Phenotyping the susceptibility od Croatian native grapevine (Vitis vinifera L.) varieties to the causal agent of downy mildew (Plasmopara viticola)

Štambuk, Petra

University of Zagreb

FACULTY OF AGRICULTURE

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Supervisors:
Professor Jasminka Karoglan Kontić, PhD
Senior Scientific Associate Ivana Tomaz, PhD

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Mentorice:

prof. dr. sc. Jasminka Karoglan Kontić

dr. sc. Ivana Tomaz, viša znanstvena suradnica

Zagreb, 2023.

Bibliography data:

Scientific area: Biotechnical sciences
Scientific field: Agriculture
Branch of science: Viticulture and Enology
Institution: University of Zagreb Faculty of Agriculture, Department of Viticulture and Enology
Supervisors of the PhD thesis: Prof. Jasminka Karoglan Kontić, PhD and Ivana Tomaz, PhD
Number of pages: 145
Number of figures: 0
Number of tables: 0
Number of appendices: 3
Number of references: 139
Date of oral examination:
The members of the PhD defence committee:
1. Assoc. Prof. Darko Preiner, PhD, University of Zagreb Faculty of Agriculture
2. Assoc. Prof. Darko Vončina, PhD, University of Zagreb Faculty of Agriculture
3. Prof. Danijela Ašperger, PhD, University of Zagreb Faculty of Chemical Engineering and Technology

Rad je pohranjen u:
Nacionalnoj i sveučilišnoj knjižnici u Zagrebu, Ulica Hrvatske bratske zajednice 4 p.p. 550, 10 000 Zagreb,
Knjižnici Sveučilišta u Zagrebu Agronomskog Fakulteta, Svetošimunska cesta 25, 10 000 Zagreb.
The doctoral thesis is stored in:
National and University Library in Zagreb, Ulica Hrvatske bratske zajednice 4 p.p. 550, 10 000 Zagreb,
Central Agricultural Library, University of Zagreb Faculty of Agriculture, Svetošimunska cesta 25, 10 000 Zagreb.

The subject of the thesis was accepted at the session of the Faculty Council of the University of Zagreb Faculty of Agriculture, held on July 6th, 2021, and approved at the session of the Senate of the University of Zagreb, held on November 16th, 2021.

UNIVERSITY OF ZAGREB

FACULTY OF AGRICULUTRE

DECLARATION OF ORIGINALITY

I, **Petra Štambuk**, declare that I have composed solely by myself the thesis titled:

PHENOTYPING THE SUSCEPTIBILITY OF CROATIAN NATIVE GRAPEVINE (*Vitis vinifera* L.) VARIETIES TO THE CAUSAL AGENT OF DOWNY MILDEW (*Plasmopara viticola*)

With my signature I confirm that:

- I am the sole author of this thesis;

- this thesis is an original report of my research, and due references have been provided on all supporting literatures and resources;

- I am familiar with the provisions of the Code of Ethics of the University of Zagreb (Article 19).

Zagreb, _____ 2023

_____

PhD Candidate signature

Doctoral thesis grade

Doctoral thesis was defended at the University of Zagreb Faculty of Agriculture on _____ 2023 in front of the PhD defence committee comprised of:

1. Assoc. Prof. Darko Preiner, PhD _____

University of Zagreb Faculty of Agriculture

2. Assoc. Prof. Darko Vončina, PhD _____

University of Zagreb Faculty of Agriculture

3. Prof. Danijela Ašperger, PhD _____

University of Zagreb Faculty of Chemical Engineering and Technology

Information about the supervisors

Professor Jasminka Karoglan Kontić, PhD, was born on December 4, 1963, in Zagreb, where she completed primary and secondary education. She graduated from the Faculty of Agriculture University of Zagreb (FAZ) in 1986. She has been employed at the Department of Viticulture and Enology of FAZ since 1987. In 2000, she was elected to the position of assistant professor, in 2004 to the position of associate professor, and in 2010 to the position of full professor.

She actively participated in the creation of new study programs according to the principles of the Bologna Declaration. She teaches courses “Viticulture I” and “Basics of Viticulture” at the undergraduate study, “Biology and Ecology of the Vine” and “Ecological Viticulture” at the graduate study, and “Ampeloecology” at the postgraduate doctoral study in Agricultural Sciences. She mentors a substantial number of graduation theses, in addition to supervising two PhD theses.

Since 2005, she has held the position of head of the graduate study in Horticulture. She was a Vice Dean for Academic Affairs at the FAZ from 2015 to 2018.

She has concentrated her research interest on three main areas: organic viticulture, genetic resources of grapevine, and the impact of climate changes on viticulture. She has been involved in numerous national and international scientific research and technology projects. She is the author of more than 50 referred journals articles, two university textbooks, and several monographs and professional books, two of which have won awards. She was a member of the editorial board of the scientific journal *Agriculturae Conspectus Scientificus* (ACS) and a member of the organizational or scientific committee of international conferences in the field of viticulture.

She participated in several working groups for the harmonization of regulations at the Ministry of Agriculture of the Republic of Croatia. She is a permanent representative of the Republic of Croatia to the International Organization of Vine and Wine (OIV) in Paris. She was the head of the Department of Viticulture and Enology, a member of the Faculty Council of the Faculty of Agriculture, the Council of the Biotechnical Area of the University of Zagreb, and the Senate of the University of Zagreb.

Ivana Tomaz, PhD, was born in Zagreb on November 12, 1980. At the Faculty of Science, University of Zagreb, she earned the title of Master of Chemical Engineering in 2010. She has worked as an expert associate at the Department of Viticulture and Enology at the Faculty of Agriculture, University of Zagreb since 2011. There, she has continuously been engaged in research on the composition and content of phenolics, volatile organic compounds, carotenoids, and other compound groups in grapes, leaves, and wine. Moreover, in her work, she develops and implements various chemical methods and techniques. In 2016, she finished her doctoral studies in Chemistry at the Faculty of Science, University of Zagreb. In 2016 and 2021, she was promoted to senior expert associate and expert advisor, respectively. In 2017, she became a scientific associate, whereas, in 2021, she was promoted to senior scientific associate.

Since her employment at the Faculty of Agriculture, she has participated in the following scientific projects: “In Vitro Propagation, Cryopreservation and Biological Activity Quantification of Berry Fruit Trees and Grapevine Fruits”, “Viticulture and Climate Change in Croatia”, and “New Start for Old Croatian Grapevine Varieties”. At the postgraduate doctoral studies in Agricultural Sciences, she teaches “Methods of Ampelographic Research” classes. She participated in the implementation of two expert projects as well as in one development project. She wrote two book chapters and contributed as an author or co-author to 35 scientific papers that are listed in the Current Contents database.

The research work in this dissertation was carried out within the project KK.01.1.1.01.0005 „Biodiversity and Molecular Plant Breeding“ led by Prof. Zlatko Šatović, PhD

CroP-BioDiv

ZNANSTVENI CENTAR IZVRSNOSTI ZA BIORAZNOLIKOST I MOLEKULARNO OPLEMENJIVANJE BILJA

CENTRE OF EXCELLENCE FOR BIODIVERSITY AND MOLECULAR PLANT BREEDING

Europska unija
Zajedno do fondova EU

Republika Hrvatska

Summary

Vitis species are shown to be susceptible, tolerant, or resistant to the *Plasmopara viticola* (Berk. & M. A. Curtis; Berl. & de Toni) pathogen. The obligate, biotrophic, and polycyclic *P. viticola* oomycete can infect any green organ of the host plant, including the shoots, leaves, inflorescences, tendrils, petioles, and green berries. It can therefore have a devastating effect on the host plant. Moreover, significant economic losses occur when effective control methods are not used when growing susceptible species and varieties.

The most widespread and commercially significant *Vitis* species in the world is the grapevine (*Vitis vinifera* L.). Although the majority of grapevine varieties are susceptible to downy mildew, there are some variations in susceptibility among them.

Croatia has a centuries-old heritage of grapevine cultivation. One hundred and twenty-five native varieties were developed and are still grown in Croatia's various geographical and climatic regions. It is therefore considered that in addition to variations in a wide range of biological and economic properties, they also differ in disease susceptibility.

Aiming to determine the differences in susceptibility of Croatian native varieties to downy mildew, phenotyping of selected varieties was carried out using the following methods: leaf disc bioassay, measurement of chlorophyll fluorescence and multispectral imaging, and analysis of polyphenolic and volatile organic compounds in grapevine leaves. Polyphenolic and volatile organic compounds were analysed in leaves before and 24, 48, and 96 hours after inoculation with *P. viticola* suspension. Measurement of chlorophyll fluorescence and multispectral imaging was performed before inoculation and during six terms after inoculation.

