:932527.

Gramaje, D., Armengol, J. (2011). Fungal trunk pathogens in the grapevine propagation process: Potential inoculum sources, detection, identification, and management strategies. *Plant Disease,* 95:1040-1055.

Gramaje, D., Di Marco, S. (2015). Identifying practices likely to have impacts on grapevine trunk disease infections: a European nursery survey. *Phytopathologia Mediterranea,* 54:313-324.

Gramaje, D., Úrbez-Torres, J. R., Sosnowski, M. R. (2018). Managing grape trunk diseases with respect to etiology: current strategies and future prospects. *Plant Disease,* 102:12-39.

Gupta, B., Atreyee, S., Saha, J., Kamala, G. (2013). Plant abiotic stress: 'omics' approach. *Journal of Plant Biochemistry and Physiology,* 1:3.

Hasin, Y., Seldin, M., Lusis, A. (2017). Multi-omics approaches to disease. *Genome Biology,* 18:83.

Héloir, M. C., Li Kim Khiook, I., Lemaitre-Guillier, C., Clement, G., Jacquens, L., Bernaud, E., Trouvelot, S., Adrian, M. (2018). Assessment of the impact of PS3-induced resistance to downy mildew on grapevine physiology. *Plant Physiology and Biochemistry,* 133:134-141.

Hily, J. M., Demaneche, S., Poulicard, N., Tannières, M., Djennane, S., Beuve, M., Vigne, E., Demangeat, G. Komar, V., Gertz, C., Marmonier, A., Hemmer, C., Vigneron, S., Marais, A., Candresse, T., Simonet, P., Lemaire, O. (2018). Metagenomic-based impact study of transgenic grapevine rootstock on its associated virome and soil bacteriome. *Plant Biotechnology Journal,* 16(1):208-220.

Hu, W., Luo, H., Yang, Y., Wang, Q., Hong, N., Wang, G., Wang, A., Wang, L. (2019). Comprehensive analysis of full genome sequence and Bd-milRNA/target mRNAs to discover the mechanism of hypovirulence in *Botryosphaeria dothidea* strains on pear infection with BdCV1 and BdPV1. *IMA Fungus,* 10:3.

Jones, L., Riaz, S., Morales-Cruz, A., Amrine, K. C. H., McGuire, B., Gubler, W. D., Walker, M. A., Cantu, D. (2014). Adaptive genomic structural variation in the grape powdery mildew pathogen, *Erysiphe necator*. *BMC Genomics,* 15:1081.

Jorrín, J. V., Rubiales, D., Dumas-Gaudot, E., Recorbet, G., Maldonado, A., Castillejo, M. A., Curto, M. (2006). Proteomics: a promising approach to study biotic interaction in legumes. A review. *Euphytica,* 147:37-47.

Kankaala, P., Nandety, R. S., Mysore, K. S. (2019). Genomics of plant disease resistance in Legumes. *Frontiers in Plant Sciences,* 10:1345.

Kessle, A., Kalske, A. (2018). Plant secondary metabolite diversity and species interactions. *Annual Review of Ecology, Evolution, and Systematics,* 49:115-138.

Köhl, J., Kolnaar, R., Ravensberg, W. J. (2019). Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. *Frontiers in Plant Sciences,* 10:845.

Lee, K.-M., Cho, W. K., Yu, J., Son, M., Choi, H., Min, K., Lee, Y-W., Kim, K.-H. (2014). A comparison of transcriptional patterns and mycological phenotypes following infection of *Fusarium graminearum* by four mycoviruses. *PLoS One,* 9(6):e100989.

Lemaître-Guillier, C., Hovasse, A., Schaeffer-Reiss, C., Recorbet, G., Poinssot, B., Trouvelot, S., Daire, X., Adrian, M., Héloir, M. C. (2017). Proteomics towards the understanding of elicitor induced resistance of grapevine against downy mildew. *Journal of Proteomics,* 156:113-125.

Lemanceau, P., Barret, M., Mazurier, S., Mondy, S., Pivato, B., Fort, T., Vacher, C. (2017). Plant communication with associated microbiota in the spermosphere, rhizosphere and phyllosphere. *Advances in Botanical Research,* 82:101-133.

Marasco, R., Rolli, E., Fusi, M., Michoud, G., Daffonchio, D. (2018). Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. *Microbiome,* 6(1):3.

Martinelli, F., Scalenghe, R., Davino, S., Panno, S., Scuderi, G., Ruisi, P., Villa, P., Stroppiana, D., Boschetti, M., Goulart, L. R., Davis, C. E., Dandekar, A. M. (2014). Advanced methods of plant disease detection. A review. *Agronomy for sustainable Development,* 35(1):1-25.

Martínez-Diz, M. P., Andrés-Sodupe, M., Bujanda, R., Díaz-Losada, E., Eichmeier, A., Gramaje, D. (2019). Soil-plant compartments affect fungal microbiome diversity and composition in grapevine. *Fungal Ecology,* 41:234-244.

Massa, A. N., Childs, K. L., Buell, C. R. (2013). Abiotic and biotic stress responses in *Solanum tuberosum* group Phureja DM1-3 516 R44 as measured through whole transcriptome sequencing. *The Plant Genome,* 6(3).

Massart, S., Perazzolli, M., Höfte, M., Pertot, I., Jijakli, M. H. (2015). Impact of the omic technologies for understanding the modes of action of biological control agents against plant pathogens. *Biocontrol,* 60(6):725-746.

Merdinoglu, D., Schneider, C., Prado, E., Wiedemann-Merdinoglu, S., Mestre, P. (2018). Breeding for durable resistance to downy and powdery mildew in grapevine. *Oeno one,* 52(3):203-209.

Morales-Cruz, A., Allenbeck, G., Figueroa-Balderas, R., Ashworth, V. E., Lawrence, D. P., Travadon, R., Smith, R. J., Baumgartner, K., Rolshausen, P. E., Cantu, D. (2018). Closed-reference metatranscriptomics enables in planta profiling of putative virulence activities in the grapevine trunk disease complex. *Molecular Plant Pathology,* 19(2):490-503.

Moretti, A., Logrieco, A. F., Susca, A. (2017). "Mycotoxins: An Underhand Food Problem" In *Mycotoxigenic Fungi: Methods and Protocols,* edited by Antonio Moretti and Antonia Susca, 3-12. New York: Springer.

Müller, H., Berg, C., Landa, B. B., Auerbach, A., Moissl-Eichinger, C., Berg, G. (2015). Plant genotype-specific archaeal and bacterial endophytes but similar Bacillus antagonists colonize Mediterranean olive trees. *Frontiers in Microbiology,* 6:138.

Nejat, N., Rookes, J., Mantri, N. L., Cahill, D. M. (2017). Plant–pathogen interactions: toward development of next-generation disease-resistant plants. *Critical Reviews in Biotechnology*, 37(2):229-237.

Novello, G., Gamalero, E., Bon, E., Boatti, L., Mignone, F., Massa, N., Cesaro, P., Lingua, G., Berta, G. (2017). The rhizosphere bacterial microbiota of *Vitis vinifera* cv. Pinot Noir in an integrated pest management vineyard. *Frontiers in Microbiology,* 8:1528.

Ortíz Ríos, R. (2015). *Plant Breeding in the Omics Era*. Switzerland: Springer International Publishing.

Palmieri, M. C., Perazzolli, M., Matafora, V., Moretto, V., Bachi, A., Pertot, I. (2012). Proteomic analysis of grapevine resistance induced by *Trichoderma harzianum* T39 reveals specific defence pathways activated against downy mildew. *Journal of Experimental Botany,* 63(17):6237-6251.

Pancher, M., Ceol, M., Corneo, P. E., Oliveira Longa, C. M., Yousaf, S., Pertot, I., Campisano, A. (2012). Fungal endophytic communities in grapevines (*Vitis vinifera* L.) respond to crop management. *Applied and Environmental Microbiology,* 78(12):4308-4317.

Pandey, A., Mann, M. (2000). Proteomics to study genes and genomes. *Nature,* 405: 837-846.

Perazzolli, M., Antonielli, L., Storari, M., Puopolo, G., Pancher, M., Giovannini, O., Pindo, M., Pertot, I. (2014). Resilience of the natural phyllosphere microbiota of the grapevine to chemical and biological pesticides. *Applied Environmental Microbiology,* 80(12):3585-3596.

Perazzolli, M., Herrero, N., Sterck, L., Lenzi, L., Pellegrini, A., Puopolo, G., Van de Peer, Y., Pertot, I. (2016). Transcriptomic responses of a simplified soil microcosm to a plant pathogen and its biocontrol agent reveal a complex reaction to harsh habitat. *BMC Genomics*, 17(1):838.

Perazzolli, M., Moretto, M., Fontana, P., Ferrarini, A., Velasco, R., Moser, C., Delledonne, M., Pertot, I. (2012). Downy mildew resistance induced by *Trichoderma harzianum* T39 in susceptible grapevines partially mimics transcriptional changes of resistant genotypes. *BMC Genomics,* 13(1):1-19.

Pertot, I., Caffi, T., Rossi, V., Mugnai, L., Hoffmann, C., Grando, M. S., Gary, C., Lafond, D., Duso, C., Thiery, D., Mazzoni, V., Anfora, G. (2017). A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. *Crop Protection,* 97:70-84.

Pinto, C., Pinho, D., Sousa, S., Pinheiro, S., Egas, C., Gomes, A. C. (2014). Unravelling the diversity of grapevine microbiome. *PLoS One,* 9(1):e85622.

Popescu, S. C., Snyder, M., Dinesh-Kumar, S. P. (2007). Arabidopsis protein microarrays for the high-throughput identification of protein-protein interactions. *Plant Signal and Behaviour,* 2:416-420.

Puopolo, G., Selena, T., Pertot, I. (2018). The impact of the omics era on the knowledge and use of *Lysobacter* species to control phytopathogenic micro-organisms. *Journal of Applied Microbiology,* 124(1):15-27.

Rasmussen, S., Barah, P., Suarez-Rodriguez, M. C., Bressendorff, S., Friis, P., Costantino, P., Bones, A. M., Nielsen, H. B., Mundy, J. (2013). Transcriptome responses to combinations of stresses in Arabidopsis. *Plant Physiology,* 161(4):1783-1794.

Roossinck, M. J., Martin, D. P., Roumagnac, P. (2015). Plant virus metagenomics: Advances in virus discovery. *Phytopathology,* 105(6):716-727.

Ruano-Rosa, D., Mercado-Blanco, J. (2015). Combining biocontrol agents and organics amendments to manage soil-borne phytopathogens. In *Organic amendments and soil suppressiveness in plant disease management,* edited by Meghvansi, M. K. Varma, A. 457-478. Switzerland: Springer.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution,* 3(3):430-439.

Sham, A., Al-Azzawi, A., Al-Ameri, S., Al-Mahmoud, B., Awwad, F., Al-Rawashdeh, A., Iratni, R., AbuQamar, S. (2014). Transcriptome analysis reveals genes commonly induced by *Botrytis cinerea* infection, cold, drought and oxidative stresses in Arabidopsis. *PloS one,* 9(11).

Sharma, V., Salwan, R., Sharma, P. N., Gulati, A. (2017). Integrated translatome and proteome: approach for accurate portraying of widespread multifunctional aspects of *Trichoderma*. *Frontiers in Microbiology* 8: 1602.

Töpfer, R., Eibach, R. (2016). Pests and diseases: Breeding the next-generation disease-resistant grapevine varieties. *Wine & Viticulture Journal,* 31(5):47-49.

Töpfer, R., Hausmann, L., Harst, M., Maul, E., Zyprian, E., Eibach, R. (2011). New horizons for grapevine breeding. *Fruit, Vegetable and Cereal Science and Biotechnology,* 5:79-100.

Tracy, W. F. (2015). "Introducing to plant breeding." In *Plant Breeding in the Omics Era*, edited by Rodomiro Ortíz Ríos, 1-18. Switzerland: Springer International Publishing.

Turner, T. R., James, E. K., Pool, P. S. (2013). The plant microbiome. *Genome Biology,* 14:209.

Van Emon, J. M. (2016). The omics revolution in agricultural research. *Journal of Agricultural and Food Chemistry,* 64:36-44.

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *New Phytologist,* 206(4):1196-1206.

Vayssier-Taussat, M., Albina, E., Citti, C., Cosson, J. F., Jacques, M. A., Lebrun, M. H., Le Loir, Y., Ogliastro, M., Petit, M. A., Roumagnac, P., Candresse, T. (2014). Shifting the paradigm from pathogens to pathobiome: new concepts in the light of meta – omics. *Cellular and Infection Microbiology,* 4(29):1-7.

Wakefield, L., Gadoury, D. M., Seem, R. C., Milgroom, M. G., Sun, Q., Cadle-Davidson, L. (2011). Differential gene expression during condition in the grape powdery mildew pathogen, *Erysiphe necator*. *Phytopathology,* 101:839-846.

Walter, D. R., Ratsep, J., Havis, N. D. (2013). Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany,* 64(5):1263-1280.

Wang, L., Luo, L., Hu, W., Yang, Y., Hong, N., Wang, G., Wang, A., Wang, L. (2018). De novo transcriptomic assembly and mRNA expression patterns of *Botryosphaeria dothidea* infection with mycoviruses chrysovirus 1 (BdCV1) and partitivirus 1 (BdPV1). *Virology Journal,* 15:126.

Warren, R. L., Sutton, G. G., Jones, S. J. M., Holt, R. A. (2007). Assembling millions of short DNA sequences using SSAKE. *Bioinformatics,* 23:500-501.

Weldon, W. A., Palumbo, C. D., Kovaleski, A. P., Tancos, K., Gadoury, D. M. Osier, M. V., Cadle-Davidson, L. (2020). Transcriptomic profiling of acute cold stress-induced disease resistance (SIDR) genes and pathways in the grapevine powdery mildew pathosystems. *International Society for Molecular Plant-Microbe Interactions,* 33(2):284-295.

Yacoub, A., Gerbore, J., Magnin, N., Chambon, P., Dufour, M. C., Corio-Costet, M. F., Guyoneaud, R., Rey, P. (2016). Ability of Pythium oligandrum strains to protect *Vitis vinifera* L., by inducing plant resistance against *Phaeomoniella chlamydospora*, a pathogen involved in Esca, a grapevine trunk disease. *Biological Control,* 92:7-16.

Zarraonaindia, I., Owens, S. H., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N. A., Mills, D. A., Martin, G., Taghavi, S., van der Lelie, D., Gilbert. J. A. (2015). The soil microbiome influences grapevine-associated microbiota. *MBio,* 6(2):e02527-14.

Zheng, T., Zhang, K., Zhu, X., Guan, L., Jiu, S., Li, X., Nasim, M., Jia, H., Fang, J. (2019). Integrated metatranscriptome and transcriptome reveals the microbial community composition and physiological function of xylem sap on grapevine during bleeding period. *Genes and Genomics,* 41(9):1095-1111.

In: Vitis: Biology and Species
Editors: António M. Jordão et al.
ISBN: 978-1-53618-308-5

Chapter 10

REMOTE SENSING: IN THE DIGITAL VITICULTURE ERA

***Sergio Vélez*[1,*], *Enrique Barajas*[1], *José A. Rubio*[1], *Carlos Poblete-Echeverría*[2] and *Guillermo F. Olmedo*[3]**

[1]Instituto Tecnológico Agrario de Castilla y León, Unidad de Cultivos Leñosos y Hortícolas (ITACyL), Valladolid, España
[2]Department of Viticulture and Oenology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland, South Africa
[3]Instituto Nacional de Tecnología Agropecuaria (INTA), EEA Mendoza, Luján de Cuyo, Mendoza, Argentina

ABSTRACT

According to ESA (European Space Agency), Remote Sensing is a way of collecting and analysing data to get information about an object, without the instrument used to collect the data in direct contact with the object. This tool has proved its usefulness in a wide range of fields, including agriculture, a field in which the use of multispectral imagery has become widespread, and could become an important tool in the fight against the climate change. These images can be used alone or combined with other data to obtain better results, giving useful information about the state of the crop. Four elements are essential in remote sensing: a platform, a target object, a sensor and how to use and store the obtained information. At present, there are several platforms for obtaining information: satellites, drones, aeroplanes, land vehicles, etc. In such a way that, depending on the platform and the sensor, data will be obtained with different characteristics of spatial, temporal, spectral, and radiometric resolution and obviously, the cost will be different depending on the technology used. In this chapter, several topics related to remote sensing in viticulture will be addressed, such as how to obtain spatial data, use of UAV and satellite imagery,

---

[*] Corresponding Author's Email: velmarse@itacyl.es.

characteristics of each platform, use and calculation of vegetation indices or advanced applications in remote sensing in viticulture.

Keywords: digital viticulture, GIS (Geographic Information System), METRIC, remote sensing, sentinel, satellite, UAV (unmanned aerial vehicle), vegetation index, Vineyard.

1. INTRODUCTION

In an increasingly competitive and challenging agriculture world, it is mandatory to have the proper instruments to carry out appropriate management of our crops. Therefore, growers need new tools that allow not only to improve the quality of their products but also to increase efficiency, productivity and input reduction to make farms more competitive. To achieve this goal, the use of remote sensing could be a key tool, since it allows to obtain information quickly, accurately, objectively and non-destructively, in such a way that accurate measurements of the grapevines can be taken almost in real-time (Krishna, 2016). A practical and simple application of remote sensing is the calculation of Vegetation Indices, such as NDVI (Normalized Difference Vegetation Index), which has proved to be a useful tool in agriculture, to estimate canopy parameters in several horticultural crops (Trout et al., 2008). Research studies in viticulture show that remote sensing techniques allow to evaluate the variability of the vineyard and monitor the quality of the grapes (Johnson et al., 2001; Lamb et al., 2004; Anastasiou et al., 2018; Vélez et al., 2019). It is interesting to see how in some countries, such as Spain, investment in technological development in the agricultural sector is increasing, compared to other sectors (INE, 2018). This situation is leading to an increasing interest in precision agriculture, at least in the scientific and research field (Santesteban, 2019) and it seems to be a promising area in which currently there is a lot of progress. In this context, remote sensing could provide winegrowers and winemakers with a decision-making tool that can give information about the whole vineyard, as well as being affordable (or even free), reliable and fast.

2. REMOTE SENSING

According to ESA (European Space Agency), Remote Sensing is ‘a way of collecting and analysing data to get information about an object, without contact’. This tool has proved its usefulness in a wide range of fields, including agriculture, in which the use of multispectral imagery (contains information in bands according to several wavelengths) has become widespread, and could become an important role to mitigate climate change impacts. Multispectral images, can be used alone or combined with other data to obtain better results, giving useful information about the state of the crop.

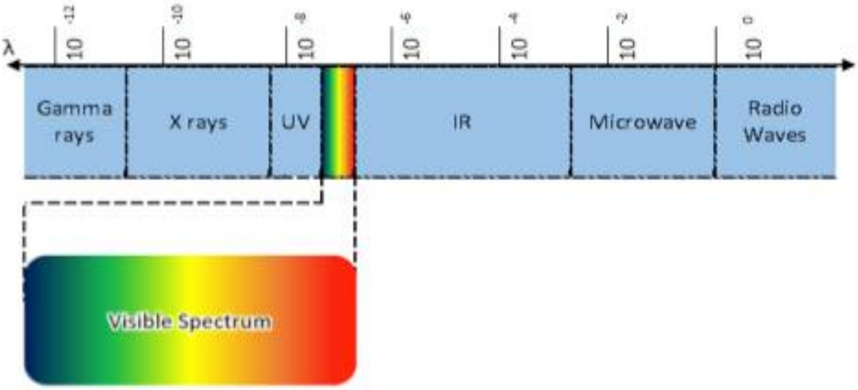
To implement remote sensing techniques, four elements are essential:

1. A platform to hold the instrument;
2. A target object to be observed;
3. An instrument or a sensor to observe the target;

And the last one, and the main purpose of remote sensing:

4. The information that is obtained from the acquired data, and how it is used and stored.

The pigments that compose the plants absorb mainly wavelengths in blue (between 420 and 480 nm) and red (between 640 and 700 nm), so the light reflected by their surface is mostly green (Evert and Eichhorn, 2012). Additionally, Kumar and Silva (1973) found that, for the same kind of crop, the near-infrared (λ = 700-1300 nm) and red (λ = 550-700 nm) reflectance differs depending on its condition. The human eye is only able to see light in the visible spectrum (Figure 1), but we can capture information from other areas of the electromagnetic spectrum using sensors mounted on various platforms, such as UAVs (Unmanned aerial vehicles), aircraft or satellites.



Adapted from Vélez et al. (2019).

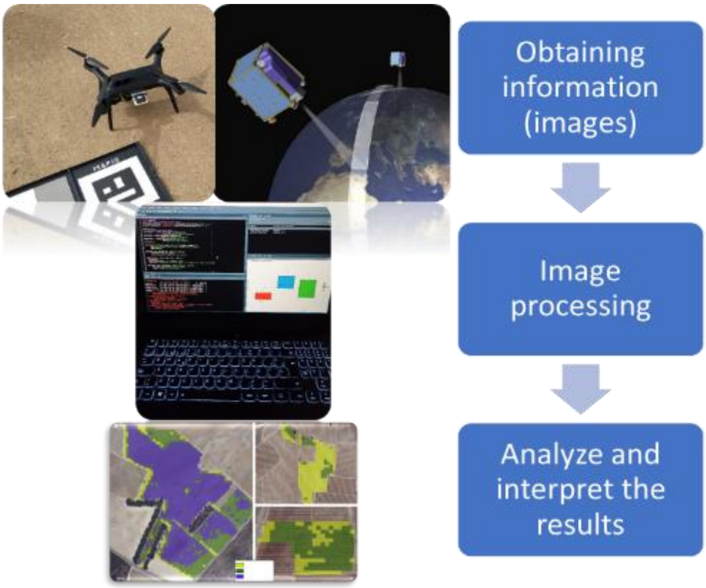
Figure 1. Electromagnetic spectrum.

The applications of remote sensing are many and varied. Some examples analyse the Earth's carbon cycle, mapping the world's forests, glacier movement assessment, climate change analysis, crop yields, changes in the atmosphere, air quality, seismic activity, assessing the impact of urban expansion, analyse natural disasters like floods, droughts, fires ... and even more applications that we have not mentioned or that are being developed today.

2.1. Remote Sensing Workflow

The remote sensing workflow has 3 basic parts (Figure 2):

1. Obtaining the information: We can acquire it from a satellite image company, download it for free, hire a UAV flight, among other sources.
2. Processing the information: We can use our computers or hire them.
3. Interpreting the results: This means, converting the remote sensing data in information to support decisions.



Source: Own elaboration.

Figure 2. Remote sensing workflow.

The interpretation is very important since many elements influence the images. When we take a picture of a crop, we get information about the surfaces of all elements within the image. Therefore, the information depends on the characteristics of the crop we are analysing (wheat is not the same as vines) and, in the case of the vineyard, we have to take into account many factors, such as the age, separation between rows and between plants, etc. This three-step process can be carried out by a technician within the winery itself, or it can be contracted to a company specialized in remote sensing applications.

3. HOW TO OBTAIN SPATIAL DATA

We can obtain spatial information in several ways, depending on the combination of sensor and platform selected. A platform is an object where the sensor is mounted. Platforms could be ground-based, often called proximal remote sensing, and usually mounted on ATV, tractor or similar vehicles. The platform could also be aerial, usually aeroplanes, but recently small unmanned aerial vehicles (UAVs) are used widely. And finally, the platform could be spaceborne, this means satellites. In this chapter, we will focus on aerial and satellite platforms since they are the most used for obtaining spatial information. Most widely used platforms for imaging are satellites, aeroplanes and UAVs, which are generally associated with low, medium and high spatial resolution, respectively. We will focus on satellites and flying drones (UAVs).

3.1. UAVs - Unmanned Aerial Vehicles

UAV - Unmanned aerial vehicle (also known just as ‘drones’ in some countries), are a good way to get high-resolution spatial information. To use UAV images, we must inevitably have a UAV to fly or hire a company to perform the flight and provide us with the images. Afterwards, we must process the image to be able to work with it. In any case, in most countries to fly a UAV requires qualified personnel with a license, and follow several requirements, which increase the cost of the operation.

3.2. Satellites

Numerous satellites in orbit are continuously generating images of the Earth's surface. There are two types of satellite constellations, depending on the availability of their images: i) paid images, provided by satellites such as GeoEye or WorldView and ii) free images, for example, Landsat or Sentinel, which we will talk about later. Usually, the paid satellites have a better spatial resolution.

3.3. Advantages and Disadvantages of Each Platform

Each platform has advantages and disadvantages, some examples are:

UAVs - Unmanned Aerial Vehicles

- The images have higher spatial resolution since they can fly closer to the target surface;
- You can choose when to fly;
- We cannot fly if there are strong winds or bad weather;
- They have a higher cost in large areas.

Satellites

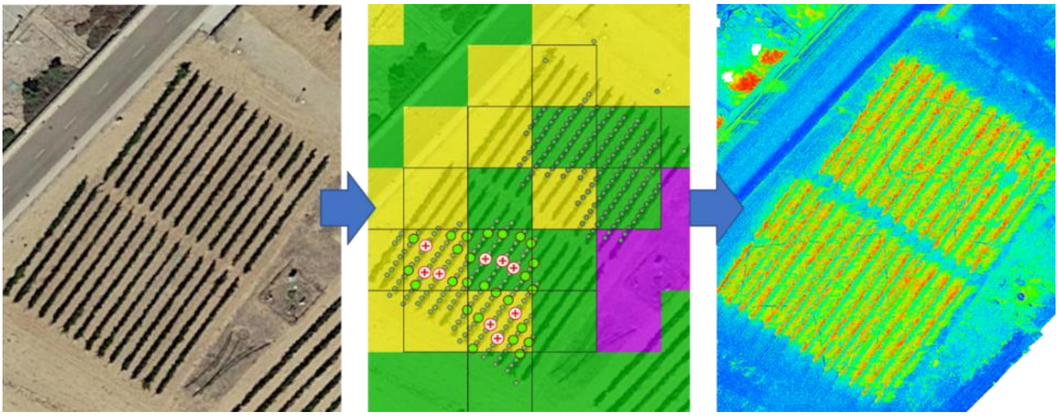
- It has a regular revisit time;
- Access to historical images;
- Even if there are strong winds, we will get the images, however, if there are clouds, we will not have any image;
- A large image of the entire area is obtained at the same time, so you can compare vineyards at the same time;
- It has a lower spatial resolution than other platforms, so each pixel contains vegetation and soil information;
- Sensors mounted on satellites usually have a better spectral and radiometric resolution. Besides, as they are not usually repairable once launched, they have better electronics and redundancy.

Additionally, it is important to highlight that within each group, there are many types of sensors and products because not all cameras, satellites or UAVs are the same. For example, when we choose to use satellite images, different resolutions are depending on the sensor chosen, and it is important to choose an appropriate image source (Sertel et al., 2012).

Which Platform to Choose

It depends on the objective of the work and the vineyard's characteristics. For example, it should be considered that if the farm is very large, the satellite imagery is the best choice because is a low-cost method compared to UAVs or ground-based robotic vehicles (Krishna, 2016).

The use of UAVs or aircraft implies an initial investment, as well as having qualified personnel capable of configuring and piloting these devices and performing subsequent processing of the data obtained (Grenzdörffer et al., 2008). Satellite imagery providers offer a ready-to-work image (maybe with a bit of processing). All of this increases the costs of some technologies over others and even a threshold value has been established: 5 hectares. A breakpoint is placed slightly above 5 ha, meaning that above such scale size the image taken by satellite may be more convenient (Matese et al., 2015). Anyway, if we can use both technologies, we should use both, because we can combine them (Figure 3) and these technologies are complementary and their combination can help in the decision-making process (Maes and Steppe, 2019).



Source: Authors.

Figure 3. Satellite and UAV image combination.

4. SENTINEL SATELLITES

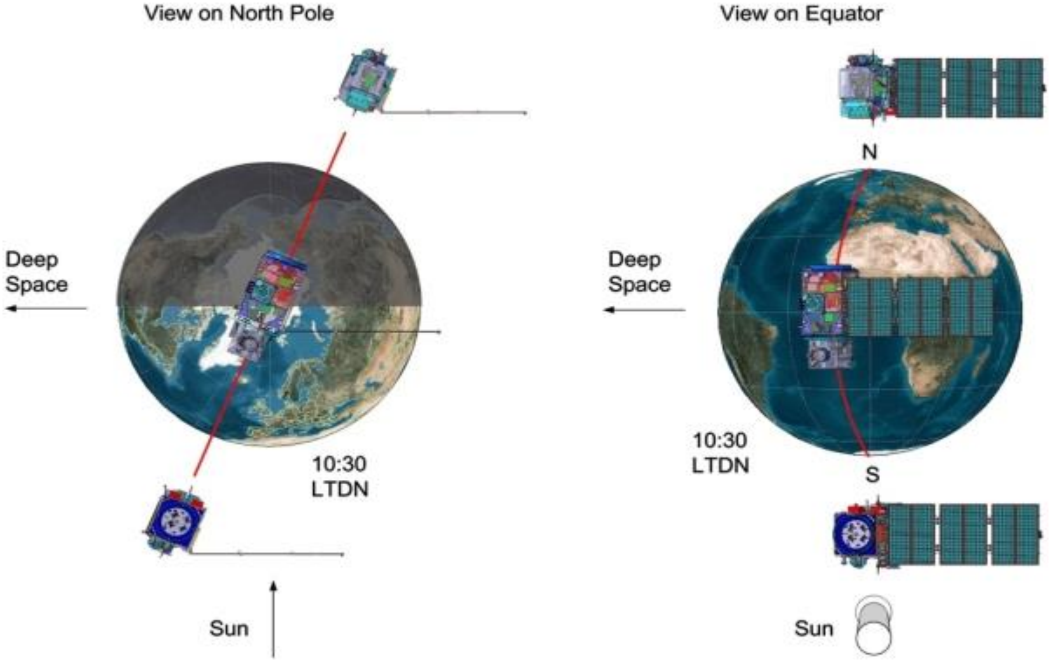
European Space Agency (ESA) is developing a new family of missions called Sentinel specifically for the operational needs of the Copernicus programme. Currently, Sentinel satellites are very important due to the usefulness they are demonstrating in several areas. Each Sentinel mission is based on a constellation of two satellites to fulfil revisit and coverage requirements, providing robust datasets for Copernicus Services.

4.1. Copernicus Programme

Copernicus is the European Union's Earth observation programme coordinated and managed by the European Commission in partnership with ESA. It provides accurate, timely and easily accessible information to improve the management of the environment, understand and mitigate the effects of climate change and ensure civil security. ESA is developing a new family of satellites, called Sentinels, specifically for the operational needs of the Copernicus programme. More information can be found on the ESA website (http://esa.int/).

4.2. Sentinel-2 Mission

According to the SENTINEL-2 User Handbook (ESA, 2015), the Sentinel-2 mission orbit is sun-synchronous. Sun-synchronous orbits are used to ensure the angle of sunlight upon the Earth's surface is consistently maintained. The mission comprises a constellation of two polar-orbiting satellites: Sentinel-2A and Sentinel-2B. They occupy the same orbit but separated by 180 degrees (Figure 4). In addition, the orbit time is 10 days and so we have an image every 5 days. The first satellite of the constellation was launched in June 2015. The Sentinel 2 satellites are placed in an orbit 786 km above the Earth's surface, with an orbit inclination of 98.62° and the Mean Local Solar Time (MLST) at the descending node is 10:30 (am). This value of MLST was chosen as a compromise between a suitable level of solar illumination and the minimization of potential cloud cover.



Source: ESA.

Figure 4. Twin-Satellite SENTINEL-2 Orbital Configuration.

Li and Roy (2017) indicate that it is possible to combine satellite images from different platforms (Sentinel-2A, Sentinel-2B and Landsat 8) to achieve an average image acquisition interval of fewer than 3 days since the MLST value in all of this cases is similar.

5. CORRECTION OF SATELLITE AND UAV DATA

It is very important to adequately prepare the images before using them. So, depending on the origin of the images, we will have to follow one process or another. For example, if we take the images with a UAV, we will obtain raw data and before we can analyse the images, we will have to calibrate them radiometrically, assemble the mosaic/orthophoto, georeferencing the image and post-process the data. Usually, this process is done using either commercial software (PIX4D, Agisoft PhotoScan, etc.) or open-source software (e.g., open drone map - https://www.opendronemap.org/). If we use satellite images, we will have other problems, such as correcting the effect of the atmosphere on the image. This process is called atmospheric correction and implies the conversion of TOA (Top-of-atmosphere) radiance values to surface reflectance. TOA reflectance is the raw reflectance of the Earth, measured from space, and is the sum of the light reflected from the surface and the atmosphere. On the other hand, the surface reflectance represents the reflectance measured on the surface. If we cannot download the corrected images, we can convert from TOA to BOA (Bottom-of-atmosphere) using physical models like Sen2cor for Sentinel.

6. USE AND CALCULATION OF VEGETATION INDICES

Multispectral images have been widely used to estimate vineyard biomass and to determine its spatial variability, caused by differences such as topography, soil characteristics, genetics, management or meso-climate. These estimates are made using vegetation indices (VI) calculated from the relationships between bands in specific sectors of the electromagnetic spectrum. These VIs are algebraic combinations designed to highlight the contrast of plant vigour and its properties (canopy biomass, absorbed radiation, chlorophyll content, etc.). These indices are in general based on the fact that plants in good health show high near-infrared reflectance and very low red reflectance (Proffitt, 2006; Bachmann et al., 2013).

6.1. Which Vegetation Index to Choose?

There are many vegetation indices. Several VI can be found in the literature listing over 500 (e.g., https://www.indexdatabase.de/db/i.php). We can choose the VIs depending on the crop or the objectives we want to achieve. Some of the most commonly VIs used are:

NDVI (Normalized Difference Vegetation Index)

The NDVI index is one of the most used indexes, due to its good results. It is a simple indicator, capable of detecting vegetation in an area and delivering a value that estimates the state of that vegetation. It relates the red and infrared band and its equation is as follows:

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)} \tag{1}$$

where:

- Red: spectral reflectance measurements acquired in the red region (visible)
- NIR: spectral reflectance measurements acquired in the near-infrared region.

SAVI (Soil Adjusted Vegetation Index)

Uses the same bands as the NDVI but tries to minimize the effect of ground reflectance on the image.

$$SAVI = \frac{(1+L)(NIR - Red)}{(NIR + Red + L)} \tag{2}$$

where:

- L: canopy background adjustment factor. If we do not have a value, we can use L = 0.5.

PVI (Perpendicular Vegetation Index)

PVI uses the perpendicular distance from each pixel coordinate to the ground line. We have to construct the ground line. It works very well in combination with others.

$$PVI = \sin(\rho) \times NIR - \cos(\rho) \times Red \tag{3}$$

where:

- ρ: The angle between the soil line and the NIR axis.

EVI (Enhanced Vegetation Index)

EVI index is designed to improve sensitivity in areas with high biomass density.

$$EVI = G \times \frac{(NIR - Red)}{(NIR + C1 \times Red - C2 \times Blue + L)} \tag{4}$$

where:

- C1 and C2: coefficients of the aerosol resistance.

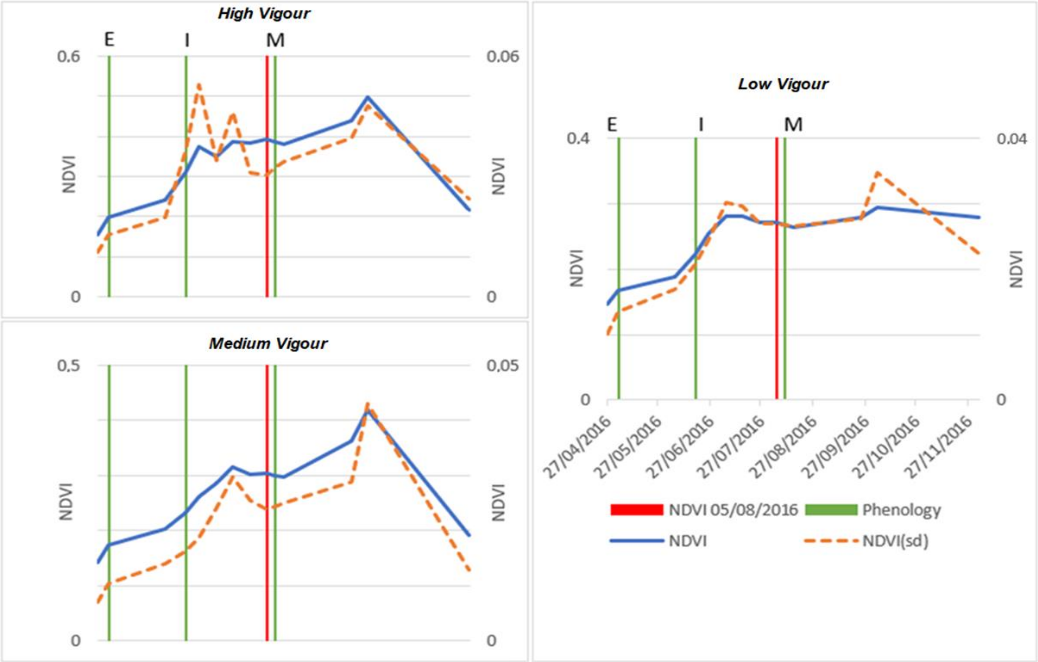
Every index has its limitations. For example, the NDVI does not consider the effect of the soil and, besides, high densities of vegetation saturate it. In contrast, the SAVI index takes into account the effect of soil, but the L factor requires calibration. PVI needs the soil line to be accurate and EVI needs the estimation of additional parameters. These indices can be combined to improve the understanding of the crop condition.

7. REMOTE SENSING IN THE DIGITAL VITICULTURE ERA

In viticulture, NDVI has proved to be a useful tool, and we can use it for many applications (Rouse et al., 1973). For example, for monitoring table grape quality characteristics (Anastasiou et al., 2018), vigour (Johnson et al., 2001), and to differentiate grape maturity and quality variables (Martinez-Casasnovas et al., 2012). The NDVI has also been related to other parameters measured in the field, such as LAI (Leaf Area Index), obtaining good results (Johnson, 2003; Towers et al., 2019), and it is even possible to use the NDVI to predict the grape yield (Sun et al., 2017). Also, the images can be used to estimate the spatial variability of the crop's vigour and have shown their usefulness in estimating the leaf structure of the plant, the phenol content and colour of the grapes (Lamb et al., 2004).

It is also possible to classify different vineyards according to their vigour to use them for the same or different winemaking processes. Vélez et al. (2019) present the classification of three commercial vineyards according to the vigour already implanted and in full production, belonging to the same winemaking group and located in the same area, but separated as far as 3.5 km. They studied the homogeneity of the vineyard to choose which image to use for classification (Figure 5). By emulating that work, we could use Sentinel-2 imagery to carry out an intra-classification, to divide the vineyard into distinct areas. This shows that remote sensors with moderate resolution allow us to obtain a lot of information about the crop, even in vineyards with low vigour. But it is important to be noted that resolutions greater than 25 cm present problems due to the discontinuous canopy of the vines. In a vineyard, only a fraction of the surface is covered by plants so the images are composed of mixed pixels, each one including vegetation, soil and shadows (Johnson and Scholasch, 2005). If the resolution is less than 0.5 m^2 it will be possible to find pixels with 100% vegetation, however, if it is 2 m^2 or more there will be only mixed pixels of vegetation, shadows and soil (Fuentes et al., 2014). Therefore, when using low-resolution imagery, the values of the pixels are an indicator of the amount of vegetation within the pixel (Vélez et al., 2020).

Because many vineyards are small areas (1 to 20 ha), an alternative is to use high spatial resolution images (less than 4 metres). In addition to the spatial resolution, the quality of the sensors must be taken into account from a radiometric point of view, because the VIs are constructed by calculating light reflectance values. Thus, the quality of a sensor should be defined in at least two terms: i) its spatial resolution and ii) its radiometric sensitivity. Vila et al. (2007) compared two multispectral sensors with different spatial resolution and radiometric sensitivity, one satellite (QuickBird) and one airborne (DMSC MKII) as vineyard biomass estimators, using NDVI and SR. The work was repeated in two conduction systems: vine arbour (horizontal canopy) and trellis (vertical canopy), due to the difficulty of working with fruit trees, since they do not have perfect Lambertian surfaces and therefore the results may be influenced by the shape of the plants and the relationship between the canopy and uncultivated soil. It was concluded that high-resolution aerial and satellite multispectral images are suitable for discriminating areas of vineyards with different biomass production. The satellite images were better because of their higher radiometric quality. Finally, it should be noted that acquiring aerial images generates a lower economic cost than acquiring satellite images, except if the satellite images are free, as is the case of Sentinel images.

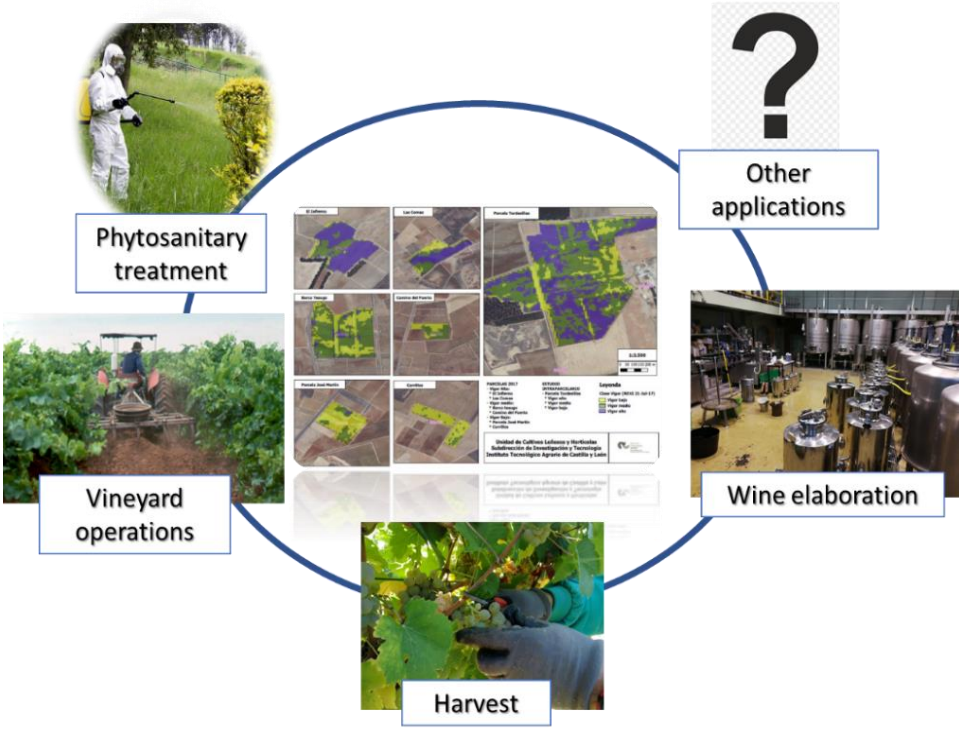


Adapted from Vélez et al. (2019).

Figure 5. Studying the variability of a vineyard over time.

Finally, we must consider UAVs, since for some authors the applicability of satellite or aerial images is limited by low revisit frequencies or low spatial resolutions, even for high-resolution images (Nebiker et al., 2008). In this sense, the use of UAVs allows the capture of very high-resolution images with low operational costs, whether fixed wings, helicopters or multi-rotors (Zhang and Kovacs, 2012). UAVs allow flying at relatively low altitudes, and this has an impact on the spatial resolution of the products obtained.

There are other applications that we have not mentioned here, which are already being carried out by remote sensing companies and wineries (Figure 6).



Source: Own elaboration.

Figure 6. Other applications of remote sensing in viticulture.