The leaf disc bioassay proved to be effective for classifying grapevine varieties into the OIV resistance classes to downy mildew. In addition, parameters of chlorophyll fluorescence, such as F_v/F_m, $F_{q'}/F_{m'}$ and ETR, were useful in distinguishing infected from non-infected leaf discs shortly after inoculation, that is, before the appearance of visible symptoms of the disease. Moreover, the $F_{q'}/F_{m'}$ and qP parameters helped distinguish between varieties with different susceptibility to *P. viticola*. The results of the polyphenolic compound analysis clearly distinguished the native varieties into the OIV resistance classes. Resistance was found to be dependent on the composition and content of polyphenolic compounds present in the leaves before inoculation. In all terms following the *P. viticola* inoculation, the stilbene resveratrol-3-*O*-glucoside content was higher in infected leaves compared to uninfected. Finally, volatile organic compound analysis can be used to distinguish infected from non-infected samples as well as resistant control genotypes (Solaris, *Vitis riparia* Michx.) from *V.*

vinifera varieties. Nevertheless, using this approach does not allow for the division of native varieties into the OIV resistance classes.

Less susceptible native grapevine varieties that belong to OIV class 5 (Malvazija istarska, Ranfol, and Teran) could be interesting to use in breeding programs aiming to produce high-quality genotypes tolerant to main fungal diseases.

Keywords: grapevine, downy mildew, leaf disc bioassay, chlorophyll fluorescence, multispectral indices, polyphenolic compounds, volatile organic compounds

Proširenisažetak

Vrste roda *Vitis* (loze) osjetljive su, tolerantne ili otporne na uzročnika plamenjače *Plasmopara viticola* (Berk. & M. A. Curtis; Berl. & de Toni). Vinova loza (*Vitis vinifera* L.) najuzgajanija je vrsta loza u svijetu iako je većina sorata vinove loze osjetljiva na plamenjaču. Ova bolest ima izrazito devastirajuće djelovanje kad se prilikom uzgoja ne primjenjuju kemijska sredstva za zaštitu bilja.

Oomiceta *P. viticola* je obligatni, biotrofni i policiklički patogen koji može inficirati sve zelene organe biljke domaćina, kao što su mladice, listovi, cvatovi, vitice, peteljke i zelene bobe. Optimalni uvjeti za rast i razvoj ovog patogena su visoka vlažnost i umjerena temperatura zraka. U takvim uvjetima patogen razvija nekoliko ciklusa vegetativnog razmnožavanja, uzrokujući značajan pad prinosa i kvalitete grožđa. Zbog toga je primjena fungicida neizbježan tehnološki postupak pri uzgoju osjetljivih sorata vinove loze, iako te agrokemikalije mogu štetno djelovati na okoliš. *Plasmopara viticola* podrijetlom je s američkog kontinenta pa su američke vrste (*Vitis labrusca* L., *Vitis riparia* Michx., *Vitis rupestris* Scheele i *Muscadinia rotundifolia* Small*)* koevolucijom na istom području razvile visoku ili potpunu otpornost. Donedavno se smatralo da je europska loza *V. vinifera* neotporna na plamenjaču i pepelnicu, odnosno da ne postoji značajna genetska varijabilnost u otpornosti unutar sorata vinove loze. Međutim, u novije su vrijeme kod nekih od njih, kao što su 'Kishmish vatkana', 'Dzhandzhal kara' i 'Mgaloblishvili', identificirani geni otpornosti, što ih čini vrijednim izvorom gena za oplemenjivačke programe s ciljem stvaranja visokokvalitetnih sorata otpornih na ekonomski najznačajnije bolesti.

Pretpostavlja se da, zbog višestoljetnog uzgoja vinove loze u Hrvatskoj i njezine prilagodbe specifičnim okolišnim uvjetima, među hrvatskim autohtonim sortama također postoje razlike u osjetljivosti na bolesti. S ciljem utvrđivanja osjetljivosti hrvatskih autohtonih sorata na plamenjaču, provedeno je istraživanje koje je obuhvatilo odabrane sorte te nekoliko otpornih i osjetljivih kontrolnih genotipova koristeći sljedeće metode fenotipizacije: metoda lisnih diskova, mjerenje fluorescencije klorofila i multispektralno snimanje lisnih diskova te analiza polifenolnih i hlapljivih organskih spojeva u listovima.

Metoda lisnih diskova provedena je u kontroliranim laboratorijskim uvjetima sukladno deskriptoru 452-1 Međunarodne organizacije za vinovu lozu i vino (engl. *International Organisation of Vine and Wine*, OIV). Na temelju toga, svaki je genotip svrstan u odgovarajući razred otpornosti pri čemu su genotipovi svrstani u razred 1 najosjetljiviji, a oni svrstani u razred 9 potpuno otporni na plamenjaču. Mjerenje fluorescencije klorofila i multispektralno snimanje provedeno je pomoću instrumenta CropReporter™ tijekom sedam

termina. Prvo mjerenje provedeno je na netretiranim lisnim diskovima, a završno kad su se razvili vidljivi simptomi bolesti (sporulacija).

Polifenolni spojevi analizirani su tekućinskom kromatografijom visoke djelotvornosti (engl. *high-performance liquid chromatography*, HPLC), a hlapljivi organski spojevi primjenom vezanog sustava plinski kromatograf-spektrometar masa (engl. *gas chromatography-mass spectrometry*, GC-MS). U tu svrhu, analizirani su listovi uzorkovani prije tretiranja te 24, 48 i 96 sati nakon inokulacije *P. viticola* suspenzijom. Lisni diskovi i listovi inokulirani su suspenzijom koncentracije 2×10^5 spora/mL raspršivanjem po naličju. Suspenzija je dobivena umakanjem listova sa svježe razvijenom sporulacijom u ultračistu vodu do zamućenja.

Utvrđeno je da je metoda lisnih diskova učinkovita za razvrstavanje sorata vinove loze u OIV razrede otpornosti na uzročnika plamenjače vinove loze. Parametri fluorescencije klorofila, odnosno F_v/F_m, $F_{q'}/F_{m'}$ i ETR, razlikovali su inficirane od neinficiranih lisnih diskova, a pomoću parametara $F_{q'}/F_{m'}$ i qP moguće je razlikovati slabije i jače osjetljive sorte ubrzo nakon zaraze, odnosno prije pojave vidljivih simptoma bolesti. Rezultati analize polifenolnih spojeva jasno su razdvojili autohtone sorte u razrede otpornosti pri čemu je utvrđeno da otpornost ovisi o sastavu i sadržaju polifenolnih spojeva u listovima prije inokulacije. Povećan sadržaj stilbena resveratrol-3-*O*-glukozida razlikovao je inficirane od neinficiranih listova tijekom svih termina nakon inokulacije. Sadržaj piceatanola i ukupnih stilbena razlikovao je potpuno otporan OIV razred 9 (*Vitis riparia* Michx.) od ostalih OIV razreda čime su potvrđena njihova snažna antimikrobna svojstva. Na temelju polifenolnih profila *V. vinifera* sorata, utvrđeno je da su uglavnom flavonol glikozidi odgovorni za manju osjetljivost sorata koje sadrže višu koncentraciju ovih spojeva. Analizom hlapljivih organskih spojeva moguće je razlikovati otporne kontrolne genotipove od *V. vinifera* sorata kao i inficirane od neinficiranih uzoraka. Iako ovom metodom nije moguće razdvojiti autohtone sorte u razrede otpornosti, rastući sadržaj nekoliko detektiranih hlapljivih spojeva nakon inokulacije zajedničko je svojstvo manje osjetljivih autohtonih sorata i otpornih kontrolnih genotipova ('Solaris', *Vitis riparia* Michx.).

Manje osjetljive autohtone sorte koje pripadaju OIV razredu 5 ('Malvazija istarska', 'Ranfol' i 'Teran') mogle bi biti zanimljive za korištenje u oplemenjivačkim programima kojima je cilj proizvesti visokokvalitetne sorte tolerantne na najznačajnije gljivične bolesti.

Ključne riječi: vinova loza, plamenjača, metoda lisnih diskova, fluorescencija klorofila, multispektralni indeksi, polifenolni spojevi, hlapljivi organski spojevi

Table of contents

List of abbreviations

APA - absolute peak area

ARI - anthocyanin reflection index

BCE - before current era

CHI - chlorophyll index

dpi - day(s) post-inoculation

ETR - electron transport rate

FarRed - far red reflectance

$\mathbf{F_{q'}/F_{m'}}$ - effective quantum yield of photosystem II (PSII) electron transport

$\mathbf{F_v/F_m}$ - maximum quantum yield of photosystem II (PSII) electron transport

GC-MS - gas chromatography-mass spectrometry

hpi - hour(s) post-inoculation

HPLC - high-performance liquid chromatography

Hue - colour appearance parameter

MAS - marker-assisted selection

NDVI - normalised difference vegetation index

NIR - near-infrared reflectance

NPQ - non-photochemical quenching

OIV - International Organisation of Vine and Wine

OIV resistance classes - 1, 3, 5, 7, and 9; class 1 is the most susceptible whereas class 9 is completely resistant to *Plasmopara viticola*

PCA - principal component analysis

PSII - photosystem II

Ren - resistance to *Erysiphe necator*

Rpv - resistance to *Plasmopara viticola*

Run - resistance to *Uncinula necator*

qP - photochemical quenching

$\mathbf{T_0 – T_6}$ - terms of leaf discs imaging and/or terms of leaves sampling for the analyses of secondary metabolites

List of appendices

Appendix 1. Scientific paper: Štambuk P., Šikuten I., Preiner D., Nimac A., Lazarević B., Marković Z., Maletić E., Karoglan Kontić J., Tomaz I. (2021). Screening of Croatian Native Grapevine Varieties for Susceptibility to *Plasmopara viticola* Using Leaf Disc Bioassay, Chlorophyll Fluorescence and Multispectral Imaging. Plants 10 (4): 661. doi: 10.3390/plants10040661

Appendix 2. Scientific paper: Štambuk P., Šikuten I., Karoglan Kontić J., Maletić E., Preiner D., Tomaz I. (2022). Leaf Polyphenolic Profile as a Determinant of Croatian Native Grapevine Varieties' Susceptibility to *Plasmopara viticola*. Frontiers in Plant Science 13: 836318. doi: 10.3389/fpls.2022.836318

Appendix 2A. Supplement material related to scientific paper Štambuk P., Šikuten I., Karoglan Kontić J., Maletić E., Preiner D., Tomaz I. (2022). Leaf Polyphenolic Profile as a Determinant of Croatian Native Grapevine Varieties' Susceptibility to *Plasmopara viticola*. Frontiers in Plant Science 13: 836318. doi: 10.3389/fpls.2022.836318

Appendix 3. Scientific paper: Štambuk P., Šikuten I., Preiner D., Maletić E., Karoglan Kontić J., Tomaz I. (2023). Croatian Native Grapevine Varieties' VOCs Responses upon *Plasmopara viticola* Inoculation. Plants 12(2): 404. doi: 10.3390/plants12020404

Appendix 3A. Supplement material related to scientific paper Štambuk P., Šikuten I., Preiner D., Maletić E., Karoglan Kontić J., Tomaz I. (2023). Croatian Native Grapevine Varieties' VOCs Responses upon *Plasmopara viticola* Inoculation. Plants 12(2): 404. doi: 10.3390/plants12020404

List of scientific papers

Published scientific papers				
Scientific paper	Database	Category	Quartile	Impact factor (IF)
1. Štambuk P., Šikuten I., Preiner D., Nimac A., Lazarević B., Marković Z., Maletić E., Karoglan Kontić J., Tomaz I. (2021). Screening of Croatian Native Grapevine Varieties for Susceptibility to *Plasmopara viticola* Using Leaf Disc Bioassay, Chlorophyll Fluorescence and Multispectral Imaging. Plants 10 (4): 661.	WoS	A_1	Q_1	4,658
2. Štambuk P., Šikuten I., Karoglan Kontić J., Maletić E., Preiner D., Tomaz I. (2022). Leaf Polyphenolic Profile as a Determinant of Croatian Native Grapevine Varieties' Susceptibility to *Plasmopara viticola*. Frontiers in Plant Science 13: 836318.	WoS	A_1	Q_1	6,627
3. Štambuk P., Šikuten I., Preiner D., Maletić E., Karoglan Kontić J., Tomaz I. (2023). Croatian Native Grapevine Varieties' VOCs Responses upon *Plasmopara viticola* Inoculation. Plants, 12(2): 404.	WoS	A_1	Q_1	4,658

Explanation of the connection between research hypotheses and published scientific papers	
Research hypothesis	Explanation of the connection between the hypothesis and the scientific paper
H1. Croatian native grapevine varieties are phenotypically different in susceptibility to downy mildew.	Leaf disc bioassay was used to determine differences in the level of sporulation on leaf discs of native and control genotypes inoculated with the downy mildew causal agent in the scientific paper number 1. As a result, the native varieties were classified into different resistance classes based on the International Organization of Vine and Wine 452-1 descriptor, which confirmed H1.
H2. Downy mildew infection differently affects the photosynthetic activity of varieties with different susceptibility levels.	The measurement of photosynthetic activity and multispectral imaging of leaf discs were described in the scientific paper number 1. Differences in certain parameters of photosynthetic activity, were determined between the downy mildew inoculated and non-inoculated samples, such as F_v/F_m, $F_{q'}/F_{m'}$ and ETR, as well as in varieties of different resistance classes, such as $F_{q'}/F_{m'}$ and qP, thus confirming H2.
H3. Differences between varieties in susceptibility to downy mildew are associated with differences in the composition and content of polyphenolic and volatile organic compounds.	The results of the principal component analysis in the scientific paper numbered 2 show the separation of the native varieties' resistance classes based on the constitutive composition and content of polyphenolic compounds in the leaves. Thus, it was determined that it is possible to classify native varieties into the corresponding resistance classes based on the constitutive composition and content of polyphenolic compounds in the leaves,

	as determined by the leaf disc bioassay described and carried out in the scientific paper number 1. Certain volatile compounds were distinguished between inoculated and non-inoculated samples in the scientific paper number 3. The specific reaction of volatile organic compounds in the leaves of native varieties of different resistance classes was not determined. The response of less susceptible native varieties and resistant control genotypes, on the other hand, was associated with an increase in the content of certain compounds after inoculation. Based on these studies, H3 is partially confirmed.

1. INTRODUCTION

Along with wheat, barley, and olive trees, the grapevine (*Vitis vinifera* L.) is one of the world's earliest domesticated plant species (Grassi and Arroyo-Garcia, 2020). In 2021, the global vineyard surface area covered 7.3 million ha, whereas world wine production was estimated at 260 million hl. In the same year, the combined production of wine in Italy, France, and Spain accounted for 47 % of the global total production (OIV, 2022).

Many economically important diseases and pests that affect the grapevine pose a constant risk of losing all or a part of the yield, making grapevine cultivation extremely challenging. Regular use of plant protection products is necessary, although it significantly increases production costs and represents a potential danger to the environment (Wilson and Tisdell, 2001; Buonassisi et al., 2017). The root-louse (phylloxera) (*Daktulosphaira vitifoliae* Fitch), which destroys the root system of the vine, alongside powdery mildew (*Erysiphe necator* Schw., syn. *Uncinula necator* Schw.; Burr.), grey mold (*Botrytis cinerea* Pers; Fr.), and downy mildew (*Plasmopara viticola* Berk. & M. A. Curtis; Berl. & de Toni), which attack the above-ground organs of the vine, are known as the most important pest and diseases of the grapevine. *Plasmopara viticola*, *Erysiphe necator* and *Daktulosphaira vitifoliae* were introduced from North America into Europe during the second half of the 19th century, resulting in massive destruction of vineyards and a demographic catastrophe in wine-producing nations. Since then, efforts have been made to find effective methods to control the aforementioned diseases and pests (Gessler et al., 2011).

Coevolving in the same geographic area, American vine species such as *Vitis cinerea* Engelm., *Vitis riparia* Michx., *Vitis labrusca* L., *Muscadinia rotundifolia* Small (previously *Vitis rotundifolia* Michx.) developed high or complete resistance to downy mildew, powdery mildew, and phylloxera. Consequently, soon after their introduction to the European continent, interspecies breeding began with the aim of obtaining offspring that would inherit the resistance of the American species, thus enhancing the quality of the European grapevine. The problem of phylloxera was solved relatively quickly by grafting noble vines on the rootstock of American species whose roots were resistant to phylloxera. Nevertheless, it took more than a century of persistent breeding work to produce varieties of satisfactory quality and high resistance to fungal diseases. Simultaneously with the breeding work, efforts were made to find effective means of controlling fungal diseases. The discovery of fungicidal effects of sulphur and copper was a watershed moment in viticulture, making

chemical protection of the grapevine an unavoidable technological procedure during its cultivation (Toepfer et al., 2011).

In 2020, the European Commission adopted the “Farm to Fork Strategy”, intending to transform European agricultural production into a more sustainable, environmentally, and human health-friendly system. The goal of this strategy is to reduce pesticide use by 50 %, fertiliser use by 20 %, and increase organic agricultural areas from 8 % to 25 % by 2030 (European Commission, 2020).