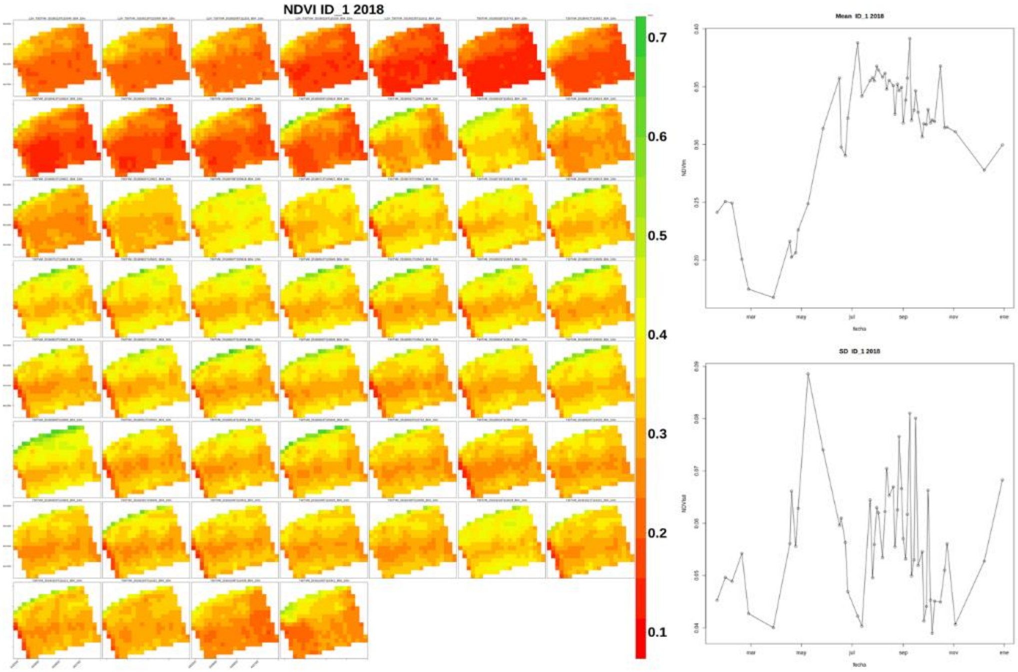
For example, they use these tools to:

- Classify according to quality and wine elaboration objectives;
- Establish the optimum time for harvesting;
- Determine water needs and assess the nutritional status of the crop;
- Missing plant detection;
- Adjust phytosanitary treatments;
- Estimate personnel for pruning;
- Etc.

To conclude this section, we would like to point out that i) it is difficult for a single image to solve our problems. We must combine it with other sources of information, and ideally with other images over time, ii) to develop its potential we must have adequate training/personnel trained in these tools and iii) we must adapt operations to the use of this type of tool (for example, removing weeds). Therefore, we must continue to go to the vineyard on our own foot.

8. ADVANCED APPLICATIONS OF REMOTE SENSING IN VITICULTURE

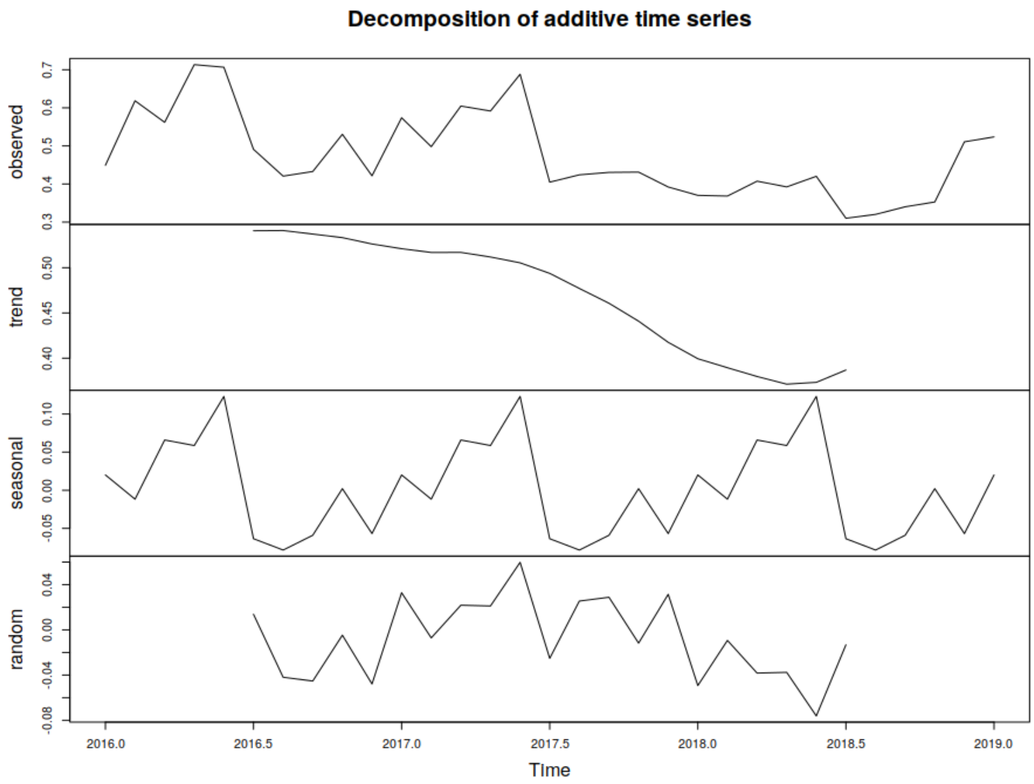
In research centres and universities, like the Agricultural Technology Institute of Castilla y León (ITACyL), in Spain; the Agricultural Experimental Station of INTA in Mendoza, Argentina, and; the University of Stellenbosch, in South Africa, we are currently working to find new ways of using remote sensing in vineyards.



Source: Own elaboration.

Figure 7. Example of NDVI evolution in a vineyard.

In this way, we are carrying out tasks related to what has already been explained in this chapter, and others such as crop prediction, evaluation of the impact of climatic events, classification based on agronomic and quality parameters, etc. For example, several satellite images could be used to build time series and analyse their evolution (Figure 7) or it can be evaluated its relationship with bio-physic parameters (Vélez et al., 2019b). If we have a database of our vineyard, we could compare a given year with another reference year and assess whether there is a problem in our vineyard. We could even use different techniques to create a model of our vineyard and decompose our data to see if the data for a given moment fits the model or if, on the contrary, there has been a problem, for example, a frost or poor phytosanitary treatment (Figure 8).

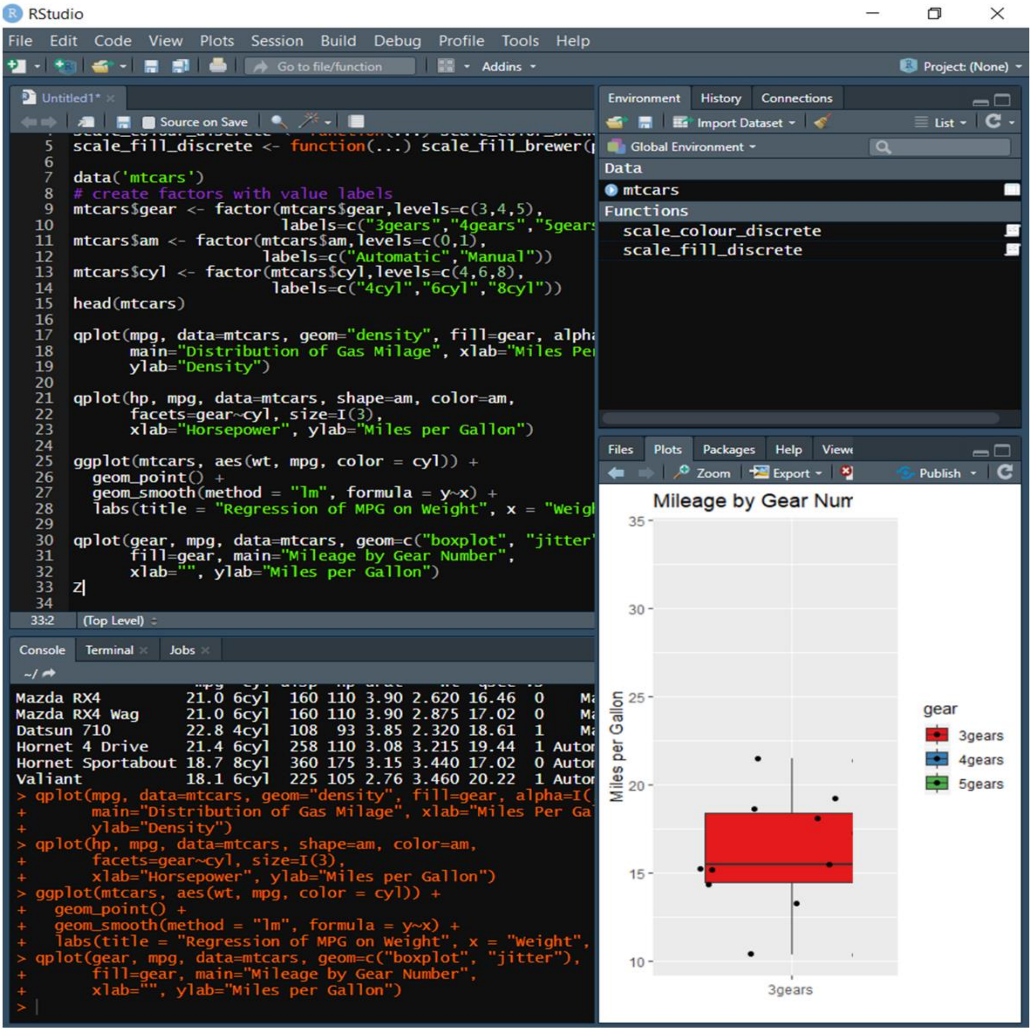


Source: Own elaboration.

Figure 8. Decomposition of data into components.

Since it is not easy to work with large volumes of data, programming languages such as R or Python can be used to assist in processing the data (Figure 9).

Related to UAVs, we can use more complex algorithms and more advanced models to check which variables have more influence on our vineyard and make more accurate classifications. Poblete-Echeverría et al. (2017) tested the efficiency of four classification methods (Spectral index (SI), K-means, Artificial Neural network (ANN) and Random Forest), for the detection of vine canopies using very high-resolution RGB images mounted on a low-cost UAV (Figure 10). In this work, it was demonstrated that it is possible to perform a segmentation of the vine canopies from very high-resolution RGB images. In particular, the RGB spectral index methods (Richardson et al., 2007) complemented with the Otsu method (Otsu, 1979; Huang et al., 2011) of threshold detection had a very high precision and stability for different moments of the crop cycle. These methods allow to obtain information about the fraction of coverage and to monitor the development of the vineyard.



```
scale_fill_discrete <- function(...) scale_fill_brewer(

data('mtcars')
# create factors with value labels
mtcars$gear <- factor(mtcars$gear,levels=c(3,4,5),
                      labels=c("3gears","4gears","5gears
mtcars$am <- factor(mtcars$am,levels=c(0,1),
                    labels=c("Automatic","Manual"))
mtcars$cyl <- factor(mtcars$cyl,levels=c(4,6,8),
                     labels=c("4cyl","6cyl","8cyl"))
head(mtcars)

qplot(mpg, data=mtcars, geom="density", fill=gear, alpha
      main="Distribution of Gas Milage", xlab="Miles Per
      ylab="Density")

qplot(hp, mpg, data=mtcars, shape=am, color=am,
      facets=gear~cyl, size=I(3),
      xlab="Horsepower", ylab="Miles per Gallon")

ggplot(mtcars, aes(wt, mpg, color = cyl)) +
  geom_point() +
  geom_smooth(method = "lm", formula = y~x) +
  labs(title = "Regression of MPG on Weight", x = "Weigh

qplot(gear, mpg, data=mtcars, geom=c("boxplot", "jitter"
      fill=gear, main="Mileage by Gear Number",
      xlab="", ylab="Miles per Gallon")
z
```

```
Mazda RX4          21.0 6cyl  160 110 3.90 2.620 16.46  0    Ma
Mazda RX4 Wag      21.0 6cyl  160 110 3.90 2.875 17.02  0    Ma
Datsun 710         22.8 4cyl  108  93 3.85 2.320 18.61  1    Ma
Hornet 4 Drive     21.4 6cyl  258 110 3.08 3.215 19.44  1 Autor
Hornet Sportabout  18.7 8cyl  360 175 3.15 3.440 17.02  0 Autor
Valiant            18.1 6cyl  225 105 2.76 3.460 20.22  1 Autor
> qplot(mpg, data=mtcars, geom="density", fill=gear, alpha=I(
+       main="Distribution of Gas Milage", xlab="Miles Per Ga
+       ylab="Density")
> qplot(hp, mpg, data=mtcars, shape=am, color=am,
+       facets=gear~cyl, size=I(3),
+       xlab="Horsepower", ylab="Miles per Gallon")
> ggplot(mtcars, aes(wt, mpg, color = cyl)) +
+   geom_point() +
+   geom_smooth(method = "lm", formula = y~x) +
+   labs(title = "Regression of MPG on Weight", x = "Weight",
> qplot(gear, mpg, data=mtcars, geom=c("boxplot", "jitter"),
+       fill=gear, main="Mileage by Gear Number",
+       xlab="", ylab="Miles per Gallon")
> |
```

Source: Own elaboration.

Figure 9. Programming language R.

Another application of remote sensing in vineyards corresponds to the calculation of water needs, which are a key factor in agriculture. These are usually estimated from crop evapotranspiration (ETc). An adequate quantification of ET allows the development of irrigation strategies, improves water use efficiency, and increases the irrigated area and production (Ferreyra et al., 1985; Baruch and Fisher, 1991; Millar, 1993).

Traditional methods to estimate ET are based on direct measurements with sophisticated instruments, such as lysimeters (Payero and Irmak, 2008), micro-meteorological stations of eddy covariance (Parent and Anctil, 2012; Poblete-Echeverria and Ortega-Farias, 2013), Bowen's ratio (Cragoa and Brutsaert, 1996); or on empirical methods, such as FAO-56 (Allen et al., 1998). Although all these methods may be accurate, it is difficult to extrapolate them to the plot level or regional levels since they do not take into account the effect of spatial-temporal variability of soil, climate or crop on ET (Richard et al., 2011).

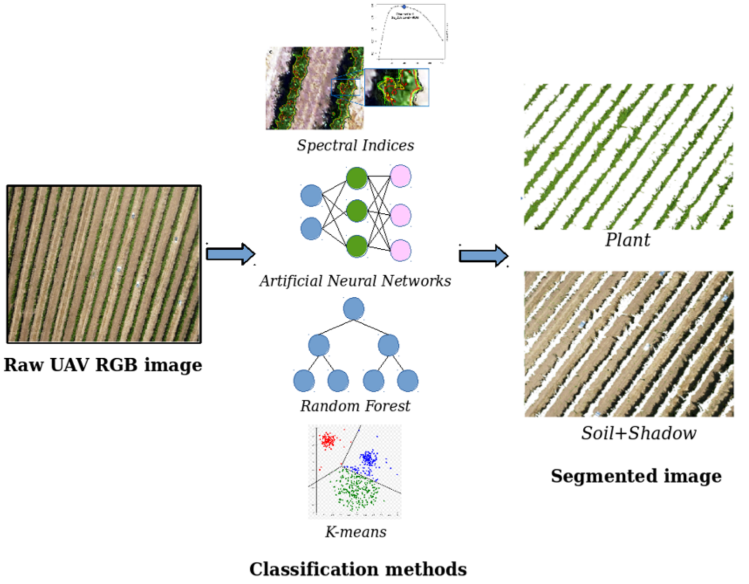


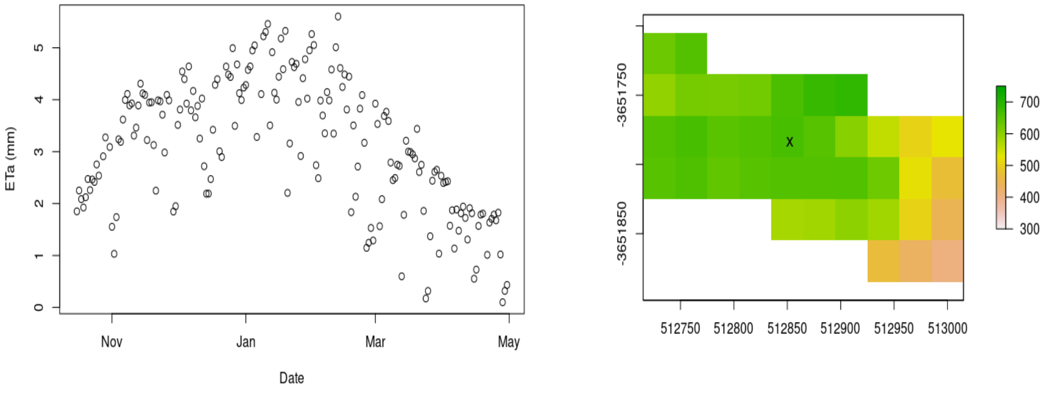
Figure 10. Classification methods for the segmentation of vineyard images. Adapted from Poblete-Echeverría et al. (2017).

To solve this, physical-mathematical methods can be used to estimate evapotranspiration using remote sensors. One of the first methods developed and applied on a global scale was SEBAL (Surface Energy Balance Algorithms for Land) (Bastiaanssen et al., 1998). This model estimates evapotranspiration from the surface energy balance equation. To calibrate the method, 2 pixels selected in the image (anchor pixels) are used, under conditions of maximum and minimum evapotranspiration, adjusting a linear relationship between surface temperature and the difference between surface temperature and air temperature. Based on this, Richard et al. (2007) developed the METRIC model (Mapping EvapoTranspiration at high Resolution with Internalized Calibration. METRIC estimates ETc as a residual of the energy balance equation, using data from satellite images and a weather station located within the study area (Figure 11).

$$LE = R_n - G - H \tag{5}$$

where:

- LE, latent heat flux consumed in the evapotranspiration process ($W \cdot m^{-2}$)
- R_n, net radiation ($W \cdot m^{-2}$)
- G, soil heat flux ($W \cdot m^{-2}$)
- H, sensible heat flux transmitted by convection to the air ($W \cdot m^{-2}$).



Source: Own elaboration.
Figure 11. Daily actual vineyard evapotranspiration (left) and total water consumption (in mm) during the entire vine cycle (right), estimated using the METRIC model and R water package. Pixel size: 30x30 meters.

The major difference between the two models is that METRIC uses the reference evapotranspiration of a weather station, considering the climatic conditions, while SEBAL uses the potential evapotranspiration of a water body in the image, where the sensible and soil heat fluxes are considered to be zero. One way to use these models is by using the water package in R, created by Olmedo et al. (2016).

CONCLUSION

There are many practical ways to incorporate remote sensing techniques to manage the vineyards, such as employing NDVI according to vigour to divide the vineyard into subzones or estimating quality and agronomic parameters in the field. In the research area, complex algorithms have been used to forecast yields or to model crop development. Soon, these applications will be extended to the productive sector, to benefit from them. Remote sensing is an essential tool to improve vineyard sustainability. It should be noted that the term "remote sensing" covers several technologies and these can be used in the vineyard for several applications. It is possible to use these techniques by investing in equipment or by paying for satellite images, however, also free valuable information is available, such as Sentinel-2 images. The challenge for this "Digital era" is to have professionals with enough training to use this kind of technology to provide practical solutions to the winegrowers.

REFERENCES

Allen, R.G., Pereira, L.S., Howell, T.A. Jensen, M.E. (2011). Evapotranspiration information reporting: I. Factors governing measurement accuracy. *Agricultural Water Management,* 98 (6):899-920.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M. (1998). Crop evapotranspiration - Guidelines for computing crop water requirements. *Food & Agriculture Org.* Vol. 56.

Anastasiou, E., Balafoutis, A., Darra, N., Psiroukis, V., Biniari, A., Xanthopoulos, G., Fountas, S. (2018). Satellite and proximal sensing to estimate the yield and quality of table grapes. *Agriculture,* 8:94.

Bachmann, F., Herbst, R., Gebbers, R., Hafner, V.V. (2013). Micro UAV based georeferenced orthophoto generation in VIS + NIR for precision agriculture. *International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences*, Volume XL-1/W2, UAV-g2013 XL (September):11-16.

Baruch, Z., Fisher, M. (1991). Factores climáticos Y de competencia que afectan el desarrollo de la planta en el establecimiento de una pastura. *Establecimiento Y Renovación de Pasturas.* CIAT. Cali, Colombia, 103–42. [Climate and competition factors affecting plant development in the establishment of a pasture. *Establishment and Renovation of Pastures.* CIAT. Cali, Colombia, 103-42.]

Bastiaanssen, W.G.M., Meneti, M., Feddes, R.A., Holtslag, A.A.M. (1998). A remote sensing surface energy balance algorithm for land (SEBAL): 1. Formulation. *Journal of Hydrology,* 212-213:198-212.

Caruso, G., Tozzini, L., Rallo, G., Primicerio, J., Moriondo, M., Palai, G., Gucci, R. (2017). Estimating biophysical and geometrical parameters of grapevine canopies ('Sangiovese') by an unmanned aerial vehicle (UAV) and VIS-NIR cameras. *Vitis,* Vol 56, No 2.

Cragoa, R., Brutsaert, W. (1996). Daytime evaporation and the self-preservation of the evaporative fraction and the bowen ratio. *Journal of Hydrology,* 178 (1–4):241-255.

European Space Agency (2015). SENTINEL-2 User Handbook. *ESA Standard Document.*

Evert, R.F., Eichhorn, S.E. (2012). Raven biology of plants. *Freeman and Company Worth Publishers*. ISBN: 9781429219617.

Ferreyra, R., Sellés, G., Tosso, J. (1985). Efecto de diferentes alturas de agua sobre el cultivo del pimiento. I. Influencia de los excesos de humedad. *Agricultura Técnica,* 45 (1):47–51. [Effect of different water heights on pepper cultivation. I. Influence of Excess Moisture. *Technical Agriculture,* 45 (1):47-51.]

Fuentes, S., Poblete-Echeverría, C., Ortega-Farias, S., Tyerman, S., De Bei, R. (2014). Automated estimation of leaf area index from grapevine canopies using cover photography, video and computational analysis methods: New automated canopy vigour monitoring tool. *Australian Journal of Grape and Wine Research,* 20:465-473.

Grenzdörffer, G.J., Engel, A., Teichert, B. (2008). The photogrammetric potential of low-cost UAVs in forestry and agriculture. *The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences.* Vol. XXXVII. Part B1.

Huang, D.Y., Lin, T.W., Hu, W.C. (2011). Automatic multilevel thresholding based on two-stage otsu's method with cluster determination by valley estimation. *International Journal of Innovative Computing, Information and Control*, 7 (10):5631–5644.

Instituto Nacional de Estadística INE, 2018. *España en cifras 2018*. ISSN: 2255-0410. [National Institute of Statistics INE, *2018 Spain in numbers*. ISSN: 2255-0410]

Johnson, L.F., Bosch, D.F., Williams, D.C., Lobitz, B.M. (2001). Remote sensing of vineyard management zones: implications for wine quality. *Applied Engineering in Agriculture*, 17. Vol. 17(4):557-560.

Johnson, L.F., Roczen, D.E., Youkhana, S.K., Nemani, R.R., Bosch, D.F. (2003). Mapping vineyard leaf area with multispectral satellite imagery. *Computers and Electronics in Agriculture*, 38 (1):33-44.

Johnson, L., Scholasch, T. (2005). Remote sensing of shaded area in vineyards. *Horttech*, 15:859–863.

Krishna, K.R. (2016). *Push button agriculture: Robotics, UAVs, satellite-guided soil and crop management*. Apple Academic Press, Inc. CRC Press, Taylor & Francis Group. ISBN-13, 978-1-77188-305-4.

Kumar, R., Silva, L. (1973). Light ray tracing through a leaf cross-section. *Appl. Optics,* 12:2950-2954.

Lamb, D.W., Weedon, M.M., Bramley, R.G.V. (2004). Using remote sensing to predict grape phenolics and colour at harvest in a Cabernet Sauvignon vineyard: Timing observations against vine phenology and optimising image resolution. *Australian Journal of Grape and Wine Research*, 10:46-54.

Li, J., Roy, D. (2017). A global analysis of sentinel-2A, sentinel-2B and landsat-8 data revisit intervals and implications for terrestrial monitoring. *Remote Sensing,* 9, 9:902.

Maes, W.H., Steppe, K. (2019). Perspectives for remote sensing with unmanned aerial vehicles in precision agriculture. *Trends in Plant Science*, 24, n.º2:152-164.

Martinez-Casasnovas, J.A., Agelet-Fernandez, J., Arno, J., Ramos, M.C. (2012). Analysis of vineyard differential management zones and relation to vine development, grape maturity and quality. *Spanish Journal of Agricultural Research*, 10:326-337.

Matese, A., Toscano, P., Di Gennaro, S., Genesio, L., Vaccari, F., Primicerio, J., Belli, C., Zaldei, A., Bianconi, R., Gioli, B. (2015). Intercomparison of UAV, aircraft and satellite remote sensing platforms for precision viticulture. *Remote Sensing,* 7:2971-2990.

Millar, A.A. (1993). Manejo de Agua Y Producción Agrícola. *Instituto Interamericano de Cooperación para la Agricultura. IICA*. [Water Management and Agricultural Production. *Inter-American Institute for Cooperation on Agriculture. IICA*].

Nebiker, S., Annen, A., Scherrer, M., Oesch, D. (2008). A Light-weight multispectral sensor for micro Uav - opportunities for very high resolution airborne remote sensing. *The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences* XXXVII (December):1193-1200.

Olmedo, G.F., Ortega-Farías, S., De la Fuente-Sáiz, D., Fonseca, D., Fuentes-Peñailillo, F. (2016). Water: tools and functions to estimate actual evapotranspiration using land surface energy balance models in R. *The R Journal,* 8 (2):352-369.

Otsu, N. (1979). A Threshold selection method from gray-level histograms. *IEEE Transactions on Systems Man, and Cybernetics,* 9:62-66.

Parent, A.C., Anctil, F. (2012). Quantifying evapotranspiration of a rainfed potato crop in South-Eastern Canada using eddy covariance techniques. *Agricultural Water Management,* 113:45-56.

Payero, J.O., Irmak, S. (2008). Construction, installation, and performance of two repacked weighing lysimeters. *Irrigation Science,* 26 (2):191-202.

Poblete-Echeverría, C.A., Ortega-Farias, S.O. (2013). Evaluation of single and dual crop coefficients over a drip-irrigated Merlot vineyard (*Vitis vinifera* L.) using combined measurements of sap flow sensors and an eddy covariance system. *Australian Journal of Grape and Wine Research,* 19(2):249-260.

Poblete-Echeverría, C., Olmedo, G.F., Ingram, B., Bardeen, M. (2017). Detection and segmentation of vine canopy in ultra-high spatial resolution RGB imagery obtained from unmanned aerial vehicle (UAV): a case study in a commercial vineyard. *Remote Sensing,* 9 (3).

Proffitt, A.P.B. (2006). Precision Viticulture: A new era in vineyard management and wine production. *Ashford, S. Aust.: Winetitles.*

Richardson, A.D., Jenkins, J.P., Braswell, B.H., Hollinger, D.Y., Ollinger, S.V. Smith, M.L. (2007). Use of digital webcam images to track spring green-up in a deciduous broadleaf forest. *Oecologia,* 152 (2):323-334.

Rouse, J.W. Jr., Haas, R.H., Schell, J.A., Deering, D.W. (1973). Monitoring vegetation systems in the great plains with ERTS. *Proceedings of the Third ERTS Symposium, NASA SP-351 1.* U.S. Government Printing Office, Washington DC (United States), pp. 309-317.

Santesteban, L.G. (2019). Precision viticulture and advanced analytics. A short review. *Food Chemistry,* 279:58-62.

Sertel, E., Seker, D.Z., Yay, I., Ozelkan, E., Saglam, M., Boz, Y., Gunduz, A. (2012). Vineyard mapping using remote sensing technologies. *FIG Working Week 2012.* Rome, (Italy).

Sun, L., Gao, F., Anderson, M.C., Kustas, W., Alsina, M., Sanchez, L., Sams, B. (2017). Daily mapping of 30 m LAI and NDVI for grape yield prediction in California vineyards. *Remote Sensing,* 9 (4):317.

Towers, P.C., Strever A., Poblete-Echeverria C. (2019). Comparison of vegetation indices for leaf area index estimation in vertical shoot positioned vine canopies with and without grenbiule hail-protection netting. *Remote Sensing,* 11:1073-1089.

Trout, T.J., Johnson, L.F., Gartung, J. (2008). Remote sensing of canopy cover in horticultural crops. *HortScience,* 43:333-337.

Vélez, S., Rubio, J.A., Andrés, M.I., Barajas, E. (2019a). Agronomic classification between vineyards (‘Verdejo’) using NDVI and Sentinel-2 and evaluation of their wines. *Vitis,* 58, nº 5 (Special Issue):33-38.

Vélez, S., Rubio, J.A., Andrés, M.I., Barajas, E. (2019b). Evolución del NDVI calculado a partir de imágenes Sentinel-2 y su relación con los parámetros bio-físicos en un viñedo de la variedad Verdejo durante 3 años. *XVIII Congreso de la Asociación Española de Teledetección.* Valladolid (España). 24-27 Septiembre de 2019. p 67-70. ISBN: 978-84-1320-038-5. [Evolution of NDVI calculated from Sentinel-2 images and its relationship with biophysical parameters in a Verdejo vineyard for 3 years. *XVIII Congress of the Spanish Association of Remote Sensing*. Valladolid (Spain). 24-27 September 2019. p 67-70. ISBN:978-84-1320-038-5.]

Vélez, S., Barajas, E., Rubio, J.A., Vacas, R., Poblete-Echeverría, C. (2020) Effect of Missing Vines on Total Leaf Area Determined by NDVI Calculated from Sentinel Satellite Data: Progressive Vine Removal Experiments. *Appl. Sci*., 10, 3612.

Vila, H., Perez Peña, J., García, M., Vallone, R.C., Mastrantonio, L., Olmedo, G.F., Rodríguez, L., Salcedo, C. (2007). Estimación de la biomasa en viñedos mediante imágenes satelitales y aéreas de Mendoza, Argentina. *Congreso Internacional de La AET. Teledetección Hacia Un Mejor Entendimiento de La Dinámica Global Y Regional.* Editado por Rivas, R., Grisotto, R. and Sacido, A. 51 to 58. [Biomass estimation in vineyards using satellite and aerial images of Mendoza, Argentina. *AET International Congress. Remote Sensing Toward a Better Understanding of Global and Regional Dynamics.* Edited by Rivas, R., Grisotto, R. and Sacido, A. 51 to 58.]

Zhang, C., Kovacs, J.M. (2012). The application of small unmanned aerial systems for precision agriculture: A review. *Precision Agriculture,* 13 (6):693-712.

In: Vitis: Biology and Species
Editors: António M. Jordão et al.
ISBN: 978-1-53618-308-5

Chapter 11

BIOSYNTHESIS AND PROFILING OF GRAPE VOLATILE COMPOUNDS

Iva Šikuten[1,2], Marina Anić[1], Petra Štambuk[1,2], Ivana Tomaz[1,*], Domagoj Stupić[1], Željko Andabaka[1], Zvjezdana Marković[1], Jasminka Karoglan Kontić[1,2], Edi Maletić[1,2], Marko Karoglan[1] and Darko Preiner[1,2]

[1]Department of Viticulture and Enology,
University of Zagreb Faculty of Agriculture, Zagreb, Croatia
[2]Centre of Excellence for Biodiversity and Molecular Plant Breeding,
University of Zagreb Faculty of Agriculture, Zagreb, Croatia

ABSTRACT

Volatile compounds are secondary metabolites that play a key role in the organoleptic properties of the grapes and wine. To this day, more than 700 volatile compounds have been identified in grapes and wine. The most important groups of volatile compounds are monoterpenes, C_{13}-norisoprenoids, sesquiterpenes, alcohols, esters, ketones, aldehydes, fatty acids, methoxypyrazines and polyfunctional thiols. All volatile compounds are contained in the pericarp and their largest proportion is in the grape skin (exocarp). In grapes, they can be present in the free form and in the form of glycosides, most often in the form of diglucosides, with glucose as a unit directly linked to a non-sugar molecule, i.e., an aglycone. The aglycones can be monoterpenes, C_{13}-norisoprenoids and volatile phenols. They are released from the berry during grape crushing by enzymatic hydrolisis or during vinification by chemical hydrolisis, and as such, in free form give the aroma of grapes and wines. Monoterpenes and C_{13}-norisopreinods are synthesized in plastids but stored in vacuoles in the form of glycosides, which, unlike aglycones, are stable, soluble in water and can only be stored in aqueous media. Grapevines volatile composition is determined

---

[*] Corresponding Author's Email: itomaz@agr.hr.

by the variety and can be influenced by biotic and abiotic factors and by different vineyard management practices. This chapter mainly concentrates in uniting the exisitng knowledge of the biosynhtesis, composition and content of volatile compounds in different berry tissue and grape varieties.

Keywords: biosynthesis, grape varieties, volatile compounds

1. INTRODUCTION

The aromatic profile of grapes is very complex because it includes a large number of different compounds which can act synergistically or antagonistically and will define sensory properties of wines. In essence, wine producers are selling a sensory experience to the consumer, which rely not on bold consitent flavours, but upon a subtle array of shifting sensations (Bisson et al., 2002). Thus, the aroma and flavour of wine are one of the main characteristics that define the differences between the vast array of wines and wine styles produced throughout the world (Swiegers et al., 2005). Today wine can be considered as an integral part of the culture in many countries, a form of entertainment with the others, or a drink of choice that benefits health (Bisson et al., 2002). Furthermore, the grapevine is one of the most important horticultural crops in the world. According to the OIV statistical report from 2019, in 2018 the worldwide grape production was 77.8 million tons and wine production 292 million hectolitres, while the estimated market value was 31 billion Euros. In order to suceed on this growing and competitive wine market, it is necessary to ensure a distiguishable product.

The grape quality is influenced by multiple factors, such as climate conditions, agronomic and vineyard management practices, harvest dates, grape variety, etc. A very important parameter of quality is the berry size and content, which mainly depend on water, sugars (glucose and fructose), organic acids (malic and tartaric acids), phenolic compounds and aroma precursors (Fontes et al., 2011). Advances in analytical, sensory, and statistical analysis have been critical for understanding the relationships between grape and wine composition and sensory perception (Robinson et al., 2014a). Initially, the sensory analysis was used simply as a component of quality control, hence to make sure that a product did not contain repellent odours or flavours that would negatively impact the consumers. Through years the field has developed and relys on the use of human tasters as analytical tools. This poses significant challenges given the diversity of human experiences and preferences for various aromas, as well as complex aroma profiles comprising a wide range of detectable scents and odours. However, with the advances in analytical tools, today it is possible to detect the trace compounds present at nanomolar concentrations, which contributes in understaning the subtle nuances associated with varietal wine flavour (Bisson et al., 2002). For characterizing the volatile composition of grape and wine multiple techniques have been used, including flame atomic absorption spectrophotometry and flame atomic emission spectrometry, liquid chromatography (LC), gas chromatography (GC), UV, visible, near-infrared, and mid-infrared spectroscopy, and electronic nose (Robinson et al., 2014b). Knowing the identitiy of compounds that are responsible for wine aroma and flavour, and their origin, formation and degradation pathways are very important for better understanding the impact of climate changes, seasonal conditions, planting decisions and for improving the grapegrowing and winemaking practices (Parker et al., 2018).

2. VOLATILE COMPOUNDS IN GRAPES

It is widely acknowledged that wine aroma is not a result of any single dominating compound, yet wines will gain their aroma character from a multitude of aroma compounds. Volatile compounds in wine can come from several sources: directly from grape berry, from alcoholic fermentation through yeast and bacterial metabolism, malolactic fermentation, or from ageing wine in wooden barrels, and from chemical reactions upon storage (Francis and Newton, 2005). Although a number of the odorant compounds contributing to wine quality are produced from nonvolatile compounds by the yeasts during fermentation or are derived from precursors during wine ageing, a number of compounds are present in the grape and come through fermentation unaltered or with only minor modifications (Gonzalez-Barreiro et al., 2015). The major groups of aroma and flavour compounds sythesized in grapes are terpenoids, norisoprenoids (mainly C_{13}-norisoprenoids), volatile phenols, alcohols, esters, methoxypyrazines and polyfunctional thiols. There are clear sensory differences in the aromas of most grape varieties, however, the overall volatile composition of most varieties is similar with the varietal aroma deriving largely from differences in relative ratios of many volatile compounds (Polaskova et al., 2008).

2.1. Terpenoids

Terpenoids are an extremely diverse and abundant group with over 40 000 individual compounds and have been the most extensively studied among varietal compounds in *Vitis vinifera* grapes (Yu and Utsumi, 2009; Gonzalez-Barreiro et al., 2015). In plants, multiple processes utilize terpenoids, such as photosynthesis, membrane construction, and growth regulation (Linet al., 2019). Also, these compounds are involved in the chemical communication between plants and other organisms as attractants, deterrents, or for signaling (Black et al., 2015). They are often associated with a floral scent that attracts appropriate pollinators, next they can contribute to the volatile emissions from fruit that enables seed dispersal, or can have antimicrobial activities to deter herbivores both above and underground (Dunlevy et al., 2009). In grapes, compounds of this family are largely responsible for fruity (citric) and floral aromas, though a number have resin-like odours (Gonzalez-Barreiro et al., 2015). Major representatives of terpenoids in grape and wine are monoterpenes, sesquiterpenes, and indirectly carotenoids (Linet al., 2019).

2.1.1. Monoterpenes

Monoterpenes are a class of compounds that give rise to Muscat characteristic floral aroma. A number of surveys have been made of monoterpene concentrations in different grape varieties and a general classification can be made: 1) intensely flavoured Muscats, with free monoterpene concentrations up to 6 mg/L; 2) non-muscat but aromatic varieties with total monoterpene concentrations of 1-4 mg/L; 3) neutral varieties not dependent upon monoterpenes for their flavour (Mateo and Jimenez, 2000). In grape berries, monoterpenes can be found both in skins and pulp with different distribution, depending on the compound (Luan and Wust, 2002). Monoterpenes are built from two isoprenoid units, which constitute a backbone of 10 carbon atoms (Figure 1).

They can be found either as free volatile compounds or as non-volatile glycosidically bound compounds. There are 22 different monoterpene molecules identified in grapes and wines (Ilc et al., 2016). The main representatives and most odoriferous are monoterpene alcohols, notably linalool, α-terpineol, nerol, geraniol, citronellol, and hotrienol (Gonzalez-Barreiro et al., 2015). These compounds can be linked to the sugar moiety, that can be glucose or another sugar molecule, such as arabinose, rhamnose, apiose, may be added at the glucose to form higher glycosides (Liu et al., 2017). Usually, the glycosylated monoterpenes are 3 to 10 times more abundant than free aglycones and the proportion of glycosidic aroma substances in the total content of aroma substances changes among grape varieties (D'Onofrio, 2013; Liu et al., 2017). In grapes of ‘Muscat of Alexandria’ approximately 90% of the monoterpenes were glycosidically bound (Park et al., 1991), while in ‘Muscat blanc’ and ‘Gewürztraminer’ 80% and 90% were monoterpene glycosides, respectively (Li et al., 2017). In a study on six important grape varieties (‘Muscat of Alexandria’, ‘Muscat Hamburg’, ‘Chardonnay’, ‘Pinot Noir’ and ‘Cabernet Sauvignon’) three types of monoterpenyl glycosides predominate in the glycoside profile: monoterpenol hexose-pentoses, malonylated glucosides, and monoterpendiol hexose-pentoses. Also, it was observed that during maturation changes in glycosylation patterns occur, predominantly post-vérasion. This was observed for two Muscat varieties which were not differentiated at the earlier stages of development but could be differentiated post-vérasion. In contrast, similarities between ‘Chardonnay’ and ‘Pinot Noir’ glycoside profiles developed post-vérasion. These results indicate that glycoside diversity and abundance may be used to differentiate among grape cultivars (Godshaw et al., 2019). Glycosidically bound monoterpenes can be released by hydrolysis, which can occur under acidic conditions or enzymatically (Liu et al., 2017).

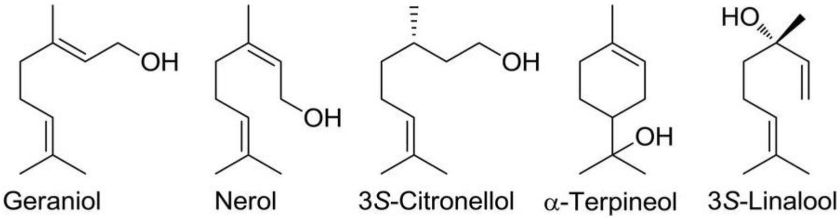


Figure 1. Most abundant monoterpenes contained in grapes.

Besides free and glycosylated compounds, monoterpenes can be found in forms as polyhydroxylated compounds or as free odourless polyols. The first hydroxylated forms discovered in grapes were linalool derivatives (Williams et al., 1980a). Even though these forms make no direct contribution to the aroma, some of them are able to spontaneously transform or break down and give rise to pleasant and potent volatiles (Mateo and Jimenez, 2000; Ilc et al., 2016). This was shown in model experiments with linalool derivatives, where under acidic conditions these compounds rearranged to give hotrienol and nerol oxide. In a feeding experiment of cultivar Scheurebe with deuterium labeled geraniol, the grape mesocarp was identified as a site of monoterpene secondary transformations. It was also revealed that geraniol can be converted to nerol by an unknown isomerase. Furthermore, geraniol can be stereoselecively reduced to (*S*)-citronellol, which can be cyclized to *cis*- and *trans*-rose oxide. The activity of these secondary transformations is dependent on the ripening stage (Figure 2) and can be distinguished from the development of the primary monoterpene synthase activity by the sharp increase at the end of the ripening period (Luan et al., 2005).

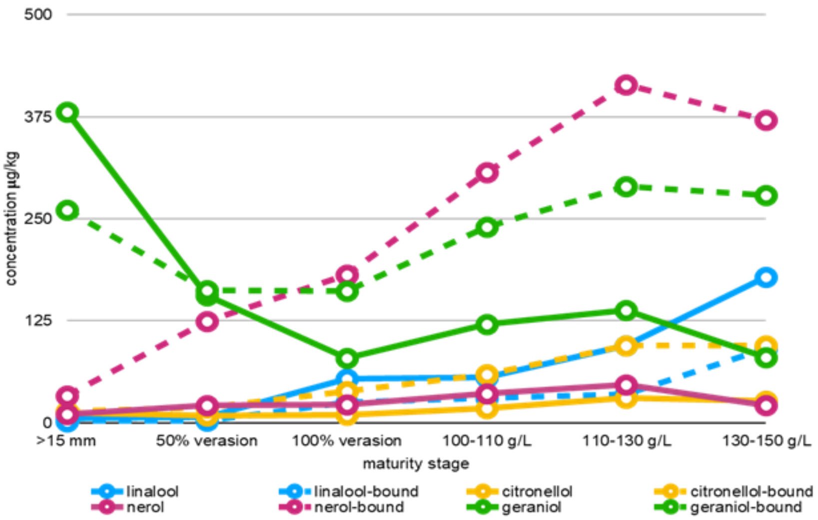


Figure 2. The evolution of free and glycosidically bound monoterpenes in developing Muscat Hamburg grapes (Fenoll et al., 2009).

Regarding free monoterpenes, it was observed that total content decreases from early berry development onwards (Zhang et al., 2016). For example, terpinenes, like α-terpinene, γ-terpinene, terpinolene, showed a declining tendency during berry ripening (Luo et al., 2019). Similar, free hydroxy-diendiol I and hydroxyl-trienol were highly concentrated before vérasion and decreased during berry ripening in ‘Moscato Bianco’. In contrast, the concentrations of linalool, geraniol and nerol, which mainly contribute to Muscat aroma, were low before vérasion and then increased during ripening (Costantini et al., 2017). This trend was also observed in ‘Shiraz’, ‘Cabernet Sauvignon’, ‘Chardonnay’ and ‘Pinot Gris’ grapes, where linalool and geraniol showed an increasing trend from fruit-set towards harvest (Luo et al., 2019). In a study by Noguerol-Pato et al. (2012) on ‘Brancellao’ grapes, it was observed that free monoterpenes were associated primarily with the skins of the berries and their concentrations increased during maturation, which was conditioned by an increase in geraniol. The bound form of monoterpenes was dominant in the flesh and reached higher values than free forms throughout the whole maturation period. Furthermore, the tips of the clusters showed a larger content of monoterpenes than the shoulders, but mainly in bound form. In Table 1 is presented the content of monoterpenes in different parts of grape berry in several grapevine cultivars.

2.1.2. Sesquiterpenes

Grape sesquiterpenes are generally considered far less volatile and aroma-active than monoterpenes and much less is known about the chemistry and biochemistry of these compounds (May and Wust, 2012; Black et al., 2015). In a review by Petronilho et al. (2014), authors reported 91 sesquiterpenic compounds in grapes, musts, wines and grape distillates. Out of these, 57 compounds were identified in grape berries. Grape sesquiterpenes can be divided into four chemical families: hydrocarbons, as the major group, ketones, oxides and alcohols (Petronilho et al., 2014). One additional problem that can occur during the chemical analysis of these compounds is the close similarity of both GC-MS elution times and mass spectra of many sesquiterpenes, thus emphasizing the careful use of reference standards for correct identification of individual compounds (Black et al., 2015). The importance and research interest have grown in recent years due to the discovery of sesquiterpene rotundone as an impact aroma compound with a strong ‘black pepper’ aroma sensory descriptor, characteristic for Australian ‘Shiraz’ wines (Wood et al., 2008). Rotundone (Figure 3) is an important aroma compound of *Piper nigrum* (black pepper) and has been found in other plants such as nut grass (*Cyperus rotundus*), marjoram (*Origanum majorana*) and rosemary (*Rosmarinus officinalis*).