Among crops, the grapevine is one of the neediest when it comes to the amount of plant protection products used. In the European Union, 40 % of all pesticides and 70 % of all fungicides are applied to grapevines in agriculture, although only 3 % of arable land is used for vineyards (Eurostat, 2007).

Plant protection products allowed in organic farming are still insufficiently effective, therefore the use of traditional fungicides based on sulphur and copper is still permitted. However, sulphur has a negative effect both on human health and the ecosystem, while copper is a non-degradable heavy metal that accumulates in groundwater and soil. In toxic concentrations, copper causes plant stress and reduces soil fertility. Due to the aforementioned reasons, it is necessary to develop other control strategies, especially for downy and powdery mildew, one of which is the cultivation of varieties resistant to fungal diseases (Pedneault and Provost, 2016).

Environmental concern has increased the interest in the creation and breeding of resistant varieties. Breeding programmes were restarted in Italy and France in addition to Germany and Hungary, where they had been carried out even after the discovery of powerful effective fungicides that became the main method of disease control. Their aim is to create high-quality varieties that at the same time possess high or complete and permanent resistance to the most important fungal diseases. A good example of such a breeding program is the French ResDur (an acronym for Durable Resistance), whose goal is to create varieties with polygenic resistance by combining different resistance genes from different sources. (Schneider et al., 2019). New molecular-genetic methods, such as marker-assisted selection, enable faster development of varieties with desired characteristics (Merdinoglu et al., 2018).

Resistant grapevine varieties are often associated with undesirable ampelographic and oenological traits of the initial hybrids, whose genomes contained a high percentage of genes from American species. However, contemporary breeding programs implement numerous backcrosses with *V. vinifera* varieties to ensure that the offspring not only have resistance but also satisfactory quality. In recent years, wine-producing nations in Europe

and West Asia have carried out thorough characterizations of grapevine genetic resources. Contrary to popular belief, *V. vinifera* varieties also contain resistance genes, indicating that they are not entirely susceptible to downy and powdery mildew. Namely, in 2009, a gene for resistance to powdery mildew was identified in the Kishmish vatkana and Dzhandzhdal kara varieties (Coleman et al., 2009), while in 2020, three genes for resistance to downy mildew were identified in the Georgian Mgaloblishvili variety (Sargolzaei et al., 2020). These findings indicate that grapevine varieties, especially local, less explored ones, could be interesting for breeding and used as potential sources of genes for resistance. Phenotypic research should also include susceptibility screening for the most important diseases. Such research was carried out among Spanish and Georgian grapevine varieties, in which marked differences in susceptibility to downy mildew were defined. Finally, the aforementioned resistance genes were identified among Georgian varieties (Sargolzaei et al., 2021).

In addition to genotyping, phenotypic evaluation methods are still a very important and inevitable step in the selection of the parental cross-combination as well as in the selection of new varieties. Therefore, the International Organization of Vine and Wine (OIV) has prescribed three descriptors related to the evaluation of grapevine varieties in susceptibility to downy mildew. The 452 descriptor refers to the susceptibility of leaves in field conditions, the 452-1 descriptor serves when evaluating leaf discs in controlled laboratory conditions, while the 453 descriptor refers to the sensitivity of inflorescences in field conditions (OIV, 2009). Recently, the 453-1 descriptor was proposed to evaluate the susceptibility of inflorescences under controlled conditions (Buonassisi et al., 2018).

In addition to monitoring visible changes in the most susceptible organs of the grapevine, further sophisticated methods have been developed to monitor changes in the primary and secondary metabolism after the downy mildew pathogen inoculation, that is, before the development of visible symptoms of infection. They include the measurement of chlorophyll fluorescence and multispectral imaging which describe the level of stress of a plant or an individual organ by monitoring changes in photosynthetic activity (Cséfalvay et al., 2009), and the analysis of secondary metabolites, the most important of which are polyphenolic and volatile organic compounds (Chitarrini et al., 2017; Ricciardi et al., 2021).

The domestication of the grapevine, one of the traditional plant species in Croatia, dates back to the establishment of Greek towns on the Adriatic coast and islands in the 4th century BCE, as well as the arrival of the Celts in the region of continental Pannonia (Maletić et al., 2015a). The peak of viticulture production is associated with the late 19th and early 20th century when around 400 grapevine varieties were planted in Croatia on an area of 200,000 ha. The decline of native varieties was brought on by the introduction of American pests and

turbulent historical events. Consequently, today's Croatian assortment contains 125 varieties. Their ampelographic characteristics, genetic determination, and kinship analysis have all been described to date (Maletić et al., 2015b; Žulj Mihaljević et al., 2020). However, the differences in susceptibility to the most important diseases have not yet been investigated. The objective of this dissertation is to evaluate the phenotypic differences of native grapevine varieties in susceptibility to downy mildew to define their full biological and economic potential. The leaf disc bioassay and sophisticated methods, such as multispectral imaging, chlorophyll fluorescence, and the analysis of polyphenolic and volatile organic compounds, were used to carry this out.

1.1. Research hypotheses and objectives

Hypotheses:

1. Croatian native grapevine varieties are phenotypically different in susceptibility to downy mildew.

2. Downy mildew infection differently affects the photosynthetic activity of varieties with different susceptibility levels.

3. Differences between varieties in susceptibility to downy mildew are associated with differences in the composition and content of polyphenolic and volatile organic compounds.

Objectives:

1. To determine the phenotypic differences between Croatian native grapevine varieties in susceptibility to downy mildew.

2. To determine the changes in photosynthetic activity due to downy mildew infection in varieties of different susceptibility.

3. To determine the composition and content of polyphenolic and volatile organic compounds in leaves infected with downy mildew in varieties of different susceptibility.

2. OVERVIEW OF RELEVANT LITERATURE

2.1. Differences in *Plasmopara viticola* susceptibility among *Vitis* species and grapevine varieties

The domestication of grapevines (*Vitis vinifera* L.) over more than ten thousand years in nearly 90 countries (Villano and Aversano, 2020) resulted in a large number of grapevine genotypes with a wide range of morphological and genetic traits (This et al., 2006). Most genotypes were produced through spontaneous hybridization, however, humans selected those that were worth cultivating and propagating based on their desirable biological and economic characteristics, such as high and stable yields, favourable chemical compositions, the size of the berries and clusters, and preferred inflorescence morphologies, among others. Admittedly, the issue of pathogen resistance was long ignored as a result of the development of effective fungicides (Grassi and Arroyo-Garcia, 2020). We are currently faced with the challenging task of supplying enough food, in adequate amounts, and of acceptable quality for the growing human population while having the least possible impact on the environment. Therefore, plant breeding initiatives become crucial as they enable the development of resistant, high-quality varieties that could withstand the hazards of major diseases (Merdinoglu et al., 2018).

Plasmopara viticola (Berk. & M. A. Curtis; Berl. & de Toni) and *Erysiphe necator* Schw. are the most destructive grapevine pathogens which cause downy and powdery mildew, respectively. Since both pathogens were introduced to Europe in the second half of the 19th century, most European grapevine varieties are susceptible to them. On the other hand, species from North America like *V. riparia* Michx., *V. labrusca* L., *V. rupestris* Scheele, and *Muscadinia rotundifolia* Small coevolved in the same geographical area as the aforementioned pathogens. Consequently, they are highly or completely resistant to mildews (Jürges et al., 2009, Gessler et al., 2011). The Asian *V. amurensis* Rupr. species developed resistance coevolving with the pathogens *Plasmopara cissi* Vienn.-Bourg and *Plasmopara amurensis* Prots which are closely related to *P. viticola* (Dick, 2002).

Vine's genetic background of resistance to downy and powdery mildew is related to quantitative trait loci (QTLs) that are responsible for defence mechanisms. To date, 31 loci of resistance to downy mildew have been identified in the *Vitis* genus germplasm. They are listed in the international *Vitis* International Variety Catalogue (VIVC) database (https://www.vivc.de/, access date: January 15, 2023). The majority of these *Rpv* (Resistance to *Plasmopara viticola*) loci have been found in the genomes of several North American and Asian *Vitis* species as well as *Muscadinia* subgenus species. They are not

only resistant to downy mildew but also to powdery mildew. For instance, the genome of *Muscadinia rotundifolia* contains the *Run1* and *Run2* loci, which confer resistance to *Uncinula necator*. Despite initial speculation to the contrary, genes for resistance to downy and powdery mildew were eventually found in the *V. vinifera* genome as well. The Georgian variety Mgaloblishvili contained the final three *Rpv* loci, *Rpv29*, *Rpv30*, and *Rpv31* (Sargolzaei et al., 2020). Compared to the response of susceptible grapevine varieties, these loci induce partial plant resistance by significantly reducing the pathogen development and sporulation (Toffolatti et al., 2016; Toffolatti et al., 2018). Moreover, *Ren1* (Resistance to *Erysiphe necator*) was identified in the Kishmish vatkana and Dzhandzhal kara varieties (Coleman et al., 2009) which originate in Central Asia. To conclude, these results reaffirm the importance of evaluating and preserving local varieties that are grown in limited areas.

The resistance to downy mildew can be based on constitutive traits, such as structural obstacles, leaf surface covered with hydrophobic trichomes, phytoanticipins, and/or induced defence mechanisms, such as programmed cell death, the synthesis of reactive oxygen species, phytoalexins and pathogenesis-related proteins. The combination of these traits was found in grapevine rootstocks SO4 and Kober 5BB (the hybrids of *V. berlandieri* and *V. riparia*) which proved to be efficient in resistance to *P. viticola*. After their leaves were inoculated with *P. viticola* suspension, small necrotic spots and/or sparse sporulation developed. On the other hand, the susceptible Pinot noir variety (*V. vinifera*) developed sporulation on both leaf surfaces, petioles and stems (Algarra Alarcon et al., 2015). The presence of the inner cuticular rim, which is a constitutive trait regardless of infection, is a significant morphological trait of the *V. riparia* species in the resistance to *P. viticola* (Jürges et al., 2009).

The appearance of necrotic spots on the leaves of North American and Asian *Vitis* species following the *P. viticola* suspension inoculation is one of the earliest detectable phenotypic differences between susceptible and resistant genotypes. These necrotic spots are attributed to a hypersensitive reaction which causes programmed cell death. Consequently, the symptoms of infection are reduced (Bellin et al., 2009). Similarly, the highly resistant interspecific Regent and Solaris varieties develop small brown spots which are localised necrosis at the infection site. Nevertheless, the sparse sporulation that eventually appears on the leaf tissue implies that only some cells have undergone programmed cell death (Bellin et al., 2009; Oerke et al., 2016).

Although the selection of resistant varieties, application of plant extracts and biological agents are more sustainable methods of plant protection, chemical control remains the most effective strategy for protecting grapevines under favourable conditions for disease

development in the field. The oldest plant protection products used in viticulture are copper-based fungicides. Despite their high efficacy against *P. viticola*, chemical control is associated with some challenges, such as the accumulation of contact fungicides in the waxy cuticle, required multiple applications, the harmful effect on the environment, and the potential emergence of fungicide-resistant pathogenic strains. Multiple applications of copper compounds, such as hydroxide, oxychloride and sulphate oxide, are the most broadly used technique for downy mildew control. A water solution of copper sulphate ($CuSO_4$) and lime [$Ca(OH)_2$], known as Bordeaux mixture, has been used since 1882. Pre-infection contact fungicides are based on copper compounds, whereas the most widely applied systemic fungicides are phenylamides and aluminium. Phenylamide compounds provide both preventive and curative protection against *P. viticola* (Gessler et al., 2011; Massi et al., 2021; Koledenkova et al., 2022).

Organic disease management involves the application of plant, microbial, animal and mineral products and is based on several modes of action: induction of resistance, antibiosis (the antagonism resulting from the toxicity of secondary metabolites by one organism to other organisms) and hyperparasitism (the process in which a parasite itself is attacked by another parasite). For example, the application of propolis suppressed the pathogen growth, whereas oligochitosan triggered plant defence responses (Dagostin et al., 2011).

Plant extracts (e.g., extracts of *Betula pendula*, *Calendula officinalis*, *Origanum vulgare*) have a wide range of protective properties that are effective under controlled conditions. However, in commercial production under field conditions, their usage is restricted by expensiveness and low durability since they are highly soluble in water (Koledenkova et al., 2022).

Biological control is part of the integrated pest management system. Specialised fungi and bacteria are beneficial microorganisms that control plant diseases by attacking their causal agents. For example, there are many *Bacillus* species that synthesise antifungal metabolites, inhibit zoospore release and induce resistance, whereas *Fusarium* species synthesise lytic enzymes (glucanase, chitinase, protease), degrade the pathogen cell wall, and establish hyperparasitism (Koledenkova et al., 2022).

Breeding programmes that aim to produce resistant varieties by hybridizing European grapevines (high quality) with North American species (resistant to diseases) were initiated after the introduction of *E. necator* and *P. viticola* in Europe. The first generations of interspecific hybrids were highly resistant, but their quality was poor. Thus, they gained a bad reputation. Nevertheless, breeding programmes have been continuously carried out in Germany and Hungary (Toepfer et al., 2011). High-quality resistant varieties were obtained

through repeated backcrosses with *V. vinifera* varieties (Yobrégat, 2018). The production of these varieties began to spread, and legal obstacles to their cultivation were gradually eliminated. Nowadays, the demand for more sustainable agricultural production and the adoption of strategic and implementation documents lead to an increasing interest in resistant grapevine varieties (European Commission, 2020). Breeding programmes have also been launched in other countries, such as France and Italy, resulting in dozens of newly registered varieties (Bavaresco et al., 2015; Schneider et al., 2019).

Due to the conservative attitude of wine producers and consumers and in order to maintain the authenticity of their wines, traditional native varieties grown in specific regions are often used as progenitors in these crossings. In addition, knowing the progenitors' characteristics, including their disease resistance, is crucial for selecting the best ones. This is undoubtedly one of the factors that have made recent phenotypic research on grapevine varieties intriguing. Furthermore, species that are notable locally or regionally are particularly appealing as a source of features that could be useful in adapting to changing environmental conditions (Sargolzaei et al., 2021).

Boso et al. (2011) found significant differences between Spanish *V. vinifera* varieties in susceptibility to downy mildew. Varieties cultivated in a certain area for more than 300 years proved to be less susceptible. On the other hand, the highest susceptibility was found in varieties that have been cultivated for less than 150 years. These results will be a useful guide for the selection of parental varieties in the breeding programmed aiming to create authentic varieties resistant to downy mildew.

Bitsadze et al. (2015) evaluated 61 Georgian grapevine genotypes (55 native varieties and six wild accessions) using the leaf disc bioassay. As a result, the varieties were grouped into resistance classes according to the 452-1 International Organisation of Vine and Wine (OIV) descriptor. Twenty genotypes were assigned to OIV resistance classes 7 or 9 meaning that they are highly or completely resistant to downy mildew. Thus, it is necessary to investigate them in more detail to include them in further breeding programmes. Furthermore, among the 13 accessions that showed high resistance, three genotypes belong to the wild grapevine (*V. vinifera* L. ssp. *sylvestris* Gmelin; Hegi). Consequently, it is plausible that careful evaluation of the more than 500 different grapevine genotypes that Georgia has in its assortment could reveal more genotypes with high resistance.

Croatia has a long history of growing grapevines, and numerous native varieties have been created in its various climatically diverse regions. The introduction of phylloxera, powdery mildew, and downy mildew at the end of the 19th century gradually caused the erosion of the preceding germplasm. As a result, today's collection of native varieties counts 125 varieties

(Maletić et al., 2015a; Žulj Mihaljević et al., 2020). Due to centuries of grapevine cultivation and their adaptation to different environmental conditions, it is assumed that there are different responses to diseases among native varieties, which have not yet been investigated.

2.2. Biology of the *Plasmopara viticola* pathogen

The causal agent of downy mildew is *Plasmopara viticola,* which is one of the most harmful pathogens affecting viticulture production in all regions of the world (Armijo et al., 2016). As an obligate biotrophic oomycete, *P. viticola* feeds on living tissue, and through its haustoria, penetrates the host cell and uses plant metabolites for its own development and reproduction (Glazebrook, 2005). Its sporangia are lemon- or almond-shaped and contain four to six nuclei (Riemann et al., 2002). During the grapevine growing season, when conditions are favourable for the development of downy mildew (air temperature 20 ± 2 °C, humidity > 80 %), symptoms of infection appear on green organs, such as leaves, tendrils, inflorescences, shoots, and green berries (Gessler et al., 2011). During the first four to five days after inoculation, downy mildew is latent, although it progressively develops within the leaf tissue. The first visible symptoms appear on the leaf's upper (adaxial) surface in the form of yellow-brownish oil spots (lesions), which are followed by sporulation on the leaf's bottom (abaxial) surface forming the "white downy" appearance (Rumbolz et al., 2002).