Table 1. Content of monoterpenes quantified in different parts of grape berry in several grapevine cultivars

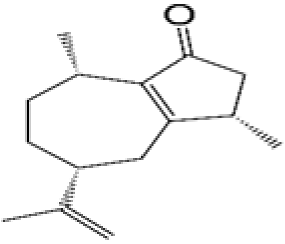
Cultivar	Berry fraction/ juice	Nerol	Geraniol	Linalool	Hhotrienol	Citronellol	Rose oxide	*t*-furan linalool oxide	*c*-furan linalool oxide	*t*-pyran linalool oxide	*c*-pyran linalool oxide	Geranic acid	α-terpineol	Reference
Muscat of Alexandria	berry mesocarp	10.0^a	37.0^a	28.0^a	1.5^a	10.0^a	n.a.*	tr**	n.d.	24.0^a	tr	2.4^a	n.d***	Park et al. (1991)
	skin	9.0^a	45.0^a	4.5^a	n.d.	1.5^a	n.a.	tr	n.d.	3.5^a	n.d.	n.d	n.d.	
Riesling	whole berries	n.a.	n.d.	22.0^b	n.a.	n.a.	n.a.	n.d.	n.d.	84^b	n.d.	n.a.	n.d.	Friedel et al. (2016)
Moscatel Rosada	juice	n.a.	169.7^c	656.5^c	61.5^c	28.5^c	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	347.2^c	Belancic et al. (2003)
Alvarinho	whole berries	1.6^c	25.1^c	10.7^c	0.3^c	1.3^c	n.a.	0.2^c	n.d.	6.0^c	0.4^c	4.4^c	0.4^c	Oliveira et al. (2004)
Amaral	whole berries	n.d.	7.8^c	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Avesso	whole berries	1.5^c	13.0^c	n.d.	n.d.	1.3^c	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Loureiro	whole berries	0.2^c	3.6^c	239.0^c	n.d.	n.d.	n.a.	4.6^c	3.2^c	44.8^c	8.4^c	n.d.	3.5^c	
Vinhão	whole berries	0.3^c	n.d.	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.	0.3^c	n.d.	n.d.	n.d.	
Aglianico	skin	4.29^d	2.59^d	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.d.	Genovese et al. (2013)
Uva di Troia	skin	5.35^d	15.10^d	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.d.	
Pinot Noir	whole berry	1.02^b	4.62^b	0.76^b	n.a.	1.08^b	n.a.	n.d.	2.19^b	n.a.	n.a.	n.a.	n.d.	Yuan and Qian (2016)
	juice	3.74^c	8.35^c	0.36^c	n.a.	1.06^c	n.a.	0.09^c	n.a.	n.a.	n.a.	n.a.	0,12^c	Fang and Qian (2012)
	juice	2.84^c	8.51^c	0.54^c	n.a.	1.10^c	n.a.	0.06^c	n.a.	n.a.	n.a.	n.a.	0.18^c	
Moscato Bianco	juice	9.7^c	52.7^c	458^c	0.04^c	0.10^c	14.4^c	3.7^c	3.3^c	n.a.	n.a.	n.a.	0.08^c	Torchio et al. (2016)

Table 1. (Continued)

Cultivar	Berry fraction/ juice	Nerol	Geraniol	Linalool	Hhotrienol	Citronellol	Rose oxide	*t*-furan linalool oxide	*c*-furan linalool oxide	*t*-pyran linalool oxide	*c*-pyran linalool oxide	Geranic acid	α-terpineol	Reference
Muscat Hamburg	whole berries	19.87^{d}	66.19^{d}	201.39^{d}	n.a.	23.36^{d}	2.01^{d}	n.a.	n.a.	n.a.	n.a.	n.a.	3.14^{d}	Fenoll et al. (2009)
Pedro Gimenez	juice	n.a.	65.25^{c}	49.34^{c}	14.38^{c}	23.38^{c}	0.62^{c}	n.a.	35.29^{c}	n.a.	n.a.	n.a.	29.28^{c}	(Maturano et al. 2018)
Viogner	whole berries	n.a.	6.99^{e}	30.33^{e}	n.a.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	44.52^{e}	Wang et al. (2019)
Riesling	whole berries	n.d.	n.d.	25.2^{d}	n.d.	n.d.	n.a.	n.d.	n.d.	23.5^{d}	7.7^{d}	n.d	n.d.	Ghaste et al. (2015)
Gewurztraminer	whole berries	22.0^{d}	187.0^{d}	n.d.	n.d.	n.d.	n.a.	n.d.	n.d.	2.7^{d}	1.6^{d}	92.0^{d}	n.d.	
Moscato Rosa	whole berries	18.2^{d}	140^{d}	124^{d}	n.d.	n.d.	n.a.	n.d.	10.6^{d}	44.4^{d}	50.2^{d}	40.4^{d}	n..d.	
Aleatico	whole berries	154.1^{f}	561.41^{f}	24.04^{f}	0.97^{f}	5.35^{f}	n.a.	1.28^{f}	0.55^{f}	13.25^{f}	3.78^{f}	734.18^{f}	2.83^{f}	D'Onofrio et al. (2017)
Brachetto	whole berries	159.8^{f}	291.81^{f}	3.71^{f}	1.02^{f}	9.51^{f}	n.a.	0.95^{f}	2.87^{f}	5.78^{f}	2.64^{f}	441.42^{f}	2.38^{f}	
Candia aromatica	whole berries	87.0^{f}	536.71^{f}	96.31^{f}	3.2^{f}	4.93^{f}	n.a.	10.23^{f}	4.69^{f}	57.05^{f}	31.63^{f}	142.22^{f}	3.91^{f}	
Moscato Bianco	whole berries	165.2^{f}	219.85^{f}	109.59^{f}	4.90^{f}	10.06^{f}	n.a.	11.54^{f}	18.41^{f}	81.41^{f}	58.99^{f}	352.10^{f}	6.07^{f}	
Shiraz	whole berries	n.a.	4.99^{g}	12.06^{g}	0.49^{g}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	6.43^{g}	Luo et al. (2019)
Cabernet Sauvignon	whole berries	n.a.	1.43^{g}	4.72^{g}	tr	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	4.58^{g}	
Riesling	whole berries	n.a.	10.32^{g}	58.55^{g}	35.5^{g}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	21.76^{g}	

Cultivar	Berry fraction/ juice	Nerol	Geraniol	Linalool	Hhotrienol	Citronellol	Rose oxide	*t*-furan linalool oxide	*c*-furan linalool oxide	*t*-pyran linalool oxide	*c*-pyran linalool oxide	Geranic acid	α-terpineol	Reference
Chardonnay	whole berries	n.a.	2.22^{g}	5.29^{g}	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	2.4^{g}	
Pinot Gris	whole berries	n.a.	3.2^{g}	3.96^{g}	tr	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	2.14^{g}	
Fiano	juice	n.d.	7^{c}	5^{c}	n.d.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	Ugliano and Moio (2008)
Airen	skin	1.8^{d}	13.2^{d}	n.a.	n.a.	2.7^{d}	n.a.	n.a.	n.a.	0.5^{d}	n.d.	31.6	n.a.	Garcia et al. (2003)
Chardonnay	skin	1.0^{d}	6.6^{d}	n.a.	n.a.	n.d.	n.a.	n.a.	n.a.	0.6^{d}	7.5^{d}	19.6^{d}	n.a.	
Macabeo	skin	tr	4.3^{d}	n.a.	n.a.	n.d.	n.a.	n.a.	n.a.	0.4^{d}	17.6^{d}	9.7^{d}	n.a.	
Aglianico	skin	24.8^{d}	3.1^{d}	n.a.	n.a.	1.4^{d}	n.a.	1.1^{d}	37.9^{d}	n.a.	n.a.	n.d.	1.5^{d}	Genovese et al. (2013)
Chardonnay	juice	n.a.	2.80^{c}	n.a.	n.a.	n.a.	n.d.	7.04^{c}	10.3^{c}	n.a.	n.a.	n.a.	0.80^{c}	Hernandez -Orte et al. (2015)
Gewurztraminer	juice	n.a.	3.64^{c}	n.a.	n.a.	n.a.	0.75^{c}	0.83^{c}	4.51^{c}	n.a.	n.a.	n.a.	33.1^{c}	
Tempranillo	juice	n.a.	0.95^{c}	n.a.	n.a.	n.a.	0.02^{c}	1.18^{c}	1.72^{c}	n.a.	n.a.	n.a.	0.54^{c}	
Merlot	juice	n.a.	0.79^{c}	n.a.	n.a.	n.a.	0.02^{c}	1.05^{c}	1.07^{c}	n.a.	n.a.	n.a.	2.53	

^{a} µg/kg of pericarp; ^{b} ng/berry; ^{c} µg/L; ^{d} µg/kg; ^{e} µg/kg berry fresh weight; ^{f} ng/g; ^{g} µg/g grape sample; * n.a. - not analyzed; ** tr - trace; *** n.d. - not detected.



Source: https://en.wikipedia.org.

Figure 3. Chemical structure of rotundone.

In a research by Wood et al. (2008), it was shown that rotundone is very potent aroma compound with detection threshold of 16 ng/L in red wine and 8 ng/L in water. During the sensory testing of rotundone, it was shown that 20% of sensory panel could not detect this compound, even at concentrations much higher than that found in red wine.

Besides Shiraz, some other grape varieties can contain higher concentrations of rotundone, such as ‘Duras’ (Geffroy et al., 2019), ‘Gamay’ (Geffroy et al., 2016), ‘Schippettino’, ‘Vespolina’ and ‘Grüner Veltliner’ (Mattivi et al., 2011). In a study by Caputi et al. (2011), it was shown that rotundone, like other sesquiterpenes, is accumulated in grape berry exocarp from vérasion to harvest. Zhang et al. (2016) showed that grapes accumulate rotundone pre-vérasion and the concentrations gradually decrease till 80% vérasion, and then gradually increase until harvest, showing a ‘U’ shaped production pattern. Furthermore, the highest rotundone concentrations are observed at the top and shaded bunch sectors, and in the southern-facing vines in the vineyard (Zhang et al., 2015). During fermentation, rotundone is released to wine in a small percentage, suggesting that skin contact could be used to modulate the peppery character of red wine (Caputi et al., 2011). Besides grape berries, rotundone was also found in flower caps, with concentrations significantly higher than in grape berries at all stages before harvest, and similar to those in harvested grapes (Zhang et al., 2016).

Regarding other sesquiterpenes, there are not many studies about their profiles in grapes. May and Wüst (2012) explored sesquiterpene profiles in several grape cultivars, such as ‘Gewürztraminer’, ‘Riesling’, ‘Yellow Muscat’, ‘Shiraz’, ‘Lemberger’ and ‘Cabernet Sauvignon’. It was shown that grapes emit numerous sesquiterpene hydrocarbons and the sesquiterpene profile depends on the grape variety and the developmental stage. In general, bicyclic sesquiterpenes with a cadinane backbone increased during ripening, while acyclic sesquiterpenes decreased. In particular, Gewürztraminer differed from other varieties due to the high content of acyclic sesquiterpenes, namely (*E,E*)-*α*-farnesene, (*3E,6Z*)-*α*-farnesene and (*E*)-*β*-methylfarnesoate. In contrast to other varieties, Cabernet Sauvignon showed almost no increase of sesquiterpenic compounds and its comparatively low sesquiterpene hydrocarbon amounts were dominated by (*E*)-*β*-caryophyllene, *α*-humulene and an unidentified sesquiterpene. Sesquiterpene profiles of other varieties were dominated by bi-and polycyclic sesquiterpenes. In similar research (Luo et al., 2019), the evolution of sesquiterpenes and other terpene compounds was investigated on five cultivars (‘Shiraz’, ‘Cabernet Sauvignon’, ‘Riesling’, ‘Chardonnay’ and ‘Pinot Gris’). During maturation, the sesquiterpene concentration had a decreasing trend that was observed from pre-vérasion to vérasion, and a slightly increase afterwords, giving an overall ‘U’ shape pattern of accumulation. The predominant sesquiterpene in the study was 7-epi-α-cadrene, which was found at relatively high concentrations during grape berry maturation following an overall decreasing pattern from fruit-set to harvest. This pattern was also found in γ-eudesmol and nearly all germacrene D derivatives.

2.1.3. Norisoprenoids

Norisoprenoids are carotenoid-derived aroma compounds and can be found in many plants. These compounds consist of a megastigmane carbon skeleton and have oxygen function located in a different location, either attached to carbon 7 (damascene family), attached to carbon 9 (ionone family), or absent (megastigmanes). The most widespread are C_{13} norisoprenoids (Figure 4), although compounds with 9 to 11 carbons are frequently found in plants (Winterhalter and Rouseff, 2001). Even though a large number of norisoprenoids have been identified in grapes and wines, only a few of these compounds have sensory significance (Figure 3). The most important norisoprenoids in grapes and wines are β-damascenone, β-ionone, vitispirane, TDN (1,1,6-trimethyl-1,2-dihydronaftalene), TPB (4-(2,3,6-trimethyl)-buta-1,3-diene), and TCH (2,2,6-trimethylcyclohexanone) (Gonzalez-Barreiro et al., 2015; Lin et al., 2019). TCH, the only C_9 compound, has been identified in Port wines and is described to have rock-rose aroma (De Freitas et al., 1999). TDN is known to contribute to the aroma of aged ‘Riesling’ wines giving characteristic petrol or kerosene aromas, but in high levels can contribute negatively to the wine aroma. Odour threshold of TDN in model wine is 2 μg/L (Sacks et al., 2012). TPB was recently identified as a potent odourant in ‘Semillon’, ‘Chardonnay’ and ‘Riesling’ wines and gives aromas described as green or cut grass, but in high concentrations is unpleasant (Mendes-Pinto, 2009). The odour threshold in a model wine is 40 ng/L, while in wines concentrations can range 50-210 ng/L (Janusz et al., 2003). β-damascenone and β-ionone are ketones that have very low odour threshold of 2 ng/L and 7 ng/L in water, respectively. β-damascenone contributes with aromas described as cooked apple, floral, quince, while β-ionone contributes with aromas described as violet, woody, raspberry (Mendes-Pinto, 2009). Vitispirane has two chiral carbons and can exist in four stereoisomeric forms, contributing to camphorous and eucalyptus aromas (Mendes-Pinto, 2009). Like monoterpenes, many norisoprenoids are present in grapes as non-volatile glycosides and can be released by hydrolysis during fermentation and storage (Gonzalez-Barreiro et al., 2015). In Table 2 are listed the contents of norisoprenoids in some grapevine cultivars.

Table 2. Content of norisoprenoids quantified in different grapevine cultivars

Cultivar	β-damascenone	α-ionone	β-ionone	TDN	TPB	TCH	Vitispirane	Reference
Riesling	0.5[a]	n.a.*	n.a.	12a	n.a.	n.a.	15[a]	Strauss et al. (1987)
Pinot Noir	9[b]	0.5b	[1b]	12b	n.a.	n.a.	7[b]	Yuan and Qian (2016)
Merlot	3[c]	n.a.	n.a.	n.d.**	n.a.	n.d.	n.d.	Sefton (1998)
Cabernet Sauvignon	0.12[d]	n.a.	n.a.	0.7[d]	n.a.	n.a.	0.6[d]	Lee et al. (2007)
Cabernet Sauvignon	30.44[e]	n.a.	17.11[e]	5.90[e]	n.a.	n.a.	n.a.	Bindon et al. (2007)
Cabernet Sauvignon	34.80[e]	n.a.	12.57[e]	4.19[e]	n.a.	n.a.	n.a.	
Touriga Femea	0.02[f]	0.22[f]	0.24[f]	0.01[f]	n.a.	0.08[f]	n.d.	Oliveira et al. (2006)
Tinta Barroca	0.11[f]	0.50[f]	0.95[f]	0.02[f]	n.a.	0.18[f]	n.d.	
Tinta Amarela	0.12[f]	0.12[f]	0.56[f]	0.03	n.a.	0.15[f]	n.d.	
Sousão	1.84[f]	0.19[f]	0.34[f]	0.05[f]	n.a.	0.21[f]	0.06[f]	
Touriga Franca	0.11[f]	0.48[f]	0.47[f]	0.05[f]	n.a.	n.d.	n.d.	
Touriga Nacional	0.17[f]	0.64[f]	0.29[f]	0.04[f]	n.a.	0.04[f]	n.d.	
Tinta Roriz	0.05[f]	0.20[f]	0.39[f]	0.03[f]	n.a.	0.05[f]	n.d.	
Tinta Cão	0.07[f]	0.24[f]	0.16[f]	0.04[f]	n.a.	0.10	n.d.	
Pedro Gimenez	12.82[c]	13.69[c]	n.d.	n.a.	n.a.	n.a.	n.a.	Maturano et al. (2018)
Pedro Gimenez	23.68[c]	17.18[c]	15.45[c]	n.a.	n.a.	n.a.	n.a.	

[a] mg/L; [b] μg/kg berry; [c] μg/L; d μg/mL; [e] ng/g; f μg/L of equivalents of β-damascenone; * n.a. - not analysed; ** n.d. - not detected; TDN (1,1,6-trimethyl-1,2-dihydronaftalene); TPB (4-(2,3,6-trimethyl)-buta-1,3-diene); TCH (2,2,6-trimethylcyclohexanone).

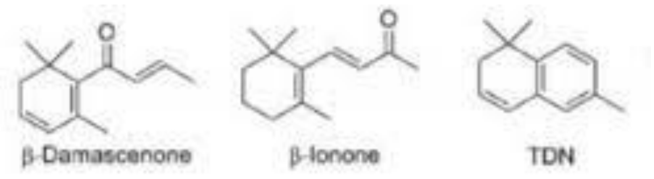


Figure 4. Chemical structures of some C_{13}-norisoprenoids.

Norisoprenoids are formed through the biodegradation of carotenoids by enzymatic or non-enzymatic reactions (Winterhalter and Rouseff, 2002). Carotenoids are more abundant in the skin than the pulp or juice. The abundance of carotenoids in berries is approximately 100-times lower than in leaves, but the ratios of the photosynthetic pigments in berries are similar (Yuan and Qian, 2016). In general, the carotenoid content in grapes decreases, while C_{13}-norisoprenoid content increases. In 'Pinot Noir' grapes a larger decrease in the level of major carotenoids was observed before vérasion, and the decrease continued during ripening. The fastest degradation for all the carotenoids happened a week before vérasion. The content of C_{13}-norisoprenoids increased during berry ripening and correlated with a carotenoid breakdown (Yuan and Qian, 2016).

During berry development carotenoids are mostly synthesized from the first stage of fruit formation until vérasion, and then degrade between vérasion and maturity to produce glycosidically bound norisoprenoids (Baumes et al., 2002). This was shown on 'Shiraz' and 'Muscat of Alexandria' cultivars, where the glycosylated norisoprenoids, detected at low levels before vérasion, increased significantly after vérasion. Free norisoprenoids in 'Muscat of Alexandria' berries were only detected from one week after vérasion and onwards. Furthermore, a region-specific oxygenase (CCD) was identified, which suggests that carotenoid breakdown occurs under enzymatic reactions (Mathieu et al., 2005).

In a study on 'Pinot Noir' grapes, the correlation between carotenoids and C_{13} norisoprenoids was compound-dependent, indicating that carotenoid cleavage is dependent on enzymatic activity and gene regulation. Total C_{13} norisoprenoids increased during berry ripening, but much more *β*-damascenone was synthesized and stored as precursors in grape berries than other norisoprenoids. Change of carotenoids (neochrome *b* and violaxanthin) were synthesized from the first stage of fruit formation until vérasion and then degraded between vérasion and maturity, and some of them (lutein, *β*-carotene, neochrome *a*, and neoxanthin) might be synthesized even earlier (Yuan and Qian, 2016). Luo et al. (2019) observed a consistent pattern of norisoprenoid evolution, which was characterized by the gradual increase from the initial stage to either vérasion or two weeks after vérasion followed by a decline until two weeks before harvest and then increased again toward harvest.

2.2. Volatile Phenols

Recently much attention has been given to this class of compounds due to their association with smoke taint aromas in grapes and wines. Bushfires are occurring more often and in very close proximity to vineyards, for example in California, USA, Australia and Europe. After smoke exposure, wine producers have reported the occurrence of smoky, dirty, and burnt aromas accompanied by lingering ash flavour in some of the produced wines (Parker et al., 2012).

Volatile phenols have been shown to contribute to this orto- and retro-nasal aromas in wine. The most known compounds to contribute to these off flavours (Figure 5) are guaiacol and 4-methylguaiacol, along with cresol, syringol, 4-methylsyringol, 4-vinilyguaiacol, phenol (Parker et al., 2012; Caffrey et al., 2019). Besides from smoke exposure, volatile phenols can originate from fermentation, where certain yeast strains can produce vinylphenols, which in high concentrations can give heavy and medicinal odours (Chatonnet et al., 1993). Furthermore, *Brettanomyces* yeast is known to produce volatile phenols, such as 4-ethylphenol and 4-ethylguaiacol, which are associated with undesirable "Brett" character described as manure, horse sweat, stable, or leather (Madsen et al., 2017).

Not all volatile phenols are associated with undesirable aromas, in fact, many of them are common plant volatiles, derived from ferulic acid or related metabolites, and contribute to pleasant spicy aroma notes. Also, many of these compounds can originate from oak barrels (Ilc et al., 2016). Due to this spicy nuances, volatile phenols may play a major sensory role in neutral grapevine varieties containing trace amounts of terpenes, for example, eugenol (clove descriptor), 4-vinylguaiacol (phenolic descriptor), and vanillin (vanilla descriptor) (Noguerol-Pato et al., 2012). Thus, volatile phenols showed to be important aroma contributors to young red wines of the grape cultivars 'Grenache', 'Tempranillo', 'Cabernet Sauvignon' (Lopez et al., 1999; Ferreira et al., 2000; Lopez et al., 2004), as well as 'Chardonnay' (Sefton et al., 1993).

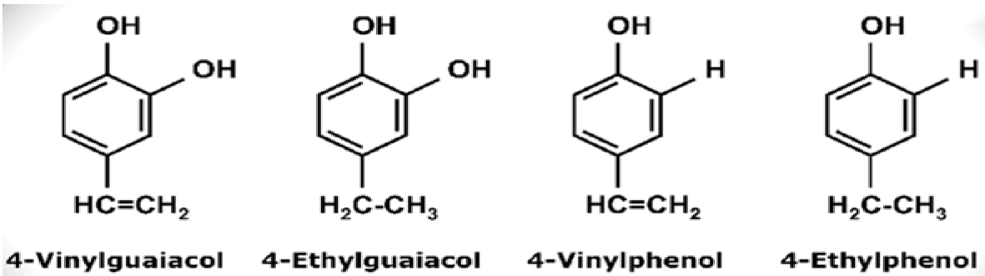


Figure 5. Some volatile phenols that contribute to off-flavours of wines.

Table 3. Content of volatile phenols quantified in some grapevine cultivars

Cultivar	Vanillin	Benzaldehyde	Benzyl alcohol	Phenylacetaldehyde	2-phenylethanol	Phenol	4-vinylguiacol	4-vinyl-2-methoxy-phenol	4-ethyl-phenol	Reference
Airen	14.0^a	0.1^a	67.6^a	0.218^a	50.5^a	n.a.*	n.a.	n.a.	n.a.	Garcia et al. (2003)
	2.2^b	1.1^b	3.0^b	tr***	144.8^b	n.a.	n.a.	n.a.	n.a.	
Chardonnay	29.2^a	4.4^a	360.6^a	0.591^a	246.0^a	n.a.	n.a.	n.a.	n.a.	
	3.3^b	1.4^b	9.9^b	0.575^b	26.1^b	n.a.	n.a.	n.a.	n.a.	
Macabeo	22.1^a	0.2^a	116.0^a	0.141^a	89.0^a	n.a.	n.a.	n.a.	n.a.	
	tr	1.3^b	0.6^b	0.595^b	tr	n.a.	n.a.	n.a.	n.a.	
Brancelao	7.4^c	3.8^c	183^c	n.a.	49^c	n.a.	0.21^c	n.a.	n.d.**	Noguerol-Pato et al. (2012)
	16^c	4.8^c	32^c	n.a.	24^c	n.a.	n.d.	n.a.	n.d.	
Pinot Noir	59.7^d	n.a.	271^d	n.a.	75.3^d	4.46^d	n.a.	12^d	n.a.	Yuan and Qian (2016)
Pinot Noir	18^b	n.a.	1506^b	n.a.	34^e	7.5^b	6.5^b	n.a.	0.20^b	Feng et al. (2017)
Merlot	n.a.	16.27^b	157.13^b	n.a.	285.59^b	1.76^b	n.a.	n.a.	n.a.	Xie et al. (2018)
Cabernet Gernischt	n.a.	15.06^b	158.74^b	n.a.	307.22^b	0.28^b	n.a.	n.a.	n.a.	
Muscat 'a Petite Grain'	n.a.	6.9^b	46.2^b	n.a.	26.6^b	n.a.	n.d.	n.a.	n.a.	Palomo et al. (2006)
Fiano	52^b	12^b	32^b	n.a.	130^b	n.a.	38^b	n.a.	n.a.	Ugliano and Moio (2008)
Listan Blanco	n.a.	0.039^f	0.062^f	n.a.	31.2^f	n.a.	0.869^f	n.a.	0.289^f	Rodriguez-Bencomo et al. (2008)
Carmenere	n.a.	19.5^a	70.7^a	n.a.	98.8^a	n.a.	21.9^a	n.a.	n.a.	De Torres et al. (2010)
Airen	n.d.	31.8^b	50.9^b	n.a.	119.5^b	n.a.	n.d.	n.a.	n.a.	Vazquez et al. (2002)
Macabeo	5.7^b	12.8^b	98^b	n.a.	181.1^b	n.a.	1.3	n.a.	n.a.	
Chardonnay	7.7^b	5.2^b	257.5^b	n.a.	256.7^b	n.a.	n.d.	n.a.	n.a.	
Muscat 'a Petite Grains'	5.0^b	5.3^b	202.0^b	n.a.	162.3^b	n.a.	1.3^b	n.a.	n.a.	
Maria Gomes	Tr	10.06^b	78.0^b	n.a.	191.2^b	n.a.	n.a.	n.d.	n.a.	Rocha et al. (2000)
Bical	Tr	2.2^b	153.4^b	n.a.	298.2^b	n.a.	n.a.	128.6^b	n.a.	

^{a} μg/kg; ^{b} μg/L; ^{c} ng/g; d μg/kg berry; ^{e} mg/L; f μg/kg of must; * n.a. - not analysed; ** n.d. - not detected; *** tr - traces.

In grape berries, volatile phenols can be found as glycosidically bound compounds. In 'Brancellao' grapes a high content of bound volatile phenols was found in the flesh of grape berries, especially in the tips of the clusters, while the skin was richer in free volatile phenols (Noguerol-Pato et al., 2012). Furthermore, in 'Syrah' berries, the content of total volatile phenols was lower in shaded bunches than in sun-exposed berries (Bureau et al., 2000). In white varieties, such as 'Chardonnay', 'Macabeo' and 'Airén' the identified volatile phenols were benzaldehyde, phenylacetaldehyde, benzyl alcohol, 2-phenylethanol and vanillin. Among these varieties, 'Chardonnay' exhibited the highest benzaldehyde, phenylacetaldehyde and benzyl alcohol levels. During the ripening period, the quantities of volatile phenols tended to decrease more or less constantly (Garcia et al., 2003). In 'Pinot Noir' berries, the volatile phenols found were phenol, 4-vinylphenol, 4-vinyl-methoxy-phenol, vanillin, and methyl anthranillate. The maximum concentration of these compounds was observed at vérasion, followed by a quick drop within one week after vérasion, and slow increased during ripening. Vanillin and methyl anthranillate concentrations reached the highest amount at vérasion, decreased in about one week, and then slowly increased during ripening. The concentrations of all these compounds were much lower than their odour threshold (Yuan and Qian, 2016). In Table 3 are shown several volatile phenol compounds quantified in some grapevine cultivars.

2.3. Aliphatic Compounds

This group is very diverse and includes aldehydes, alcohols, esters, and acids, which are mostly produced during alcoholic fermentation by yeast metabolism, and contribute differently to the aroma and flavours of the wine.

2.3.1. Aldehydes and Alcohols

C_6 aldehydes and alcohols are a diverse group of compounds originating both from grapes and yeast fermentation. They are responsible for green, herbaceous and sometimes bitter aromas of wine (Garcia et al., 2003; Ilc et al., 2016). Also, C_6 and C_9 aldehydes and alcohols play an important role in the plants' defense strategies and pest resistance (Schwab et al., 2008). In grape berries during ripening the content of C_9 aldehydes, compared with C_6 aldehydes, is extremely low and is represented by (*E*)-2-nonenal and (*E,Z*)-2,6-nonadienal (Zhu et al., 2012). Aldehydes formed in grape berries are products of hydroperoxide lyase that catalyzes the cleavage of fatty acid hydroperoxides to produce C_6 compounds (Zhu et al., 2012). In 'Cabernet Sauvignon' grapes aldehydes were prevalent throughout berry development, with (*E*)-2-hexenal and heptanal characteristic of all developmental stages. The patterns of (*E*)-2-hexenal and hexanal productions were similar. Both compounds showed a significant increase after vérasion followed by a decrease in the harvest. The decrease in concentrations of these compounds could be associated with the reduction of aldehydes to alcohols, which were characteristic of late berry development represented by (*Z*)-3-hexen-1-ol and hexan-1-ol (Kalua and Boss, 2009). In the skins of several white grape varieties ('Chardonnay', 'Macabeo' and 'Airén') the dominant aldehydes were hexanal and (*E*)-2-hexenal. During the ripening period, the concentrations of these compounds tended to increase, stabilize and then even decrease. The alcohols hexanol, (*E*)-2-hexenol and (*Z*)-3-hexenol come from the reduction of their respective aldehydes, with no clear pattern of accumulation (Garcia et al., 2003). In 'Brancellao' grapes the aldehydes were mainly present in the skins of the berries. In the berry flesh, these compounds decreased during ripening in the tips and increased in the shoulders of the clusters. The C_6 alcohols showed the highest concentrations in the flesh, whereas in the skin the berries from the shoulders were those with the highest concentrations (Noguerol-Pato et al., 2012).

2.3.2. Aliphatic Esters

A group of volatile compounds that contribute to and enhance sweet-fruity notes of wine and fermented beverages are esters (Robinson et al., 2014a). Esters are powerful odourants with very low odour threshold, and even small variations in their concentrations can change the aromatic perception of wine (Pineau et al., 2009). Most esters are secondary metabolites produced by *S. cerevisiae* during fermentation, with their synthesis linked to the lipid and acetyl-CoA metabolism (Swiegers and Pretorius, 2005). The two main groups of esters in fermented beverages, including wine, are acetate esters and ethyl esters. The group of acetate esters include ethyl acetate (solvent-like aroma), isoamyl acetate (banana), isobutyl acetate (fruity aroma), and phenyl acetate (roses, honey). The second group are ethyl esters of medium-chain fatty acids, which includes ethyl hexanoate (aniseed, apple-like aroma), and ethyl octanoate (sour apple aroma) (Saerens et al., 2010). The production of acetate esters is largely determined by the activity of the synthesizing enzymes, while ethyl esters are dependent on the concentration of the fatty acid precursors (Saerens et al., 2010). This was observed for the concentrations of ethyl hexanoat and ethyl octanoate, which are strongly positively correlated with the concentrations of their precursors, hexanoic and octanoic acids (Ilc et al., 2016). Furthermore, C_9 and C_{12} compounds may also positively influence ethyl ester production (Boss et al., 2015). Regarding the acetate esters, Dennis et al. (2012) observed that production of certain acetate esters during fermentation is dependent on the presence of the precursors in the musts. The C_6 compounds hexan-1-ol, hexanal, (*E*)-2-hexen-1-ol and (*E*)-2-hexenal are all precursors of hexyl acetate, and octanol and benzyl alcohols are precursors of octyl acetate and benzyl acetate. A positive linear relationship exists between the pre-fermentation must concentrations of the alcohol/aldehyde substrates and the post-fermentation concentrations of the corresponding acetate esters in the model alcoholic ferments. In 'Cabernet Sauvignon' grapes, the esters were identified from early physiological developmental stages. The dominant esters were those with a (*Z*)-3-hexenyl moiety. During all developmental stages, (*Z*)-3-hexenyl acetate was detected, with significantly increased concentrations early in berry development, followed by a significant drop in concentrations at vérasion. This implies that the alcohol acetyltransferase enzyme (AAT) was active during early berry development, while it was less active toward late berry development, which may explain why esters are rarely detected in ripe grapes (Kalua and Boss, 2009).

2.3.3. *Aliphatic Acids*

Volatile acidity describes a group of volatile organic acids of short carbon chain-length (Swiegers et al., 2005). Aliphatic acids are biosynthesized during fermentation by yeasts and bacteria from primary metabolites. Thus, long aliphatic acids (C_6 and above) are derived from fatty acids, while short and branched aliphatic acids are derived from amino acids. In addition, acetic acid is derived from sugar (Ilc et al., 2016). Usually, the content of volatile acids in wine is between 500 and 1000 mg/L, of which acetic acid constitutes about 90% (Swiegers et al. 2005). Volatile fatty acids at concentrations at or below the sensory threshold contribute to the complexity of wine bouquet, although generally, the aroma of fatty acids is unpleasant, ranging from sweaty and cheesy to goaty and rancid (Olivero and Trujillo, 2011; Ilc et al., 2016). However, aliphatic acids can be transformed into compounds such as esters and lactones, which contribute positively to wine aroma (Ilc et al., 2016). Acetic acid is of particular importance in the production of several acetate esters, which give pleasant fruity aromas to the wine, but at elevated concentrations, acetic acid contributes to the vinegar-like character of wine (Swiegers et al., 2005; Olivero and Trujillo, 2011).

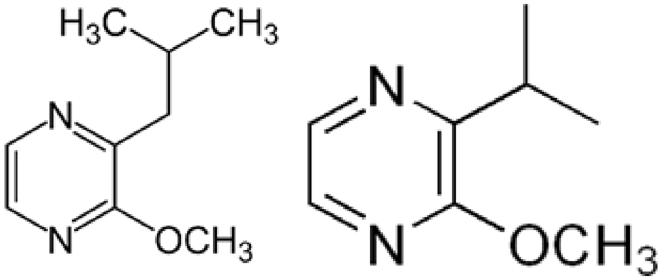
Potentially positive contribution to the wine flavour has short-chain fatty acids, such as branched-chain isobutyric and isovaleric, and straight-chained butyric and propanoic acids (Robinson et al., 2014a). Isobutyric and isovaleric acids are considered as important markers of ‘Brett’ character and may have a masking effect on the perception of ‘Brett’ character (Romano et al., 2009). The medium-chain fatty acids (C_6, C_8 and C_{10}) in higher concentrations can stop fermentation by inhibiting yeast metabolism, while long-chain fatty acids, when unsaturated (oleic and linoleic acids), are fermentation activators, especially in anaerobic conditions (Olivero and Trujillo, 2011). In Table 4 are presented some major aliphatic compounds present in grape berries of some grapevine cultivars.

2.4. Methoxypyrazines

Methoxypyrazines (MPs), or precisely 3-alkyl-2-methoxypyrazines, are a class of nitrogenated heterocyclic compounds found in many plants. These compounds are products of amino acid metabolism which originate in grapes and are associated with vegetal characteristics of wines (Gonzalez-Barreiro et al., 2015; Lei et al., 2018). MPs are found in grapes as free volatile compounds mostly located in skins and have extremely low odour threshold of 2-16 ng/L in wine. Although they can be perceived negatively when present in high concentrations, MPs are desirable in small quantities (Lin et al., 2019). The most important MPs in grapes and wines are 3-isobutyl-2-methoxypyrazine (IBMP), 2-isopropyl-2-methoxypyrazine (IPMP), and 3-sec-butyl-2-methoxypyrazine (SBMP) (Figure 6). IBMP aroma is described as bell peppers or green gooseberries, that of IPMP as asparagus or green bean, and aroma of SBMP as pea or bell pepper (Gonzalez-Barreiro et al., 2015).

Due to their low concentrations, the analytical detection of these compounds is often difficult (Robinson et al., 2014a), however, it is known that MPs play an important role in the aroma of musts and wines of the following grapes: 'Sauvignon Blanc' (Allen et al., 1991; Lacey et al., 1991; Lund et al., 2009), 'Cabernet Sauvignon' (Noble et al., 1995; De Boubee et al., 2000; 2002), 'Cabernet Franc', 'Merlot' (De Boubee et al., 2000) and 'Carmenere' (Belancic and Agosin, 2007).

In a study on 'Cabernet Sauvignon' grapes, it was observed that in bunches the highest content of MPs was located in the stem, while in grape berries the most of MPs are located in skins and seeds, containing 67% and 32%, respectively, while flesh contains only 1% of MPs. During ripening, a decrease in the proportion of IBMP in the stems and seeds was observed, with an accompanying increase in the skins (De Boubee et al., 2002). In another study on 'Cabernet Sauvignon' grapes, it was observed that the amount of IBMP in the berries was always greater than that of IPMP. The levels of IBMP increased in a period 2-8 weeks after flowering and then started to decrease until 16 weeks after flowering. The greatest concentrations of IBMP were found in the skin and the lowest in the seeds at all stages of berry development. IPMP was only found above trace amounts in the berry skins at 4 and 6 weeks after flowering, and no IPMP was found in the flesh or seeds (Dunlevy et al., 2010).



Source: https://en.wikipedia.org.

Figure 6. Chemical structure of IBMP (3-isobutyl-2-methoxypyrazine) and IPMP (2-isopropyl-2-methoxypyrazine).

Table 4. Content of aliphatic compounds quantified in some grapevine cultivars.

Cultivar	Hexanal	E-2-hexenal	1-hexanol	E-3-hexen-1-ol	Z-3-hexen-1-ol	E-2-hexen-1-ol	Z-2-hexen-1-ol	Reference
Monastrell	200^a	258a	87^a	n.a.*	21^a	236^a	n.a.	Gomez et al. (1994)
Cabernet Sauvignon	142^a	$>1^a$	312^a	n.a.	$>1^a$	25	n.a.	
Tempranillo	83^a	21^a	161^a	n.a.	11^a	71	n.a.	
Airen	1.800^b	6.478^b	0.916^b	21.3^c	0.857^b	1.588^b	6.2^c	Garcia et al. (2003)
Chardonnay	4.961^b	6.161^b	0.692^b	22.3^c	0.065^b	0.375^b	7.9^c	
Macabeo	3.281^b	4.751^b	0.815^b	21.5^c	0.322^b	0.402^b	8.7^c	
Mencia	234.5^c	50.3^c	363.7^c	2.4^c	6.0^c	128.7^c	0.8^c	Canosa et al. (2011)
Espadeiro	94.1^c	75.4^c	164.5^c	1.2^c	11.2^c	95.2^c	0.6^c	
Caino Redondo	276.6^c	181.7^c	452.3^c	3.3^c	14.5^c	128.6^c	1.1^c	
Pedral	88.4^c	72.4^c	249.3^c	2.0^c	26.5^c	140.6^c	1.8^c	
Sousão	93.9^c	56.8^c	358.9^c	5.7^c	6.1^c	151.8^c	1.3^c	
Loureiro	618.4^c	1290.0^c	10.9^c	n.a.	2.8^c	20.8^c	n.a.	Oliveira et al. (2000)
Alvarinho	720.8^c	1165.4^c	22.3^c	n.a.	8.5^c	20.7^c	n.a.	
Brancellao	53^d	105^d	1094^d	12d	16^d	883^d	n.a.	Noguerol-Pato et al. (2012)
Pinot Noir	86.8^c	34.5^c	61.7^c	8.13^c	25.5^c	288^c	n.a.	Fang and Qian (2012)
	53.5^c	43.4^c	27.2^c	6.30^c	37.5^c	316^c	n.a.	
	550e	907^e	344^e	n.a.	31.1^e	133^e	n.a.	Yuan and Qian (2016)
Aleatico	n.a.	9.55^f	65.18^f	n.a.	46.08^f	116.63^f	n.a.	D'Onofrio et al. (2017)
Brachetto	n.a.	7.26^f	38.88^f	n.a.	37.18^f	132.54^f	n.a.	
Malvasia di Candia aromatica	n.a.	16.61^f	167.73^f	n.a.	12.21^f	227.91^f	n.a.	
Moscato Bianco	n.a.	9.23^f	126.44^f	n.a.	68.34^f	197.86^f	n.a.	
Aglianico	362.02a	332.07^a	134.89^a	n.a.	12.83^a	405.13^a	n.a.	Genovese et al. (2013)
Uva di Troia	158.74a	301.06^a	114.63^a	n.a.	16.02^a	701.46^a	n.a.	

^{a} µg/kg; ^{b} mg/kg; ^{c} µg/L; ^{d} ng/g of berry; ^{e} µg/kg berry; ^{f} ng/g; * n.a. - not analysed.

2.5. Volatile thiols

Volatile thiols belong to a group of volatile sulfur compounds along with sulfides, polysulfides, heterocyclic compounds, and thioesters, all of which vary widely in their sensory properties (Swiegers et al., 2005). Sulfur compounds can range from intensely unpleasant aromas (cabbage, rotten eggs, garlic, onion and sulfurous) to pleasantly fruity (strawberry, passionfruit and grapefruit) and are important contributors to the aromas of many plants such as passionfruit, black currant, garlic and asparagus (Swiegers et al., 2005; Lin et al., 2019).

Volatile thiols are crucial components of 'varietal character' in some varieties and originate from precursors found in the berry. Thus, volatile thiols are generated during fermentation by yeast from these precursors present in the musts (Coetzee and Du Toit, 2012; Lin et al., 2019). The conjugated thiol precursors are produced in the grape berries, but little is known about the mechanism involved in their biosynthesis (Robinson et al., 2014a). In wines, the concentration of volatile thiols is related to the concentration of their precursors, but only a small proportion of these precursors release the aromatic thiol (Coetzee and Du Toit, 2012). The most important thiols are 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), 3-mercaptohexyl acetate (3MHA), 4-mercapto-4-methylpentan-2-ol (4MMPOH). They all have extremely low odour thresholds expressed in ng/L, for example, odour threshold of 4MMP is 3 ng/L, of 3MH is 60 ng/L and that of 3MHA is 4 ng/L (Swiegers et al., 2005). The aroma of 4MMP is described as a box tree, passionfruit, broom and black currant bud. 3MH and 3 MHA are responsible for the passionfruit, grapefruit and citrus aroma, as well as 4MMPOH, although its role is limited due to low concentrations (Coetzee and Du Toit, 2012). These compounds are important for the aroma of wines from the following grapes: 'Sauvignon Blanc', 'Gewürztraminer', 'Riesling', 'Colombard', 'Petit Manseng' (Tominaga et al., 1998; Tominaga et al., 2004), 'Chardonnay' (Kobayashi et al., 2012), 'Merlot' and 'Cabernet Sauvignon' (Bouchilloux et al., 1998).

Although the volatile thiols are practically nonexistent, Capone et al. (2011) analyzed the content of 3MH during various grape maturity stages of 'Sauvignon Blanc'. They observed that concentrations of 3MH were undetectable at vérasion and then relatively static, at approximately 100 ng/L, until harvest. As mentioned above, volatile thiols originate from precursors found in grapes, of which the most well-known are *S*-gluthatione and *S*-cysteine conjugates. The aromatic compound can be released by the action of β-lyase enzymes from yeasts, while 3MHA requires, in addition, the acetylation of the 3MH by acetotransferase, also from yeast (Vanzo et al., 2017; Ferreira and Lopez, 2019).

In grape berries, the precursors of volatile thiols are mostly located in the skin. A study on the 'Sauvignon Blanc' grape berries showed that S-cysteine conjugates are not distributed uniformly, with their distribution differing according to the type of precursor, but is independent of the ripening stage. The content of 4MMP and 4MMPOH precursors

(P-4MMP, P-4MMPOH) were mainly located in the juice, while the 3MH precursor (P-3MH) was equally distributed between the juice and skins. During ripening, the berry contained no P-3MH at the beginning of the vérasion, but after midvérasion, the distribution of P-3MH between juice and skin (50:50) remained constant until maturity (Des Gachons et al. 2002). Similar observations were reported by Roland et al. (2011) in 'Sauvignon Blanc' and 'Melon Blanc' grape berries, where the precursors were also mostly located in the skin. In 'Sauvignon Blanc' grape berries the cysteine precursor of 3MH (Cys3MH) was mostly present in the skin (78%), whereas glutathione precursor (G3MH) was more or less equally distributed in the skin (57%) and the pulp (43%). Regarding 'Melon Blanc' berries, only G3MH was found in the pulp, whilst other precursors were exclusively found in the skin. Also, it was observed that glutathione precursors were more abundant than cysteine precursors. In a study by Cerreti et al. (2015) the evolution of volatile thiol precursors in grapes from the cultivars 'Sauvignon Blanc', 'Grechetto' and 'Malvasia del Lazio' was studied. In the precursor-rich 'Sauvignon Blanc', during ripening, the concentration of precursors increased significantly until 22°Brix. Some precursors increased until grapes reach 19°Brix and decreased in the later stages of ripening. A similar trend was observed for 'Grechetto', while no trend was observed for 'Malvasia del Lazio'. Furthermore, Kobayashi et al. (2012) observed that even during the day the concentrations of precursors can vary. In 'Chardonnay' grape berries, the accumulation of 3MH precursor peaked early in the morning and decreased during the day, but again at night reaccumulated.

3. BIOSYNTHESIS OF VOLATILE COMPOUNDS

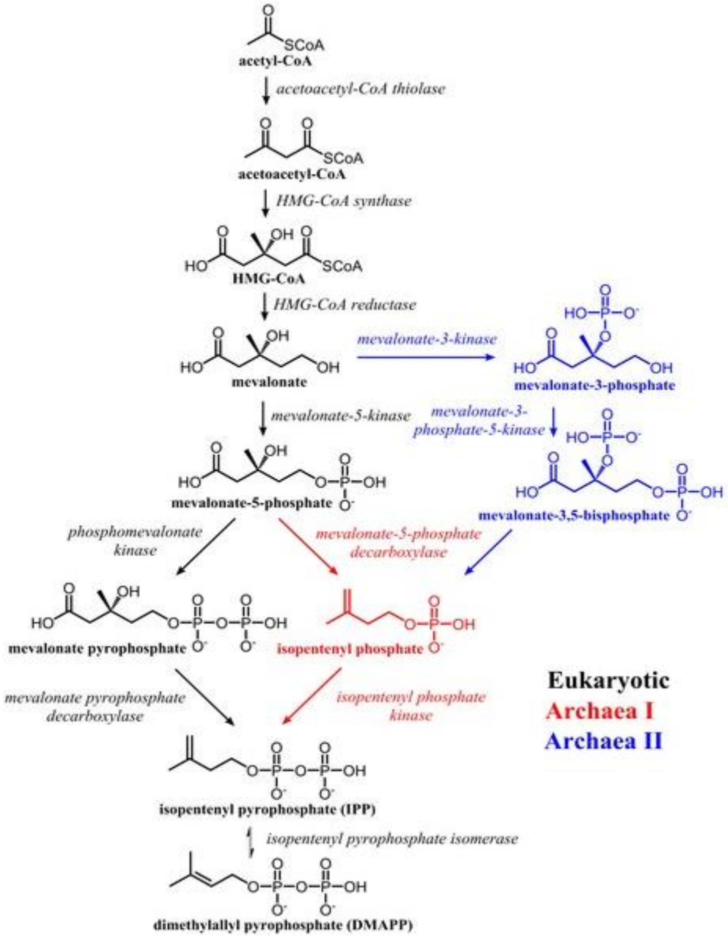
3.1. Monoterpene and Sesquiterpene Biosynthesis

Monoterpenes and sesquiterpenes are biosynthesised from their universal C_5 precursor isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl pyrophosphate (DMAPP). There are two independent pathways for the biosynthesis of IPP and DMAPP, the mevalonic acid pathway (MVA) and the methylerythritol phosphate pathway (MEP) (Schwab and Wust, 2015) (Figure 7 and 8).

The elongation of IPP and DMAPP by prenyl transferase (PT) yields geranyl diphosphate (GPP), farnesyl disphosphate (FPP), and geranylgeranyl diphosphate (GGPP). These compounds are substrates for families of terpene synthase (TPS) enzymes and serve as immediate precursors for all monoterpenes (C_{10}), sesquiterpenes (C_{15}) and diterpenes (C_{20}) (Bohlmann and Keeling, 2008).

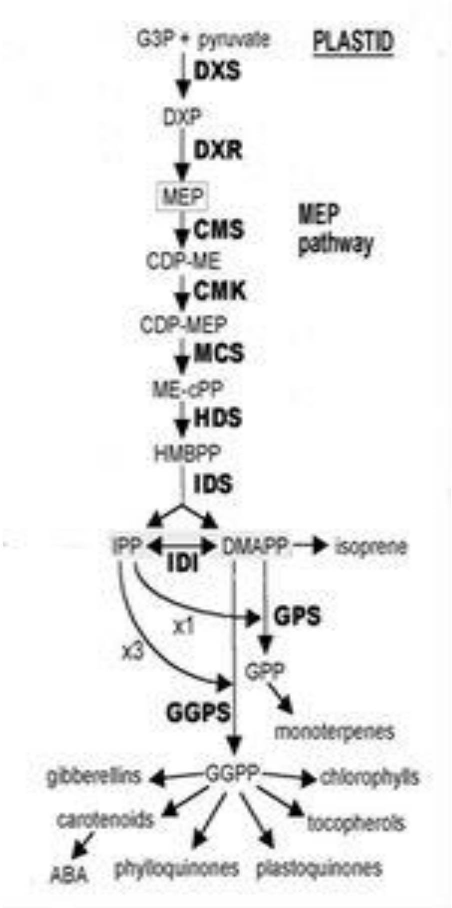
In plants, biosynthesis of monoterpenes and diterpenes occurs in plastids via the MEP pathway (Figure 8), while sesquiterpenes are biosyntesized in the cytosol via MVA patway (Bohlmann and Keeling, 2008). In grape berries, the monoterpenes are biosynthesized via

1-deoxy-D-xylulose-5-phosphate/2C-methyl-D-erythritol 4 phosphate (DOXP/MEP) pathway, which was demonstrated in a feeding experiment on Muscat Ottonel grapes by Luan and Wust (2002). Furthermore, results showed that monoterpene metabolism is compartmenalised between different berry tissue. Thus, geraniol is mainly synthesized in grape berry exocarp, while linalool is synthesized in exocarp as well as in mesocarp. In another feeding experiment by May et al. (2013) on cultivar Lemberger, the biosynthesis of sesquiterpenes was observed. The results showed that the MVA and DOXP/MEP pathways both contribute to sesquterpene biosynthesis in berry exocarp. The exchange process generates a homogenous, cytosolic pool of IPP/DAMPP from both cytosolic and plastidial precursors, which is then further metabolized to sesquiterpenes. Furthermore, there appears to be only very limited import of cytosolic IPP/DAMPP into plastids for the biosynthesis of monoterpenes, indicating that transport of precursors occurs primarily in the direction of cytosol.



Source: https://en.wikipedia.org/wiki/Mevalonate_pathway.

Figure 7. Monoterpenes and sesquiterpenes biosynthesis: mevalonate pathway.



Source: http://www.ice.mpg.de/ext/index.php?id=750.

Figure 8. Monoterpenes and sesquiterpenes byosinthesis: methylerythritol phosphate pathway.

After the biosynthesis of IPP and DMAPP, the immediate precursor molecules for monoterpenes and sesquiterpenes are syntesized. In the plastids, the C_{10} GPP is produced by geranyl diphosphate synthase (GPPS), which condenses one molecule of IPP and one molecule of DMAPP in a head-to-tail orientation. The C_{15} FPP is produced in cytosol by the consecutive condenstaion of IPP to DMAPP and then to geranyl diphosphate (GPP) product by FPP synthase (Dunlevy et al., 2009). The final monoterpene and sesquiterpene biosynthesis are under regulation of different terpene synthase (TPS) enzymes that compete for GPP and FPP.

In grapevine 69 putatively functional VvTPS genes have been identified and divided into five out of seven TPS gene subfamilies, TPS-a through TPS-g, except TPS-f and TPS-d. The gene subfamilies were characterized previously by Martin et al. (2010). The majority of VvTPS genes belong to the TPS-a subfamily, characterized as sesquiterpene synthases, which produce a vast array of sesquiterpenes with FPP as a substrate (Martin et al., 2010). Sesquiterpenes are derived from FPP via farnesyl carbocation and the downstream pathway can be grouped into four major categories: (*E,E*)-humulyl carbocation pathway, acyclic cyclization pathway, (*E,E*)-germacradienyl cation pathway, and nerolidyl cation pathway (Li et al., 2020). The characterized sesquiterpene synthases do not produce the entire array of sesquiterpenes found in grape berries, and it is likely that some compounds are produced by acid catalyzed rearrangements of the acid- and thermo-labile sesquiterpenes (Schwab and Wust, 2015; Lin et al., 2019). The TPS-b subfamily produces many of the acyclic and cyclic monoterpene hydrocarbons and a few of the monoterpene alcohols, while TPS-g subfamily, greatly expanded in *Vitis vinifera,* produces exclusively acyclic terpene alcohols.

The synthesized monoterpenes can undergo secondary transformations, like hydroxylation, reduction or dehydration (Schwab and Wust, 2015). This was investigated in a feeding experiment with deuterium labeled geraniol. It was demonstrated that the enzymatic reduction of the C2/C3 double bond of geraniol to (*S*)-citronellol is a stereoselective process. Furthemore, an *E/Z*-isomerization to nerol was demonstrated, indicating the presence of an isomerase. The oxidation to neral/geranial and glycosylation of the corresponding monoterpene alcohols was demonstrated as well. The labeled geraniol could also be converted to *cis*- and *trans*-rose oxide, and it is highly probable that rose oxide is generated from geraniol and/or nerol by a reaction sequence: after stereoselective reduction, the generated (*S*)-citronellol is cyclized to *cis*- and *trans*-rose oxide (Luan et al., 2005). Like geraniol, linalool as well can be transformed. It was shown that the stereoselective transformation of linalool generates diendiol II, furanoid and pyranoid linalool oxides. Other metabolites could be detected as well, like diendiol I, hotrienol, and 8-hydroxy linalool. The time course studies revealed that the activity of these secondary transformations is dependent on the grape ripening stage (Luan et al., 2006).

3.2. Norisoprenoid Biosynthesis

This class of compounds is derived from carotenoids and their biosynthesis strats with carotenoid biosynthesis. The general biosynthetic pathway begins with cleavage of the carotenoid by a deoxygenase, followed by an enzymatic transformation of cleavage products to non-volatile precursors and ultimately conversion of the precursors to volatile norisoprenoids through acid catalyzed reactions (Lin et al., 2019). The complexity of chemical reactions that may occur to norisoprenoids and their precursors, make the research on their biosynthesis difficult and complex (Dunlevy et al., 2009).

Carotenoids are synthesized in the plastid via the MEP pathway, like monoterpenes. The IPP and DMAPP undergo consecutive enzymatic condensations to form phytoene, the basic carotenoid, which is subjected to a number of desaturation steps resulting in the addition of four double bonds to form lycopene. The non-cyclic lycopene is further transformed into cyclic carotenoids (Dunlevy et al., 2009). The most common carotenoids are *β*-carotene and lutein, accompanied by minor carotenoids such as neoxanthin, violaxanthin, lutein-5,6-epoxide, zeaxanthin, neochrome, flavoxanthin and luteoxanthin (Baumes et al., 2002). Carotenoids can be degraded through enzymatic and non-enzymatic reactions, yielding norisoprenoids (Mendes-Pinto, 2009). The enzymes that are responsible for initial cleavage of carotenoids are carotenoid cleavage dioxygenases (CCD), which are able to cleave carotenoids at the 9,10 and 9,'10' double bonds to release C_{13}-noisoprenoids (Dunlevy et al., 2009). There are nine CCD members but only CCD1 and CCD4 have shown to cleave carotenoids at 9,10 and 9,'10' double bonds to yield two C_{13} ketone end groups and one C_{14} aldehyde (Lin et al., 2019).

Mathieu et al. (2005) identified and characterized *VvCCD1* gene, which encoded a functional CCD. The recombinant *VvCCD1* catalyzed cleavage of zeaxanthin and lutein to generate 3-hydroxy-*β*-ionone, but no cleavage was detected when *β*-carotene was used as a substrate. Also, significant induction of gene expression approching vérasion was observed and was maintained throughout the ripening stage. Besides *VvCCD1* gene, Lashbrooke et al. (2013) identified *VvCCD4a* and *VvCCD4b* genes. The *VvCCD4a* expression is predominant in leaves and peaked at vérasion, while *VvCCD4a* was dominant in berries and increased dramatically throughout berry ripening. It appears that *VvCCD4a* is most likely responsible for catalysing carotenoid cleavage in ripening berries. In a study by Meng et al. (2019) it was shown that *VvCCD1* and *VvCCD4b* could cleave *β*-carotene at the 7,8(7,'8') position to produce *β*-cyclocitral. Furthermore, the two enzymes could also cleave *β*-carotene at the 9,10(9,'10') position to produce *β*-ionone and cleave lycopene 5,6(5,'6') position to form 6-methyl-5-hepten-2-one (MHO).

Only *β*-ionone is a direct cleavage product of *β*- and *α*-carotene, while other norisoprenoids require further chemical modifications, like oxidation and reduction (Mathieu et al., 2005; Dunlevy et al., 2009). Furthermore, glycosyltransferases also have a role in norisoprenoid modifications, since most of the compounds detected are found in their glycosylated form (Mathieu et al., 2005). In a cell suspension of cultivar 'Gamay', an ability to metabolize *β*-ionone was studied. The study showed that *β*-ionone was totally metabolized yielding 14 metabolites, mainly dioxygenated compounds, of which 3-oxo-*α*-ionol and 4-oxo-*β*-ionol were dominant (Mathieu et al., 2009).

The *β*-damascenone is formed from its precursor grasshoper ketone, which is the primary oxidative product of neoxanthin. The precursor undergoes enzymatic reduction to megastigma-6,7-diene-3,5,9-triol followed by acid-catalyzed conversion into *β*-damascenone (Mendes-Pinto, 2009). TDN (1,1,6-trimethyl-1,2-dihydronaftalene), TPB (4-(2,3,6-trimethyl)-buta-1,3-diene) and vitispirane are formed by acid hydrolysis of the glycosylated precursors during wine fermentation and ageing. Although a number of precursors have been isolated, the mechanism of their synthesis is still unexplored (Mendes-Pinto, 2009; Lin et al., 2019).

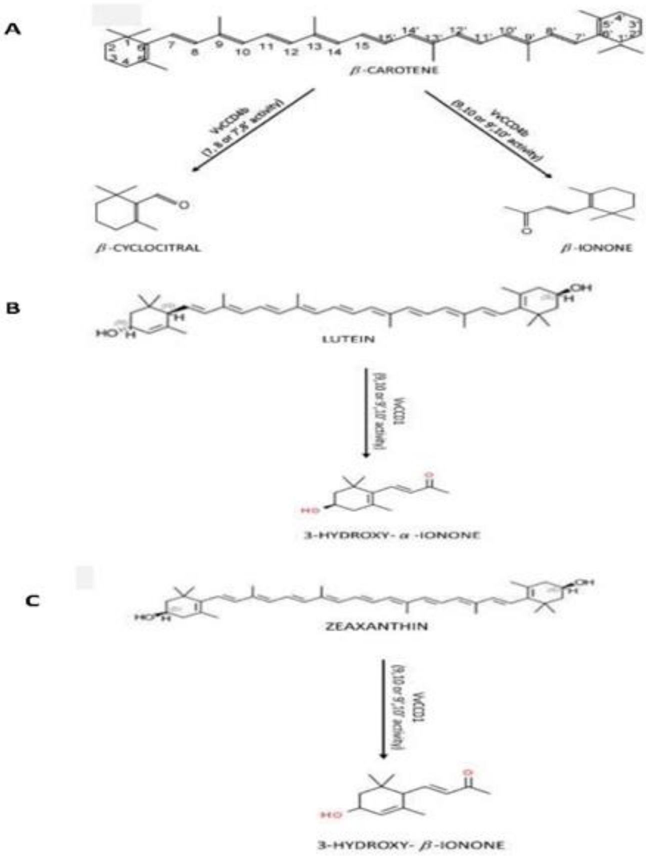


Figure 9. Possible biosynthetic pathways for norisoprenoid compounds.

3.3. Biosynthesis of Volatile Phenols

The phenylpropanoid pathway is an important biosynthetic pathway in the production of secondary plant metabolites. In grapes, the phenylpropanoid pathway leads to the production of flavanoides, anthocynanins, stilbenes, hydrocinnimates, and other compounds, including volatile phenols. The specific mechanisms that lead to the production of volatile compounds have not been well studied in grape cultivars. Since these compounds are not unique for *Vitis* genus, it can be hypothesized that biosynthesis is similar to processes in other higher plants (Dunlevy et al., 2009).

The biosynthesis of phenylpropanoids and benzenoids have a starting point in the shikimate pathway, where in a sequence of seven metabolic steps, phosphoenol-pyruvate and erythrose-4-phosphate are converted to chorismate, the precursor of the aromatic amino-acids and many secondary aromatic metabolites (Herrmann and Weaver, 1999). The phenilalanin (Phe), the product of the shikimate pathway, is biosynthesized in plastids, but the conversion to volatile compounds is outside this organelle (Dudareva et al., 2013). The first step in the synthesis of phenylpropanoids is the conversion of Phe into *E*-cinnamic acid by the activity of *L*-phenylalanine amonia lyase (PAL). In grape berries, the PAL is mainly present in the cell walls, secondarily thickened walls and the parenchyma cells of the berry mesocarp cells (Chen et al., 2006).

Benzoides (C_6-C_1) are formed by shortening of the propyl side chain taking place in peroxysomes by the *β*-oxidation sequence (Wust, 2017). The pathway begins with esterification of cinnamic acid to its CoA tioester, which is hydrated, oxidized and degraded to benzyl-CoA. The hydrolysis of benzyl-CoA will give rise to benzoic acid (Dunlevy et al., 2009; Dudareva et al., 2013). Another, non *β*-oxidative, the pathway has been suggested, with benzaldehyde as an intermediate oxidized to benzoic acid (Dudareva et al., 2013). Phenyl-propenes (C_6-C_3), like eugenol, require the elimination of the oxygen functionality at the C-9 position, which is achieved by the reduction of the corresponding acetates (Wust, 2017). Coniferyl alcohol is converted by alcohol acetytransferase to coniferyl acetate, which is reduced to eugenol and isoeugenol by eugenol and isoeugenol synthase. In contrast to benzenoids, the biosynthesis of phenylacetaldehyde and 2-phenylethanol does not occur via cinnamic acid and competes with PAL for Phe utilization (Dudareva et al., 2013).

During alcholic fermentation *p*-coumaric and ferulic acids are decarboxylated in a non-oxidative process by *Saccharomyces cerevisiae* to form the volatile phenols, such as, 4-vinylguaiacol and 4-vinylphenol. Phenolic acids can be decarboxylated to volatile phenols, first to 4-vinyl derivatives and then reduced to 4-ethyl derivatives through phenolic acid decarboxylases, which are found in *S. cerevisiae* and several bacterias (Swiegers et al., 2005).

3.4. Biosynthesis of Aliphatic Compounds

Most of the plant volatiles originate from saturated and unsaturated fatty acids. Compounds derived from fatty acids are C_6 and C_9 alcohols, and aldehydes, formed by *α*- and *β*-oxidation, and the lipoxygenase pathway (Schwab et al., 2008). In grapes, major aliphatic compounds are products of the lipoxygenase pathway (Dunlevy et al., 2009).

The fatty acids are biosynthesized from a plastidic pool of acetyl-CoA generated from pyruvate. The unsaturated C_{18}-fatty acids, linoleic and linolenic, are precursors for volatile aliphatic compounds (Wust, 2017). In the lipoxygenase pathway, these fatty acids are oxidized by lipoxygenases and modified to aldehydes by hydroperoxide lyase, followed by reduction to alcohol by alcohol dehydrogenase (Dunlevy et al., 2009). The first enzymes that initiate the degradation of fatty acids are lipoxygenases (LOXs). In plants, they can be classified by their target oxygenation site into 9-LOX or 13-LOX. The 13-LOX can be further classified according to the absence (Type I) or presence (Type II) of a plastidic transit peptide (Lin et al., 2019). LOXs are non-hem iron-containing dioxygenases that oxygenate unsaturated fatty acids to produce 13(S)- or 9(S)-fatty acid hydroxyperoxides, which are rapidly converted into oxylipins (Podolyan et al., 2010). In grapes, Podolyan et al. (2010) detected 18 individual members of the LOX family. Phylogenetic analysis placed all identified LOXs into either Type II 13-LOX or 9-LOX group. There are four LOX genes, two are identified as 13-LOX (*VvLOXA*, *VvLOXO*), and one as 9-LOX (*VvLOXC*). The *VvLOXD* differs significantly from other LOX genes. The gene expression of four identified LOXs varied across berry tissue and development. The most abundant was *VvLOXA* expressed predominantly in berry skins and throughout all developmental stages. The *VvLOXC* and *VvLOXD* were evenly distributed between seeds, pulp and skin, while *VvLOXO* was mostly expressed in the seeds.

After the formation of hydroxyperoxides, hydroxyperoxide lyases (HPL) catalize a multistep reaction sequence yielding short-chain aldehydes and ω-oxo acids (Wust, 2017). HPL is a member of cytochrome P450 family CYP74B/C that act on a hydroperoxide functional group. Like LOX, HPL can be divided into 9-HPL or 13-HPL, with the addition of 9/13-HPL, which can cleave both 9- and 13-hydroperoxides (Schwab et al., 2008; Dunlevy et al., 2009). Zhu et al. (2012) identified and characterized two genes encoding HPLs, *VvHPL1* and *VvHPL2*. *In vitro* enzyme assay showed that *VvHPL1* encodes 13-HPLs producing C_6 aldehydes, while *VvHPL2* encodes 9/13-HPL producing both C_6 and C_9 aldehydes. During development, expression of both genes was characterized by a rapid increase after vérasion and a decrease at maturity, which corresponded to the accumulation of their volatile compounds.

The formed aldehydes can be further metabolized by alcohol dehydrogenase (ADH) to form corresponding alcohols (Schwab et al., 2008), which can be further converted to their esters by alcohol acetyltransferase (Dudareva et al., 2013). The ADH enzymes are encoded by a small multi-gene family. There are six isoforms of *VvADH* identified, with only *VvADH1-3* expressed in grape berries (Lin et al., 2019).

3.5. Biosynthesis of Methoxypyrazines

There are two proposed biosynthetic pathways for the methoxypyrazine (MP) biosynthesis. Only the final step *O*-methylation has been confirmed in grapevine, while other steps are still unknown (Lei et al., 2018). One biosynthetic pathway begins with the reaction of leucin and glyoxal leading to the formation of a 3-alkyl-2-methoxypyrazine (HP) intermediate, which is then enzymatically methylated to form MPs. The second pathway includes leucine and glycine as initial building blocks, which undergo dehydration and methylation to produce IBMP (3-isobutyl-2-methoxypyrazine), while aminoacids valin and isoleucin are proposed precursors of IPMP (3-isopropyl-2-methoxypyrazine) and

SBMP (3-sec-butyl-2-methoxypyrazine) (Dunlevy et al., 2013). Still, neither of these pathways have been demonstrated in plants (Lin et al., 2019).

The final step of MP biosynthesis has been demonstrated in grapes, and it includes the *O*-methylation of 2-hydroxy-3-alkylpyrazine (Lei et al., 2018). Hashizume et al. (2001) isolated an *O*-methyltransferase (OMT) from 'Cabernet Sauvignon' berries, capable of converting 3-hydroxy-3-isobutylpyrazine (IBHP) and 2-hydroxy-3-isopropylpyrazine (IPHP) to IBMP and IPMP, respectively. They also observed that the levels of HP and OMT activity were closely related to the level of MPs in grapes. Dunlevy et al. (2010) identified and characterized the gene encoding the previously isolated OMT, *VvOMT1*, along with similar gene sequence *VvOMT2*. Recombinant *VvOMT1* and *VvOMT2* both encoded OMTs able to methylate HPs to produce MPs, however, both showed greatest acitivty towards flavonol quercetin. Higher activity of *VvOMT1* was shown to IBHP, while *VvOMT2* had higher activity towards IPHP. In 'Cabernet Sauvignon' berries, the *VvOMT1* expression was observed in skin and flesh and was correlated with the MP accumulation in these tissues. The expression of *VvOMT2* was greatest in roots, which were found to contain high levels of MPs. Additional two genes were identified, *VvOMT3* and *VvOMT4*. *VvOMT3* is suggested to play an important role in MP biosynthesis in grapes and has high affinity HP precursors of methoxypyrazines (Dunlevy et al., 2013; Guillaumie et al., 2013). In 'Cabernet Sauvignon' berries the expression of *VvOMT3* was high at pre-vérasion and coincided with the major period of IBMP accumulation; while in 'Pinot Noir' berries, the *VvOMT3* expression was low (Dunlevy et al., 2013). Similar results were obtained by Guillaumie et al. (2013). In high MP cultivar 'Carmenere', the expression of *VvOMT3* was high at pre-vérasion, whereas the expression in 'Petit Verdot', low MP cultivar, was low at the same stage.

4. ABIOTIC FACTORS AFFECTING THE GRAPE VOLATILE COMPOSITION

4.1. Sunlight Exposure

Grape development and ripening is greatly influenced by canopy microclimate, which in turn influences its flavour and aroma potential, and is ultimately reflected in wines quality. Grapevine growth and berry development are mainly dependent on leaf photosynthesis, which is greatly affected by sunlight exposure. Grape bunches that grow in low sunlight exposure compared with grapes that grow under high sunlight exposure are reported to have delayed ripenning, along with lower soluble solids, higher acidity and lower pH (Smart et al., 1988; Dokoozlian and Kliewer, 1996). Furthermore, volatile compounds in grapes are also highly influenced by the sunlight exposure, so higher sun exposure within the canopy is accompanied with higher monoterpene and norisoprenoid concentrations, while IBMP (3-isobutyl-2-methoxypyrazine) concentrations are lower (Marais et al., 1999; Young et al., 2016). Increased sunlight exposure before vérasion reduces methoxypyrazine accumulation in grapes (Marais et al., 1999; Koch et al., 2012; Gregan and Jordan, 2016). For example, increased sunlight exposure resulted in a decrease of IBMP accumulation until vérasion, after which the sunlight does not affect the degradation of this compound (Ryona et al., 2008; Mosetti et al., 2016). Grapes exposed to the sunlight had the higher formation of methoxypyrazines in unripe berries, while photodegradation of these compounds was promoted in ripe berries, and the final concentration of methoxypyrazines is the result of these two events (Hashizume and Samuta, 1999).

Carotenoids act as photosynthetic pigments and their biosynthesis in grapes occurs from early stages of berry development until vérasion. Similar to methoxypyrazines, carotenoid concentration increases until vérasion and is promoted by sunlight, and after vérasion, the sunlight promotes their degradation (Razungles et al., 1996). Since carotenoids are substrate in C_{13}-norisoprenoid biosynthesis (Young et al., 2012), the higher concentration of carotenoids can lead to higher concentrations of C_{13}-norisoprenoid precursors in sun exposed grapes (Mendes-Pinto, 2009). In general, direct sunlight exposure favoures the accumulation of norisoprenoids (Lee et al., 2007; Meyers et al., 2013; Feng et al., 2015), but not all compounds behave in that way. For example, it was reported that sunlight exposure have various impact on β-damascenone concentrations, either improving the concentrations (Meyers et al., 2013; Feng et al., 2015), having no effect on the concentration (Marais et al., 1992), or promoting higher concentrations in grapes (Lee et al., 2007). On the other hand, the concentrations of TDN and vitispirane precursors increased and were significantly higher in sun exposed grapes compared to the shaded ones (Lee et al., 2007; Meyers et al., 2013; Schuttler et al., 2015).

Increase in total bound and free terpenoids (Skinkis et al., 2010; Feng et al., 2015; Young et al., 2016) in grapes was positively correlated with increased sunlight exposure in later stages of berry development after vérasion (Young et al., 2016), with linalool showing highest sensitivity to sunlight exposure, while free and bound nerol and geraniol displayed different responses to sunlight (Erpeng et al., 2017). Monoterpenes are involved in the protection of berry tissue from UV-B radiation and other abiotic and biotic stresses (Gil et al., 2013; Joubert et al., 2016). Thus grape sunlight exposure stimulates the expression of monoterpene metabolic genes in berries (Friedel et al., 2016). Furthermore, the UV radiation has shown to increase concentration of volatile thiols in grapes (Kobayashi et al., 2011) and wines (Suklje et al., 2014; Suklje et al., 2016), as well as regulate the metabolism of C_6 compounds (hexanal and *trans*-2-hexanal) in grape berries (Joubert et al., 2016).

4.2. Soil and Cover Crop

Impact of the soil on the grape chemical composition is mostly associated with its ability to generate different levels of grapevine vigour and yield. Increased shoot growth, influenced by high amount of water and nutrients in the soil, promotes shading of the grape bunches, and consequently increase the concentration of methoxypyrazines (De Boubee et al., 2000; Scheiner et al., 2012), and decreases the concentration of monoterpenes and C_{13}-norisoprenoids (Marais et al., 1999). Clay soils are usually deeper, more fertile and have a higher retention capacity, so wines obtained from grapes grown on such soils are usually attributed to vegetative aromas (De Boubee et al., 2000) due to the increased shoot growth and cluster shading. Stony and sandy soils are associated with lower water retention and nutrient availability, so grapes and wines have generally more fuity aromas (Des Gachons et al., 2005). Water also has an important role in the production of high quality grapes. Grapevine grown under water deficit conditions are usually less vigorous, thus exposing grape bunches to more snlight, which leads to higher concentrations of norisoprenoids and monoterpenes (Deluc et al., 2009; Koundouras et al., 2009; Song et al., 2012), and lower concentrations of negative volatile compounds, such as C_6 compounds, rotundone, and methoxypyrazines, leading to an increase in fruity aromas in grapes and wine (Zhanget al., 2015; Brillante et al., 2018).

Cover crops in the vineyard are a powerful technique for controlling water and nutrient level in the soil, which helps to contol the grapevine vigour (Monteiro and Lopes, 2007), thus modifying canopy microclimate. For example, permanent grass cover consisting of hard red fescue (*Festuca rubra spp. Rubra*) has to lead to a decrease in *β*-damascenone concentration in grapes and corresponding wines (Yuan et al., 2015).

4.3. Defoliation

Basal leaf removal is one of the most important ampelotechnical procedures used for modifying canopy microclimate, yield, grape composition and wine quality, and it can be applied from pre-bloom to full vérasion. Climate plays an important role when considering leaf removal operations. In hot climates grapes can exibit sunburns and degradation of phenolic compounds, mainly anthocyanin, due to the increase in berry temperature (Bergqvist et al., 2001; Spayd et al., 2002).

By improving microclimate of the vine, basal leaf removal promotes the formation of positive volatile compounds, such as monoterpenes and C_{13}-norisoprenoids (Hernandez-Orte et al., 2015; Young et al., 2016; Alessandrini et al., 2018), as well as reducing negative volatile compounds, such as methoxypyrazines (Gregan and Jordan, 2016; Ferrari et al., 2017; Sivilotti et al., 2016). Basal leaves contain high concentrations of methoxypyrazines, and their removal affects the concentration in the grapes (Darriet et al., 2012). On the other hand, leaf removal had no influence on C_6-alcohols and aldehydes in grapes (Feng et al., 2015), which can have a detrimental effect on the wine aroma. Regarding norisoprenoids, leaf removal was shown to result in lower (Gerdes et al., 2001) or higher (Lee et al., 2007; Schuttler et al., 2015) concentrations of TDN and vitispirane in berries. Exposing bunches to sunlight by removing basal leaves, can lead to an increase in *β*-damascenone (Feng et al., 2015), can have no effect on concentrations of *β*-damascenone and *β*-ionone in grapes (Ristic et al., 2010), or higher concentrations of *β*-damascenone can be found in shaded grapes (Lee et al., 2007). These different observations arise from differences in climate conditions in which the experiments were carried out.

Timing of basal leaf removal also plays an important role in the concentration of volatile compounds in grapes, since different compounds are biosynthesized during various stages in berry development (Wang et al., 2018). A better understanding of key periods during berry development in which sun exposure increases desired volatile compounds could assist growers in making appropriate decisions about canopy management for producing grapes with a specific flavour profile. For example, leaf removal performed pre-bloom improves grape composition at harvest by increasing total soluble solids, anthocyanin and phenolic content, reducing fruit set and berry weight, and increasing the canopy porosity and skin-to-berry ratio (Verdenal et al., 2017; Hickey and Wolf, 2018; Vander Weide et al., 2018). Pre-bloom leaf removal was shown to increase glycoside aroma precursors, in particular glycoside terpenols and norisoprenoids (Komm and Moyer, 2015; Alessandrini et al., 2018), as well as thiol precursors, like glutathionyl-4-methylpentan-2-one and 3-*S*-glutathionylhexan-1-ol (Sivilotti et al., 2017). On the other hand, when performed at the pea size, leaf removal did not affect the concentration of varietal thiol precursors in grapes (Bubola et al., 2019) but can increase the concentration of free and bound *β*-damascenone and some bound terpenoid compounds (Feng et al., 2015).

CONCLUSION

The reasearch in the filed of volatile compounds from grape and wines has evolved significantly in the past 20 years and is continuing to do so. Even though the development of analytical tools has enabled researchers to analyze and identify volatile compounds, including those occuring in trace amounts, as well as analyzing their sensory properties, there are still questions about their biosytnhesis and secondary trasformations in grapes, as well as processes during alcoholic fermentations and wine maturation. These complex pathways are also influenced by external factors, such as growing and weather conditions, as well as current climate change. Thus, it is sometimes difficult to distinguish the influence of individual factor from another. However, with developing genetic research and the research of grapevine genome, it could be possible to find the missing links by identifying the genes responsible for activating certain biosynthetic pathways. All these researches are necessary to understand the nature of volatile compounds, thus enabling the producers to maximize the grape aromatic potential and provide even better sensory experience to the consumer.

REFERENCES

Alessandrini, M., Battista, F., Panighel, A., Flamini, R., Tomasi, D. (2018). Effect of pre-bloom leaf removal on grape aroma composition and wine sensory profile of Semillon cultivar. *Journal of the Science of Food and Agriculture,* 98 (5):1674-1684. doi: 10. 1002/jsfa.8638.

Allen, M. S., Lacey, M. J., Harris, R. L. N., Brown, W. V. (1991). Contribution of Methoxypyrazines to Sauvignon Blanc wine aroma. *American Journal of Enology and Viticulture,* 42 (2):109-112.

Baumes, R., Wirth, J., Bureau, S., Gunata, Y., Razungles, A. (2002). Biogeneration of C-$_{13}$-norisoprenoid compounds: experiments supportive for an apo-carotenoid pathway in grapevines. *Analytica Chimica Acta,* 458 (1):3-14. doi: 10.1016/s0003-2670(01) 01589-6.

Belancic, A., Agosin, E. (2007). Methoxypyrazines in grapes and wines of *Vitis vinifera* cv. Carmenere. *American Journal of Enology and Viticulture,* 58 (4):462-469.

Belancic, A., Gunata, Z., Vallier, M. J., Agosin, E. (2003). Beta-glucosidase from the grape native yeast Debaryomyces vanrijiae: Purification, characterization, and its effect on monoterpene content of a Muscat grape juice. *Journal of Agricultural and Food Chemistry,* 51 (5):1453-1459. doi: 10.1021/jf025777l.

Bergqvist, J., Dokoozlian, N., Ebisuda, N. (2001). Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California. *American Journal of Enology and Viticulture,* 52 (1):1-7.

Bindon, K. A., Dry, P. R., Loveys, B. R. (2007). Influence of plant water status on the production of C-13-norisoprenoid precursors in *Vitis vinifera* L. Cv. Cabernet Sauvignon grape berries. *Journal of Agricultural and Food Chemistry,* 55 (11):4493-4500. doi: 10.1021/jf063331p.

Bisson, L. F., Waterhouse, A. L., Ebeler, S. E., Walker, M. A., Lapsley, J. T. (2002). The present and future of the international wine industry. *Nature,* 418 (6898):696-699. doi: 10.1038/nature01018.

Black, C. A., Parker, M., Siebert, T. E., Capone, D. L., Francis, I. L. (2015). Terpenoids and their role in wine flavour: recent advances. *Australian Journal of Grape and Wine Research,* 21:582-600. doi: 10.1111/ajgw.12186.

Bohlmann, J., Keeling, C.I. (2008). Terpenoid biomaterials. *Plant Journal,* 54 (4):656-669. doi: 10.1111/j.1365-313X.2008.03449.x.

Boss, P. K., Pearce, A. D., Zhao, Y. J., Nicholson, E. L., Dennis, E. G., Jeffery, D. W. (2015). Potential grape-derived contributions to volatile ester concentrations in wine. *Molecules,* 20 (5):7845-7873.

Bouchilloux, P., Darriet, P., Henry, R., Lavigne-Cruege, V., Dubourdieu, D. (1998). Identification of volatile and powerful odorous thiols in Bordeaux red wine varieties. *Journal of Agricultural and Food Chemistry,* 46 (8):3095-3099. doi: 10.1021/jf971027d.

Brillante, L., Martinez-Luscher, J., Kurtural, S. K. (2018). Applied water and mechanical canopy management affect berry and wine phenolic and aroma composition of grapevine (*Vitis vinifera* L., cv. Syrah) in Central California. *Scientia Horticulturae,* 227:261-271. doi: 10.1016/j.scienta.2017.09.048.

Bubola, M., Lukic, I., Radeka, S., Sivilotti, P., Grozic, K., Vanzo, A., Bavcar, D., Lisjak, K. (2019). Enhancement of Istrian Malvasia wine aroma and hydroxycinnamate composition by hand and mechanical leaf removal. *Journal of the Science of Food and Agriculture,* 99 (2):904-914. doi: 10.1002/jsfa.9262.

Bureau, S. M., Baumes, R. L., Razungles, A. (2000). Effects of vine or bunch shading on the glycosylated flavor Precursors in grapes of *Vitis vinifera* L. Cv. Syrah. *Journal of Agricultural and Food Chemistry,* 48 (4):1290-1297. doi: 10.1021/jf990507x.

Caffrey, A., Lerno, L., Rumbaugh, A., Girardello, R., Zweigenbaum, J., Oberholster, A., Ebeler, S. E. (2019). Changes in smoke-taint volatile-phenol glycosides in wildfire smoke-exposed Cabernet Sauvignon grapes throughout winemaking. *American Journal of Enology and Viticulture,* 70 (4):373-381. doi: 10.5344/ajev.2019.19001.

Canosa, P., Oliveira, J. M., Masa, A., Vilanova, M. (2011). Study of the volatile and glycosidically bound compounds of minority *Vitis vinifera* red cultivars from NW Spain. *Journal of the Institute of Brewing,* 117 (3):462-471. doi: 10.1002/j.2050-0416.2011.tb00494.x.

Capone, D. L., Sefton, M. A., Jeffery, D. W. (2011). Application of a modified method for 3-mercaptohexan-1-ol determination to investigate the relationship between free thiol and related conjugates in grape juice and wine. *Journal of Agricultural and Food Chemistry,* 59 (9):4649-4658. doi: 10.1021/jf200116q.

Caputi, L., Carlin, S., Ghiglieno, I., Stefanini, M., Valenti, L., Vrhovsek, U., Mattivi, F. (2011). Relationship of changes in rotundone content during grape ripening and winemaking to manipulation of the ‘Peppery’ character of wine. *Journal of Agricultural and Food Chemistry,* 59 (10):5565-5571. doi: 10.1021/jf200786u.

Cerreti, M., Esti, M., Benucci, I., Liburdi, K., de Simone, C., Ferranti, P. (2015). Evolution of S-cysteinylated and S-glutathionylated thiol precursors during grape ripening of *Vitis vinifera* L. cvs Grechetto, Malvasia del Lazio and Sauvignon Blanc. *Australian Journal of Grape and Wine Research,* 21 (3):411-416. doi: 10.1111/ajgw.12152.

Chatonnet, P., Dubourdieu, D., Boidron, J. N., Lavigne, V. (1993). Synthesis of volatile phenols by *saccharomyces cerevisiae* in wines. *Journal of the Science of Food and Agriculture*, 62(2):191-202.

Chen, J. Y., Wen, P. F., Kong, W. F., Pan, Q. H., Wan, S. B., Huang, W. D. (2006). Changes and subcellular localizations of the enzymes involved in phenylpropanoid metabolism during grape berry development. *Journal of Plant Physiology,* 163 (2):115-127. doi: 10.1016/j.jplph.2005.07.006.

Coetzee, C., Toit, W.J. du (2012). A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. *Food Research International,* 45 (1):287-298. doi: 10.1016/j.foodres.2011.09.017.

Costantini, L., Kappel, C. D., Trenti, M., Battilana, J., Emanuelli, F., Sordo, M., Moretto, M., Camps, C., Larcher, R., Delrot, S., Grando, M. S. (2017). Drawing links from transcriptome to metabolites: the evolution of aroma in the ripening berry of Moscato Bianco (*Vitis vinifera* L.). *Frontiers in Plant Science,* 8:24. doi: 10.3389/fpls.2017.00780.

D'Onofrio, C. (2013). Changes in volatile compounds. In *Sweet, Reinforced and Fortified Wines*, 91-103. John Wiley & Sons, Ltd.

D'Onofrio, C., Matarese, F., Cuzzola, A. (2017). Study of the terpene profile at harvest and during berry development of *Vitis vinifera* L. aromatic varieties Aleatico, Brachetto, Malvasia di Candia aromatica and Moscato bianco. *Journal of the Science of Food and Agriculture*, 97 (9):2898-2907. doi: 10.1002/jsfa.8126.

Darriet, P., Thibon, C., Dubourdieu, D. (2012). Aroma and aroma precursors in grape berry. Edited by H. Geros, M. M. Chaves and S. Delrot, *Biochemistry of the Grape Berry*. Sharjah: Bentham Science Publ.

De Boubee, D. R., Cumsille, A. M., Pons, M., Dubourdieu, D. (2002). Location of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon grape bunches and its extractability during vinification. *American Journal of Enology and Viticulture,* 53 (1):1-5.

De Boubee, D. R., Van Leeuwen, C., Dubourdieu, D. (2000). Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *Journal of Agricultural and Food Chemistry,* 48 (10):4830-4834.

De Freitas, V. A. P., Ramalho, P. S., Azevedo, Z., Macedo, A. (1999). Identification of some volatile descriptors of the rock-rose-like aroma of fortified red wines from Douro demarcated region. *Journal of Agricultural and Food Chemistry,* 47 (10):4327-4331. doi: 10.1021/jf9901035.

De Torres, C., Diaz-Maroto, M. C., Hermosin-Gutierrez, I., Perez-Coello, M. S. (2010). Effect of freeze-drying and oven-drying on volatiles and phenolics composition of grape skin. *Analytica Chimica Acta,* 660 (1-2):177-182. doi: 10.1016/j.aca.2009.10.005.

Deluc, L. G., Quilici, D. R., Decendit, A., Grimplet, J., Wheatley, M. D., Schlauch, K. A., Merillon, J. M., Cushman, J. C., Cramer, G. R. (2009). Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *Bmc Genomics,* 10:33. doi: 10.1186/1471-2164-10-212.

Dennis, E. G., Keyzers, R. A., Kalua, C. M., Maffei, S. M., Nicholson, E. L., Boss, P. K. (2012). Grape contribution to wine aroma: production of hexyl acetate, octyl acetate, and benzyl acetate during yeast fermentation is dependent upon precursors in the must. *Journal of Agricultural and Food Chemistry,* 60 (10):2638-2646. doi: 10.1021/jf2042517.

Des Gachons, C. P., Tominaga, T., Dubourdieu, D. (2002). Localization of S-cysteine conjugates in the berry: Effect of skin contact on aromatic potential of *Vitis vinifera* L. cv. Sauvignon blanc must. *American Journal of Enology and Viticulture*, 53 (2):144-146.

Des Gachons, C. P., Van Leeuwen, C., Tominaga, T., Soyer, J. P., Gaudillere, J. P., Dubourdieu, D. (2005). Influence of water and nitrogen deficit on fruit ripening and aroma potential of *Vitis vinifera* L. cv Sauvignon blanc in field conditions. *Journal of the Science of Food and Agriculture*, 85 (1):73-85. doi: 10.1002/jsfa.1919.

Dokoozlian, N. K., Kliewer, W. M. (1996). Influence of light on grape berry growth and composition varies during fruit development. *Journal of the American Society for Horticultural Science*, 121 (5):869-874. doi: 10.21273/jashs.121.5.869.

Dudareva, N., Klempien, A., Muhlemann, J. K., Kaplan, I. (2013). Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytologist,* 198 (1):16-32. doi: 10.1111/nph.12145.

Dunlevy, J. D., Dennis, E. G., Soole, K. L., Perkins, M. V., Davies, C., Boss, P. K. (2013). A methyltransferase essential for the methoxypyrazine-derived flavour of wine. *Plant Journal,* 75 (4):606-617. doi: 10.1111/tpj.12224.

Dunlevy, J. D., Soole, K. L., Perkins, M. V., Dennis, E. G., Keyzers, R. A., Kalua, C. M., Boss, P. K. (2010). Two O-methyltransferases involved in the biosynthesis of methoxypyrazines: grape-derived aroma compounds important to wine flavour. *Plant Molecular Biology*, 74 (1-2):77-89. doi: 10.1007/s11103-010-9655-y.