The life cycle of *P. viticola* proceeds in two main stages. In the generative (sexual) stage, the pathogen overwinters and produces oospores, which are the source of primary infections and new genotypes. This stage is followed by the vegetative (asexual) stage, during which numerous cycles of vegetative reproduction take place. During asexual reproduction, two phases occur: sporangiogenesis (formation of multinucleate sporangia), and zoosporogenesis (formation of motile biflagellate zoospores). The zoospores are propagules that infect the grapevine host causing secondary infections. The pathogen's mycelium (filamentous vegetative body) grows intercellularly and uptakes nutrients by parasitizing the plant cells via haustoria. When the mycelium reaches the substomatal cavity, it forms a cushion from which sporangiophores arise. On abaxial leaf surfaces and stems they arise through stomata, whereas in young berries they arise through lenticels and form sporangia. The sporangiophores' branching is vertical and monopodial, meaning that the growth of the main branch continues, while the lateral branch stays fixed. The terminal branches called sterigmata are usually trichotomous in the genus *Plasmopara*. Sporangia are spores that germinate indirectly since they produce and release thin-walled zoospores. Two heterokont flagella make the zoospores motile. The anterior flagellum is longer, hairy, and moves a zoospore forward, whereas the posterior flagellum is shorter, glabrous and changes the

direction of zoospore movement. The nature of *P. viticola* is polycyclic, where both types of spores (oospores and sporangia) are responsible for its proliferation (Burruano, 2000; Gobbin et al., 2005; Judelson, 2009; Koledenkova et al., 2022).

The main climatic factors influencing the severity of primary infection are precipitation and temperature (Rouzet and Jacquin, 2003). More than 50 years ago, a model also known as Müller forecasting method was developed to predict the occurrence of primary infections that is still applicable today. According to the model, primary infections occur under conditions of a minimum air temperature of 10 °C, 10 mm of rain has fallen during the last two days, and shoots are about 10 cm in length (Gessler et al., 2011). These conditions are usually met in the spring. The pathogen overwinters as oospores (dormant thick-walled sexual spores) in leaf litter, shoots and soil, and as dormant mycelia in infected leaves and twigs. These both structures initiate primary infections in the spring. Oospores can survive under severe conditions (e.g., drought, extreme temperatures, harsh chemicals) and remain dormant for several years until conditions become favourable for their germination. Unlike sporangia that are formed on the plant surface, oospores are formed inside the host tissue. In spring, the oospores germinate by producing the macrosporangium, which releases zoospores under wet conditions. Dispersed by rain or airborne, the zoospores reach the vine organs (Armijo et al., 2016; Buonassisi et al., 2017).

Zoospores encyst and produce germ tubes in water droplets on the plant surface in the vicinity of stomatal complex. The germ tube forms an appressorium that penetrates plant tissue through the stomata using physical (pressure) and chemical mechanisms (cell wall-degrading enzymes). The appressorium produces intercellular or invasive hyphae that degrade the plant cell wall, grow through, and invade the plasma membrane. The pathogen colonizes the leaf parenchyma that uptakes nutrients from host cells using intracellular haustoria. As a result, the pathogen establishes a parasitic relationship with the host plant. Successful colonisation occurs when the host plant does not induce the defence mechanism and the infection culminates in *P. viticola* sporulation (Armijo et al., 2016; Buonassisi et al., 2017; Koledenkova et al., 2022).

P. viticola is a heterothallic oomycete with two mating types, P1 and P2, which means that fertilisation occurs when haploid nuclei of the opposite mating type come into contact. The male antheridium produces a fertilisation tube that penetrates the female oogonium. Single haploid antheridial and oogonial nuclei fuse to form a diploid oospore, which is the source of *P. viticola* genetic variation (Burruano, 2000; Wong et al., 2001).

Due to the leaves' size, the volume of cells for haustorial expansion, the number of stomata and the deficiency of protection against *P. viticola* invasion, they are the main source of

spores necessary for further disease development. Infection of leaves results in discoloration, necrosis, and defoliation, leading to a reduction of sugar accumulation in berries, yield, nutrient composition, and bud overwintering ability. In leaves and young berries, mycelium causes chlorophyll degradation, destroying cells and turning them brown. On infected mature leaves, a mosaic pattern appears, confined by leaf veins. As the berries ripen, they become less susceptible to the pathogen, although infection can spread through the rachis (Gessler et al., 2011; Fröbel and Zyprian, 2019).

2.3. Anatomy of a grapevine leaf

Since the first host plant-pathogen interaction occurs in the leaves, the *P. viticola* infection symptoms manifest mainly in these organs. The intensity and appearance of symptoms depend on the susceptibility degree of the particular variety or vine species (Nascimento-Gavioli et al., 2020). Leaf morphology and anatomy often play an important role in this (Boso Alonso et al., 2010). Moreover, specific defence responses such as programmed cell death or increased synthesis of some secondary metabolites are induced in the leaves of resistant varieties (Chitarrini et al., 2017). *P. viticola* infection also affects physiological processes in the leaf, especially photosynthesis (Nogueira et al., 2020). Therefore, the methods used to evaluate the resistance of genotypes to downy mildew, used in this research, are primarily performed on the leaf, which requires a good knowledge of its structure and function.

Vitis species and varieties can be differentiated by leaf polymorphism and by the density and length of trichomes that may be present on the leaf's lower epidermis (Karabourniotis et al., 1999). Trichomes help reduce water loss by regulating the temperature of the leaf surface. Moreover, they repel insects, protect the leaf lamina from harmful ultraviolet radiation due to phenolic compounds that absorb and disperse radiation, and partially isolate the leaf from other environmental stressors (Keller, 2020).

The leaf lamina is adapted to absorb solar energy and convert it into chemical energy, which is used to synthesize numerous organic compounds. The upper and lower epidermis act as the outer protective layers of the leaf, and they are directly exposed to environmental conditions. The outer cell walls of epidermal cells contain cutin, which is a strong and elastic biopolyester. The outer epidermal cells are covered with an extracellular membrane called the cuticular membrane or cuticle. As a specialized modification of the cell wall, the cuticle consists of cutin, polysaccharides, phenols (especially hydroxycinnamic acids and flavonoids), and lipids (Bargel et al., 2006). The epidermis and cuticle are the first mechanical barriers against pathogens and physical or chemical damage. Due to their

hydrophobic properties, they repel fungal spores and dust particles and serve as insulation against extreme temperatures (Keller, 2020).

Between the epidermal layers is the mesophyll, which is the most active photosynthetic tissue. The mesophyll consists of one layer of elongated cells called palisade parenchyma and four to six layers of sparsely placed cells of irregular shape that form the spongy parenchyma. Both cell types contain a large number of chloroplasts, unlike the epidermal cells in which they are absent. The chloroplasts are the centres of photosynthesis and assimilation. The palisade parenchyma is located below the upper epidermis and has small intercellular spaces, while the spongy parenchyma is located next to the lower epidermis and has large intercellular spaces (Keller, 2020). This tissue provides optimal conditions for *P. viticola* mycelium formation and spreading (Jürges et al., 2009).

Stomata are openings that are located on the lower epidermis in *Vitis* species. Stomata act as valves that are responsible for regulating gas exchange (absorption of carbon dioxide for photosynthesis, oxygen for respiration, and release of water vapour through transpiration) between the leaf and the atmosphere (Lawson, 2009; Keller, 2020). *P. viticola* zoospores enter the leaf tissue through the stomata. Their secretory behaviour, such as callose synthesis in the infected stomata, could act as a mechanical barrier inhibiting the pathogen from completing its life cycle, which has been described for the resistant variety Solaris (Gindro et al., 2003).

2.4. Leaf disc bioassay

Young leaves are almost always the first to show signs of infection among the other green organs that can be infected by *P. viticola*. The International Organisation of Vine and Wine (OIV) recognised this issue and accordingly, in “Descriptor List of Grapevine Varieties and Vitis Species”, prescribed two descriptors related to the evaluation of leaves infected with *P. viticola*. The 452 descriptor (Leaf: degree of resistance to *Plasmopara*) is used to evaluate disease severity on leaves in field conditions. To avoid the impact of outdoor stressors, the 452-1 descriptor [Leaf: degree of resistance to *Plasmopara* (leaf disc test)] is carried out in controlled laboratory conditions (OIV, 2009). This method is known as the leaf disc bioassay and is widely accepted among plant breeders and phytopathologists who are aiming to define the level of a particular variety's susceptibility to *P. viticola* (Gómez-Zeledón et al., 2017; Vezzulli et al., 2018; Bove et al., 2019; Nascimento-Gavioli et al., 2020).