Dunlevy, J. D., Kalua, C. M., Keyzers, R. A., Boss, P. K. (2009). The production of flavour & aroma compounds in grape berries. In *Grapevine Molecular Physiology & Biotechnology*, edited by K.A. Roubelakis-Angelakis, 293-340. Dordrecht: Springer Netherlands.

Erpeng, Z. P., Chai, F. M., Zhang, H. H., Li, S. H., Liang, Z. C., Fan, P. G. (2017). Effects of sunlight exclusion on the profiles of monoterpene biosynthesis and accumulation in grape exocarp and mesocarp. *Food Chemistry,* 237:379-389. doi: 10.1016/j.foodchem.2017.05.127.

Fang, Yu, Qian, M. C. (2012). Development of C6 and other volatile compounds in Pinot Noir grapes determined by stir bar sorptive extraction-GC-MS. In *Flavor Chemistry of Wine and Other Alcoholic Beverages,* 81-99. American Chemical Society.

Feng, H., Skinkis, P. A., Qian, M. C. (2017). Pinot Noir wine volatile and anthocyanin composition under different levels of vine fruit zone leaf removal. *Food Chemistry,* 214:736-744. doi: 10.1016/j.foodchem.2016.07.110.

Feng, H., Yuan, F., Skinkis, P. A., Qian, M. C. (2015). Influence of cluster zone leaf removal on Pinot Noir grape chemical and volatile composition. *Food Chemistry,* 173:414-423. doi: 10.1016/j.foodchem.2014.09.149.

Fenoll, J., Manso, A., Hellin, P., Ruiz, L., Flores, P. (2009). Changes in the aromatic composition of the *Vitis vinifera* grape Muscat Hamburg during ripening. *Food Chemistry,* 114 (2):420-428. doi: 10.1016/j.foodchem.2008.09.060.

Ferrari, V., Disegna, E., Dellacassa, E., Coniberti, A. (2017). Influence of timing and intensity of fruit zone leaf removal and kaolin applications on bunch rot control and quality improvement of Sauvignon blanc grapes, and wines, in a temperate humid climate. *Scientia Horticulturae,* 223:62-71. doi: 10.1016/j.scienta.2017.05.034.

Ferreira, V., Lopez, R. (2019). The actual and potential aroma of winemaking grapes. *Biomolecules,* 9 (12):818. doi: 10.3390/biom9120818.

Ferreira, V., Lopez, R., Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture,* 80 (11):1659-1667. doi: 10.1002/1097-0010(20000901)80:11<1659::Aid-jsfa693>3.0.Co;2-6.

Fontes, N., Geros, H., Delrot, S. (2011). Grape berry vacuole: A complex and heterogeneous membrane system specialized in the accumulation of solutes. *American Journal of Enology and Viticulture,* 62 (3):270-278. doi: 10.5344/ajev.2011.10125.

Francis, I. L., Newton, J .L. (2005). Determining wine aroma from compositional data. *Australian Journal of Grape and Wine Research,* 11 (2):114-126. doi: 10.1111/j.1755-0238.2005.tb00283.x.

Friedel, M., Frotscher, J., Nitsch, M., Hofmann, M., Bogs, J., Stoll, M., Dietrich, H. (2016). Light promotes expression of monoterpene and flavonol metabolic genes and enhances flavour of winegrape berries (*Vitis vinifera* L.) cv. Riesling. *Australian Journal of Grape and Wine Research,* 22 (3):409-421. doi:10.1111/ajgw.12229.

Garcia, E., Chacon, J. L., Martinez, J., Izquierdo, P. M. (2003). Changes in volatile compounds during ripening in grapes of Airen, Macabeo and Chardonnay white varieties grown in La Mancha Region (Spain). *Food Science and Technology International,* 9 (1):33-41. doi: 10.1177/1082013203009001006.

Geffroy, O., Buissiere, C., Lempereur, V., Chatelet, B. (2016). A sensory, chemical and consumer study of the peppery typicality of French Gamay wines from cool-climate vineyards. *Journal International Des Sciences de la Vignee et du Vin,* 50 (1):35-47.

Geffroy, O., Descotes, J., Levasseur-Garcia, C., Debord, C., Denux, J. P., Dufourcq, T. (2019). A 2-year multisite study of viticultural and environmental factors affecting rotundone concentration in Duras red wine. *Oeno One,* 53 (3):457-470. doi: 10.20870/ oeno-one.2019.53.3.2341.

Genovese, A., Gambuti, A., Lamorte, S. A., Moio, L. (2013). An extract procedure for studying the free and glycosilated aroma compounds in grapes. *Food Chemistry,* 136 (2):822-834. doi: 10.1016/j.foodchem.2012.08.061.

Genovese, A., Lamorte, S. A., Gambuti, A., Moio, L. (2013). Aroma of Aglianico and Uva di Troia grapes by aromatic series. *Food Research International,* 53 (1):15-23. doi: 10.1016/j.foodres.2013.03.051.

Gerdes, S. M., Winterhalter, P., Ebeler, S. B. (2001). Effect of sunlight exposure on norisoprenoid formation in white Riesling grapes. In *Carotenoid-Derived Aroma Compounds,* 262-272. American Chemical Society.

Ghaste, M., Narduzzi, L., Carlin, S., Vrhovsek, U., Shulaev, V., Mattivi. F. (2015). Chemical composition of volatile aroma metabolites and their glycosylated precursors that can uniquely differentiate individual grape cultivars. *Food Chemistry,* 188:309-319. doi: 10.1016/j.foodchem.2015.04.056.

Gil, M., Bottini, R., Berli, F., Pontin, M., Silva, M.F., Piccoli, P. (2013). Volatile organic compounds characterized from grapevine (*Vitis vinifera* L.) cv. Malbec berries increase at pre-harvest and in response to UV-B radiation. *Phytochemistry,* 96:148-157. doi: 10.1016/j.phytochem.2013.08.011.

Godshaw, J., Hjelmeland, A. K., Zweigenbaum, J., Ebeler, S. E. (2019). Changes in glycosylation patterns of monoterpenes during grape berry maturation in six cultivars of *Vitis vinifera*. *Food Chemistry,* 297:9. doi: 10.1016/j.foodchem. 2019.05.195.

Gomez, E., Martinez, A., Laencina, J. (1994). Localization of free and bound aromatic compounds among skin, juice and pulp fractions of some grape varieties. *Vitis,* 33 (1):1-4.

Gonzalez-Barreiro, C., Rial-Otero, R., Cancho-Grande, B., Simal-Gandara, J. (2015). Wine aroma compounds in grapes: A critical review. *Critical Reviews in Food Science and Nutrition,* 55 (2):202-218. doi: 10.1080/10408398.2011. 650336.

Gregan, S. M., Jordan, B. (2016). Methoxypyrazine accumulation and O-methyltransferase gene expression in Sauvignon blanc grapes: The role of leaf removal, light exposure, and berry development. *Journal of Agricultural and Food Chemistry,* 64 (11):2200-2208. doi: 10.1021/acs.jafc. 5b05806.

Guillaumie, S., Ilg, A., Rety, S., Brette, M., Trossat-Magnin, C., Decroocq, S., Leon, C., Keime, C., Ye, T., Baltenweck-Guyot, R., Claudel, P., Bordenave, L., Vanbrabant, S., Duchene, E., Delrot, S., Darriet, P., Hugueney, P., Gomes, E. (2013). Genetic analysis of the biosynthesis of 2-methoxy-3-Isobutylpyrazine, a major grape-derived aroma compound impacting wine quality. *Plant Physiology,* 162 (2):604-615. doi: 10.1104/ pp.113.218313.

Hashizume, K., Samuta, T. (1999). Grape maturity and light exposure affect berry methoxypyrazine concentration. *American Journal of Enology and Viticulture,* 50 (2):194-198.

Hashizume, K., Tozawa, K., Endo, M., Aramaki, I. (2001). S-adenosyl-L-methionine-dependent O-methylation of 2-hydroxy-3-alkylpyrazine in wine grapes: A putative final step of methoxypyrazine biosynthesis. *Bioscience Biotechnology and Biochemistry,* 65 (4):795-801. doi: 10.1271/bbb.65.795.

Hernandez-Orte, P., Concejero, B., Astrain, J., Lacau, B., Cacho, J., Ferreira, V. (2015). Influence of viticulture practices on grape aroma precursors and their relation with wine aroma. *Journal of the Science of Food and Agriculture,* 95 (4):688-701. doi: 10.1002/jsfa.6748.

Herrmann, K. M., Weaver, L. M. (1999). The shikimate pathway. *Annual Review of Plant Physiology and Plant Molecular Biology,* 50:473-503. doi: 10.1146/annurev.arplant. 50.1.473.

Hickey, C. C., Wolf, T. K. (2018). Leaf removal effects on Cabernet franc and Petit Verdot: I. Crop yield components and primary fruit composition. *American Journal of Enology and Viticulture,* 69 (3):221-230. doi: 10.5344/ajev.2018. 17106.

Ilc, T., Werck-Reichhart, D., Navrot, N. (2016). Meta-analysis of the core aroma components of grape and wine aroma. *Frontiers in Plant Science,* 7:15. doi: 10.3389/ fpls.2016.01472.

Janusz, A., Capone, D. L., Puglisi, C. J., Perkins, M. V., Elsey, G. M., Sefton, M. A. (2003). (E)-1-(2,3,6-Trimethylphenyl)buta-1,3-diene: A potent grape-derived odorant in wine. *Journal of Agricultural and Food Chemistry,* 51 (26):7759-7763. doi: 10.1021/ jf0347113.

Joubert, C., Young, P. R., Eyeghe-Bickong, H. A., Vivier, M. A. (2016). Field-grown grapevine berries use carotenoids and the associated xanthophyll cycles to acclimate to UV exposure differentially in high and low light (Shade) conditions. *Frontiers in Plant Science,* 7:17. doi: 10.3389/fpls.2016.00786.

Kalua, C. M., Boss, P. K. (2009). Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.). *Journal of Agricultural and Food Chemistry,* 57 (9):3818-3830. doi: 10.1021/jf803471n.

Kobayashi, H., Matsuyama, S., Takase, H., Sasaki, K., Suzuki, S., Takata, R., Saito, H. (2012). Impact of harvest timing on the concentration of 3-mercaptohexan-1-ol precursors in *Vitis vinifera* berries. *American Journal of Enology and Viticulture,* 63 (4):544-548. doi: 10.5344/ajev.2012.12051.

Kobayashi, H., Takase, H., Suzuki, Y., Tanzawa, F., Takata, R., Fujita, K., Kohno, M., Mochizuki, M., Suzuki, S., Konno, T. (2011). Environmental stress enhances biosynthesis of flavor precursors, S-3-(hexan-1-ol)-glutathione and S-3-(hexan-1-ol)-L-cysteine, in grapevine through glutathione S-transferase activation. *Journal of Experimental Botany,* 62 (3):1325-1336. doi: 10.1093 /jxb/erq376.

Koch, A., Ebeler, S. E., Williams, L. E., Matthews, M. A. (2012). Fruit ripening in *Vitis vinifera*: light intensity before and not during ripening determines the concentration of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon berries. *Physiologia Plantarum,* 145 (2):275-285. doi: 10.1111/j.1399-3054. 2012.01572.x.

Komm, B. L., Moyer, M. M. (2015). Effect of early fruit-zone leaf removal on canopy development and fruit quality in Riesling and Sauvignon blanc. *American Journal of Enology and Viticulture,* 66 (4):424-434. doi: 10.5344/ajev.2015.15007.

Koundouras, S., Hatzidimitriou, E., Karamolegkou, M., Dimopoulou, E., Kallithraka, S., Tsialtas, J. T., Zioziou, E., Nikolaou, N., Kotseridis, Y. (2009). Irrigation and rootstock effects on the phenolic concentration and aroma potential of *Vitis vinifera* L. cv. Cabernet Sauvignon grapes. *Journal of Agricultural and Food Chemistry,* 57 (17):7805-7813. doi: 10.1021/jf901063a.

Lacey, M. J., Allen, M. S., Harris, R. L. N., Brown, W. V. (1991). Methoxypyrazines in Sauvignon Blanc grapes and wines. *American Journal of Enology and Viticulture,* 42 (2):103-108.

Lashbrooke, J. G., Young, P. R., Dockrall, S. J., Vasanth, K., Vivier, M. A. (2013). Functional characterisation of three members of the *Vitis vinifera* L. carotenoid cleavage dioxygenase gene family. *Bmc Plant Biology,* 13:17. doi: 10.1186/1471-2229-13-156.

Lee, S. H., Seo, M. J., Riu, M., Cotta, J. P., Block, D. E., Dokoozlian, N. K., Ebeler, S. E. (2007). Vine microclimate and norisoprenoid concentration in cabernet sauvignon grapes and wines. *American Journal of Enology and Viticulture,* 58 (3):291-301.

Lei, Y. J., Xie, S., Guan, X. Q., Song, C. Z., Zhang, Z. W., Meng, J. F. (2018). Methoxypyrazines biosynthesis and metabolism in grape: A review. *Food Chemistry,* 245:1141-1147. doi: 10.1016/j.foodchem.2017.11.056.

Li, X. Y., Wen, Y. Q., Meng, N., Qian, X., Pan, Q. H. (2017). Monoterpenyl glycosyltransferases differentially contribute to production of monoterpenyl glycosides in two aromatic *Vitis vinifera* varieties. *Frontiers in Plant Science,* 8:13. doi: 10.3389/fpls.2017.01226.

Li, Z. Z., Howell, K., Fang, Z. X., Zhang, P. Z. (2020). Sesquiterpenes in grapes and wines: Occurrence, biosynthesis, functionality, and influence of winemaking processes. *Comprehensive Reviews in Food Science and Food Safety,* 19 (1):247-281. doi: 10. 1111/1541-4337.12516.

Lin, J., Massonnet, M., Cantu, D. (2019). The genetic basis of grape and wine aroma. *Horticulture Research,* 6:24. doi: 10.1038/s41438-019-0163-1.

Liu, J. B., Zhu, X. L., Ullah, N., Tao, Y. S. (2017). Aroma glycosides in grapes and wine. *Journal of Food Science,* 82 (2):248-259. doi: 10.1111/1750-3841. 13598.

Lopez, R., Ezpeleta, E., Sanchez, I., Cacho, J., Ferreira, V. (2004). Analysis of the aroma intensities of volatile compounds released from mild acid hydrolysates of odourless precursors extracted from Tempranillo and Grenache grapes using gas chromatography-olfactometry. *Food Chemistry,* 88 (1):95-103. doi: 10.1016/j.foodchem.2004.01.025.

Lopez, R., Ferreira, V., Hernandez, P., Cacho, J. F. (1999). Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: a comparative study. *Journal of the Science of Food and Agriculture,* 79 (11):1461-1467. doi: 10.1002/(sici)1097-0010(199908)79:11<1461:Aid-jsfa388>3.0.Co;2-k.

Luan, F., Mosandl, A., Degenhardt, A., Gubesch, M., Wust, M. (2006). Metabolism of linalool and substrate analogs in grape berry mesocarp of *Vitis vinifera* L. cv. Morio Muscat: demonstration of stereoselective oxygenation and glycosylation. *Analytica Chimica Acta,* 563 (1-2):353-364. doi: 10.1016/j.aca.2006.01.001.

Luan, F., Mosandl, A., Munch, A., Wust, M. (2005). Metabolism of geraniol in grape berry mesocarp of *Vitis vinifera* L. cv. Scheurebe: demonstration of stereoselective reduction, E/Z-isomerization, oxidation and glycosylation. *Phytochemistry,* 66 (3):295-303. doi: 10.1016/j.phytochem.2004.12.017.

Luan, F., Wust, M. (2002). Differential incorporation of 1-deoxy-D-xylulose into (3S)-linalool and geraniol in grape berry exocarp and mesocarp. *Phytochemistry,* 60 (5):451-459. doi: 10.1016/s0031-9422(02)00147-4.

Lund, C. M., Thompson, M. K., Benkwitz, F., Wohler, M. W., Triggs, C. M., Gardner, R., Heymann, H., Ncolau, L. (2009). New Zealand Sauvignon blanc distinct flavor characteristics: sensory, chemical, and consumer aspects. *American Journal of Enology and Viticulture,* 60 (1):1-12.

Luo, J. Q., Brotchie, J., Pang, M., Marriott, P. J., Howell, K., Zhang, P. Z. (2019). Free terpene evolution during the berry maturation of five *Vitis vinifera* L. cultivars. *Food Chemistry,* 299:13. doi: 10.1016/j.foodchem.2019.125101.

Madsen, M. G., Edwards, N. K., Petersen, M. A., Mokwena, L., Swiegers, J. H., Arneborg, N. (2017). Influence of *Oenococcus oeni* and *Brettanomyces bruxellensis* on hydroxycinnamic acids and volatile phenols of aged wine. *American Journal of Enology and Viticulture,* 68 (1):23-29. doi: 10.5344/ajev. 2016.16015.

Marais, J., Hunter, J. J, Haasbroek, P. D. (1999). Effect of canopy microclimate, season and region on Sauvignon blanc grape composition and wine quality. *South African Journal of Enology and Viticulture,* 20 (1):19-30. doi: 10.21548/20-1-2223.

Marais, J., Van Wyk, C. J., Rapp, A. (1992). Effect of sunlight and shade on norisoprenoid levels in maturing Weisser Riesling and Chenin blanc grapes and Weisser Riesling wines. *South African Journal of Enology and Viticulture,* 13 (1):23-32. doi: 10.21548/13-1-2191.

Martin, D. M., Aubourg, S., Schouwey, M. B., Daviet, L., Schalk, M., Toub, O., Lund, S. T., Bohlmann, J. (2010). Functional annotation, genome organization and phylogeny of the grapevine (*Vitis vinifera*) terpene synthase gene family based on genome assembly, FLcDNA cloning, and enzyme assays. *Bmc Plant Biology,* 10:22. doi: 10.1186/1471-2229-10-226.

Mateo, J. J., Jimenez, M. (2000). Monoterpenes in grape juice and wines. *Journal of Chromatography A,* 881 (1-2):557-567. doi: 10.1016/s0021-9673(99)01342-4.

Mathieu, S., Terrier, N., Procureur, J., Bigey, F., Gunata, Z. (2005). A carotenoid cleavage dioxygenase from *Vitis vinifera* L.: functional characterization and expression during grape berry development in relation to C-13-norisoprenoid accumulation. *Journal of Experimental Botany,* 56 (420):2721-2731. doi: 10.1093/jxb/eri265.

Mathieu, S., Wirth, J., Sauvage, F. X., Lepoutre, J. P., Baumes, R., Gunata, Z. (2009). Biotransformation of C-13-norisoprenoids and monoterpenes by a cell suspension culture of cv. Gamay (*Vitis vinifera*). *Plant Cell Tissue and Organ Culture,* 97 (2):203-213. doi: 10.1007/s11240-009-9516-z.

Mattivi, F., Caputi, L., Carlin, S., Lanza, T., Minozzi, M., Nanni, D., Valenti, L., Vrhovsek, U. (2011). Effective analysis of rotundone at below-threshold levels in red and white wines using solid-phase microextraction gas chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry,* 25 (4):483-488. doi: 10.1002/rcm.4881.

Maturano, Y. P., Nally, M. C., Assof, M. V., Toro, M. E., Figueroa, L. I. C. de, Jofre, V. P., Vazquez, F. (2018). Free volatile compounds of cv. Pedro Gimenez (*Vitis vinifera* L.) white grape must grown in San Juan, Argentina. *South African Journal of Enology and Viticulture,* 39 (1):27-34. doi: 10.21548/39-1-1565.

May, B., Lange, B. M., Wust, M. (2013). Biosynthesis of sesquiterpenes in grape berry exocarp of *Vitis vinifera* L.: Evidence for a transport of farnesyl diphosphate precursors from plastids to the cytosol. *Phytochemistry,* 95:135-144. doi: 10.1016/j.phytochem.2013.07.021.

May, B., Wust, M. (2012). Temporal development of sesquiterpene hydrocarbon profiles of different grape varieties during ripening. *Flavour and Fragrance Journal,* 27 (4):280-285. doi: 10.1002/ffj.3096.

Mendes-Pinto, M. M. (2009). Carotenoid breakdown products the-norisoprenoids-in wine aroma. *Archives of Biochemistry and Biophysics,* 483 (2):236-245. doi: 10.1016/j.abb.2009.01.008.

Meng, N., Yan, G.L., Zhang, D., Li, X. Y., Duan, C. Q., Pan, Q. H. (2019). Characterization of two *Vitis vinifera* carotenoid cleavage dioxygenases by heterologous expression in Saccharomyces cerevisiae. *Molecular Biology Reports,* 46 (6):6311-6323. doi: 10.1007/s11033-019-05072-3.

Meyers, J. M., Sacks, G. L., Vanden Heuvel, J.E. (2013). Glycosylated aroma compound responses in 'Riesling' wine grapes to cluster exposure and vine yield. *Horttechnology,* 23 (5):581-588. doi: 10.21273/horttech.23.5.581.

Monteiro, A., Lopes, C. M. (2007). Influence of cover crop on water use and performance of vineyard in Mediterranean Portugal. *Agriculture Ecosystems & Environment,* 121 (4):336-342. doi: 10.1016/j.agee.2006.11.016.

Mosetti, D., Herrera, J. C., Sabbatini, P., Green, A., Alberti, G., Peterlunger, E., Lisjak, K., Castellarin, S. D. (2016). Impact of leaf removal after berry set on fruit composition and bunch rot in 'Sauvignon blanc.' *Vitis,* 55 (2):57-64. doi: 10.5073/vitis.2016.55.57-64.

Noble, A. C., Elliottfisk, D. L., Allen, M. S. (1995). Vegetative flavor and methoypyrazines in Cabernet-Sauvignon. In *Fruit Flavors: Biogenesis, Characterization, and Authentication,* edited by R .L. Rouseff and M. M. Leahy, 226-234. Washington: Amer Chemical Soc.

Noguerol-Pato, R., Gonzalez-Barreiro, C., Cancho-Grande, B., Santiago, J. L., Martinez, M. C., Simal-Gandara, J. (2012). Aroma potential of Brancellao grapes from different cluster positions. *Food Chemistry,* 132 (1):112-124. doi: 10.1016/j.foodchem.2011.10.042.

OIV (2019). *2019 Statistical report on world vitiviniculture*. Internationl Organisation of Vine and Wine.

Oliveira, C., Barbosa, A., Ferreira, A. C. S., Guerra, J., Pinho, P. G. de (2006). Carotenoid profile in grapes related to aromatic compounds in wines from Douro region. *Journal of Food Science,* 71 (1):S1-S7. doi: 10.1111/j.1365-2621.2006.tb12398.x.

Oliveira, J. M., Araujo, I. M., Pereira, S. M., Maia, J. S., Amaral, A. J., Maia, M. O. (2004). Characterization and differentiation of five "Vinhos Verdes" grape varieties on the basis of monoterpenic compounds. *Analytica Chimica Acta,* 513 (1):269-275. doi: 10.1016/j.aca.2003.10.020.

Oliveira, J. M., Maia, M. O., Baumes, R., Bayonove, C. L. (2000). *Free and bound aromatic components of Loureiro and Alvarinho grape varieties from the vinhos verdes region.*

Olivero, S. J. P., Trujillo, J. P. P. (2011). A new method for the determination of short-chain fatty acids from the aliphatic series in wines by headspace solid-phase microextraction-gas chromatography-ion trap mass spectrometry. *Analytica Chimica Acta*, 696 (1-2):59-66. doi: 10.1016/j.aca.2011.03.063.

Palomo, E. S., Perez-Coello, M., Diaz-Maroto, M. C., Vinas, M. A. G., Cabezudo, M.D . (2006). Contribution of free and glycosidically-bound volatile compounds to the aroma of muscat "a petit grains" wines and effect of skin contact. *Food Chemistry,* 95 (2):279-289. doi: 10.1016/j.foodchem.2005.01.012.

Park, S. K., Morrison, J. C., Adams, D. O., Noble, A. C. (1991). Distribution of free and glycosidically bound monoterpenes in the skin and mesocarp of Muscat of Alexandria grapes during development. *Journal of Agricultural and Food Chemistry,* 39 (3):514-518. doi: 10.1021/jf00003a017.

Parker, M., Capone, D. L., Francis, I. L., Herderich, M .J. (2018). Aroma precursors in grapes and wine: flavor release during wine production and consumption. *Journal of Agricultural and Food Chemistry,* 66 (10):2281-2286. doi: 10.1021/acs.jafc.6b05255.

Parker, M., Osidacz, P., Baldock, G. A., Hayasaka, Y., Black, C. A., Pardon, K. H., Jeffery, D. W., Geue, J. P., Herderich, M. J., Francis, I. L. (2012). Contribution of several volatile phenols and their glycoconjugates to smoke-related sensory properties of red wine. *Journal of Agricultural and Food Chemistry,* 60 (10):2629-2637. doi: 10.1021/jf2040548.

Petronilho, S., Coimbra, M. A., Rocha, S. M. (2014). A critical review on extraction techniques and gas chromatography based determination of grapevine derived sesquiterpenes. *Analytica Chimica Acta,* 846:8-35. doi: 10.1016/j.aca.2014. 05.049.

Pineau, B., Barbe, J. C., Van Leeuwen, C., Dubourdieu, D. (2009). Examples of perceptive interactions involved in specific “Red-“ and “Black-berry” aromas in red wines. *Journal of Agricultural and Food Chemistry,* 57 (9):3702-3708. doi: 10.1021/jf803325v.

Podolyan, A., White, J., Jordan, B., Winefield, C. (2010). Identification of the lipoxygenase gene family from Vitis vinifera and biochemical characterisation of two 13-lipoxygenases expressed in grape berries of Sauvignon Blanc. *Functional Plant Biology,* 37 (8):767-784. doi: 10.1071/fp09271.

Polaskova, P., Herszage, J., Ebeler, S. E. (2008). Wine flavor: chemistry in a glass. *Chemical Society Reviews,* 37 (11):2478-2489. doi: 10.1039/b714455p.

Razungles, A. J., Babic, I., Sapis, J. C., Bayonove, C. L. (1996). Particular behavior of epoxy xanthophylls during veraison and maturation of grape. *Journal of Agricultural and Food Chemistry,* 44 (12):3821-3825. doi: 10.1021/jf960260t.

Ristic, R., Bindon, K., Francis, L. I., Herderich, M. J., Iland, P. G. (2010). Flavonoids and C-$_{13}$-norisoprenoids in Vitis vinifera L. cv. Shiraz: relationships between grape and wine composition, wine colour and wine sensory properties. *Australian Journal of Grape and Wine Research,* 16 (3):369-388. doi: 10.1111/j.1755-0238.2010.00099.x.

Robinson, A. L., Boss, P. K., Solomon, P. S., Trengove, R. D., Heymann, H., Ebeler, S. E. (2014a). Origins of grape and Wwne aroma. Part 1. Chemical components and viticultural impacts. *American Journal of Enology and Viticulture,* 65 (1):1-24. doi: 10.5344/ajev.2013.12070.

Robinson, A. L., Boss, P. K., Solomon, P. S., Trengove, R. D., Heymann, H., Ebeler, S. E. (2014b). Origins of grape and wine aroma. Part 2. Chemical and sensory analysis. *American Journal of Enology and Viticulture,* 65 (1):25-42. doi: 10.5344/ajev.2013.13106.

Rocha, S., Coutinho, P., Barros, A., Coimbra, M. A., Delgadillo, I., Cardoso, A. D. (2000). Aroma potential of two Bairrada white grape varieties: Maria Gomes and Bical. *Journal of Agricultural and Food Chemistry,* 48 (10):4802-4807. doi: 10.1021/jf000175s.

Rodriguez-Bencomo, J. J., Mendez-Siverio, J. J., Perez-Trujillo, J. P., Cacho, J. (2008). Effect of skin contact on bound aroma and free volatiles of Listan blanco wine. *Food Chemistry,* 110 (1):214-225. doi: 10.1016/j.foodchem.2008.01. 037.

Roland, A., Schneider, R., Charrier, F., Cavelier, F., Rossignol, M., Razungles, A. (2011). Distribution of varietal thiol precursors in the skin and the pulp of Melon B. and Sauvignon Blanc grapes. *Food Chemistry,* 125 (1):139-144. doi: 10.1016/j. foodchem.2010.08.050.

Romano, A., Perello, M. C., Lonvaud-Funel, A., Sicard, G., Revel, G. de (2009). Sensory and analytical re-evaluation of “Brett character.” *Food Chemistry,* 114 (1):15-19. doi: 10.1016/j.foodchem.2008.09.006.

Ryona, I., Pan, B. S., Intrigliolo, D. S., Lakso, A. N., Sacks, G. L. (2008). Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. Cv. Cabernet Franc). *Journal of Agricultural and Food Chemistry,* 56 (22):10838-10846. doi: 10.1021/jf801877y.

Sacks, G. L., Gates, M. J., Ferry, F. X., Lavin, E. H., Kurtz, A. J., Acree, T. E. (2012). Sensory threshold of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and concentrations in young Riesling and non-Riesling wines. *Journal of Agricultural and Food Chemistry,* 60 (12):2998-3004. doi: 10.1021/jf205203b.

Saerens, S. M. G., Delvaux, F. R., Verstrepen, K. J., Thevelein, J. M. (2010). Production and biological function of volatile esters in *Saccharomyces cerevisiae*. *Microbial Biotechnology,* 3 (2):165-177. doi: 10.1111/j.1751-7915.2009. 00106.x.

Scheiner, J. J., Vanden Heuvel, J. E., Pan, B., Sacks, G. L. (2012). Modeling Impacts of viticultural and environmental factors on 3-isobutyl-2-methoxypyrazine in Cabernet franc grapes. *American Journal of Enology and Viticulture,* 63 (1):94-105. doi: 10. 5344/ajev.2011.11002.

Schuttler, A., Guthier, C., Stoll, M., Darriet, P., Rauhut, D. (2015). Impact of grape cluster defoliation on TDN potential in cool climate Riesling wines. In *38th World Congress of Vine and Wine,* edited by A. JeanMarie. Cedex A: EDP Sciences.

Schwab, W., Davidovich-Rikanati, R., Lewinsohn, E. (2008). Biosynthesis of plant-derived flavor compounds. *Plant Journal,* 54 (4):712-732. doi: 10.1111/j.1365-313X. 2008.03446.x.

Schwab, W., Wust, M. (2015). Understanding the constitutive and induced biosynthesis of mono- and sesquiterpenes in grapes (*Vitis vinifera*): A key to unlocking the biochemical secrets of unique grape aroma profiles. *Journal of Agricultural and Food Chemistry,* 63 (49):10591-10603. doi: 10.1021/acs.jafc.5b04398.

Sefton, M. A. (1998). Hydrolytically-released volatile secondary metabolites from a juice sample of *Vitis vinifera* grape cvs Merlot and Cabernet Sauvignon. *Australian Journal of Grape and Wine Research,* 4 (1):30-38. doi: 10.1111/j.1755-0238.1998.tb00132.x.

Sefton, M. A., Francis, I. L., Williams, P. J. (1993). The volatile composition of Chardonnay juices - a study by flavor precursor analysis. *American Journal of Enology and Viticulture,* 44 (4):359-370.

Sivilotti, P., Falchi, R., Herrera, J.C., Skvarc, B., Butinar, L., Lemut, M. S., Bubola, M., Sabbatini, P., Lisjak, K., Vanzo, A. (2017). Combined effects of early season leaf removal and climatic conditions on aroma precursors in Sauvignon Blanc grapes. *Journal of Agricultural and Food Chemistry,* 65 (38):8426-8434. doi: 10.1021/acs.jafc.7b03508.

Sivilotti, P., Herrera, J. C., Lisjak, K., Cesnik, H. B., Sabbatini, P., Peterlunger, E., Castellarin, S. D. (2016). Impact of leaf removal, applied before and after flowering, on anthocyanin, tannin, and methoxypyrazine concentrations in Merlot (*Vitis vinifera* L.) grapes and wines. *Journal of Agricultural and Food Chemistry,* 64 (22):4487-4496. doi: 10.1021/acs.jafc.6b01013.

Skinkis, P. A., Bordelon, B. P., Butz, E. M. (2010). Effects of sunlight exposure on berry and wine monoterpenes and sensory characteristics of Traminette. *American Journal of Enology and Viticulture,* 61 (2):147-156.

Smart, R. E., Smith, S. M., Winchester, R. V. (1988). Light quality and quantity effects on fruit ripening for Cabernet Sauvignon. *American Journal of Enology and Viticulture,* 39 (3):250-258.

Song, J.Q., Shellie, K.C., Wang, H., Qian, M.C. (2012). Influence of deficit irrigation and kaolin particle film on grape composition and volatile compounds in Merlot grape (*Vitis vinifera* L.). *Food Chemistry,* 134 (2):841-850. doi: 10.1016/j.foodchem.2012.02.193.

Spayd, S. E., Tarara, J. M., Mee, D. L., Ferguson, J. C. (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *American Journal of Enology and Viticulture,* 53 (3):171-182.

Strauss, C. R., Wilson, B., Anderson, R., Williams, P. J. (1987). Development of precursors of C_{13} nor-isoprenoid flavorants in Riesling grapes. *American Journal of Enology and Viticulture,* 38 (1):23-27.

Suklje, K., Antalick, G., Buica, A., Langlois, J., Coetzee, Z.A., Gouot, J., Schmidtke, L. M., Deloire, A. (2016). Clonal differences and impact of defoliation on Sauvignon blanc (*Vitis vinifera* L.) wines: a chemical and sensory investigation. *Journal of the Science of Food and Agriculture,* 96 (3):915-926. doi: 10.1002/jsfa.7165.

Suklje, K., Antalick, G., Coetzee, Z., Schmidtke, L. M., Cesnik, H. B., Brandt, J., Toit, W.J. du, Lisjak, K., Deloire, A. (2014). Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of *Vitis vinifera* L. cv. Sauvignon Blanc wine. *Australian Journal of Grape and Wine Research,* 20 (2):223-233. doi: 10.1111/ajgw.12083.

Swiegers, J. H., Bartowsky, E. J., Henschke, P. A., Pretorius, I. S. (2005). Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of Grape and Wine Research,* 11 (2):139-173. doi: 10.1111/j.1755-0238.2005.tb00285.x.

Swiegers, J. H., Pretorius, I. S. (2005). Yeast modulation of wine flavor. In *Advances in Applied Microbiology, Vol 57,* edited by A. I. Laskin, J. W. Bennett and G. M. Gadd, 131-175. San Diego: Elsevier Academic Press Inc.

Tominaga, T., Gachons, C. P. des, Dubourdieu, D. (1998). A new type of flavor precursors in *Vitis vinifera* L cv Sauvignon blanc: S-cysteine conjugates. *Journal of Agricultural and Food Chemistry,* 46 (12):5215-5219. doi: 10.1021/jf980481u.

Tominaga, T., Masneuf, I., Dubourdieu, D. (2004). Powerful aromatic volatile thiols in wines made from several *Vitis vinifera* grape varieties and their releasing mechanism. In *Nutraceutical Beverages: Chemistry, Nutrition, and Health Effects*, edited by F. Shahidi and D. K. Weerasinghe, 314-337. Washington: Amer Chemical Soc.

Torchio, F., Giacosa, S., Vilanova, M., Segade, S. R., Gerbi, V., Giordano, M., Rolle, L. (2016). Use of response surface methodology for the assessment of changes in the volatile composition of Moscato bianco (*Vitis vinifera* L.) grape berries during ripening. *Food Chemistry,* 212:576-584. doi: 10.1016/j.foodchem.2016. 05.191.

Ugliano, M., Moio, L. (2008). Free and hydrolytically released volatile compounds of *Vitis vinifera* L. cv. Fiano grapes as odour-active constituents of Fiano wine. *Analytica Chimica Acta,* 621 (1):79-85. doi: 10.1016/j.aca.2008.03.002.

Vander Weide, J., Medina-Meza, I. G., Frioni, T., Sivilotti, P., Falchi, R., Sabbatini, P. (2018). Enhancement of fruit technological maturity and alteration of the flavonoid metabolomic profile in Merlot (*Vitis vinifera* L.) by early mechanical leaf removal. *Journal of Agricultural and Food Chemistry,* 66 (37):9839-9849. doi: 10.1021/acs. jafc.8b02709.

Vanzo, A., Janes, L., Pozgan, F., Bolta, S. V., Sivilotti, P., Lisjak, K. (2017). UHPLC-MS/MS determination of varietal thiol precursors in Sauvignon Blanc grapes. *Scientific Reports,* 7:11. doi: 10.1038/s41598-017-13273-8.

Vazquez, L. C., Perez-Coello, M. S., Cabezudo, M. D. (2002). Effects of enzyme treatment and skin extraction on varietal volatiles in Spanish wines made from Chardonnay, Muscat, Airen, and Macabeo grapes. *Analytica Chimica Acta,* 458 (1):39-44.

Verdenal, T., Zufferey, V., Dienes-Nagy, A., Gindro, K., Belcher, S., Lorenzini, F., Rosti, J., Koestel, C., Spring, J. L., Viret, O. (2017). Pre-flowering defoliation affects berry structure and enhances wine sensory parameters. *Oeno One,* 51 (3):263-275. doi: 10. 20870/oeno-one.2017.51.2.1808.

Wang, J. F., Abbey, T., Kozak, B., Madilao, L. L., Tindjau, R., Del Nin, J., Castellarin, S. D. (2019). Evolution over the growing season of volatile organic compounds in Viognier (*Vitis vinifera* L.) grapes under three irrigation regimes. *Food Research International,* 125:14. doi: 10.1016/j.foodres.2019. 108512.

Wang, Y., He, L., Pan, Q. H., Duan, C. Q., Wang, J. (2018). Effects of basal defoliation on wine aromas: a meta-analysis. *Molecules,* 23 (4):18. doi: 10.3390/molecules23040779.

Williams, P. J., Strauss, C. R., Wilson, B. (1980a). New linalool derivatives in Muscat of Alexandria grapes and wines. *Phytochemistry,* 19 (6):1137-1139. doi: 10.1016/0031-9422(80)83070-6.

Williams, P. J., Strauss, C. R., Wilson, B. (1980b). Hydroxylated linalool derivatives as precursors of volatile monoterpenes of Muscat grapes. *Journal of Agricultural and Food Chemistry,* 28 (4):766-771. doi: 10.1021/jf60230a037.

Winterhalter, P., Rouseff, R. (2001). Carotenoid-derived aroma compounds: an introduction. In *Carotenoid-Derived Aroma Compounds*, 1-17. American Chemical Society.

Wood, C., Siebert, T. E., Parker, M., Capone, D. L., Elsey, G. M., Pollnitz, A. P., Eggers, M., Meier, M., Vossing, T., Widder, S., Krammer, G., Sefton, M. A., Herderich, M. J. (2008). From wine to pepper: rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. *Journal of Agricultural and Food Chemistry,* 56 (10):3738-3744. doi: 10.1021/jf800183k.

Wust, M. (2017). Biosynthesis of plant-derived odorants. Edited by A. Buettner, *Springer Handbook of Odor*. Cham: Springer International Publishing Ag.

Xie, S., Tang, Y. H., Wang, P., Song, C. Z., Duan, B. B., Zhang, Z. W., Meng, J. F. (2018). Influence of natural variation in berry size on the volatile profiles of *Vitis vinifera* L. cv. Merlot and Cabernet Gernischt grapes. *Plos One,* 13 (9):14. doi: 10.1371/journal.pone.0201374.

Young, P. R., Eyeghe-Bickong, H. A., Plessis, K. du, Alexandersson, E., Jacobson, D. A., Coetzee, Z., Deloire, A., Vivier, M. A. (2016). Grapevine plasticity in response to an altered microclimate: Sauvignon Blanc modulates specific metabolites in response to increased berry exposure. *Plant Physiology,* 170 (3):1235-1254. doi: 10.1104/pp.15.01775.

Young, P. R., Lashbrooke, J. G., Alexandersson, E., Jacobson, D., Moser, C., Velasco, R., Vivier, M. A. (2012). The genes and enzymes of the carotenoid metabolic pathway in *Vitis vinifera* L. *Bmc Genomics,* 13:17. doi: 10.1186/1471-2164-13-243.

Yu, F. N. A., Utsumi, R. (2009). Diversity, regulation, and genetic manipulation of plant mono- and sesquiterpenoid biosynthesis. *Cellular and Molecular Life Sciences,* 66 (18):3043-3052. doi: 10.1007/s00018-009-0066-7.

Yuan, F., Feng, H., Qian, M. C. (2015). C-13-norisoprenoids in grape and wine affected by different canopy management. In *Advances in Wine Research,* edited by S. B. Ebeler, G. Sacks, S. Vidal and P. Winterhalter, 147-160. Washington: Amer Chemical Soc.

Yuan, F., Qian, M. C. (2016). Development of C_{13}-norisoprenoids, carotenoids and other volatile compounds in Vitis vinifera L. Cv. Pinot noir grapes. *Food Chemistry,* 192:633-641. doi: 10.1016/j.foodchem.2015.07.050.

Zhang, P. Z., Barlow, S., Krstic, M., Herderich, M., Fuentes, S., Howell, K. (2015). Within-vineyard, within-vine, and within-bunch variability of the rotundone concentration in berries of *Vitis vinifera* L. cv. Shiraz. *Journal of Agricultural and Food Chemistry,* 63 (17):4276-4283. doi: 10.1021/acs.jafc.5b00590.

Zhang, P. Z., Fuentes, S., Siebert, T., Krstic, M., Herderich, M., Barlow, E. W. R., Howell, K. (2016). Terpene evolution during the development of *Vitis vinifera* L. cv. Shiraz grapes. *Food Chemistry,* 204:463-474. doi: 10.1016/j.foodchem.2016.02.125.

Zhang, P. Z., Howell, K., Krstic, M., Herderich, M., Barlow, E. W. R., Fuentes, S. (2015). Environmental factors and seasonality affect the concentration of rotundone in *Vitis vinifera* L. cv. Shiraz wine. *Plos One,* 10 (7):21. doi: 10.1371/journal.pone.0133137.

Zhu, B. Q., Xu, X. Q., Wu, Y. W., Duan, C. Q., Pan, Q. H. (2012). Isolation and characterization of two hydroperoxide lyase genes from grape berries. *Molecular Biology Reports,* 39 (7):7443-7455. doi: 10.1007/s11033-012-1577-0.

In: Vitis: Biology and Species
Editors: António M. Jordão et al.
ISBN: 978-1-53618-308-5

Chapter 12

PHENOLIC CHARACTERIZATION OF DIFFERENT PORTUGUESE GRAPE VARIETIES (*VITIS VINIFIERA* L.)

Ana C. Correia[1], Fernanda Cosme[2,3]
and António M. Jordão[1,3,*]
[1]Department of Food Industries, Agrarian Higher School,
Polytechnic Institute of Viseu, Viseu, Portugal
[2]Department of Biology and Environmental,
School of Life Science and Environment,
University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[3]Chemistry Research Centre of Vila Real (CQ-VR),
Food and Wine Chemistry Laboratory, Vila Real, Portugal

ABSTRACT

Grapes, especially from *Vitis vinifera* L. species, have long been appreciated for their high content in phenolic compounds such as gallic acid, (+)-catechin, anthocyanins, resveratrol, and proanthocyanidins. Phenolic compounds, especially, anthocyanins, flavonols, catechins and other flavonoids, are very important for wine quality. These compounds are responsible for most of the wine sensory characteristics, particularly the color and astringency. Also, these compounds have demonstrated to have a wide range of biochemical and pharmacological effects, including anticarcinogenic, antiatherogenic, anti-inflammatory, antimicrobial and antioxidant activities. The concentration and composition of phenolic compounds in grapes differ with the grape variety, but also by other important factors such as climatic, geological and soil conditions, the altitude of the wine region, vineyard management and crop level. Thus, each grape variety produced in a

---

[*] Corresponding Author's Email: antoniojordao@esav.ipv.pt.

specific *terroir* reflects the locality in its chemical composition. In this context, Portugal shows a high diversity of *terroirs* throughout its territory, from the south of the country to the north. In all regions of the country, grape and wine production play a key role in the country's economic activity. Today, Portugal is one of the countries with the largest number of grape varieties suitable for wine production (around 340 different varieties). Most of the grape varieties cultivated in Portugal are native varieties with specific characteristics, namely in terms of their phenolic composition. Thus, the main purpose of this chapter is to show a review about the characterization and quantification of phenolic compounds, mainly the anthocyanins, proanthocyanidins and other individual phenolics from diverse grapes varieties produced in the different Portuguese wine regions, particularly for the native grape varieties cultivated.