For this purpose, artificial inoculation with a *P. viticola* suspension is performed on excised parts of young, healthy leaves that have not been treated with chemical protection. The fourth and fifth leaves from the shoot apex should be sampled since they are the most

susceptible to *P. viticola*. Leaf discs are excised with a cork borer and placed on wet filter papers in Petri dishes with the abaxial side up, since the zoospores penetrate through the stomata. The leaves are inoculated with a highly concentrated *P. viticola* suspension. For the first 24 hours, closed and sealed Petri dishes need to be placed in a climate chamber in the dark with optimal conditions for the development of downy mildew (20 °C air temperature, 80 % humidity). After that, a photoperiod of 16 hours is applied to mimic outdoor conditions (OIV, 2009; Bellin et al., 2009; Vezzulli et al., 2018).

On the sixth or seventh day following the inoculation, the sporulation typically appears on the abaxial surfaces of susceptible genotypes. The disease severity is visually assessed by the percentage of the abaxial leaf surface covered with sporulation (EPPO, 2001). The OIV 425-1 descriptor uses five classes of resistance. Odd numbers from 1 to 9 are used to identify the five classes. Class 1 represents the most susceptible genotypes, whose leaf discs are completely covered with dense *P. viticola* sporulation (e.g., Müller-Thurgau), whereas resistant genotypes belong to class 9 (e.g., Kober 5BB). Sporulation is not developed on their leaf discs, although necrotic spots can be observed as a symptom of a hypersensitive response (OIV, 2009; Bellin et al., 2009). Because of this, the leaf disc bioassay is often used as an indicator of a particular variety's susceptibility to *P. viticola* in field conditions (Calonnec et al., 2013).

2.5. Determination of leaf photosynthesis efficiency by measuring chlorophyll fluorescence and multispectral imaging

Plants use solar energy during photosynthesis, a crucial component of their primary metabolism, to create organic compounds, primarily carbohydrates. What is more, by limiting nutrient availability, plants can use this process as a defence mechanism against pathogens. Therefore, photosynthesis inhibition is seen as one of the first signs of plant stress (Pérez-Bueno et al., 2019).

Along with the visual detection and evaluation of *P. viticola* infection, there are novel techniques that determine the level of plant stress by measuring photosynthetic efficiency. These techniques include the measurement of chlorophyll fluorescence and multispectral imaging, which are the tools of precision phenotyping. They were previously used for the evaluation of *V. vinifera* varieties' susceptibility to downy mildew (Cséfalvay et al., 2009; Nogueira Júnior et al., 2020) and also for the quantification of other plant diseases (Chaerle et al., 2007; Bürling et al., 2010). One of their biggest advantages is the detection of infection prior to the appearance of visible symptoms (Rolfe and Scholes, 2010).

Pathogenesis induces changes in plant primary metabolism. Thus, the measurement of chlorophyll fluorescence is applied to determine the temporal and spatial changes of photosynthesis inside the plant tissue with high precision (Lenk et al., 2006; Prokopová et al., 2010). The remote sensing of vegetation and imaging spectroscopy has recently become widely applied in precision viticulture. Non-destructive determination of leaf and canopy senescence, chlorophyll content, green biomass, and plant water status can be quantified by vegetation indices. These findings can be used in field studies and breeding programmes for high-throughput phenotyping (Li et al., 2014).

Photon energy transfer can result in three different outcomes: thermal dissipation, photochemistry, and chlorophyll fluorescence emission. Thus, a decrease in photosynthesis or thermal dissipation is indicated by an increase in chlorophyll fluorescence emission. (Rosenqvist and van Kooten, 2003). Some of the most commonly applied chlorophyll fluorescence parameters are the maximum (F_v/F_m) and effective ($F_{q'}/F_{m'}$) quantum yields of photosystem II (PSII) electron transport, electron transport rate (ETR), non-photochemical quenching (NPQ), and photochemical quenching (qP), whereas multispectral imaging includes far-red reflectance (Far Red), near-infrared reflectance (NIR), chlorophyll index (CHI), anthocyanin reflection index (ARI), normalised difference vegetation index (NDVI), and Hue (proportion of total chlorophyll content) (Baker and Oxborough, 2004). They were all used in this research, which aimed to define the differences between non-inoculated and inoculated leaf discs as well as between genotypes differently susceptible to *P. viticola*.

2.6. Secondary metabolites in grapevine leaves

Once the grapevine leaves are infected with *P. viticola*, structural and metabolomic changes occur within the leaf tissue, and these changes are dependent on a specific grapevine-downy mildew interaction. The interaction can either be compatible, allowing the pathogen to complete its infection cycle, or incompatible, which is usually associated with wild *Vitis* species and interspecific hybrids (Kranz, 2003). Plants employ defence mechanisms to prevent the development of pathogenic diseases. Constitutive defence includes constant resistance, for which physical obstacles (e.g., trichomes, thick cell walls, wax layers) and chemical compounds (e.g., antimicrobial secondary metabolites) are responsible (Kono and Shimizu, 2020). These compounds are also known as phytoanticipins, which exist in healthy plants even before the attack of a pathogen (Tiku, 2020). On the other hand, induced defence is triggered by biotic and abiotic stressors (Muganu and Paolocci, 2013). The compounds that are then synthesized are called phytoalexins (Jeandet, 2015).

Unlike primary metabolism, which is directly involved in the growth, development, and reproduction of plants, secondary metabolism is responsible for plant adaptability by participating in defence mechanisms against biotic and abiotic stresses and in signalling pathways (Thirumurugan et al., 2018). Primary metabolites include carbohydrates, organic acids, amines, amino acids, and lipids, whereas the most important secondary metabolites included in grapevine defence against *P. viticola* are phenols and volatile organic compounds (Chitarrini et al., 2017).

The main structural difference between phenolic and polyphenolic compounds is that phenolics, regardless of their number, contain an aromatic ring, whereas polyphenolics contain several aromatic rings with one or more hydroxyl groups attached. They come from the shikimate/phenylpropane pathway (Quideau et al., 2011). According to this definition, stilbenes and flavonoids (anthocyanins, flavonols and flavan-3-ols) are polyphenols, whereas phenolic acids (hydroxycinnamic and hydroxybenzoic acids) are phenols.

During the early stage after inoculation, the primary metabolites are usually affected, whereas the changes in secondary metabolites, such as phenylpropanoids and flavonoids, are more pronounced during later stages (Chitarrini et al., 2017). These compounds differentiated the susceptible variety Trincadeira from the resistant variety Regent (Ali et al., 2012). A higher concentration of the stilbene *trans*-resveratrol was detected in the leaves of the resistant variety Bianca soon after inoculation. The *trans*-resveratrol oligomers, such as *trans*-*ε*-viniferin, *α*-viniferin and pallidol accumulated later (Chitarrini et al., 2017).

Although volatile organic compounds constitute only 1% of plant secondary metabolites, these compounds are important because they act as mediators of interactions within the plant and between neighbouring plants. Therefore, they regulate plant responses to biotic stress. Volatile organic compounds have a low molecular weight and a high vapour pressure, meaning they easily diffuse into the environment and pass through biological membranes. In most cases, plants induce the synthesis of methyl salicylate, heterocyclic compounds, mono- (C_{10}) and sesquiterpenes (C_{15}), green leaf volatiles (aldehydes, alcohols and esters), and ketones in response to pathogen attack (Lazazzara et al., 2018).

The accumulation of volatile organic compounds in vines proves their defensive role after *P. viticola* infestation (Lazazzara et al., 2021). For example, higher accumulation of 2-phenylethanol, (*E*)-2-pentenal, *β*-cyclocitral, *β*-caryophyllene, and *β*-selinene was detected in the leaves of resistant genotypes (BC4, SO4, Kober 5BB, and Solaris) compared to susceptible variety Pinot noir (Lazazzara et al., 2018). Terpenes with direct antimicrobial activity, such as farnesene, nerolidol, ocimene, and valencene, contributed to the defence mechanism of the resistant varieties Mgaloblishvili (*V. vinifera*) and Bianca (interspecific

hybrid) (Ricciardi et al., 2021). Some compounds, including benzaldehyde, farnesene, linalool, neral, and (*E*)-nerolidol differentiated resistant genotypes (Bianca, Solaris, BC4, F12P160 and F12P60) from the susceptible Pinot noir variety (Ciubotaru et al., 2021).

The emission of sesquiterpenes was higher in *in vitro* plantlets of the resistant genotypes SO4 and Kober 5BB compared to Pinot noir. The emission of monoterpenes was detected only in inoculated samples of SO4, which indicated that *P. viticola* infection triggered their synthesis. Although both vines and pathogens can synthesize these compounds, their emissions were high even in plantlets with sparse sporulation. This suggests that they are mainly synthesized by plant cells (Algarra Alarcon et al., 2015).