Keywords: anthocyanins, grape varieties, phenolic compounds, Portugal, proanthocyanidins

1. INTRODUCTION

Grapes from *Vitis vinifera* L. belong to the world's largest fruit crops, and are consumed by population and applied, mainly, on wine production, but also consumed in fresh as table grapes and in the form of dried grapes. Grapevine is composed of a large number of cultivars and is one of the oldest crops in the world. According to the International Organization of Vine and Wine (OIV) 2019 report, the world's winegrowing area represents 7.4 mha, while in 2018, the global production of fresh grapes (grapes intended for all types of use) is almost 78 mt. Most of the grapes produced are for wine and juice production and represents around 44.5 mt (57% of total grape production), while the remaining grape production is intended for fresh consumption, as table grape with 28.0 mt (36% of total grape production), and also for dried grape production with 5.4 mt (7% of total production).

In China, despite an 11% drop in production in 2018, the country was the world's leading grape producer with 11.7 mt (15% of global grape production), followed by Italy (8.6 mt), USA (6.9 mt), Spain (6.9 mt) and France (5.5 mt). The top three European producers (Italy, Spain and France) recorded a 28% increase in grape production. Concerning to table grape production, the production doubled in the last twenty years, contributing significantly to the increase in total grape production over the period. According to the last OIV 2019 data report, in 2018, China remains the world's leading producer of table grape (9.5 mt), followed by Turkey (1.9 mt) and India (1.9 mt). Finally, dried grape production was estimated at 1.3 mt in 2018. This level is in line with its 10 year average. Turkey (381 kt) and the United States (263 kt) are still the two largest producers in the world and account for almost 50% of global production of dried grapes. The major countries producers by type of grapes (wine grape, dried grape and table grape), are shown in Figure 1.

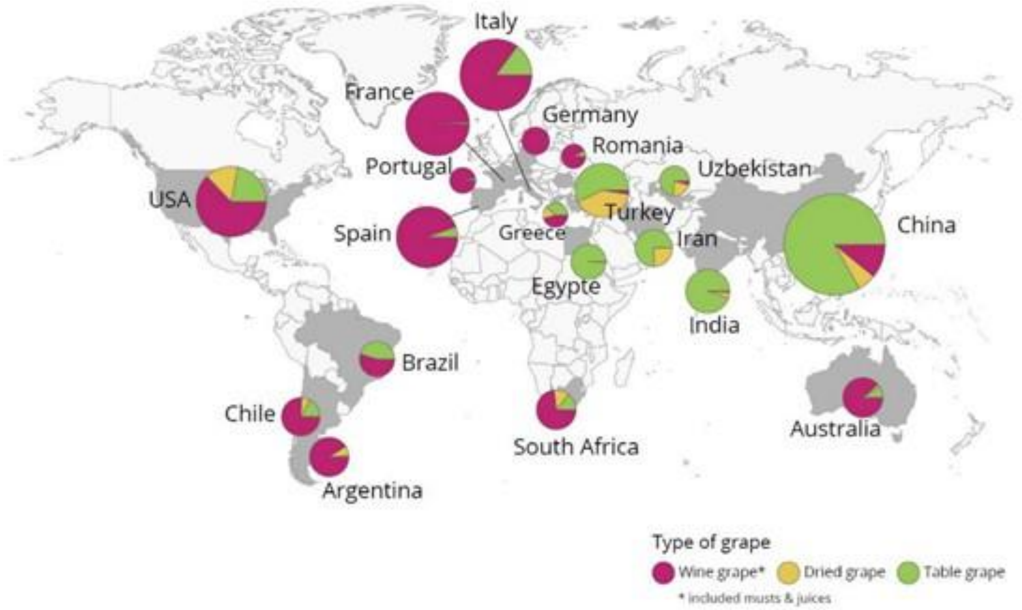


Figure 1. Major countries producers by type of grapes: wine grape; dried grape and table grape (OIV, 2019).

In past years, an increase in the vineyard area occurred. Thus, the size of the global vineyard area (regardless of the final destination of the grapes and including vines not yet in production) in 2018 reached 7.4 mha and is slightly higher than in 2017 (+24 kha). Spain remains the leading country for area cultivated with 969 kha, followed by China (875 kha) and France (793 kha). In addition, the Chinese winegrowing area continues to increase (+10 kha between 2017 and 2018). On the other hand, the European Union's vineyards seem to have curbed their rate of decline and stood at 3,324 kha in 2018 (+10 kha/2017) (OIV, 2019).

In Portugal, the wine sector has an important role in economic stability and growth and also a relevant social impact on the different Portuguese's regions (including Madeira and Azores islands). Grapevines covering an area of nearly 192,000 ha (3% of the global vineyard surface area) and producing approximately 6.1×10^6 hL of wine in 2018 corresponding to the 11th position among the main wine producing countries in the world. Roughly, half of this production is exported, corresponding to more than 670×10^6 €. Also, in 2018 Portugal was the ninth largest wine producer/exporter in the world (OIV, 2019). The consumption of grapes and wines is very much rooted in Portuguese food habits, being an important component of the Mediterranean diet.

Grapes and wines, particularly red grape varieties and their wines are an important source of phenolic compounds. These compounds, also called polyphenols, constitute a diverse group of secondary metabolites which exist in grapes, mainly in the grape berry skins and seeds. In general, the average concentration of total phenolic compounds in the different grape berry fractions are around 2178.8 mg/g gallic acid equivalent in seeds, 374.6 mg/g gallic acid equivalent in skins, and 23.8 mg/g gallic acid equivalent in pulps (Pastrana-Bonilla et al., 2003). According to several works published, grape phenolic compounds play an important role in human health, such as an important role in the oxidation inhibition of low-density lipoproteins (LDLs) (Ghiselli et al., 1998; Serafini et al., 2000; Xia et al., 2010), a decrease of inflammatory and carcinogenic processes (Tapiero et al., 2002) and inhibition of platelet aggregation (Gryglewski et al., 1987; Escarpa and Gonzalez, 2001). Specifically, in red wine and other grape products, phenolic compounds are considered to be the most important components, as a consequence of their direct relationship with color, astringency, bitterness, and susceptibility to oxidation reactions.

Phenolic composition of grapes is determined by different factors related with the soil and climatic conditions of the diverse wine country regions, viticulture techniques, grape physiology and also taking into consideration the different grape varieties and genetic diversity. In this circumstance, Portugal is an excellent example of a wine country with a great diversity of soils and climates, also presenting a high diversity of native grape varieties. Portugal is very rich in grapevine biodiversity with 341 different grape cultivars officially authorized for wine production. Therefore, all these facts are crucial in the composition of the grapes, in particular for the phenolic composition. Thus, all of these aspects related to the phenolic composition and its diversity in the different Portuguese grape varieties will be covered in this chapter.

2. DIVERSITY OF GRAPE VARIETIES CULTIVATED IN PORTUGAL AND THEIR WINE REGIONS

Grapes have been cultivated in Portugal for a long time, with evidence that *Vitis* expansion in the region now known as Portugal occurred some 5000 years ago and that the Romans played an important role in the Lusitanian viticulture, namely by the introduction of new different cultivars.

Portugal is very rich in grapevine biodiversity and in the XIX century, 1482 different cultivar names were detected (Almadanim et al., 2007). Nowadays, many cultivars are hardly used or at extinction risk and although 341 of them are officially authorized for wine production, today only 31 are relevant for wine production. Table 1 shows the main 25 grape varieties cultivated in Portugal in terms of vine area occupied. By analyzing the data shown in Table 1, it is clear that the majority of the grape varieties are autochthones grape varieties and only four grape varieties (Syrah, Alicante Bouschet, Cabernet Sauvignon and Caladoc) are international varieties from French origin. In addition, modern Portuguese vineyards are mainly planted with 16 different grape varieties (representing 90% of the total area), while in the oldest vineyards it is still possible to identify a greater number of grape varieties all of them mixed (Carvalho et al., 2017). Indeed, Portuguese native grape cultivars represent the majority of those presently used for wine production, namely, Alvarinho, Antão Vaz, Fernão Pires, Arinto or Loureiro for white grape cultivars, and Touriga Franca, Touriga Nacional, Trincadeira or Baga for red grape varieties. However, it is important to note that considering the global area under different grape varieties, the data reveal an extension of the varietal concentration in the world's vineyard over the decade of 2010. According to Anderson and Aryal (2013), half the world's plantings in 2000 were accounted for 21 grape varieties but, by 2010, that total had dropped to 15 grape varieties. At the same time, French grape varieties increased their share of the old world's vineyards, from 20% to 27% over that decade. This evolution occurred also in Portugal, where several grape varieties from French origin have been introduced in different Portuguese wine regions, such as Cabernet Sauvignon, Syrah, Merlot or Chardonnay, especially in the south of the country. However, the tendency for reduction of diversity could lead to an irreversible loses dangerously shrinks the genetic pool, and, consequently, increasing the crop vulnerability to climate changes and new pests and diseases (Almadanim et al., 2007). Fraga et al. (2016; 2017) studied the climatic suitability of Portuguese grapevine varieties and climate change adaptation and concluded that Portuguese varieties have high adaptability because they are grown over a large range of thermal conditions, although the climate data indicated a tendency for a strong warming and drying trends in the future. However, the future use of Portuguese grape varieties are also deeply depending on the interest of winemakers and global market policies for the production of wines with specific sensory profiles.

This high number of grape cultivars used in Portugal and their dissemination all over the country resulted also in different names being attributed to genetically identical plants (synonymous). According to Eiras-Dias et al. (1988), traditionally cultivar characterization relied on plant morphological description. However, these observations are time consuming and error-prone due to environmental variations that may alter the expression of the measured characteristics. In addition, for Veloso et al. (2010), the preservation of the high genetic resources detectable in Portuguese grape varieties are expensive and require considerable land area.

Portugal comprises a total of 14 different wine regions (mainland Portugal, Azores and Madeira islands), which include 31 Protected Denominations of Origin (Figure 2). In the north, the Douro Demarcated Region, with almost 1.5 MhL of total wine production and 45.000 ha of vineyards, is the oldest and one of the most important wine regions of the country. Douro region is a very mountainous region, characterized by the steep slopes of the Douro Valley. This region, famous for its Port wine, is responsible for one fourth of all wine produced in Portugal, and its vineyard landscape is also considered World Heritage by the UNESCO since 2001 (IVV, 2017).

According to several authors (Santos et al., 2013; Fraga et al., 2014), Douro region and their Valley dominated by Douro river contains a significant part of the country's vineyard land cover and comprises a large of bioclimatic regions due to the complex topography of the Douro Valley. In this region, native grape varieties dominate, such as Touriga Franca, Tinta Roriz and Touriga Nacional for red varieties and Códega, Rabigato and Malvasia Fina for white varieties. Also in the north of Portugal, particularly in the northwestern region, near to the maritime area, the Minho region ("Vinhos Verdes" region) produces predominantly white wines from native grape varieties, namely, Loureiro, Perdenã and Alvarinho, characterized by a typical freshness and slightly higher acidity. This region is characterized by relatively high annual precipitation (above 1.200 mm) and also relatively mild summers (Santos et al., 2013).

Table 1. Main grape varieties cultivated in Portugal in terms of vine area occupied (data from IVV, 2017)

Grape variety	Red/white Variety	Area (ha)*	%**
Aragonez/Tinta Roriz/Tempranillo	R	20.884	11
Touriga Franca	R	13.445	7
Touriga Nacional	R	13.032	7
Fernão Pires/Maria Gomes	W	12.052	6
Castelão/João de Santarém/Periquita	R	9.130	5
Trincadeira/Tinta Amarela/Trincadeira Preta	R	8.413	4
Baga	R	8.851	3
Loureiro	W	8.851	3
Arinto/Pedernã	W	5.778	3
Syrah/Shiraz	R	5.674	3
Síria/Roupeiro/Códega	W	5.432	3
Alicante Bouschet	R	4.888	3
Vinhão/Sousão	R	3.894	2
Tinta Barroca	R	3.790	2
Jaen/Mencia	R	3.789	2
Rufete/Tinta Pinheira	R	3.422	2
Alvarinho	W	3.187	2
Caladoc	R	2.667	1
Malvasia Fina/Boal	W	2.605	1
Marufo/Mourisco Roxo	R	2.122	1
Cabernet Sauvignon	R	1.752	1
Rabigato	W	1.592	1
Malvasia Rei	W	1.584	1
Trajadura/Treixadura	W	1.564	1
Malvasia	W	1.488	1

R - red grape varieties; W - white grape varieties; * thousands; ** % total vineyard area.

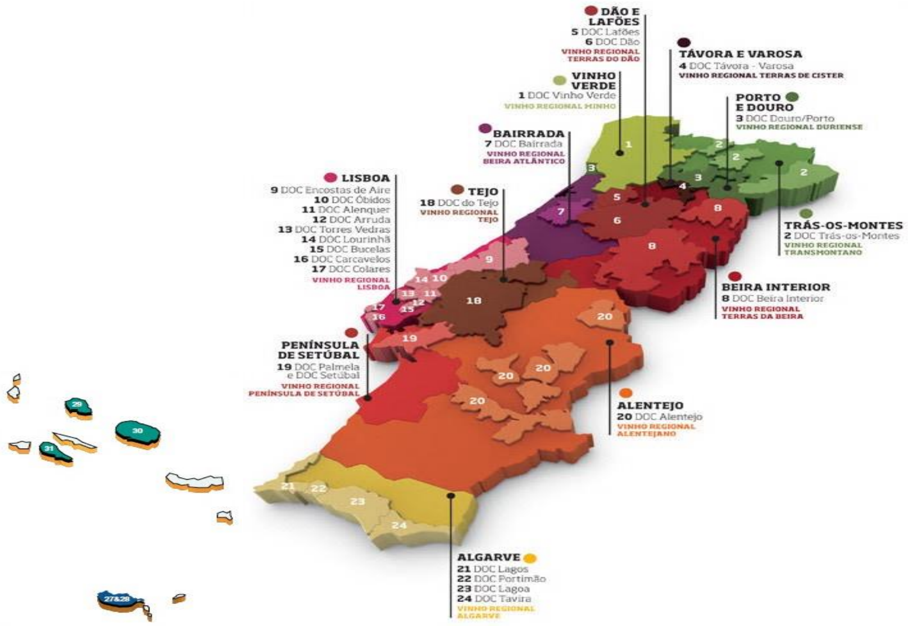


Figure 2. Portuguese wine regions (including Madeira and Azores islands) and respective protected designations of origin (IVV, 2017).

Portugal, despite having a small total area of 92,212 km^2, has a high diversity of climatic and soil characteristics (Fraga et al., 2012). Thus, in the south of the country, Alentejo is a distinct wine region mostly flatland with a typical Mediterranean climate, i.e., with a relatively homogenous warm and dry climate. This region during the grape ripening it's characterized by high temperatures, sometimes above of 40ºC. In this region dominate the red grape varieties, Tinta Roriz/Aragonês, Trincadeira, Alicante Bouschet and Syrah and for white grape varieties have preponderance Portuguese native grape varieties, namely, Antão Vaz, Arinto and Fernão Pires.

It is important to emphasize that it is in the south of the country that the international varieties, namely from French origin and particularly for red varieties, have greater use, being in some regions among the most used grape varieties. Thus, Caladoc and Syrah are one of the most varieties cultivated in Lisboa wine region, occupied the first and third position respectively, in the most cultivated grape varieties in this region, while Syrah represents around 5% of the total vine area in Tejo wine region. Also, Alicante Bouschet and Syrah represent 12% and 9%, respectively, of the total vine area in the Alentejo wine region, which corresponds to the third and the fourth position among the most planted grape varieties in this wine region (IVV, 2017).

Finally, in Madeira and Azores, Portuguese islands located in the Atlantic Ocean, there is also an important wine activity with some tradition, particularly for Madeira wine. In general, Madeira wines are produced in a variety of styles ranging from dry wines which can be consumed on their own as an aperitif to sweet wines usually consumed with dessert.

Tinta Negra (red grape) is the main variety cultivated in the Madeira island; however, there are also other grape varieties considered as noble varieties, namely, Sercial, Verdelho, Boal and Malvasia (Perestrelo et al., 2012). For Azores wines, wine production its located mainly, on three of the 9 islands of this archipelago (Pico, Terceira and Graciosa islands). According to Eiras-Dias et al. (2006), the majority of the varieties are traditional Portuguese autochthones white grapes, namely, Verdelho, Arinto (Terrantez da Terceira), Terrantez do Pico e Boal (Malvasia Fina). In last years, the wine production from these islands (particularly, from Pico island) showed a great increase.

Considering the great diversity of varieties grown in Portugal, namely native varieties, will have a strong reflection on the diversity of their characteristics, particularly in terms of their phenolic composition that will be presented in the following items.

3. CHEMICAL STRUCTURE OF MAIN GRAPE PHENOLIC COMPOUNDS

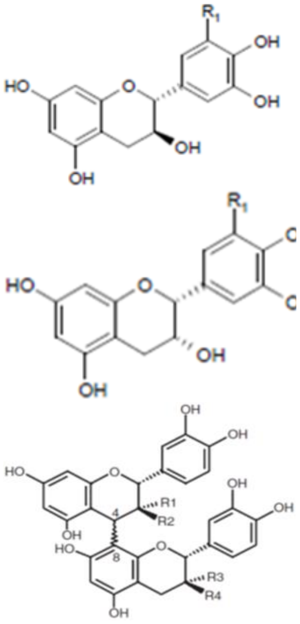
Grape total phenolic compounds are classified according to their chemical structure into flavonoids (anthocyanins, flavonols and flavan-3-ols) and non-flavonoid compounds (phenolic acids and stilbenes), these compounds are synthesized in the grape berry. Flavonoids are found mainly in grape (*Vitis vinifera* L.) seeds and skins and non-flavonoids in the grape skins.

Grape proanthocyanidins, also named as condensed tannins or flavanols are oligomers and polymers of flavan-3-ols monomeric units (such as (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-*O*-gallate, and (-)-epigallocatechin). In grape seed proanthocyanidins are oligomers and polymers of the monomeric flavan-3-ols (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-*O*-gallate linked by C_4-C_8 and/or C_4-C_6 bonds (B-type) (Prieur et al., 1994). In grape skin, their monomers are [(+)-catechin, (-)-epicatechin, (+)-gallocatechin and (-)-epigallocatechin units] (Figure 3), so in grape skin, it is also possible to detect (-)-epigallochatechin (Genebra et al., 2014). Therefore, in grape seed, there are only procyanidins and in grape skins, both procyanidins and prodelphinidins could be detected (Souquet et al., 1996).

Procyanidins are dimers resulting from the union of monomeric units of flavanols [(+)-catechin, (−)-epicatechin] by C_4–C_8 (procyanidin B1 to B4) or C_4–C_6 (procyanidin B5 to B8) interflavane linkage. Among grape varieties, there are differences in procyanidins concentrations, but their profile remains unchanged among grape varieties; procyanidin B1 is usually more abundant in the skin while B2 is more abundant in seeds (Fuleki and Ricardo-da-Silva, 2003). Proanthocyanidins (procyanidins and prodelphinidins) are the major phenolic compounds in grape seed and skins (Fuleki and Ricardo-da-Silva, 2003), about 60-70% of the total polyphenols are stored in grape seeds.

An important structural characteristic related to proanthocyanidins is their degree of polymerization (dimers, trimers, oligomers and polymers) (Cosme et al., 2009). In grape the main form of proanthocyanidins are polymeric (60-80%), followed by oligomeric (15-30%) and monomeric flavan-3-ols that represent less than 10% (Sun et al., 2001). Depending on the grape variety and on the grape fraction there is a high range of degree of polymerization of proanthocyanidins. According to Cosme et al. (2009) in grape varieties cultivated in Portugal, such as Touriga Nacional, Trincadeira, Castelão, Syrah and Cabernet Sauvignon; it was determined 77-85% of polymeric fractions in seeds and 91-99% of polymeric fractions in skins.

In *Vitis vinifera* L. grape skins, it is important to notice that each grape species and variety has its unique set of anthocyanins. *Vitis vinifera* grape varieties have only one molecule of glucose at the carbon 3 position forming the 3-*O*-monoglycosides anthocyanins. Position 6 of glucose, part of the anthocyanins, can both be unsubstituted or esterified (acylated anthocyanins as esters of acetic acid -acetylated derivatives, *p*-coumaric acid-*p*-coumaroylated derivatives, or caffeic acid-caffeoylated derivatives (Mazza et al., 1999; He et al., 2010; Squadrito et al., 2010). Grape anthocyanins are flavonoids, and the monomeric anthocyanins occur as 3-*O*-glucosides (anthocyanins), substituted with two (di-oxygenated: cyanidin- and peonidin-3-*O*-glucosides) or three (tri-oxygenated: delphinidin-, petunidin-, and malvidin-3-*O*-glucosides) hydroxyl (-OH) and/or methoxyl (-OCH$_3$) groups in the side-ring (B) of the flavonoid structure (Figure 4).

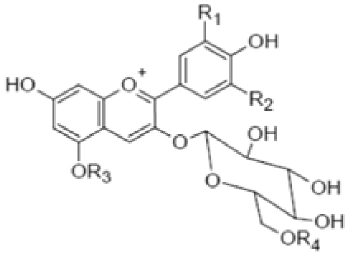


Flavan-3-ols	**R$_1$**
(+)-catechin	H
(+)-gallocatechin	OH

Flavan-3-ols	**R$_1$**
(+)-epicatechin	H
(+)-epigallocatechin	OH

Dimers (C$_4$–C$_8$)	**R$_1$**	**R$_2$**	**R$_3$**	**F**
Procyanidin B1	OH	H	H	O
Procyanidin B2	OH	H	OH	I
Procyanidin B3	H	OH	H	O
Procyanidin B4	H	OH	OH	I

Figure 3. Monomeric flavanols and proanthocyanidin structures found in grapes.

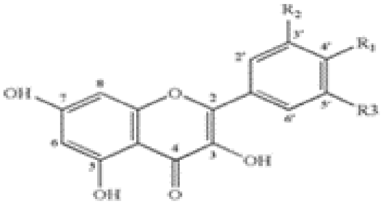


Anthocyanins	**R_1**	**R_2**
Pelargonidin-3-*O*-glucoside	H	H
Petunidin-3-*O*-glucoside	OCH_3	OH
Peonidin-3-*O*-glucoside	OCH_3	H
Malvidin-3-*O*-glucoside	OCH_3	OCH_3
Cyanidin-3-*O*-glucoside	H	OH
Delphinidin-3-*O*-glucoside	OH	OH

R_3 = H, glucose
R_4 = H, acetyl, *p*-hydroxyxinnamoyl, caffeoyl.

Figure 4. Anthocyanins structure found in *Vitis vinifera* red grape varieties.

The flavonols present in the grapes are mainly represented by the most common aglycones including kaempferol, quercetin and myricetin, and by simple *O*-methylated forms such as isorhamnetin (Makris et al., 2006) (Figure 5). The conjugates are exclusively 3-*O*-glycosides, whereas sugar attachment on other positions of the flavonol skeleton has never been reported. For isorhamnetin, only glucose derivatives have been identified, but myricetin, quercetin and kaempferol may also occur as glucuronides (Makris et al., 2006).



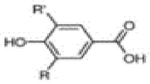
	R_1	R_2	R_3
Quercitin	OH	OH	H
Myricetin	OH	OH	OH
Isorhamnetin	OH	OCH_3	H
Kaempferol	OH	H	H

Figure 5. Chemical structures of grape flavonols.

Phenolic acids are divided into the benzoic acids that contain seven carbon atoms (C_6-C_1) and the cinnamic acids with nine carbon atoms (C_6-C_3), they exist mainly as hydroxybenzoic and hydroxycinnamic acids, in either the free or the conjugated form. In grapes, phenolic acids are mainly hydroxycinnamic acids found in the skins and pulps, in the form of tartaric esters (Jin et al., 2009). The most important benzoic acids are vanillic, syringic and salicylic acids, which appear to be bound to the cell walls and, in particular, gallic acid, which is in the form of the ester of flavanols. Other benzoic acids that are present in lesser amounts are the protocatechuic and *p*-hydroxybenzoic acids. The structure of these acids varies according to the number and position of hydroxylic and methoxylic groups attached to these benzoic ring (Figure 6). These acids can be found in bound forms, such as esters or glycosides, or in free forms. The most important cinnamic acids are ferulic, *p*-coumaric and caffeic acids (Baderschneider and Winterhalter, 2001; Jin et al., 2009) (Figure 6).

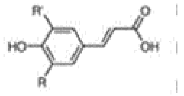
Grapes also contain C_6-C_3-C_6 stilbenes, they are phenolic compounds comprising two aromatic rings linked by a methylene bridge. The main grape stilbenes are *cis*- and *trans*-resveratrol, resveratrol-3-*O*-β-d-glucopyranoside (piceid), piceatannol and resveratrol dimers (viniferins) (Bavaresco et al., 2002; Vitrac et al., 2005). *Trans-resveratrol* is the simplest stilbene, which is a precursor for the synthesis of other stilbenes. *Cis-resveratrol* is a less stable isomer. Glycosylation is one of the possible modifications of stilbenes. *trans*- and *cis*- resveratrol and *trans*- and *cis*- piceid glucoside derivatives (Jang et al., 1997).

A



	R`	R
p-Hydroxybenzoic acid	H	H
Protocatechuic acid	H	OH
Gallic acid	OH	OH
Vanillic acid	H	OCH_3
Syringic acid	OCH_3	OCH_3

B



	R`	R
p-Coumaric acid	H	H
Caffeic acid	H	OH
Ferulic acid	H	OCH_3

Figure 6. Chemical structure of different hydroxybenzoic (A) and hydroxycinnamic acids (B).

4. PHENOLIC CHARACTERIZATION OF MAIN PORTUGUESE GRAPE VARIETIES

The grape phenolic composition and profile, which comprises detailed qualitative data, is determined by a great number of factors. Thus, grape phenolic content is influenced by the plant yield (Cortell et al., 2005; Peña-Neira et al., 2007; Junquera et al., 2012), leaf area/berry ratio (Susaj et al., 2013; Song et al., 2018), and by other factors that modulate the berry development, such as soil (Yokotsuka et al., 1999; Morlat and Bodin, 2006; Wang et al., 2015), geographic location (Mateus et al., 2002; Costa et al., 2014; 2015a; 2015b), climatic conditions (Bergqvist et al., 2001; Downey et al., 2006; Mori et al., 2007) and viticulture practices (Ollé et al., 2011; Fanzone et al., 2011; Portu et al., 2015; Yu et al., 2016; Wang et al., 2018), and also vintage (Liang et al., 2012; Costa et al., 2015b; Hernández et al., 2017). In addition, the potential phenolic content and profile characteristic by each grape varieties will be another important factor which determines the grape phenolic composition in the different grape berry fractions (Obreque-Slier et al., 2010; Fanzone et al., 2011; Hanlin et al., 2011; Perestrelo et al., 2012; Costa et al., 2014; 2015a;

Favre et al., 2018; Fu-Xiang et al., 2019; Li et al., 2019). On the other hand, it is important to note that the grape phenolic content changes also during ripening (Jordão et al., 1998; 2001a; 2001b; 2012; Kennedy et al., 2001; Obreque-Slier et al., 2010; Jordão and Correia, 2012).

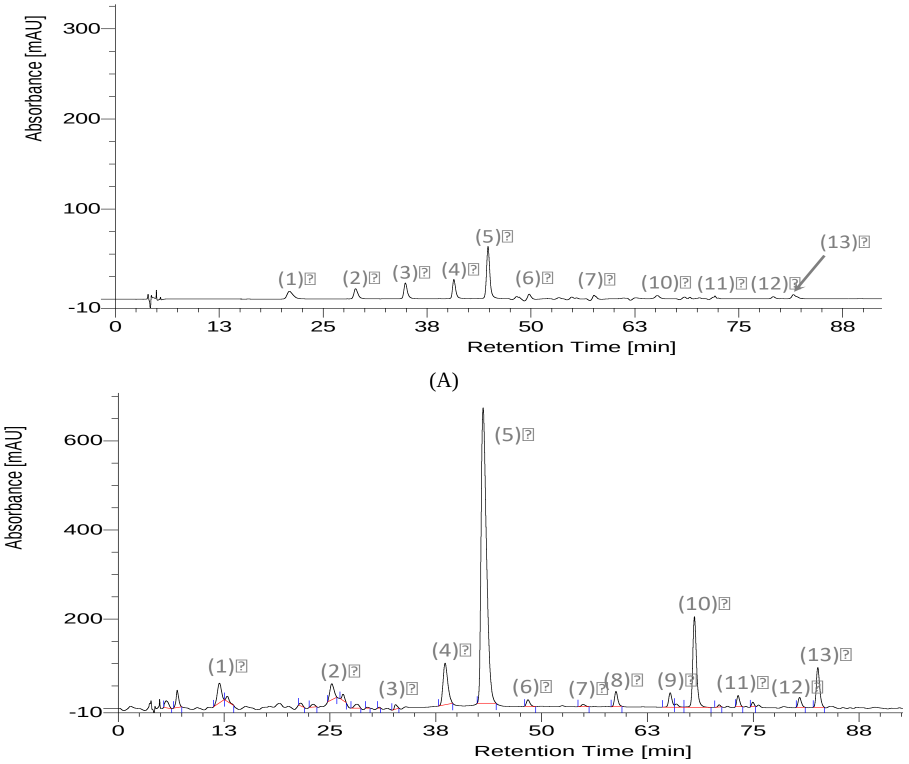
Portugal its characterized by a high diversity of wine regions with different climatic, topography and soil characteristics, besides a large number of cultivated grape varieties, the majority of them autochthonous varieties (see examples in Table 1). Thus, all of these factors induce a potential high variability of phenolic composition of the different grape varieties cultivated.

4.1. Anthocyanins

In general, grape anthocyanin content and also the profile of different monomeric anthocyanins are influenced mostly by a high number of different factors, namely, the geographical origin of the grapes, climatic characteristics of individual years, type and intensity of agronomical measures practices and also determined by the different clones of each grape variety (Rogero et al., 1986; Ojeda et al., 2002; Downey et al., 2006; Geana et al., 2011; Soubeyrand et al., 2014; Costa et al., 2015a; 2015b; Xing et al., 2015; Coklar, 2017). In addition, according to several published works, anthocyanin content of grapes appears to be related to genetic factors which could induce potential differences between several grape varieties (Revilla et al., 2001; Wu and Prior, 2005; Dopico-García et al., 2008; Rababah et al., 2008; Zhang et al., 2019). Another important point that sometimes induces a potential difference, namely in a quantitative point of view for the different grapes varieties are related with different methods used for anthocyanins extractions, including during the fermentation process of red wines. This factor will be another source of the variability in the color capacity of grapes (Guidoni et al., 2002; Fournand et al., 2006; Li et al., 2010; Ćurko et al., 2017; Sommer and Cohen, 2018). In general, the evolution of anthocyanins during grape ripening is characterized by an increase which begins at véraison, even 2 or 3 weeks before the color of grapes being visible. From véraison to complete maturity, in general, the development of anthocyanins is characterized by three phases: the first one presents a slow increase, followed by a rapid increase (20 to 35 day after véraison) ending in a stabilization phase before a decrease at the end of ripening and/or during over-ripening (González-San José et al., 1990; Jordão et al., 1998a; 1998b; 2012; Fournand et al., 2006). Concerning to the individual anthocyanins, anthocyanin-3-monoglucosides are the most abundant pigment group, while malvidin-3-monoglucoside is the major individual anthocyanin detected during the grape ripening (Jordão et al., 1998a; 2012; Brar et al., 2008; Costa et al., 2014). Finally, several authors described that acetylglucoside and coumaroylglucoside derivatives groups, are found in a very small percentage at *vérasion* and in the first one or two weeks after *vérasion* (Jordão et al., 1998a;

1998b; Fournand et al., 2006; Jordão and Correia, 2012; Sousa, 2020). Figure 7 shows an example of a monomeric anthocyanins profile from a Portuguese grape variety, Touriga Nacional, at two maturation stages.

Considering the studies published, where native Portuguese grape varieties were analyzed, Jordão and Correia (2012) reported for two important grape varieties, Touriga Nacional and Tinta Roriz, that the group of anthocyanin-3-*O*-glucoside were the most abundant, followed by the acetylglucoside derivatives and finally by the coumaroylglucoside derivatives. In contrast, for several international red grape varieties from French origins, such as, Cabernet-Sauvignon, Cabernet Franc, Syrah and Petit Verdot, Zhang et al. (2019) reported that the acylated anthocyanins were the major anthocyanins that differentiated these grape cultivars.



(A)

(B)

Figure 7. HPLC-DAD chromatogram at 520 nm of monomeric anthocyanins profile of grape must from Portuguese grape variety, *Touriga Nacional*, at two maturation stages: (A) first week of véraison; (B) grape harvest at technological maturation (adapted from Sousa, 2020).

Compounds: (1) delphinidin-3-*O*-monoglucoside; (2) cyanidin-3-*O* -monoglucoside; (3) petunidin-3--*O* monoglucoside; (4) peonidin-3-*O* -monoglucoside; (5) malvidin-3-*O* -monoglucoside; (6) cyanidin-3-*O* -acetylglucoside; (7) petunidin-3-*O* -acetylglucoside, (8) peonidin-3-*O* -acetylglucoside; (9) delphinidin-3-*O* -acetylglucoside; (10) malvidin-3-*O* -acetylglucoside; (11) petunidin-3-*O* -*p*-coumaroylglucoside; (12) peonidin-3-*O* -*p*-coumaroylglucoside; (13) malvidin-3-*O* -*p*-coumaroylglucoside.

Costa et al. (2015b) in order to deepen the knowledge of the phenolic composition, particularly for anthocyanin content, between several French (Alicante Bouschet, Cabernet Sauvignon, Merlot, Pinot Noir and Syrah) and Portuguese (Tinta Roriz and Touriga Nacional) grape varieties grown in two different Portuguese's wine regions studied the three main groups of anthocyanins. Thus, in descending order of global average values of the three anthocyanin groups, these were grouped as glucosylated, *p*-coumaroylated, and acetylated for the grape varieties studied. Also, Silva et al. (2016) described the anthocyanin profile of five different Portuguese native grape varieties (Jaen, Touriga Nacional, Alfrocheiro and Tinta Roriz) and also confirm glucosides group as the main anthocyanin group, followed by the *p*-coumaroylated derivatives. In addition, the results obtained by these authors showed that Jaen grape variety was the poorest variety in individual monomeric anthocyanin content (total of 1359.6 mg/kg of lyophilized sample), while Touriga Nacional showed remarkable higher amounts (total of 5336.3 mg/kg of lyophilized sample), which was at least more then twice higher than the other variety analyzed. The tendency for high values of anthocyanin content detected in Touriga Nacional was also described by Agudelo-Romero et al. (2013) compared to the values detected in other Portuguese grape varieties such as Trincadeira and Aragonês (also called Tinta Roriz in Northern of Portugal).

For individual anthocyanin content, the majority of the works published reported malvidin-3-monoglucoside as the major individual anthocyanin quantified in the different *Vitis vinifera* grape varieties, including for the Portuguese native varieties (Dallas and Laureano, 1994; Costa et al., 2014; 2015a; 2015b; Jordão et al., 1998a; 1998b; Novaka et al., 2008; 2012; Jordão and Correia, 2012; Mateus et al., 2002; Silva et al., 2016). However, for a reduced number of *Vitis vinifera* species, such as for example Graciano variety (Spanish origin), cyanidin derivatives are the most abundant forms (Núñez et al., 2004; Liang et al., 2008). Novaka et al., (2008) studied four Portuguese native grape varieties (Alfrocheiro, Jaen, Touriga Nacional and Tinta Roriz) from the Dão wine demarcated region and found that for all studied grape varieties, malvidin-3-monoglucoside was the most abundant anthocyanin in all varieties analyzed. Concentrations of malvidin-3-monoglucoside obtained ranged from 414.7 μg/g in Jaen to 2232 μg/g in Touriga Nacional grape varieties. In addition, the next most abundant anthocyanin was petunidin-3-monoglucoside, followed by delphinidin-3-monoglucoside and peonidin-3-monoglucoside, whose content was quite small, except for 'Touriga Nacional' grapes. Very small amounts of cyanidin-3-monoglucoside were found, being negligible in 'Jaen' grapes. This is logical if one bears in mind that cyanidin is a precursor in the biosynthesis of the other anthocyanins in the *Vitaceae* family. In a large study where the anthocyanin profile of 18 autochthonous grape varieties from Portuguese origin were analyzed, Costa et al., (2014) reported that malvidin-3-monoglucoside as the major individual anthocyanin (ranging from 0.62 to 6.09 mg/g of skin) in all varieties studied except for Alvarilhão and Rufete, where the major individual anthocyanins were peonidin-3-glucoside (1.04 mg/g of skin) and malvidin-3-*p*-coumaroylglucoside (1.48 mg/g of skin), respectively. In addition, malvidin-3-*p*-coumaroyl glucoside was the second main individual anthocyanin for the majority of the varieties analyzed and the values ranged from 0.12 to 3.44 mg/g of skin. Mateus et al. (2001) also reported for one of the most important Portuguese grape variety cultivated in Douro Region, Touriga Franca variety, that malvidin-3-monoglucoside and its respective acylated esters (acetyl, coumaroyl and caffeoyl esters) were the most relevant anthocyanins found in grape skins.

Tinta Negra is the main variety cultivated in the Madeira Island (around 90%), a Portuguese island located in the Atlantic Ocean and is used to produce the world-famous Madeira wine. According to Perestrelo et al., (2012), malvidin-3-monoglucoside is the predominant individual phenolic compound detected in this red grape variety at *véraison*, representing 73% of the phenolic composition quantified. At maturity, malvidin-3-monoglucoside, malvidin acetylglucoside, malvidin coumarylglucoside, and rutin represent 82% of the total phenolic composition.

In last years, Touriga Nacional, some of the one most important Portuguese red grape variety, has shown an expansion in its cultivation in several countries, namely in the southern hemisphere. Oliveira et al., (2018) reported the anthocyanin profile of Touriga Nacional grape variety cultivated in a semi-arid tropical region of Brazil, during four harvest seasons. According to these authors, non-acylated anthocyanins were pigments with the highest concentrations within the group of monomeric anthocyanins, while malvidin-3-monoglucoside was the most abundant individual monomeric anthocyanin quantified. Table 2, summarize the variability of the several individual anthocyanins quantified in different Portuguese red grape varieties.

4.2. Flavan-3-ols and Individual Procyanidins

In the grape berries, the flavan-3-ols ((+)-catechin and proanthocyanidins) are present in the solid parts of grapes (stems, skins and particularly in seeds) and exists with different degrees of polymerization (dimers, trimers, tetramers and polymers). These compounds also called condensed tannins and are transferred from solid parts of the grapes (skins, seeds and stems) into the must during winemaking process (crushing, maceration and fermentation).

In grapes, the quantity and the chemical structure of proanthocyanidins differ, depending on their localization in the grape tissue (Jordão et al., 2001a; Bordiga et al., 2011). In grape berries, they are mainly located in seeds and contribute to approximately 50% of the flavan-3-ols quantified in red wines (Sun et al., 1999). Such as anthocyanins, proanthocyanidins play a relevant role in the sensory characteristics of red wines, contribute to the astringency and bitterness of grapes and consequently of the wines. In addition, they have a crucial role in wine color stabilization (Canals et al., 2005). Indeed, proanthocyanidins interact with the glycoproteins of saliva and cause the sensation of astringency (Santos-Buelga and Scalbert, 2000). In addition, during winemaking and wine aging, these compounds undergo important structural transformations through oxidation and polymerization reactions, which lead to the characteristic color and sensorial changes. Flavan-3-ols are also involved in the formation of new pigments during wine aging (Kennedy, 2008). In general, the evolution and the quantification of these compounds during grape maturation are characterized by high values prior to *véraison*, following by a decrease during the ripening (Jordão et al., 1998; 2001a; 2001b; Jordão and Correia, 2012; Asproudi et al., 2015; Allegro et al., 2016).

Procyanidins and (+)-catechin concentration and distribution, in grapes are depended on different factors, such as climatic factors and the geographical location of the vineyards (Mateus et al., 2001; Hanlin and Downey, 2009; Lorrain et al., 2011) and also the vintage effect (Hernández et al., 2016; Oliveira et al., 2018). The composition and content of flavan-3-ols in grapes is also a consequence of the many viticulture factors, such as pruning system management (Ó-Marques et al., 2005), leaf removal (Bogicevic et al., 2015), vine production level (Gil et al., 2013), fertilizer and sometimes by the water regimes (Genebra et al., 2014; Kyraleou et al., 2016).

Grape varieties, their genetic characteristics combined with the vintage effect are also one of the main factors that determine the proanthocyanidin content and their structural characteristics in grapes. This point has been evidenced in a great number of published works (Sun et al., 1998; Jordão et al., 1998b; 2001a; 2011b; Monagas et al., 2003; Cosme et al., 2009; Obreque-Slier et al., 2010; Bautista-Ortín et al., 2013; Oliveira et al., 2018).

For the levels of individual flavan-3-ols and procyanidins quantified in several grape varieties, a high range of values have been quantified in published works. Specifically, for the Portuguese grape varieties, the number of published studies on the evaluation of the content and the structural characteristics diversity of these phenolic compound groups is still not very high. However, in Table 3 different flavan-3-ols and some individual procyanidins quantified in several Portuguese grape varieties are shown. According to several published works for a diversity of Portuguese grapevine varieties on average and basis of fresh weight, the concentrations of proanthocyanidins are as follows: total monomers ((+)-catechin and (-)-epicatechin), 2-12 mg/g in seeds and 0.1-0.7 mg/g in skins; total oligomers, 19-43 mg/g in seeds and 0.8-3.5 mg/g in skins and total polymers, 45-78 mg/g in seeds, 2-21 mg/g in skins and 28.0-35.8 mg/g in stems (Jordão et al., 2001a; 2001b; Sun et al., 2001). Nevertheless, the levels of individual oligomeric procyanidins in the different grape fraction (seeds, skins and stems) are very different. Thus, procyanidin dimer B1 is the major component in stems and skins, while procyanidin dimer B2 has its highest concentration in seeds (Jordão et al., 1998b; 2001b; Mateus et al., 2001).

Table 2. Several monomeric anthocyanins quantified in some Portuguese red grape varieties

Grape variety	Monomeric anthocyanins										
	Delp gluc	Cyan gluc	Petu gluc	Peo gluc	Malv gluc	Cyanacet-gluc	Petuacet gluc	Peo acet gluc	Malv acet-gluc	Malvcou-gluc	Ref.
Castelão	6.2	2.6	8.5	11.7	59.2	0.2	0.4	0.5	4.0	4.6	Jordão et al. (1998a) (a)
Touriga Franca	0.9	0.1	2.5	3.6	46.3	0.1	2.5	3.6	12.9	23.4	
Alfrocheiro	142	0.33	617	139	1152	n.a.	n.a.	n.a.	n.a.	n.a.	Novak et al. (2008) (b)
Jaen	10.3	n.a.	95.1	61.3	414	n.a.	n.a.	n.a.	n.a.	n.a.	
Tinta Roriz	123	0.17	418	338	887	n.a.	n.a.	n.a.	n.a.	n.a.	
Touriga Nacional	691	0.73	1761	542	2232	n.a.	n.a.	n.a.	n.a.	n.a.	
Camarate	n.a.	0.05	0.22	0.49	5.08	0.02	0.02	0.12	1.29	0.90	Costa et al. (2014) (c)
Monvedro	0.60	0.01	0.94	0.30	6.09	0.05	0.14	0.01	1.65	1.48	
Moreto Boal	0.08	0.01	0.06	0.49	3.96	0.01	0.06	0.11	1.01	1.27	
Negro Mole	0.04	0.01	0.24	0.51	5.92	0.02	0.21	0.11	1.43	2.21	
Negro Mouro	0.03	n.a.	0.21	0.54	5.88	0.02	0.08	0.10	1.45	2.23	
Alfrocheiro	0.03	n.a.	0.16	0.14	2.90	n.a.	n.a.	0.04	0.18	1.46	
Alvarilhão	0.08	0.24	0.12	1.04	0.99	0.01	0.01	0.06	0.05	0.12	
Bastardo	0.02	0.03	0.01	0.13	0.62	n.a.	n.a.	0.02	0.05	0.16	
Jean	0.09	0.02	0.20	0.27	2.75	0.02	0.04	0.03	0.89	0.77	
Malvasia Preta	0.21	0.06	0.31	0.55	2.32	n.a.	0.01	0.01	0.10	0.28	
Rufete	n.a.	n.a.	n.a.	0.01	1.39	n.a.	n.a.	0.02	0.14	1.48	
Sousão	0.21	0.09	0.33	1.34	2.76	n.a.	n.a.	n.a.	0.06	0.18	
Tinta Amarela	0.58	0.07	0.74	0.69	2.91	0.02	0.04	0.03	0.10	0.65	
Tinta Barca	0.34	0.04	0.46	0.23	2.41	n.a.	n.a.	0.05	0.13	0.98	
Tinta Barroca	0.21	0.06	0.41	0.57	4.39	n.a.	n.a.	0.07	0.26	1.94	
Tinta Miúda	0.20	0.01	0.25	0.64	2.31	n.a.	0.02	0.04	0.26	0.41	
Tinto Cão	0.27	0.01	0.39	0.13	2.65	n.a.	0.01	0.16	0.37	2.57	

Table 2. (Continued)

Grape variety	Monomeric anthocyanins										Ref.
	Delp gluc	Cyan gluc	Petu gluc	Peo gluc	Malv gluc	Cyanacet-gluc	Petuacet gluc	Peo acet gluc	Malv acet-gluc	Malvcou-gluc	
Alfrocheiro	111	21.3	146	244	1128	n.a.	n.a.	n.a.	n.a.	132	Silva and Queiroz (2016) [(d)]
Jaen	92.6	16.5	112	129	842	n.a.	n.a.	n.a.	n.a.	132	
Tinta Roriz	425	45.4	376	107	1260	n.a.	n.a.	n.a.	n.a.	287	
Touriga Nacional	609	75.9	644	427	2800	n.a.	n.a.	n.a.	n.a.	573	
Touriga Nacional [(f)]	14.9	13.0	12.9	20.4	60.4	0.95	3.65	20.4	8.4	6.0	Oliveira et al. (2018) [(e)]

n.a. not analyzed; Delp gluc, delphinidin-3-monoglucoside; Cyan gluc, cyanidin-3-monoglucoside; Petu gluc, petunidin-3-monoglucoside; Peo gluc, peonidin-3-monoglucoside; Malv gluc, malvidin-3-monoglucoside; Cyan acet-gluc, cyanidin-3-acetyl glucoside; Petu acet-gluc, petunidin-3-acetyl glucoside; Peo acet-gluc, peonidin-3-acetyl glucoside; Malv acet-gluc, malvidin-3-acetyl glucoside; Malv cou-gluc, malvidin-3-*p*-coumaroyl glucoside. (a) values in % weight of anthocyanins/weight grape; (b) values in µg/g of skin; (c) values in mg/g of skin; (d) values in mg/Kg of lyophilized sample; (e) values in mg/g of berry; (f) average values of four harvests.

Grape skins have a relatively low content of procyanidin dimers relative to that in seeds, the latter containing relatively high concentrations of procyanidin dimer B4, while in skins it is not possible to detect this dimer. According to De Freitas et al. (2000), in French 'Merlot' grape variety, procyanidin dimer B4 may be used as a chemical marker in musts and wines to quantify the contribution of the seeds. However, Lorrain et al. (2011) identified and quantified several oligomers (B1, B2 and B3 and trimer T) including also dimer B4, in seeds and skins at harvest in 'Merlot' and 'Cabernet Sauvignon' grapes from Bordeaux region. Procyanidin dimer B7 was only detected in low concentration in the seeds (average value of 9.7 mg/g of berry) of Portuguese Touriga Nacional grape variety (Mateus et al., 2001). Recently, Oliveira et al. (2018) also for 'Touriga Nacional' cultivated in Brazil, reported on grape seeds for (+)-catechin and (-)-epicatechin values between 1038.1 and 701.5 mg/kg of seeds, respectively. In addition, for several dimeric procyanidins the values varied from 5.7 to 11.5 mg/kg of seeds for dimer B1, from 219 to 429.8 mg/kg of seeds for dimer B2, from 5.7 to 64.6 mg/kg of seeds for dimer B3 and from 6.5 to 19.7 mg/kg of seeds for dimer B4. These authors also reported several values for grape skins. Thus, for (+)-catechin and (-)-epicatechin, in the second harvest season of 2016, 72.8 mg/kg and 117.4 mg/kg of skins were quantified, respectively. In addition, for procyanidin dimers, B2 was the predominant form in the skins (ranging from 26.8 to 74.2 mg/kg of skins).

4.3. Phenolic Acids and Other Phenolic Compounds

In grapes, phenolic acids are frequently divided into two main groups, hydroxycinnamic and hydroxybenzoic acids. Hydroxycinnamic acids are aromatic compounds characterized by a C_6-C_3 structure, being the most common in grapes the following ones: caffeic, ferulic, *p*-coumaric, sinapic, caftaric, coutaric and fertarique acids (Lu and Foo, 1999). They are mainly in the *trans* form but also exist in the *cis* form. They are found mainly in the grape skin and pulp as tartaric acid esters (Baderschneider and Winterhalter, 2001).

Comparative analysis of hydroxycinnamic esters content shows higher concentrations in the skin fresh material than in the pulp (Romeyer et al., 1985). However, in the grape pulp, high content of gallic acid, *p*-hydroxybenzoic, protocatechuic, vanillic and syringic acids could be found (Bravo, 1998; Lu and Foo, 1999). These compounds appear to be bound to the cell walls and, in particular, gallic acid, which is in the form of the ester of flavanols. According to Ribéreau-Gayon et al. (2005), in grapes, the most important cinnamic acids are ferrulic, *p*-coumaric and caffeic acids.

Table 3. Flavan-3-ols and some individual procyanidins quantified in several Portuguese grape varieties

Grape variety	Individual flavan-3-ols and procyanidins						
	(+)-Cat.	(-)-Epic.	Procy B3	Procy B1	Procy B4	Procy B2	Ref.
Stems							
Castelão	n.a.	n.a.	0.11	0.79	0.01	0.04	Ricardo-da-Silva et al. (1992) (a)
Vital	n.a.	n.a.	0.70	2.51	0.07	0.15	
Touriga Franca	0.11	1.33	0.89	6.59	0.13	0.27	Jordão et al. (2001b) (b)
Castelão	0.07	1.13	0.04	1.93	0.14	0.10	
Skins							
Castelão	n.a.	n.a.	0.008	0.314	0.004	0.007	Ricardo-da-Silva et al. (1992) (a)
Vital	n.a.	n.a.	0.015	0.105	0.001	0.007	
Touriga Nacional	0.8-1.1	n.a.	0.9	12.8-14.2	n.a.	1.4	Mateus et al. (2001) (c)
Touriga Franca	0.5	0.5	n.a.	4.8-5.9	n.a.	0.6-0.7	
Alfrocheiro	11.2	n.a.	n.a.	n.a.	n.a.	n.a.	Novak et al. (2008) (d)
Jaen	3.64	n.a.	n.a.	n.a.	n.a.	n.a.	
Tinta Roriz	2.92	n.a.	n.a.	n.a.	n.a.	n.a.	
Touriga Nacional	7.46	n.a.	n.a.	n.a.	n.a.	n.a.	
Touriga Nacional (g)	48.1	140.7	3.3	8.8	2.9	43.4	Oliveira et al. (2018) (e)
Seeds							
Castelão	n.a.	n.a.	0.78	0.83	1.64	2.67	Ricardo-da-Silva et al. (1992) (a)
Vital	n.a.	n.a.	0.43	0.40	0.70	1.31	
Touriga Franca	3.30	2.30	0.33	0.54	0.59	1.35	Jordão,et al. (2001a) (f)
Castelão	2.11	4.55	0.52	0.08	2.64	2.02	
Touriga Nacional	24.5-30.4	52-66	19.3-36.0	81.5-136	53.2-27.7	167-198	Mateus, et al. (2001) (c)
Touriga Franca	26.6-50.1	33.8-63.8	14.6-23.3	44.8-60.6	21.1-36.3	56.8-74.9	
Touriga Nacional (g)	793.3	563.2	23.8	34.8	27.0	305.7	Oliveira et al. (2018) (e)

n.a. not analyzed; (a) values in g/kg of stems, seeds or skins; (b) values in mg/g of stems; (c) values in mg/g of berry; (d) values in µg/g of skins; (e) values in mg/kg of skins or seeds; (f) values in mg/g of seeds; (g) average values of four harvests.

In grapes, it is also detected several C_6-C_3-C_6 stilbenes, such as *trans*-resveratrol, *cis*-resveratrol, and *trans*-resveratrol glucoside (Jackson, 2008). For Sautter et al. (2005), the concentration of *trans*-resveratrol in grape juice ranged from 0.19 to 0.90 mg/L. This stilbene is considered as the most abundant form in grapes, including also the glucosyl derivatives: pieced and ε-viniferin (dimeric resveratrol) (Jeandet et al., 1991; Romero-Pérez et al., 1999). Nicoletti et al., (2008) quantified in several Italian red grape varieties values that ranged between 3.6 and 63.0 mg/kg of berry dry weight for *trans*-resveratrol. In addition, for the Portuguese red grape variety Castelão, Sun et al., (2006) reported for grape stems average values of 145.5 mg/kg of dry stems. Also, Souquet et al., (2000) reported that grape stems are an important source of phenolic acids and flavanols, such as astilbin. In that case, this last individual phenolic compound was identified for the first time in stems by these authors. For Sercial white grape variety, Perestrelo et al., (2012), reported for skins, that caftaric acid vanilloyl pentoside, quercetin-glucuronide, quercetin-glucoside, *cis*-coutaric acid, protocatechuic acid-glucoside, and *p*-hydroxybenzoyl glucoside as the predominant phenolic compounds identified in this Portuguese grape variety. In that case, they represent about 78% of phenolic composition, at véraison and at the end of the maturity process.

Montealgre et al., (2006) analyzed non-anthocyanin phenols present in the skins and seeds of 70 grape samples belonging to 10 cultivars. Thus, these authors reported that grape skins contained tartaric esters of hydroxycinnamic (6-45 mg/kg of grape), monomeric and dimeric flavan-3-ols (9-96 mg/kg of grape) and flavonols (25-197 mg/kg of grape). Also, the seed constituents comprised almost exclusively flavan-3-ols with concentration ranges of 330-1390 mg/kg of grape. The content of these compounds was also determined by certain varietal differences, although other important factors have to be taken into accounts, such as the degree of ripeness or berry size. Lachman et al. (2004) reported significant differences in *trans*-resveratrol content between grape skins and seeds, however, no statistical differences among varieties were found. However, Faitová et al. (2004) in white ‘Riesling’ wines obtained from different vineyard sub-regions concluded that the content of *trans*-resveratrol quantified was determined by grape origin region and also by the vintages. Nevertheless, according to several authors, the phenolic profile of grapes do not change significantly through harvests (Mazza et al., 1999; Kammerer et al. 2004; Fanzone et al., 2011). Also, other authors reported that different geographical origin of grapes and the specific variety characteristics could determine the content of *trans*-resveratrol in grapes and consequently in wines (Peña-Neira et al., 2000; Sakkiadi et al., 2001; Kallithraka et al., 2001; Kallithraka et al., 2006). Also, climatic conditions could determine the content of several individual stilbenes, such as *trans*-resveratrol. Thus, intense UV irradiation leads to complete disappearance of *trans*- and *cis*-isomers of piceid and to a large decrease in resveratrol isomers content (Roggero, 2000).

Table 4. Several phenolic acids and other individual phenolic compounds quantified in several Portuguese grape varieties

	Individual phenolic compounds									
Grape variety	Gallic acid	Trans-resveratrol	Cis-resveratrol	Rutin	Quercetin-glucoside	Caftaric acid	Ferulic acid	Caffeic acid	Protocatecheuic acid	Ref.
Grape berry										
Jaen	1.5	n.a.	n.a.	n.a.	24.3	n.a.	24.3	4.2	n.a.	Silva and Queiroz (2016) (a)
Touriga Nacional	13.4	n.a.	n.a.	n.a.	97.6	n.a.	8.8	29.2	n.a.	
Alfrocheiro	3.9	n.a.	n.a.	n.a.	32.1	n.a.	n.a.	15.2	n.a.	
Tinta Roriz	11.8	n.a.	n.a.	n.a.	29.8	n.a.	n.a.	9.9	n.a.	
Skins										
Jaen	n.a.	n.a.	n.a.	29.11	n.a.	n.a.	n.a.	n.a.	n.a.	Novak et al. (2008) (b)
Touriga Nacional	n.a.	n.a.	n.a.	214.3	n.a.	n.a.	n.a.	n.a.	n.a.	
Alfrocheiro	n.a.	n.a.	n.a.	46.6	n.a.	n.a.	n.a.	n.a.	n.a.	
Tinta Roriz	n.a.	n.a.	n.a.	78.0	n.a.	n.a.	n.a.	n.a.	n.a.	
Sercial	n.a.	8.1	7.4	61.6	370.4	n.a.	n.a.	n.a.	202.4	Perestrelo et al. (2012) (c)
Tinta Negra	n.a.	9.9	8.0	428.0	245.5	n.a.	n.a.	n.a.	51.3	
Malvasia Fina	5.7	309.2 (e)	n.a.	n.a.	48.4	22.8	n.a.	n.a.	n.a.	Ferreira et al. (2016) (d)
Gouveio	8.1	809.7 (e)	n.a.	n.a.	81.3	10.5	n.a.	n.a.	n.a.	
Moscatel Galego Branco	19.2	917.0 (e)	n.a.	n.a.	51.6	24.0	n.a.	n.a.	n.a.	
Malvasia Fina Roxo	11.6	369.8 (e)	n.a.	n.a.	70.7	15.8	n.a.	n.a.	n.a.	
Moscatel Galego Roxo	19.6	836.3 (e)	n.a.	n.a.	52.7	45.4	n.a.	n.a.	n.a.	
Azal tinto	3.4 (f)	n.a.	n.a.	n.a.	n.a.	11.0 (g)	n.a.	n.a.	n.a.	Dopico-Garcia et al. (2008)
Vinhão	2.7 (f)	n.a.	n.a.	n.a.	n.a.	11.0 (g)	n.a.	n.a.	n.a.	
Rabo de Ovelha	2.6 (f)	n.a.	n.a.	n.a.	n.a.	2.6 (g)	n.a.	n.a.	n.a.	
Espadeiro	5.1 (f)	n.a.	n.a.	n.a.	n.a.	44.0 (g)	n.a.	n.a.	n.a.	

n.a. not analyzed; (a) values in mg/kg of lyophilized grape sample; (b) values in µg/g of skins; (c) values in mg/g of skin; (d) values in mg/kg of berry dry basis; (e) values of resveratrol-3-O-glucoside; (f) values in mg/kg dried matter; (g) values in mg/kg dried weight.

Finally, the role of several individual phenolic acids may also be associated with the defence of grape berries against the grey mould caused by *Botrytis cinerea* (specifically as a result of the excretion of lytic enzymes such as polyphenoloxidases or laccases). Goetz et al. (1999) reported that (+)-catechin, epicatechin-3-*O*-gallate, *trans*-caftaric, *trans*- and *cis*-coutaric and *trans*-coumaric acids, taxifoline-3-*O*-rhamnoside and quercetine-3-*O*-glucuronide were identified as potent stilbene oxidase inhibitors. Thus, high concentrations of some of those compounds could be closely involved in the persistence of the quiescent stage of *Botrytis cinerea*, between bloom and véraison in all grape varieties studied (Gamay and Gamoret varieties) and after véraison in resistant varieties.

Considering the studies published, where native Portuguese grape varieties were analyzed, few of them were published for the individual phenolic acids and other specific phenolic compounds. However, in Table 4 several results obtained for the different grape fractions from some Portuguese grape varieties are shown. The majority of the works published are related to the interest in the bioactivity of these phenolic compounds from higher plants, such vines, at least in part, to the potential health benefits of these polyphenolic components as well as to their involvement in important biological and industrial processes.

5. FINAL REMARKS AND PERSPECTIVES

The grape phenolic composition has been widely studied specifically the composition of the different grape bunch fractions (seed, skin, stem and pulp). The main phenolic compounds from grapes are flavonoids (flavonols, anthocyanins and flavan-3-ols or proanthocyanidins) and non-flavonoids (phenolic acids and stilbenes). These compounds presented important biological properties related to human health and also an important role in wine quality.

As shown in this review, the values obtained for the grape phenolic composition from the different grape bunch fractions changed as a consequence of a great number of factors namely the grape variety. In that case, Portugal shows a high diversity of terroirs throughout its territory, and at the same time also shows more than 300 different grape varieties, the majority of them only cultivated in this country. Thus, all of these native grape varieties show specific characteristics, including in terms of their phenolic composition. However, taking into account the high number of varieties and their genetic diversity, the phenolic characterization studies of these native grape varieties are still restricted to a low number of varieties.

On the other hand, due to climate change, eventually many of these native grape varieties may have characteristics that are more resistant to dryness and high temperatures that in some regions of Portugal tends to occur. In this sense, the deepening of the characterization of many of these varieties, including in terms of their phenolic composition, may in the future be a key factor in their characterization. This point could also contribute to the production of differentiated wines with specific sensory profiles to be produced by a small country such as Portugal.

REFERENCES

Agudelo-Romero, P., Erban, A., Sousa, L., Pais, M. S., Kopka, J., Fortes, A. M. (2013). Search for transcriptional and metabolic markers of grape pre-ripening and ripening and insights into specific aroma development in three Portuguese cultivars. *Plos One,* 8:e60422.

Allegro, G., Pastore, C., Valentini, G., Muzzi, E., Filippetti, I. (2016). Influence of berry ripeness on accumulation, composition and extractability of skin and seed flavonoids in cv. Sangiovese (*Vitis vinifera* L.). *Journal of the Science of Food and Agriculture,* 96:4553-4559.

Almadanim, M. C., Baleiras-Couto, M. M., Pereira, H. S., Carneiro, L. C., Fevereiro, P., Eiras-Dias, J. E., Morais-Cecilio, L., Viegas, W., Veloso, M. M. (2007). Genetic diversity of the grapevine (*Vitis vinifera* L.) cultivars most utilized for wine production in Portugal. *Vitis,* 46:116-119.

Anderson, K., Aryal, N. (2013). Where in the world are various wine grape varieties grown? Evidence from a new database. *Bulletin de l'OIV,* 992-993-994:461-484.

Asproudi, A., Piano, F., Anselmi, G., Di Stefano, R., Bertolone, E., Borsa, D. (2015). Proanthocyanidin composition and evolution during grape ripening as affected by variety: Nebbiolo and Barbera cv. *Journal International des Sciences de la Vigne et du Vin,* 49:59-69.

Baderschneider, B., Winterhalter, P. (2001). Isolation and characterization of novel benzoates, cinnamates, flavonoids, and lignans from Riesling wine and screening for antioxidant activity. *Journal of Agricultural and Food Chemistry,* 49:2788-2798.

Bautista-Ortín, A. B., Jiménez-Pascual, E., Busse-Valverde, N., López-Roca, J. M., Ros-García, J. M., Gómez-Plaza, E. (2013). Effect of wine maceration enzymes on the extraction of grape seed proanthocyanidins. *Food and Bioprocess Technology,* 6:2207-2212.

Bavaresco, L., Fregoni, M., Trevisan, M., Mattivi, F., Vrhovsek, U., Falchetti, R. (2002). The occurrence of the stilbene piceatannol in grapes. *Vitis,* 41:133-136.

Bergqvist, J., Dokoozlian, N., Ebisuda, N. (2001). Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin valley of California. *American Journal of Enology and Viticulture,* 52:1-6.

Bogicevic, M., Maras, V., Mugoša, M., Kodžulović, V., Raičević, J., Šućur, S., Failla, O. (2015). The effects of early leaf removal and cluster thinning treatments on berry growth and grape composition in cultivars Vranac and Cabernet Sauvignon. *Chemical and Biological Technologies in Agriculture*, 2:13.

Bordiga, M., Travaglia, F., Locatelli, M., Coïsson, J. D., Arlorio, M. (2011). Characterization of polymeric skin and seed proanthocyanidins during ripening in six *Vitis vinifera* L. cv. *Food Chemistry,* 127:180-187.

Brar H. S., Singh Z., Swinny E. (2008). Dynamics of anthocyanin and flavonol profiles in the 'Crimson Seedless' grape berry skin during development and ripening. *Scientia Horticulturae,* 117:349-356.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Research*, 56:317-333.

Canals, R., Llaudy, M. C., Valls, J., Canals, J. M., Zamora, F. (2005). Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *Journal of Agricultural and Food Chemistry,* 53:4019-4025.

Carvalho, L. C., Silva, M., Coito, J. L., Rocheta, M. P., Amâncio, S. (2017). Design of a custom RT-qPCR array for assignment of abiotic stress tolerance in traditional Portuguese grapevine varieties. *Frontiers in Plant Science*, 8:1835.

Coklar, H. (2017). Antioxidant capacity and phenolic profile of berry, seed, and skin of Ekşikara (*vitis vinifera L*) grape: Influence of harvest year and altitude. *International Journal of Food Properties,* 20:2071-2087.

Cortell, J. M., Halbleib, M., Gallagher, A. V., Righetti, T. L., Kennedy, J. A. (2005). Influence of vine vigor on grape (*Vitis vinifera* L. Cv. Pinot Noir) and wine proanthocyanidins. *Journal of Agricultural and Food Chemistry*, 53:5798-5808.

Cosme, F., Ricardo-Da-Silva, J. M., Laureano, O. (2009). Tannin profiles of *Vitis vinifera* L. cv. red grapes growing in Lisbon and from their monovarietal wines. *Food Chemistry*, 112:197-204.

Costa, E., Cosme, F., Jordão, A. M., Mendes-Faia, A. (2014). Anthocyanin profile and antioxidant activity from 24 grape varieties cultivated in two Portuguese wine regions. *Journal International des Sciences de la Vigne et du Vin,* 48:51-62.

Costa, E., Cosme, F., Rivero-Pérez, M. D., Jordão, A. M., González-SanJosé, M. L. (2015a). Influence of wine region provenance on phenolic composition, antioxidant capacity and radical scavenger activity of traditional Portuguese red grape varieties. *European Food Research and Technology*, 241:61-73.

Costa, E., Da Silva, J. F., Cosme, F., Jordão, A. M. (2015b). Adaptability of some French red grape varieties cultivated at two different Portuguese terroirs: comparative analysis with two Portuguese red grape varieties using physicochemical and phenolic parameters. *Food Research International*, 78:302-312.

Ćurko, N., Tomašević, M., Bubalo, M. C., Gracin, L., Redovniković, I., Ganić, K. (2017). Extraction of proanthocyanidins and anthocyanins from grape skin by using ionic liquids. *Food Technology and Biotechnology,* 55:429-437.

Dallas, C., Laureano, O. (1994). Effect of SO_2 on the extraction of individual anthocyanins and colored matter of three Portuguese grape varieties during winemaking. *Vitis,* 33:41-47.

De Freitas, V. A. P., Glories Y., Monique, A. (2000). Developmental changes of procyanidins in grapes of red *Vitis vinifera* varieties and their composition in respective wines. *American Journal of Enology and Viticulture*, 51:397-403.

Dopico-García, M. S., Fique, A., Guerra, L., Valentão, P., Andrade, P. B., Seabra, R. M., Afonso, J. M., Pereira, O. (2008). Principal components of phenolics to characterize red Vinho Verde grapes: anthocyanins or non-coloured compounds? *Talanta,* 75:1190-202.

Downey, M. O., Dokoozlian, N. K., Krstic, M. P. (2006). Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *American Journal of Enology and Viticulture,* 57:257-268.

Eiras-Dias, J. E., Pereira, C. A., Baptista da Cunha, J. P. (1988). *Catálogo das Castas: Região do Ribatejo, Oeste e Península de Setúbal* [*Grape Variety Catalog: Ribatejo Region, West and Setúbal Peninsula*]. Instituto da Vinha e do Vinho. Estação Vitivinícola Nacional.

Eiras-Dias, J. E., Paulos, V., Mestre, S., Martins, J. T., Goulart, I. (2006). The grapevine varieties of Azores region. *Ciência e Técnica Vitivinícola,* 21:99-112.

Escarpa, A., Gonzalez, M. C. (2001). An overview of analytical chemistry of phenolic compounds in foods. *Critical Reviews in Analytical Chemistry* 31: 57-139.

Faitová, K., Hejtmánková, A., Lachman, J., Pivec, V., Dudjak, J. (2004). The contents of total polyphenolic compounds and *trans*-resveratrol in white Riesling originated in the Czech Republic. *Czech Journal of Food Sciences,* 22:215-221.

Fanzone, M., Zamora, F., Jofré, V., Assof, M., Peña-Neira, A. (2011). Phenolic composition of Malbec grape skins and seeds from Valle de Uco (Mendoza, Argentina) during ripening. Effect of cluster thinning. *Journal of Agricultural and Food Chemistry,* 59:6120-6136.

Favre, G., González-Neves, G., Piccardo, D., Gómez-Alonso, S., Pérez-Navarro, J., Hermosín-Gutiérrez, I. (2018). New acylated flavonols identified in *Vitis vinifera* grapes and wines. *Food Research International,* 112:98-107.

Ferreira, V., Fernandes, F., Pinto-Carnide, O., Valentão, P., Falco, V., Martín, J. P., Ortiz, J. M., Arroyo-García, R., Andrade, P. B., Castro, I. (2016). Identification of *Vitis vinifera* L. grape berry skin color mutants and polyphenolic profile. *Food Chemistry,* 194:117-127.

Ferrer-Gallego, R., Hernández-Hierro, J. M., Rivas-Gonzalo, J.C., Escribano-Bailon, M. T. (2012). Influence of climatic conditions on the phenolic composition of *Vitis vinifera* L. cv. Graciano. *Analytica Chimica Acta,* 732:73-77.

Fournand, D., Vicens, A., Sidhoum, L., Souquet, J.-M., Moutounet, M., Cheynier, V. (2006). Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. *Journal of Agricultural and Food Chemistry,* 54:7331-7338.

Fraga, H., Santos, J. A., Malheiro, A. C., Moutinho-Pereira, J. (2012). Climate change projections for the Portuguese viticulture using a multi-model ensemble. *Ciência Técnica Vitivinicola,* 27:39-48.

Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Jones, G. V., Alves, F., Pinto, J. G., Santos, J. A. (2014). Very high resolution bioclimatic zoning of Portuguese wine regions: present and future scenarios. *Regional Environmental Change,* 14:295-306.

Fraga, H., Santos, J. A., Malheiro, A. C., Oliveira, A. A., Moutinho-Pereira, J., Jones, G. V. (2016). Climatic suitability of Portuguese grapevine varieties and climate change adaptation. *International Journal of Climatology,* 36:1-12.

Fraga, H., García de Cortázar Atauri, I., Malheiro, A. C., Moutinho-Pereira, J., Santos, J. A. (2017). Viticulture in Portugal: A review of recent trends and climate change projections. *OENO One,* 51:61-69.

Fuleki, T., Ricardo-Da-Silva, J. M. (2003). Effects of cultivar and processing method on the contents of catechins and procyanidins in grape juice. *Journal of Agricultural and Food Chemistry,* 51:640-646.

Hernández, M. M., Song, S., Menéndez, C. M. (2017). Influence of genetic and vintage factors in flavan-3-ol composition of grape seeds of a segregating *Vitis vinifera* population. *Journal of the Science of Food and Agriculture*, 97:236-243.

Lachman, J., Šulc, M., Hejtmánková, A., Pivec, V., Orsák, M. (2004). Content of polyphenolic antioxidants and *trans*-resveratrol in grapes of different varieties of grapevine (*Vitis vinifera* L.). *Horticultural Science,* 31:63-69.

Liang, Z., Wu, B., Fan, P., Yang, C., Duan, W., Zheng, X., Liu, C., Li, S. (2008). Anthocyanin composition and content in grape berry skin in *Vitis* germplasm. *Food Chemistry,* 111:837-844.

Li, F. X., Li, F. H., Yang, Y. X., Yin, R., Ming, J. (2019). Comparison of phenolic profiles and antioxidant activities in skins and pulps of eleven grape cultivars (*Vitis vinifera* L.). *Journal of Integrative Agriculture*, 18:1148-1158.

Li, Z., Pan, Q., Cui, X., Duan, C. (2010). Optimization on anthocyanins extraction from wine grape skins using orthogonal test design. *Food Science and Biotechnolog*y, 19:1047-1053.

Liang, N. N., He, F., Bi, H. Q., Duan, C. Q., Reeves, M. J., Wang, J. (2012). Evolution of flavonols in berry skins of different grape cultivars during ripening and a comparison of two vintages. *European Food Research and Technology,* 235:1187-1197.

Lorrain, B., Kleopatra, C., Teissedre, P. L. (2011). Phenolic composition of Merlot and Cabernet-Sauvignon grapes from Bordeaux vineyard for the 2009-vintage: comparison to 2006, 2007 and 2008 vintages. *Food Chemistry,* 126:1991-1999.

Garrido, J., Borges, F. (2013). Wine and grape polyphenols - A chemical perspective. *Food Research International,* 54:1844-1858.

Geana, E. I., Iordache, A. M., Ionete, R. E. (2011). Assessing the wine anthocyanin profile for red grape varieties identification. *Progress of Cryogenics and Isotopes Separation,* 14:127-133.

Genebra, T., Santos, R. R., Francisco, R., Pinto-Marijuan, M., Brossa, R., Serra, A. T., Duarte, C. M. M., Chaves, M. M., Zarrouk, O. (2014). Proanthocyanidin accumulation and biosynthesis are modulated by the irrigation regime in Tempranillo seed. *International Journal of Molecular Sciences,* 15:11862-11877.

Ghiselli, A., Nardini, M., Baldi, A., Scaccini, C. (1998). Antioxidant activity of different phenolic fractions separed from an Italian red wine. *Journal of Agricultural and Food Chemistry,* 46:361-367.

Goetz, G., Fkyerat, A., Métais, N., Kunz, M., Tabacchi, R., Pezet, R., Pont, V. (1999). Resistance factors to grey mould in grape berries: identification of some phenolics inhibitors of *Botrytis cinerea* stilbene oxidase. *Phytochemistry,* 52:759-767.

González-San José, M. L, Barron, L. J. R., Diez, C. (1990). Evolution of anthocyanins during maturation of tempranillo grape variety (*Vitis vinífera*) using polynomial regression models. *Journal of the Science of Food and Agriculture,* 51:337-343.

Gryglewski, R. J., Korbut, R., Robak, J., Swies, J., (1987). On the mechanism of antithrombotic action of flavonoids. *Biochemical Pharmacology,* 36:317-322.

Guidoni, S., Allara, P., Schubert, A. (2002). Effect of cluster thinning on berry skin anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. *American Journal of Enology and Viticulture,* 53:224-226.

Hanlin, R. L., Kelm, M. A., Wilkinson, K. L., Downey, M. O. (2011). Detailed characterization of proanthocyanidins in skin, seeds, and wine of Shiraz and Cabernet Sauvignon wine grapes (*Vitis vinifera*). *Journal of Agricultural and Food Chemistry, 59*:13265-13276.

He, F., Mu, L., Yan, G. L., Liang, N. N., Pan, Q. H., Wang, J., Reeves, M. J. Duan, C. Q. (2010). Biosynthesis of anthocyanins and their regulation in colored grapes. *Molecules,* 15:9057-9091.

Hernández, M. M., Song, S., Menéndez, C. M. (2017). Influence of genetic and vintage factors in flavan-3-ol composition of grape seeds of a segregating *Vitis vinifera* population. *Journal of the Science of Food and Agriculture*, 97:236-243.

Huang, Z., Wang, B., Williams, P., Pace, R. D. (2009). Identification of anthocyanins in muscadine grapes with HPLC-ESI-MS. *LWT- Food Science and Technology,* 42:819-824.

Jackson, R. S. *Wine science principle and applications*, 3rd ed.; Academic Press: Burlington, VT, USA, 2008.

Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W. W., Fong, H. H. S., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., Moon, R. C., Pezzuto, J. M. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*, 275:218-220.

Jeandet, P., Bessis, R., Gautheron, B. (1991). The Production of resveratrol (3,5,4'-trihydroxystilbene) by grape berries in different developmental stages. *American Journal of Enology and Viticulture,* 42:41-46.

Jin, Z. M., He, J. J., Bi, H. Q., Cui, X. Y., Duan, C. Q. (2009). Phenolic compound profiles in berry skins from nine red wine grape cultivars in northwest China. *Molecules,* 14:4922-4935,

Jordão, A. M., Ricardo-da-Silva, J. M., Laureano, O. (1998a). Evolution of anthocyanins during grape maturation of two varieties (*Vitis vinifera* L.): Castelão Francês and Touriga Francesa. *Vitis,* 37:93-94.

Jordão, A. M., Ricardo-da-Silva, J. M., Laureano, O. (1998b). Influência da rega na composição fenólica das uvas tintas da casta Touriga Francesa (*Vitis vinifera* L.). *Journal of Food,* 2:60-73.

Jordão, A. M., Ricardo-da-Silva, J. M., Laureano, O. (2001a). Evolution of catechin and procyanidin composition during grape maturation of two varieties (*Vitis vinifera* L.) Castelão Francês and Touriga Francesa. *American Journal of Enology and Viticulture,* 52:230-234.

Jordão, A. M., Ricardo-da-Silva, J. M., Laureano, O. (2001b). Evolution of proanthocyanidins in bunch stems during berry development (*Vitis vinifera* L.). *Vitis,* 40:17-22.

Jordão, A. M., Correia, A. C. (2012). Relationship between antioxidant capacity, proanthocyanidin and anthocyanin content during grape maturation of Touriga Nacional and Tinta Roriz grape varieties. *South African Journal of Enology and Viticulture,* 33:214-224.

Jordão, A. M., Correia, A. C., Gonçalves, F. J. (2012). Evolution of antioxidant capacity in seeds and skins during grape maturation and their association with proanthocyanidin and anthocyanin content. *Vitis,* 51:137-139.

Junquera, P., Lissarrague, J. R., Jiménez, L., Linares, R., Baeza, P. (2012). Long-term effects of different irrigation strategies on yield components, vine vigour, and grape composition in *cv*. Cabernet-Sauvignon (*Vitis vinifera* L.). *Irrigation Science,* 30:351-361.

Kallithraka, S., Arvanytoiannis, I., El-Zajouli, A., Kefalas, P. (2001). The application of an improved method for trans-resveratrol to determine the origin of Greek red wines. *Food Chemistry,* 75:355-363.

Kallithraka, S., Tsoutsouras, E., Tzourou, E., Lanaridis, P. (2006). Principal phenolic compounds in Greek red wines. *Food Chemistry,* 99:784-793.

Kammerer, D., Claus, A., Carle, R., Schieber, A. (2004). Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. *Journal of Agricultural and Food Chemistry,* 52:4360-4367.

Kennedy, J. A. (2008). Grape and wine phenolics: observations and recent findings. *Ciencia e Investigación Agraria,* 35:107-120.

Kennedy, J. A., Hayasaka, Y., Vidal, S., Waters, E. J., Jones, G. P. (2001). Composition of grape skin proanthocyanidins at different stages of berry development. *Journal of Agricultural and Food Chemistry,* 49:5348-5355.

Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., Zamora, F. (2010). Comparison of methods for estimating phenolic maturity in grapes: correlation between predicted and obtained parameters. *Analytica Chimica Acta*, 660:127-133.

Kyraleou, M., Kotseridis, Y., Koundouras, S., Chira, K., Teissedre, P. L., Kallithraka, S. (2016). Effect of irrigation regime on perceived astringency and proanthocyanidin composition of skins and seeds of *Vitis vinifera* L. cv. Syrah grapes under semiarid conditions. *Food Chemistry*, 203:292-300.

Lu, Y., Foo, L. Y. (1999). The polyphenol constituents of grape pomace. *Food Chemistry,* 65:1-8.

Makris, D. P., Kallithrakab, S., Kefalasa, K. (2006). Flavonols in grapes, grape products and wines: Burden, profile and influential parameters. *Journal of Food Composition and Analysis,* 19:396-404.

Mateus, N., Proença, S., Ribeiro, P., Machado, J. M., Freitas, V. De (2001). Grape and wine polyphenolic composition of red *vitis vinifera* varieties concerning vineyard altitude. *Ciencia y Tecnología Alimentaria,* 3:102-110.

Mateus, N., Machado, J. M., De Freitas, V. (2002). Development changes of anthocyanins in *Vitis vinifera* grapes grown in the Douro Valley and concentration in respective wines. *Journal of the Science of Food and Agriculture*, 82:1689-1695.

Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., Ewert, B. (1999). Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *Journal of Agricultural and Food Chemistry,* 47:4009-4017.

Monagas, M., Gómez-Cordovés, C., Bartolomé B., Laureano, O., Ricardo-da-Silva, J. M. (2003). Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *Journal of Agricultural and Food Chemistry,* 51:6475-6481.

Montealegre, R. R., Peces, R. R., Vozmediano, J. L. C., Gascueña, J. M., Romero, E. G. (2006). Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. *Journal of Food Composition and Analysis,* 19:687-693.

Mori, K., Goto-Yamamoto, N., Kitayama, M., Hashizume K. (2007). Loss of anthocyanins in red-wine grape under high temperature. *Journal of Experimental Botany,* 58:1935-1945.

Morlat, R., Bodin, F. (2006). Characterization of viticultural terroirs using a simple field model based on soil depth – II. Validation of the grape yield and berry quality in the Anjou vineyard (France). *Plant and Soil,* 281:55-69.

Nicoletti, I., Bello, C., De Rossi, A., Corradini, D., (2008). Identification and quantification of phenolic compounds in grapes by HPLC-PDA-ESI-MS on a semi micro separation scale. *Journal of Agricultural and Food Chemistry,* 56:8801-8808.

Novaka, I., Janeiro, P., Seruga, M., Oliveira-Bret, A. M. (2008). Ultrasound extracted flavonoids from four varieties of Portuguese red grape skins determined by reverse-phase high-performance liquid chromatography with electrochemical detection. *Analytica Chimica Acta,* 630:107-115.

Núñez, V., Monagas, M., Gomez-Cordovés, M. C., Bartolomé, B. (2004). *Vitis vinifera* L. cv. Graciano grapes characterized by its anthocyanin profile. *Postharvest Biology and Technology,* 31:69-79.

Obreque-Slier, E., Peña-Neira A., López-Solís, R., Zamora-Marín, F., Ricardo-da-Silva, J. M., Laureano, O. (2010). Comparative study of the phenolic composition of seeds and skins from Carménère and Cabernet Sauvignon grape varieties (*Vitis vinifera* L.) during ripening. *Journal of Agricultural and Food Chemistry,* 58:3591-3599.

Ó-Marques, J., Reguinga, R., Laureano, O., Ricardo-da-Silva, J. M. (2005). Changes in grape seed, skin and pulp condensed tannins during berry ripening: effect of fruit pruning. *Ciência e Técnica Vitivinícola,* 20:35-52.

Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A., Deloire, A. (2002). Influence of pre- and post-veraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *American Journal of Enology and Viticulture,* 53:261-267.

Oliveira, J. B., Faria, D. L., Duarte, D. F., Egipto, R., Laureano, O., Castro, R., Pereira, G. E., Ricardo-da-Silva, J. M. (2018). Effect of the harvest season on phenolic composition and oenological parameters of grapes and wines CV. Touriga Naconal (*Vitis vinifera* L.) produced under tropical semi-arid climate, semi-arid climate, in the state of Pernanbuco, Brazil. *Ciência e Técnica Vitivinícola,* 33:145-166.

Ollé, D., Guiraud, J. L., Souquet, J. M., Terrier, N., Ageorges, A., Cheynier, V., Verries C. (2011). Effect of pre- and post-veraison water deficit on proanthocyanidin and anthocyanin accumulation during Shiraz berry development. *Australian Journal of Grape and Wine Research,* 17:90-100.

Pastrana-Bonilla, E., Akoh, C. C., Sellappan, S., Krewer, G. (2003). Phenolic content and antioxidant capacity of muscadine grapes. *Journal of Agricultural and Food Chemistry,* 51:5497-5503.

Peña-Neira, A., Hernández, T., García-Valejjo, C., Suarez, J. A. (2000). A survey of phenolic compounds in Spanish wines of different geographical origin. *European Food Research and Technology,* 210:445-448.

Peña-Neira, A., Cáceres, A., Pastenes, C. (2007). Low molecular weight phenolic and anthocyanin composition of grape skins from cv. Syrah (*Vitis vinifera* L.) in the Maipo Valley (Chile): Effect of clusters thinning and vineyard yield. *Food Science and Technology International,* 13:153-158.

Perestrelo, R., Ying, L, Santos, A. O. S., Silvestre, A. J. D., Neto, C. P., Câmara, J. S., Rocha, S. M. (2012). Phenolic profile of Sercial and Tinta Negra *Vitis vinifera* L. grape skins by HPLC–DAD–ESI-MS^n: Novel phenolic compounds in *Vitis vinifera* L. grape. *Food Chemistry,* 135:94-104.

Portu, J., López-Alfaro, I., Gómez-Alonso, S., López, R., Garde-Cerdán, T. (2015). Changes on grape phenolic composition induced by grapevine foliar applications of phenylalanine and urea. *Food Chemistry,* 180:171-180.

Prieur, C., Rigaud, J., Cheynier, V., Moutounet, M. (1994). Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry,* 3:781-784.

Rababah, T. M., Ereifej K. I., Al-Mahasneh, M. A., Ismaeal, K., Hidar, A.-G., Yang, W. (2008). Total phenolics, antioxidant activities, and anthocyanins of different grape seed cultivars grown in Jordan. *International Journal of Food Properties,* 11:472-479.

Revilla, E., García-Beneytez, E., Cabello, F., Martín-Ortega, G., Ryan, J. M. A. (2001). Value of high-performance liquid chromatographic analysis of anthocyanins in the differentiation of red grape cultivars and red wines made from them. *Journal of Chromatography A,* 915:53-60.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., Lonvaud, A. (2005). *Trattato di Enologia: Microbiologia del vino e Vinificazioni* [*Treatise on Enology: Microbiology of Wine and Vinification*], 2nd ed.; Edagricole: Bologna, Italy.

Ricardo-da-Silva, J. M., Belchior, A. P., Spranger, M. I., Bourzeix, M. (1992). Oligomeric procyanidins of three grapevine varieties and wines from Portugal. *Sciences des Aliments,* 12:223-237.

Roggero, J. P., Coen, S., Ragonnet, B. (1986). High performance liquid chromatography survey on changes in pigment content in ripening grapes of Syrah. An approach to anthocyanins metabolism. *American Journal of Enology and Viticulture*, 37:77-83.

Roggero, J. P. (2000). Study of the ultraviolet irradiation of resveratrol and wine. *Journal of Food Composition and Analysis,* 13:93-97.

Romero-Pérez, A. I., Ibern-Gómez, M., Lamuela-Raventós, R. M., Torre-Boronat M. C. (1999). Piceid, the major resveratrol derivative in grape juices. *Journal of Agricultural and Food Chemistry,* 47:1533-1536.

Romeyer, F. M., Sapis, J. C., Macheix, J. J. (1985). Hydroxycinnamic esters and browning potential in mature berries of some grape varieties. *Journal of the Science of Food and Agriculture,* 36:728-732.

Sakkiadi, A. V., Haroutounian, S. A., Stavrakakis, M. N. (2001). Direct HPLC assay of five biologically interesting phenolic antioxidants in varietal Greek red wines. *Lebensmittel-Wissenschaft und -Technologie,* 34:410-413.

Santos, J. A., Grätsch, S. D., Karremann, M. K., Jones, G. V., Pinto, J. G. (2013). Ensemble projections for wine production in the Douro Valley of Portugal. *Climatic Change,* 117:211-225.

Santos-Buelga, C., Scalbert, A. (2000). Proanthocyanidins and tannin-like compounds - nature, occurrence, dietary intake and effects on nutrition and health. *Journal of the Science of Food and Agriculture,* 80:1094-1117.

Sautter, C. K., Denardin, S., Alves, A. O., Mallmann, C. A., Penna, N. G., Hecktheuer, L. H. (2005). Determinação de resveratrol em sucos de uva no Brasil [Determination of resveratrol in grape juices in Brazil]. *Ciência e Tecnologia de Alimentos,* 25:437-442.

Serafini, M., Laranjinha, J. A. N., Almeida, L. M., Maiani, G. (2000). Inhibition of human LDL lipid peroxidation by phenol-rich beverages and their impact on plasma total antioxidant in humans. *Journal of Nutritional Biochemistry,* 11:585-590.

Silva, L. R., Queiroz, M. (2016). Bioactive compounds of red grapes from Dão region (Portugal): Evaluation of phenolic and organic profile. *Pacific Journal of Tropical Biomedicine,* 6:315-321.

Sommer, S., Cohen, S. D. (2018). Comparison of different extraction methods to predict anthocyanin concentration and color characteristics of red wines. *Fermentation,* 4:39.

Song, C. Z., Wang, C., Xie, S., Zhang, Z. bW. (2018). Effects of leaf removal and cluster thinning on berry quality of *Vitis vinifera* cultivars in the region of Weibei dry land in China. *Journal of Integrative Agriculture,* 17:1620-1630.

Soubeyrand, E., Basteau, C., Hilbert, G., Van Leeuwen, C., Delrot, S., Gomès, E. (2014). Nitrogen supply affects anthocyanin biosynthetic and regulatory genes in grapevine cv. Cabernet-Sauvignon berries. *Phytochemistry,* 103:38-49.

Souquet, J. M., Labarbe, B., Le Guernevé, C., Cheynier, V., Moutounet, M. (2000). Phenolic composition of grape stems. *Journal of Agricultural and Food Chemistry,* 48:1076-1080.

Sousa, T. (2020). *Degree report of food science and technology*. Federal University of Santa Catarina, Brazil, pp: 45.

Squadrito, M., Corona, O., Ansaldi, G., Di Stefano, R. (2010). Evolution of anthocyanin profile from grape to wine. *OENO One,* 44:167-177.

Sun, B., Pinto, T., Leandro, M. C., Ricardo-da-Silva, J. M., Spranger, M. I. (1999). Transfer of catechins and proanthocyanidins from solid parts of the grape cluster into wine. *American Journal of Enology and Viticulture,* 50:179-184.

Sun, B. S., Ricardo-da-Silva, J. M., Spranger, M. I. (2001). Quantification of catechins and proanthocyanidins in several Portuguese grapevine varieties and red wines. *Ciência e Técnica Vitivinícola,* 16:23-34.

Sun, B., Ribes, A. M., Conceição, M. L., Belchior, A. P., Spranger, M. I. (2006). Stilbenes: quantitative extraction from grape skins, contribution of grape solids to wine and variation during wine maturation. *Analytica Chimica Acta,* 563:382-390.

Susaj, E., Susaj, L., Voci, F., Brahimi, F., Hodaj, B., Paçe, H., Subashi, Y., Ferraj, B. (2013). Effect of cluster thinning on productivity indicators and wine quality of the indigenous red wine grapevine cultivar ‘Kallmet’ in Mjeda, Shkodër, in north-western part of Albania. *Journal of Food, Agriculture and Environment,* 11:1009-1014.

Tapiero, H., Tew, K. D., Nguyen, B. A. G., Mathe, G. (2002). Polyphenols: do they play a role in prevention of human pathologies? *Biomedicine & Pharmacotherapy,* 56:200-207.

Veloso, M. M., Almandanim, M. C., Baleiras-Couto, M., Pereira, H. S., Carneiro, L. C., Fevereiro, P., Eiras-Dias, J. (2010). Microsatellite database of grapevine (*Vitis vinifera* L.) cultivars used for wine production in Portugal. *Ciência e Técnica Vitivinícola,* 25:53-61.

Vitrac, X., Bornet, A., Vanderlinde, R., Valls, J., Richard, T., Delaunay, J. C., Mérillon, J. M., Teissédre, P. L. (2005). Determination of stilbenes (delta-viniferin, *trans*-astringin, *trans*-piceid, *cis*- and *trans*-resveratrol, epsilon-viniferin) in Brazilian wines. *Journal of Agricultural and Food Chemistry,* 53:5664-5669.

Xia, E., Deng, G., Guo, Y., Li, H. (2010). Biological activities of polyphenols from grapes. *International Journal of Molecular Sciences,* 11:622-646.

Xing, R. R., He, F., Xiao, H. L., Duan, C. Q., Pan, Q. H. (2015). Accumulation pattern of flavonoids in Cabernet-Sauvignon grapes grown in a low-latitude and high-altitude region. *South African Journal of Enology and Viticulture,* 36:32-43.

Wang, R., Sun, Q., Chang, Q. (2015). Soil types effect on grape and wine composition in Helan mountain area of ningxia. *PLoS ONE,* 10:e0116690.

Wang, Y., He, Y. N., Chen, W. K., He, F., Chen, W., Cai, X. D., Duan, C. Q., Wang, J. (2018). Effects of cluster thinning on vine photosynthesis, berry ripeness and flavonoid composition of Cabernet Sauvignon. *Food Chemistry,* 248:101-110.

Wu, X., Prior, R. L. (2005). Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: Fruits and berries. *Journal of Agricultural and Food Chemistry,* 53:2589-2599.

Yokotsuka, K., Nagao, A., Nakazawa, K., Sato, M. (1999). Changes in anthocyanins in berry skins of Merlot and Cabernet Sauvignon grapes grown in two soils modified with limestone or oyster shell versus a native soil over two years. *American Journal of Enology and Viticulture,* 50:1-12.

Yu, R., Cook, M. G., Yacco, R. S., Watrelot, A. A., Gambetta, G., Kennedy, J. A., Kurtural, S. K. (2016). Effects of leaf removal and applied water on flavonoid accumulation in grapevine (*Vitis vinifera* L. cv. Merlot) berry in a hot climate. *Journal of Agricultural and Food Chemistry,* 64:8118-8127.

Zhang, K., Yuan, L., Li, Q., Wang, R., Zhang, Z.-Z. (2019). Comparison of the anthocyanins composition of five wine-making grape cultivars cultivated in the Wujiaqu area of Xinjiang, China. *OENO One,* 53:549-559.

In: Vitis: Biology and Species
Editors: António M. Jordão et al.

ISBN: 978-1-53618-308-5

Chapter 13

MODERN BIODYNAMIC VITICULTURE, GEOGRAPHIC INFORMATION SYSTEMS AND GRAPES WASTE RECOVERY TECHNOLOGIES

Pooja Nikhanj[*]
Punjab Horticultural Postharvest Technology Centre,
Punjab Agricultural University, Ludhiana, India

ABSTRACT

Winemakers across the globe are integrating modern technology to enhance traditional wine making. Modern wine making technology paves the way for the most wonderful wines of the future. From the cultivation and upkeep of vines, to harvesting and processing, right through to the bottling and storage of wine, there is still a great deal of manual work involved in viticulture and wine production today. In order to remain competitive today, wine producers need to optimize and automate their production line. The reasoning behind the introduction of new technology in viticulture and wine production remain the same today as they have always been namely, to help processes run more smoothly and safely, as well as saving on manpower and time, while incurring fewer costs if possible. Modern technology allows winegrowers to utilize a sophisticated strategy like biodynamic farming to yield better quality grapes with increased productivity. Biodynamic farming uses organic methods, but is more big-picture focused, treating the land and the farm's micro-climates as living things that need to be nurtured. Geographic Information Systems can provide incredibly useful information related to the cultivation of grapes to create a 3D map to identify the differences of the land that is used to grow grapes. In conclusion, many advancement technologies are reforming the wine making industry positively. However, approaches like usage of GIS systems, converting grape waste into fuel, using biodynamic systems etc. are highly appreciable aspects in terms of modern wine making that can add to viticulture to produce quality wines.

---

[*] Corresponding Author's Email: poojanikhanj@pau.edu.

Keywords: biodynamic viticulture, biofuel, geographic information systems

1. INTRODUCTION

The shift toward sustainable production of the food and wine sector is not a recent event. Sirieix and Remaud stated that since the early 1990s concerns about environmental and sustainable practices and consumption acquired increasing popularity. The reasons for this are many, as follows: the growing public concern over climate change; an even more "green" consumer; the increasing competition on the market and the consequent industries' necessity of differentiation strategy and positive corporate image and reputations. The attention toward environmentally friendly practices and green products is increasing in the worldwide consumers' choices (organic- and/or biodynamic-labeled food or showing claims on sustainability or other bio-sounding expressions and characteristics). Environmental values also represent important drivers for winegrowers and industry, because they could represent a credential to offset an environmental impact of wine chain (i.e., high food miles or a large carbon footprint caused by long transports). This is even more important if we consider that viticulture and wine industry have a strong connection with environment and a mutual influence is widely recognized not only in the phase of cultivation but also during the vinification stages.

A recent survey on consumer's perceptions describes the Italian wine market of the future as addressed toward organic-labeled, carbon-free, vegan or other environmentally friendly products. These characteristics appear to be common in the evolution of wine consumption in the Old World countries as on the New World wine markets (i.e., the USA, Chile, Australia, New Zealand and South Africa) even if the consumers' sensibility and interests are different. The biodynamic movement has strong connections with sustainability approach and is sometimes considered as an extreme evolution of organic agriculture. Organic and biodynamic techniques are strictly linked but with an important difference: organic viticulture is regulated by an official set of rules (i.e., in EU Council Regulation EC No 834/2007 and EC Regulation No 203/2012) while biodynamic regulation is still founded on a "voluntary" basis, without any public intervention.

It is worth remembering that for a long period only grapes could be called organic and wine could only be labeled as "derived from organic grapes", and this happened until 2012. Currently, on the market, the presence of several competing "green" categories with different logos and claims (natural, organic, biodynamic, sustainable wine, etc.) has created confusion among the consumers who are not well informed about these product specifications or properties; this fact has increased the consumers' uncertainty and affected their choices. A market analysis of the supply side dimensions and behavior and about the demand attributes (a large part of the surveyed literature is focused on the biodynamic wine consumers) represents the main part of the work together with a review on the association between biodynamic wine chain and some environmental issues.

Finally, some short considerations about the potentiality and needs of the biodynamic wine sector are made. It should be noted that the literature about biodynamic viticulture and winemaking is not abundant; often biodynamic is considered as a branch of sustainable approach to agriculture, and the literature mainly focuses on organic or green practices (including biodynamic but not specifically on it). Scopus classification of these documents by subject area is interesting because it shows that beyond agriculture, biodynamic viticulture and wine represent fields of work for several different disciplines such as engineering, business and management, chemistry, medicine, arts and humanities.

2. BIODYNAMIC FARMING

Biodynamic farming is one example of a 'natural farming' system. Common to all 'natural farming' systems (including organics) is the recognition that the farm is a part of the greater environment, and a focus on whole-farm ecology and soil health. Synthetic fertilizers, herbicides and pesticides are not allowed in an effort to minimize disruption to natural biological systems. The motivations for adoption of these systems vary, but a common belief is that there is potential for improving product quality by farming that uses these principles. This belief has driven the significant level of interest in Biodynamic (BD) viticulture in Australia and overseas over the last 10-15 years.

The most important objective is to highlight that although there are many observed benefits to utilizing 'natural farming' techniques, they could be more widely investigated by mainstream researchers and growers. There would be merit in a research program which seeks to understand the soil and plant science which underpin the benefits of BD. In such a way, opportunities for incorporating the best elements of BD, organic and so-called 'conventional' systems into combined production systems could potentially be identified.

A significant limitation to better understanding what is really happening in these systems is a language barrier that sometimes exists between 'natural farming' practitioners and 'conventional' growers and researchers. With a clear objective to extract the highest quality and most individual expression of fruit from each vineyard parcel, the Paxton viticulture team considered that conventional avenues for improvement had been exhausted. The shift to BD farming was sparked by the potential to further improve wine quality. A growing number of producers around the world farm using BD and are recognized for the high quality of their wines (Jacometti et al., 2000; Bekkers, 2012). A commonly held belief amongst practitioners is that fruit from BD vineyards better highlight the subtle differences of site and vintage. Many producers report an improvement in the texture and flavour profile of their wines (Reeve et al., 2005). It was considered that these observations should be investigated and BD practice commenced on all Paxton company vineyards in 2005.

Two things distinguish it from other forms of organic farming:

- The use of a complex system of herbal sprays and composting techniques, known as ‘preparations’;
- The timing of the operations on the land, which is strictly regulated by the movements of the spheres.

Biodynamics has made some high profile converts in recent years and is taken seriously by the wine industry purely on the evidence of the wines it produces.

2.1. Biodynamics - The Basics

Described and advocated by Rudolph Steiner in Europe during the 1920s, BD is an organic system of farming avoiding synthetic fertilizers and pesticides (Carpenter et al., 2000). It focuses on building fertile soils through the application of natural compost preparations. It is this link to Steiner and the anthroposophical movement that is the first level of discomfort that many have with the BD system. Steiner report was a clearly defined farming method that incorporated the key tenets of pre-industrial European farming; these being manuring, composting and respect for the soil (Carpenter et al., 2000; Reeve et al., 2005). Fundamentalist BD seems an unusual collection of rituals in today’s world, but such standardization ensures a common method and allows better comparison of results on different properties. Perhaps the Standard Operating Procedure we use today is not such a modern concept? Many of the objectives and techniques of BD are shared with organic agriculture, including the elimination of chemical fertilizers, herbicides and pesticides.

Moreover, it differs in using the application of nine soil and plant treatments or preparations in an effort to stimulate the soil and improve plant resistance and efficiency and also product quality (Bekkers, 2012). From a scientific viewpoint, whether these preparations actually augment soil or wine grape quality is unclear and controversial (Carpenter et al., 2000; Reeve et al., 2005). The preparations consist of mineral, plant or animal manure extracts, usually fermented and applied in small proportions to compost, manures, the soil, or directly onto plants, after dilution. The original biodynamic (BD) preparations are numbered 500-508. The BD 500 preparation (horn-manure) is made from cow manure (fermented in a cow horn that is buried in the soil for six months through autumn and winter) and is used as a soil spray, said to stimulate root growth and humus formation. The BD 501 preparation (horn-silica) is made from powdered quartz (packed inside a cow horn and buried in the soil for six months through spring and summer) and applied as a foliar spray in an effort to stimulate and regulate growth. In this respect the desirable outcomes of tougher leaves, more rigid growth and earlier lignification and tannin development are sought. The next six preparations, BD 502-507, are plants and are used in making compost. Finally, there is BD preparation 508 which is prepared from the silica-rich horsetail plant (*Equisetum arvense*) and used as a foliar spray and which is believed to suppress fungal diseases in plants. Table 1 shows several examples of different biodynamic spray programs for viticulture.

Table 1. Suggested biodynamic spray program for viticulture

Activity	Type of biodynamic preparation	Rate Per Ha	Stirring	Application	Times of application
Soil preparation prior to planting vines	Apply Combined soil preparation to green manure crops in area planned for vineyard	245g	1 hour flow forms or stirring machine	Soil spray droplets 35 L per ha	Moon descending or moon opposition Saturn afternoon
Apply basalt rock dust and lime or soil amendments as required and deep rip rows to plant vines in	Spreader	1-2 tons	Soil preparation and thereafter via compost		
Soil spray apply to soil in rows between vines	Combined soil spray-containing Horn Manure concentrate - Winter Horn Clay 508 – fermented8 x potency	245g	1 hour flow forms or stirring machine	Soil spray droplets 35 L per ha	Moon descending or Moon opposition Saturn afternoon
Next atmospheric spray	Horn silica 501 Summer Horn Clay 508 – fresh 8 x potency	2g $10g^{-1}$ vial per tank	1 hour flow forms or stirring machine	Air spray mist	Sunrise day after soil spray
Atmospheric Sprays	Horn silica 501 Summer horn Clay 508 – fresh 8 x potency	2g $10g^{-1}$ vial per tank	1 hour flow forms	Air spray mist	Moon opposition saturn each month sunrise or sunset
Apply monthly during growth period - spring to harvest	Fish or seaweed concentrate	2 –4 L/ha	20 minutes flow forms	Foliar spray a.m. or afternoon	Monthly 2-4 days up to full moon
Compost: apply 2 x per year – spring and autumn	Make up using the biodynamic compost preparations 502-508	1-2 shovelfuls per vine or 4-5 tons per Ha	Spread in inter-rows in autumn or under vines and then mulch in spring		
Biodynamic pasting	Biodynamic paste	20kg pail Enough for 100 vines	Mix till sloppy and either paint on or spray thinner solution	Cover trunk and over pruning cuts	At pruning (descending moon) or over winter

A more comprehensive summary of their preparation and expected outcomes can be found in Waldin (2004). Finally, in observations, the BD farm is seen as a whole entity, rather than a series of problems to be solved individually. There tends to be a great focus on soil health in an effort to encourage a natural interaction between the plant and soil. BD is more than just avoiding chemicals. It is about treating the farm as an integrated system including biofertilizers, vermicomposting and integrated pest management. Perceived benefits and motivations Practitioners of BD hold different motivations for adopting the practice, however, the most commonly cited expected outcomes from BD wine producers include improved grape and wine quality and improved whole-farm health. Various interactions in the soil as well as with plants during viticulture can help in the improvement of the wine grapes quality including high protein, sugar, reducing sugars, phenolics and antioxidants.

Improved Grape and Wine Quality

Improved fruit and wine quality is a contentious and well-defined target to say the least. A common opinion, however, is that BD, by providing a more natural interaction between the vine and the soil offers quality benefits. In particular, the notion that BD wines better capture the individual character of the parcel from which they were grown. That is, they enhance the effect of *terroir*. Commonly this is seen to be expressed as ‘brighter and more vibrant fruit’ and wines that are ‘highly textured’ or hold desirable tannin profiles (Waldin, 2004). It must be pointed out that there is little empirical evidence to support these observations, although it would not be difficult to test (Ross et al., 2009) and the anecdotal evidence is strong enough to warrant investigation.

Improved Whole-Farm Health

Possibly the most commonly reported physical effect of the BD method is an improvement in soil structure and health. The improvement of organic matter content, the elimination of chemical inputs and the aim to provide conditions favorable to soil biology are well recognized soil conservation measures (White, 2003). For some, the BD method is an extension of a greater lifestyle choice, often including a quest for self-sufficiency. Others place great importance on the elimination of chemical inputs from the view point of human health. Environmentalism and sustainability are other common motivators. Marketing is often cited as a motivation, but there is little evidence for BD resulting in better sales of product. Motivations can change over time and many practitioners find that closer observance of the farm ecosystem stimulates a greater appreciation for environmental issues.

Irrigation

Biodynamic soils retain water in the humus which is created using biodynamic practices. The biodynamic plants also develop extensive rooting systems which can access deeper water sources. Biodynamic viticulturists have found that they use 50-75% less water than neighbors. Dry land viticulture works for many biodynamic viticulturists, depending on location and personal choice (Crisp et al., 2000; Ross et al., 2009).

2.2. Fungal Disease - Prevention in Biodynamic System

Fungal disease happens when a number of climatic and plant health stresses coincide. Plant health is improved immeasurably by using the biodynamic preparations and sprays. Through keeping the sugar saps up and the sap pH at 6.4 the plant does not attract insects. Bruce Tainio, microbiologist and agricultural consultant in Washington, USA, has found that if the sap pH is higher than 6.4 the plant is susceptible to insect attack. A low, more acidic sap pH, will also see disease ensue. Fungal diseases occur also when the moisture and temperature are high. Biodynamic viticulturists watch the weather and also use the Antipodean Astro Calendar to alert them to potential climatic problems ahead. For instance, when there is a Full Moon and Moon Perigee, (which is when the Moon is closest to the earth) they know and can prepare for increased risk of fungal activity (Crisp et al., 2000). Under these circumstances the Earth is over-endowed with watery forces, and by regularly using the compost preparations and the horn silica sprays and equisetum (BD508) or Casuarina spray (which balances watery forces) it is possible to strengthen the plant against this possibility of fungal disease. The horn silica also keeps the sugar sap levels up in times of stress, so the plant is strong enough to withstand the adverse conditions.

Ideally the whole Biodynamic treatments have been applied to the vineyard earlier in the season, such as:

- Composting of vines in autumn;
- Tree pasting of the vine trunks after pruning;
- Application of the Soil Sprays - Horn Manure 500, Manure Concentrate, Winter Horn Clay and fermented 508 late spring, mid winter and monthly since August;
- Application of the Atmospheric Sprays -Horn Silica 501, Equisetum 508 (fresh) or fresh Casuarina tea and Summer Horn Clay, prior to leaf shoot;
- Seaweed Tea applications every 2nd week with added fresh Casuarina Tea as the weather is humid.

3. GEOGRAPHIC INFORMATION SYSTEMS

Geographical information system (GIS) is a technology that provides the means to collect and manage large volumes of geo-coded spatial data derived from a variety of sources. The capabilities of GIS are to accept input data allowing to store, retrieve, manipulate, analyze, overlay, and display these data based on the requirements of the user and to create both tabular and cartographic output which reflect these requirements. In a more generic sense, GIS is a software tool that allows users to create interactive queries, analyze the spatial information, edit data, maps, and present the results of all these operations. Geographic data contains not only the attribute being reported but also the spatial location of the attribute. Each measured variable from a vineyard that is geo referenced becomes a unique data layer that can be overlaid and visually and mathematically correlated or interpreted. This approach allows data to be input as separate themes (e.g., soil moisture, soil fertility, temperature, humidity, crop yield) and overlaid based on analysis requirements. The GIS software makes it possible to synthesize large amounts of different data, combining different layers of information to manage and retrieve the data in a more useful manner (An et al., 2003; Blauth and Ducati, 2010).

3.1. Components of Geographical Information System

Geographical information system (GIS) enables the user to input, manage, manipulate, analyze, and display geographically referenced data using a computerized system. To perform various operations with GIS, the components of GIS such as software, hardware, data, people and method.

Software

GIS software provides the functions and tools needed to store, analyze, and display geographic information. Key software components are: (1) a database management system (DBMS); (2) tools for the input and manipulation of geographic information; (3) tools that support geographic query, analysis, and visualization; and (4) a graphical user interface (GUI) for easy access to tools. There are many different types of GIS software available with the most common being ArcGIS and MapInfo.

Hardware

Hardware is the computer on which a GIS operates. Today, GIS runs on a wide range of hardware types, from centralized computer servers to desktop computers used in stand-alone or networked configurations.

Data

The most important component of a GIS is the data. Geographic data or spatial data and related tabular data can be collected in-house or bought from a commercial data provider. Spatial data can be in the form of a map/ remotely-sensed data such as satellite imagery and unmanned aerial vehicle photography. These data forms must be properly geo referenced (latitude/longitude) (An et al., 2003; Bramley et al., 2011).

3.2. Functions of Geographical Information System

General-purpose geographical information system software performs six major tasks such as input, manipulation, management, query, analysis, and data output.

Input

The important input data for any GIS is digitized maps, images, spatial data, and tabular data. The tabular data is generally typed on a computer using relational database management system software. Before geographic data can be used in a GIS, it must be converted into a suitable digital format. The DBMS system can generate various objects such as index generation on data items, to speed up the information retrieval by a query.

Manipulation

Geographical information system can store, maintain, distribute and update spatial data associated text data. The spatial data must be referenced to a geographic coordinate system (latitude/longitude). The tabular data associated with spatial data can be manipulated with help of data base management software.

Management

For small GIS projects it may be sufficient to store geographic information as computer files. However, when data volumes become large and the number of users of the data becomes more than a few, it is advised to use a DBMS to help store, organize, and manage data. A DBMS is a database management software package to manage the integrated collection of database objects such as tables, indexes, query, and other procedures in a database (Hall and Jones, 2010; Johnson, 2012).

Query

The stored information either spatial data or associated tabular data can be retrieved with the help of Structured Query Language (SQL). Depending on the type of user interface, data can be queried using the SQL or a menu driven system can be used to retrieve map data. For example, you can begin to ask questions such as analysis and data output: *Analysis* - Geographical information system (GIS) really comes into their own when they are used to analyze geographic data. The processes of geographic analysis often called spatial analysis or geo-processing uses the geographic properties of features to look for patterns and trends, and to undertake “what if” scenarios. *Data Output* - Geographical information system (GIS) can provide hard copy maps, statistical summaries, modeling solutions and graphical display of maps for both spatial and tabular data. For many types of geographic operations, the end result is best visualized as a map or graph (Massot et al., 2008; Hall and Jones, 2010; Hoff et al., 2017).

4. CONVERTING GRAPE WASTE INTO FUEL

The winemaking industry has been majorly positively portrayed, due to the socioeconomic and cultural benefits attributed to it (Arvanitoyannis et al., 2006; Ioannou et al., 2014). Regardless of the vast amounts of waste generated, the great use of water resources and the exhaustive land usage, the industry has not been viewed negatively by the general public. This, in turn, has encouraged its development and consequent generation of higher amounts of waste. Waste can be seen as a virtually inexhaustible resource, being utilized in industrial markets to generate combined heat and power (CHP) and fertilizers, in the affluent developed world. Within the coming decade, these markets will develop further, as well as shifting into recovering chemicals and generating energy, synthetic materials, feeds and food from the waste, in an effort to reduce the carbon footprint of their production, as a result of legislative, environmental, economic and social drivers (Arvanitoyannis et al., 2006). Utilizing natural resources will place limitations on manufacturing, but will also achieve environmental sustainability and will constitute non-solid waste safe for environmental discharge, in the form off particle, nutrient free and sterile effluents. Therefore, the utilization of waste as a valuable commodity and platform chemicals "mine" is an important step for the development and deployment of alternative sources of energy production.

Conventional treatment of waste is becoming increasingly expensive, demanding significant amounts of effort, resources and energy for safe waste discharge into the environment (Ioannou et al., 2014). Tightening legislations regarding waste disposal call for alternative solutions to methods such as landfilling, land spreading or disposal in water streams such as rivers. In the current knowledge-driven economy that aims for low carbon use, and with the growing awareness of environmental protection - due to climate change and natural resources exhaustion-, the need to recycle, reuse and recover energy and valuable chemicals from waste and wastewater becomes apparent.

4.1. The Biorefinery Idea

Using agricultural goods for the production of other products is barely a novelty. However, the use of plant biomass as a raw material for the production of numerous products using complex physicochemical processing methods, a concept similar to petroleum refinery, is a rather new idea, first initiated in the 1980's. This approach though successful to an extent has several drawbacks. Plant based biomass is a rich source of lignin, carbohydrates, proteins and fats, also containing in smaller amounts vitamins, dyes and flavors (Arvanitoyannis et al., 2006; Ioannou et al., 2014). Its utilization as bioconversion substrate requires extensive, often costly, pre-treatment in order to be processed successfully by the microorganisms. It has to be intensively cultivated and grown to produce considerable amounts of fuels, chemicals and power. This leads to land competition for crops development, potential shortage of feedstock, environmental constraints, due to excessive use of fertilizers, human food and export market, as well as possible water shortage (Fernando et al., 2006).

Therefore, in recent years there is a shift from the whole crop concept, where an entire crop of wheat, rye, barley, corn or triticale is used as feedstock, to the waste based concept mainly in lignocellulose feedstock, where hard fibrous plant materials generated from agricultural or forestry activities are used (Li et al., 2011). This approach, albeit beneficial, has been hard to apply due to the extensive demand in pre-treatment (enzymatic hydrolysis or chemical digestion) for the production of cellulosic and hemicellulose material (Tyagi and Lo, 2013). Moreover, several researchers (Zacharof and Lovitt, 2014) have highlighted the importance of recycling waste, municipal, agricultural, domestic, and industrial, through bioconversion, i.e., applying a biorefinery concept, but with waste as the main feedstock. This approach has been voiced by numerous governmental and non-governmental bodies and most importantly by the European Union which has called for the increase of the recycling and preparing for re-use of municipal waste to 70% by 2030, and has stipulated phasing out landfilling recyclable waste (including plastics, paper, metals, glass and bio-waste) in non-hazardous waste landfills, reducing landfilling to a maximum of 25% by 2025.

Waste, depending on its origin, contains various high-value chemical substances and elements, including carbon sources in the form of carboxylic and other acids, carbohydrates, proteins, nitrogen (N) as ammonia, phosphorus (P) and metals. The use of recovered materials from waste would be highly beneficial for the environment and the economy. For example; phosphate rock is a non-renewable natural resource, of critical importance because of its numerous applications including drinking water softening, feed and food additives, and fertilizers. Although its production is carbon neutral, mining P is gradually becoming more expensive and supply risks, related to environmental and socio-political issues, have risen. It has been reported that by 2035 the demand for P will outpace the supply as the finite resource becomes increasingly expensive (800% rise between 2006 ($50) and 2008 ($400), current value of over $500/tonne) On the other hand, P removal from wastewater has to improve as water discharge standards become more stringent, raising the costs of wastewater treatment. Substantial value also exists in the high content of metal ions in numerous agricultural and industrial wastes (Tyagi and Lo, 2013; Zacharof et al., 2016).

Ammonia, another resource, has a market value of $800/ton and its global consumption exceeds 150 million tons. As well as being used heavily in fertilizers, it is also an important component of various commercial and industrial products. These include fuels, antimicrobial agents, woodworking agents and cleaners. It has a large production carbon footprint (best practice being 2.2 tons of CO_2 per ton of ammonia), as during its synthesis methane is reformed to produce H_2 and CO_2. In addition, the disposal and return of ammonia to the atmosphere through nitrification and denitrification adds additional costs to wastewater treatment (Tyagi and Lo, 2013).

Therefore, reclaiming these valuable chemicals into formulated feedstock suitable for biochemical conversion to industrially relevant products, is a crucial step in improving sustainability and reducing environmental impact. Multiple benefits lie in this approach including: recycled materials will substitute newly synthesized or mined materials; the reduction in the volume and concentration of waste will reduce demand and costs in waste treatment plants and methane emissions in the landfills; recovery of ferrous and non-ferrous metals from the waste streams for recycling is more energy efficient than mining for virgin resources; electricity obtained by methane generation through anaerobic digestion offsets electricity generated from fossil fuels; valuable streams, such as formulated of nutrient streams, are created for application in agriculture and bioprocessing.

5. GRAPE WINERY WASTE: A SUSTAINABLE SUPPLY OF RESOURCES AND ENERGY

In the context of a current high energy demand economy, with growing awareness of environmental protection and the strengthening of water resource and wastewater related legislation; the need to recover and produce energy and chemicals from wastes becomes apparent (Zacharof et al., 2016). The continuously rising human population results in rising demand for food, energy and water. This growing global urbanization coupled with elevated environmental awareness, expressed by various steep legislative frameworks over waste disposal as well as public pressure, are pushing private and public waste treatment providers to review and reengineer their waste management strategies.

The development of novel, cost-effective waste management methodologies is of great interest to various groups such as contractors, engineering consultants, equipment providers, policy regulators (agencies, politicians, and think tanks), and the general public and depends on the needs of the community in a microscale but also on the general good in a macroscale. Probably not all waste types are suitable to use as biorefinery feedstock, since several complications due to their complex physicochemical nature might occur. Implications relevant to transportation or the need of extensive costly pre-treatment might hinder the use, for instance, of construction waste. Construction waste may include lignocellulosic material but due to its heavily mixed nature and current ways of collection is unsuitable for such an approach.

Waste generated by the beverage, food, feed, and agricultural industry is certainly the best candidate for the biorefinery approach, satisfying criteria such as size, continuity of supply and nutritive content. Beverage and food production have become heavily industrialized and therefore regulated generating tons of waste per annum. The food industry is shifting towards the intensive production of ready to eat foods (RTE) that are consumed in venues that have fewer conventional methods of stabilizing food, therefore resulting in even larger amounts of waste since 1990's. In addition to the directly occurring waste due to food processing (slaughterhouse, dairy, wheat and corn milling, confectionary, sugar and starch processing, vegetative processing etc.), the food industry is linked to agricultural waste produced by intensive animal and crop farming to satisfy food demand, reaching a 264,854 tons per annum in United Kingdom alone. Agricultural waste is third in terms of waste industry size, comparable only to municipal solid waste and it imposes environmental threats, since conventional treatments, such as landfilling or land spreading, may cause eutrophication and land and water toxicity, due to freely available nutrients and metals spread in water and soil. There are also human health concerns due to land related pathogenicity contained in the raw materials.

Industrial wastewaters from food processing industries, wineries, breweries and agricultural wastewater from animal confinements are ideal candidates for biotechnological production of high value substances and platform chemicals (Angenent et al., 2004; Zacharof and Lovitt, 2014), however their effective formulation remains a desideratum. These effluents, if used as nutrient media, are potentially highly profitable, especially when compared to the traditional synthetic media or that derived from food sources such as crops. For example, the cost per kilo of Man de Rogosa broth, a well-known nutrient medium used in research and development of starter cultures used in dairy industry can reach $1311 per kilo, while a formulated waste deriving nutritive effluent can cost as little as $2.4 per kilo of nutrients (acids, ammonia, phosphate) recovered (Tyagi and Lo, 2013).

Previous research has shown the strong potential of discharged waste effluents to be used as feedstock for the production of various biobased chemicals. Consequently, waste can represent an ideal feedstock, since the main focus of a biorefinery is to produce low-value, high-volume (LVHV) products to meet the global energy demand simultaneously with the production of high-value, low-volume (HVLV) products that enhance profitability, while the production of Combined heat and power (CHP) can be used to reduce the costs of processing procedures.

5.1. Grape Winery Waste

Winery waste can be divided into two main categories, solid and liquid waste. Solid waste is generated during the collection of grapes and liquid waste is generated during the wine making process. Solid winery waste, namely grape stalks, grape pomace and grape seeds, varies in chemical composition and texture. In terms of percentage it is composed of up to 7.5% grape stalks, up to 45% grape pomace, up to 6% grape seeds and various other waste sources. Grape stalks are the major by-product of vineyards with an average production of 5 tons per hectare per year. They are rich is lignin, cellulose, N and potassium (K), having a high agronomic value and are used for composting. Grape stalks have been found to be highly effective for soils, as they have low organic matter content. Grape pomace contains up to 15% sugars, 0.9% pigments and phenols, especially in the case of red grape pomace, up to 1% tartrate acid and up to 40% fiber. Grape pomace is being used as a feed additive due to its high fiber content. Grape seeds are very rich in linoleic acid and omega-6 fatty acids, with up to 17 and up to 6% phenols.

Winery waste, however, is not limited to waste generated at the first stages of grape harvesting and initial stages of wine formulation. Waste known as lees, composed of solid and liquid fractions, is generated during the fermentation and maturation stages. The solid part is comprised of the remains precipitated at the bottom of the tanks, mainly consisting of bacterial biomass, undissolved carbohydrates of hemi- or cellulosic nature, phenolic compounds, lignin, proteins, metals, inorganic salts, organic acid salts (mainly tartrates, in the case of wine lees) and other materials such as pips (tannins sustaining grape seeds), fruit skins, grains and seeds. The liquid phase is represented mainly by the spent fermentation broth, often rich in organic acids and ethanol.

Vinasses, a by-product of the wine lees, are defined as liquid fraction waste deriving from the distillation process of the wine lees, which is carried out to recover ethanol and elaborate distilled beverages. A vast amount of waste, in the form of wastewater is generated during the further stages of processing, including fermentation (vessels pre- and post washing), storage and maturation (pre- and post-washing of storage tanks, pre- and post washing of fermentation vessels, spillages), clarification (wastewater generated from filtration) decanting and bottling (spillages and cleaning of vessels and bottles). Cleaning is not only done with water (cold or lukewarm) but also with solvents, detergents and chemical agents, such as sodium hydroxide. Each wine production step generates a varying amount of wastewater, with qualitative characteristics relevant to the process stage. Both the solid and the liquid winery waste can be used successfully as feedstock for the production of high value chemicals either in a format of conventional biorefinery (lees, vinasses, marc) or as green (leafs and pomace) or a lignocellulosic (LCF) (stalks, peels, seeds, trimming vine shots, pips and pomace) biorefinery, where the effluent winery waste can be used as bioconversion feedstock. In the case of winery wastewater, the high content is organic matter expressed by the Chemical Oxygen Demand (COD).

5.2. Utilization of Grape Winery Wastewater as Substrate

Winery wastewater, i.e., the post cleaning operation (crushing, pressing etc.) wastewater, has not been widely used as biotechnological conversion feedstock. Limited studies have been conducted, with winery wastewater being used as substrate for *Gluconaceto bacterxylinus* for the production of cellulose at a 6.26 g L^{-1}. Other studies

(Angenent et al., 2004) involve the use of fungi, *Trichoderma viride, Aspergillus niger* and *Aspergillus oryzae* for the production of SCP at a 5 g L^{-1} and a simultaneous reduction of COD to 90%. The vast majority of waste distilleries have been treated using traditional wastewater treatment processes, such as land spreading or anaerobic digestion, with the focus being the treatment on Biological Oxygen Demand (BOD) and COD, rather than the production of energy or platform chemicals. Winery waste can be successfully used as feedstock in the biorefinery concept. The seasonal availability of the waste, however, demands judicious handling and treatment to achieve economic feasibility and efficiency (Angenent et al., 2004; Zacharof and Lovitt, 2014). Further research and practical experimentation is necessary since, in the case of winery waste, limited studies have been conducted and life cycle analysis regarding full economic costing of the use wine waste as a resource is needed. The currently available results on the biotechnological use of winery waste are a promising alternative to the current treatment techniques that are focusing on the waste remediation and treatment, rather than resource recovery.

CONCLUSION

It is concluded that the geospatial technologies are important in the development of viticulture, by supporting environmental diagnostics associated with the vineyard and strategic applications in agricultural management. The analytical tools and the various sensors can quickly provide accessible data to technicians and farmers, which can be viewed by customers of the production system and these technologies, can be transferred to the end user. The technology transfer can be made through free GIS software and the results may be available, either in the form of digital products or graphics through web mapping, digital download and database accessible. Regardless of the researcher's intent with viticulture, spatial variability within and between vineyards always serves as the research problem, while geospatial techniques provide the interface whereby this problem can be addressed. In the case of site location and site suitability analyses, the variability stems from the differing physical site characteristics that accommodate or restrict grapevine growth. GIS allows all of the desirable characteristics of vineyard areas to be overlaid, therefore properly evaluating terroir and predicting the best possible sites for future vineyards.

In locating vineyards with aerial or satellite imagery, the variability of the vineyard surface, vine rows of grapevine leaves and inter row spaces of bare earth or grass, creates a number of issues. Remote sensing techniques allow for the automation of this process through such creative methodologies as those presented, to inventory vineyard blocks quickly and inexpensively compared to field based methods. In the same regard, precision viticulture looks to address variability in the grape crop itself within and between specific vineyard blocks. GPS, GIS, and remote sensing, all help to study this vineyard variability.

These tools and techniques provide the spatial wherewithal to properly make management decisions that are in turn employed in-the-field to minimize expenditures while maximizing revenues. Indeed, the future looks bright for viticultural practitioners who embrace geospatial tools and techniques into their current workflows.

REFERENCES

An, K., Xie, G., Leng, Y., Xiao, Y. (2003). Design of farmland GIS for precision agriculture. *Chinese Geographical Science*, 13:20-24.

Angenent, L.T., Karim, K., Muthanna, H.A.D., Wrenn, B.A., Espinosa-Domiguez, R. (2004). Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends Biotechnol*ogy, 22:477-485.

Arvanitoyannis, I.S., Ladas, D., Mavromatis, A. (2006). Wine waste treatment methodology. *International Journal of Food Science and Technol*ogy, 41:1117-1151.

Bekkers, T. (2012). *Biodynamics in viticulture. proceedings fourteenth Australian wine industry technical conference.*

Blauth, D.A., Ducati, J.R. (2010). A web-based system for vineyards management, relating inventory data, vectors and images. *Computers and Electronics in Agriculture*, 71:182-188.

Bramley, R.G.V., Trought, M.C.T., Praat, J.P. (2011). Vineyard variability in Marlborough, New Zealand: characterizing variation in vineyard performance and options for the implementation of precision viticulture. *Australian Journal of Grape and Wine Research*, 17:72-78.

Carpenter-Boggs, L., Kennedy, A.C., Reganold, J.P. (2000). Organic and biodynamic management: effects on soil biology. *Soil Science Society of America Journal*, 64:1651-1659.

Crisp, P., Scott, E.A., Wicks, T.B. (2000). University of Adelaide, Sustainable control of grapevine powdery mildew, *Unicinulanecator*, pp- 180-186.

Fernando, S., Adhikari, S., Chandrapal, C., Murali, N. (2006). Biorefineries: current status, challenges, and future direction. *Energy Fuels*, 20:1727-1737.

Hall, A., Jones, G.V. (2010). Spatial analysis of climate in wine grape-growing regions in Australia. *Australian Journal of Grape and Wine Research*, 16:389-404.

Hoff, B.R., Ducati, J.R., Farias, A.R. (2017). GIS and remote sensing to support precision viticulture for analysis of vineyards in the campanha wine region, Brazil Rosemary. *Journal of Environmental and Agricultural Sciences*, 10:20-32.

Ioannou, L.A., Li Puma, G., Fatta-Kassinos, D. (2014). Treatment of winery wastewater by physicochemical, biological and advanced processes: a review. *Journal of Hazardous Materials*, 286:343-368.

Jacometti, M.A., Wratten, S.D., Walter, M. (2010). Review: alternatives to synthetic fungicides for *Botrytis cinerea* management in vineyards. *Australian Journal of Grape and Wine Research,* 16:154-172.

Johnson, L.F. (2012). Remote sensing for viticultural research and production. In: Dougherty, P.H. (ed.) *The geography of wine: regions, terroir, and techniques.* Netherlands: Springer Science, pp. 209–226.

Li, W.W., Yu, H.Q. (2011). From wastewater to bioenergy and biochemicals via two-stage bioconversion processes: a future paradigm. *Biotechnology Advances,* 29:972-982.

Massot, A., Peuchot, C., Mietton- Peuchot, M., Milisic, V. (2008). Nanofiltraion and reverse osmosis in wine making. *Desalination,* 231:283-289.

Reeve, J.R., Carpenter-Boggs, L., Reganold, J.P., York, A.L., McGourty, G., McCloskey, L.P. (2005). Soil and wine grape quality in biodynamically and organically managed vineyards. *American Journal of Enology and Viticulture,* 56(4):367-376.

Ross, C.F., Weller, K.M., Blue, R.B., Reganold, J.P. (2009). Difference testing of Merlot produced from biodynamically and organically grown winegrapes. *Journal of Wine Re*search 20(2):85-94.

Tyagi, V.K., Lo, S.L. (2013). Sludge: a waste or renewable source for energy and resources recovery? *Renew Sustain Energy Rev*iew, 25:708-728.

Waldin, M. (2004). *Biodynamic Wines*. London, Mitchell Beazley: 513.

White, R.E. (2003). *Soils for Fine Wines.* New York, Oxford University Press: 279.

Zacharof, M.P., Lovitt, R.W. (2014). The filtration characteristics of anaerobic digester effluents employing cross flow ceramic membrane microfiltration for nutrient recovery. *Desalination*, 341:27-37.

Zacharof, M.P., Mandale, S.J., Williams, P.M., Lovitt, R.W. (2016). Nanofiltration of treated digested agricultural wastewater for recovery of carboxylic acids. *Journal of Cleaner Production,* 112:4749-4761.

ABOUT THE EDITORS

António Manuel Santos Tomás Jordão is currently Associate Professor of Oenology at Polytechnic Institute of Viseu - School of Agriculture and member of the Chemistry Research Centre - Food and Wine Lab, Vila Real, Portugal. He is graduated in Agro-Industrial Engineering and has a Master in Food Science and Technology from Technical University of Lisbon. In 2006, obtained a PhD in Agro-Industrial Engineering at Agronomy Higher Institute of Technical University of Lisbon. In recent years, he held several academic positions (Vice-President of the School of Agriculture, Vice-President of Scientific Council and Director of Food Industries Department). Coordinator of Viticulture and Oenology Technical Course from Polytechnic Institute of Viseu. Member of several research projects in Oenology area and scientific editor of four books of Oenology and one book about Phenolic Compounds. He is author/co author of 45 articles in peer reviewer scientific journals, 15 book chapters and 97 communications in scientific congress. Member of editorial board of scientific journals. Supervisor of several Master Thesis in Oenology, Food Science and Food Technology. Develops consultancy services to wine companies and research projects evaluation for Portuguese National Innovation Agency. The main research interests are: grape and wine phenolic composition; wine technology (particularly the use of wood in wine production/aging).

Renato Vasconcelos Botelho is Associate Professor of Viticulture and Fruit Crops at the State University of Midwest of Paraná (UNICENTRO), in Guarapuava, Paraná State, Brazil, since 2003. He is Graduated in Agronomy from the Faculty of Agronomic Sciences – UNESP, in Botucatu, São Paulo State, Brazil, Master of Science and PhD in Agronomy (Horticulture) from the same institution. Developed Postdoctoral projects in the Fruit Crops Section of the Agronomic Institute of Campinas, São Paulo State, Brazil and in the Agrarian and Veterinary School of the University of Bologna, Italy. He published 110 scientific papers and he is reviewer of numerous international journals and advises several Research Funding Agencies. Develops research in the area of viticulture and fruit crops in organic system, acting mainly on the following themes: training systems, management, alternative control of vine and fruit trees diseases, dormancy release, fertilizing, post-harvest and plant physiology.

INDEX

This book is a fascinating journey into the viticultural world and biodiversity, jealously guarded by man starting from the Mediterranean, in the oceans, islands and continents. The authors narrate the symbiosis of man and *Vitis,* the most generous genus, in which rural and scientific knowledge are admirably reflected and fruitful technological innovations ripen. The reading invites, with a look at the past, an appreciation of the inestimable values preserved in the wine-growing landscapes and, at the same time, look with confidence to the future, inspired by the search for harmony between man and nature.

Professor Dr. Adamo Domenico Rombolà
Professor of Viticulture
University of Bologna, Italy

The book "*Vitis: Biology and Species*" provides an interesting and comprehensive overview of viticulture in different producing regions of the world, including different species and new technologies applied to characterize them. Addressing scientific questions for experts and professionals, but in a clear and accessible way, this book will also help technicians, producers and the general public to find answers to several aspects involving the modern cultivation of vines.

Professor Dr. Regina Vanderlinde
Institute of Biotechnology - University of Caxias do Sul, RS, Brazil
President of O.I.V. - International Organisation of Vine and Wine

nova
science publishers
www.novapublishers.com
ISBN 978-1-53618-308-5
9 781536 183085