There are many studies related to the content and composition of secondary metabolites before and after *P. viticola* inoculation in the leaves of resistant *Vitis* species and resistant genotypes compared to susceptible *V. vinifera* varieties. However, the specific metabolomic patterns among *V. vinifera* varieties of different susceptibilities are scarce. Therefore, this study will focus on native grapevine varieties from Croatia as well as both susceptible and resistant control genotypes to address this exact issue.

3. RESEARCH RESULTS AND DISCUSSION

3.1. Overview of published qualification scientific papers

3.1.1. Abstract of the scientific paper “Screening of Croatian Native Grapevine Varieties for Susceptibility to *Plasmopara viticola* Using Leaf Disc Bioassay, Chlorophyll Fluorescence and Multispectral Imaging”

In the era of sustainable grapevine production, there is a growing demand to define differences between *Vitis vinifera* varieties in susceptibility to downy mildew. Croatia, as a country with a long tradition of grapevine cultivation, preserves a large number of native grapevine varieties. A leaf disc bioassay has been conducted on 25 of them to define their response to downy mildew, according to the International Organisation of Vine and Wine (OIV) descriptor 452-1, together with the stress response of the leaf discs using chlorophyll fluorescence and multispectral imaging with 11 parameters included. Time points of measurement were as follows: before treatment (T_0), one day post-inoculation (dpi) (T_1), two dpi (T_2), three dpi (T_3), four dpi (T_4), six dpi (T_5), and eight dpi (T_6). Visible changes in form of developed *Plasmopara viticola* (*P. viticola*) sporulation were evaluated on the seventh day upon inoculation. Results show that methods applied here distinguish varieties of different responses to downy mildew. Based on the results obtained, a phenotyping model in the absence of the pathogen is proposed, which is required to confirm by conducting more extensive research.

3.1.2. Abstract of the scientific paper “Leaf Polyphenolic Profile as a Determinant of Croatian Native Grapevine Varieties’ Susceptibility to *Plasmopara viticola*”

Since grapevine is highly susceptible to various pathogens, enormous amounts of pesticides are applied each season to achieve profitable production. One of the most destructive grapevine diseases is downy mildew, and their interaction has been in the spotlight for more than a decade. When it comes to a metabolome level, phenolic compounds are relevant to investigate due to their involvement in the plant immune system and known antifungal properties. Croatian grapevine germplasm is highly heterogeneous due to its long history of cultivation in diversified geographical regions. Since it has been found that native varieties react differently to the infection of *Plasmopara viticola*, the intention of this study is to define if the chemical background of the leaves, i.e., polyphenolic composition, is responsible for these dissimilarities. Therefore, the leaves of 17 genotypes, among which 14 were native

and 3 were controls, were analyzed using high-performance liquid chromatography (HPLC) in four terms: before inoculation and 24, 48, and 96 h post inoculation (hpi). During this early phase, significant differences were found neither between the terms nor between the non-inoculated and inoculated samples, except for resveratrol-3-*O*-glucoside. By applying principal component analysis (PCA) using initial leaf polyphenolic composition, varieties of *V. vinifera* were clearly separated into three different groups corresponding to their International Organization of Vine and Wine (OIV) classes of susceptibility to *P. viticola*. Results obtained in this research suggest that the initial constitutive polyphenolic composition of the cultivar leaves has a crucial influence on their susceptibility to *P. viticola*, and this finding can be used to improve the success of grapevine breeding programs toward downy mildew resistance.

3.1.3. Abstract of the scientific paper "Croatian Native Grapevine Varieties' VOCs Responses upon *Plasmopara viticola* Inoculation"

The *Plasmopara viticola* pathogen causes one of the most severe grapevine diseases, namely downy mildew. The response to *P. viticola* involves both visible symptoms and intricate metabolomic alterations, particularly in relation to volatile organic compounds, and depends on the degree of resistance of a particular variety. There are numerous native grapevine varieties in Croatia, and they vary in susceptibility to this oomycete. As previously reported, *in vitro* leaf disc bioassay and polyphenolic compound analysis are complementary methods that can be used to separate native varieties into various resistance classes. This research used the Solid Phase Microextraction-Arrow Gas Chromatography-Mass Spectrometry method to identify the early alterations in the VOCs in the leaves after *P. viticola* inoculation. Based on the absolute peak area of sesquiterpenes, some discrepancies between the sampling terms were noticed. The presence of certain chemical compounds such as humulene, ylangene, and *α*-farnesene helped distinguish the non-inoculated and inoculated samples. Although specific VOC responses to *P. viticola* infection of native varieties from various resistance classes could not be identified, the response of less susceptible native varieties and resistant controls was associated with an increase in the absolute peak area of several compounds, including geranylacetone, *ß*-ocimene, and (*E*)-2-hexen-1-ol.

3.2. Unified discussion

When compared to other *Vitis* species, the grapevine is found to be the most cultivated since its fruits are utilised for a truly wide variety of products, including wines, jams, juices, distillates, table grapes, raisins, and so on (OIV, 2022). Since the grapevine is extremely

susceptible to diseases, attempts have been made in recent decades to identify alternate, environmentally friendly means of disease suppression. One of the approaches is the screening of the existing grapevine germplasm, selecting less susceptible and resistant varieties in order to use them as progenitors in breeding programmes (Boso Alonso and Kassemeyer, 2008; Boso et al., 2011; Toepfer et al., 2011; Gaforio et al., 2015; Toffolatti et al., 2016).

With over a hundred native grapevine varieties, Croatia is a rich source of grapevine biodiversity, which makes it attractive for breeding and the wine industry. The research efforts over the past two decades have been directed towards evaluating the production traits of native varieties (Andabaka et al., 2022), clonal selection (Pejić et al., 2015; Preiner et al., 2017), describing ampelographic features (Maletić et al., 2015a), genetic diversity, population structure, and parentage analysis (Maletić et al., 2015b; Žulj Mihaljević et al., 2020). However, to determine their full potential, it is necessary to investigate the level of their susceptibility to the most common fungal diseases, one of which is downy mildew.

Downy mildew is caused by the obligate biotrophic oomycete *Plasmopara viticola* which invades all green vine organs, primarily young leaves. Its germ tube penetrates through the stomata of the leaf and progressively develops its mycelium in the spongy parenchyma. This leaf tissue provides optimal conditions for *P. viticola* development since it is comprised of air spaces and loosely packed cells that are sources of water and nutrients for this parasite (Gessler et al., 2011; Keller, 2020). As the first visible infection symptoms usually appear on the leaves, the International Organisation of Vine and Wine (OIV) prescribed the descriptor 452-1 [Leaf: degree of resistance to *Plasmopara* (leaf disc test)] which is dedicated to differentiation and classification of *Vitis* species and grapevine varieties into different classes of resistance (OIV, 2009). This descriptor is based on the leaf disc bioassay which is applied on excised leaf parts artificially inoculated with *P. viticola* suspension and placed in optimal conditions for downy mildew development.

Previously, many authors (Bellin et al., 2009; Gómez-Zeledón et al., 2017; Buonassisi et al., 2018; Nascimento-Gavioli et al., 2020) conducted their studies by applying the leaf disc bioassay and thus, the same was used in this research aiming to classify Croatian native grapevine varieties in the resistance classes. This method was found to be a straightforward procedure that yielded results about differences in susceptibility to downy mildew in a short amount of time (typically no more than seven days), from inoculation until the emergence of *P. viticola* sporulation. Since genotypes with known levels of susceptibility or resistance were compared, interesting and reliable results were obtained. In earlier studies using leaf discs, other authors (Deglene-Benbrahim et al., 2010; Gómez-Zeledón and Kaiser, 2016; Oerke et

al., 2016; Buonassisi et al., 2018; Vezzulli et al., 2018) also included Solaris, Regent, *V. riparia*, Chardonnay, and Cabernet Sauvignon, though their measurements for determining the level of susceptibility varied slightly from one another. Nonetheless, all of their results are comparable, similar, or the same. The distinction between Regent and Solaris was that the former was classified as resistant (class > 7) (Buonassisi et al., 2018) and partially resistant (Gómez-Zeledón and Kaiser, 2016; Oerke et al., 2016; Vezzulli et al., 2018), while the latter as partially resistant (Oerke et al., 2016; Vezzulli et al., 2018). *V. riparia*, *V. aestivalis*, and *V. rupestris* were found to be the most resilient species among the North American species. *V. riparia* allowed no sporulation and seldom showed necrotic spots (Gómez-Zeledón and Kaiser, 2016), which is in agreement with this research. These North American species, which coevolved on the same continent as downy mildew, were exposed to the same stressful stimulus and developed epigenetic modifications that are the basis of their defence mechanisms (Kumar et al., 2020). In response to the *P. viticola* infection, different passive mechanisms (such as dense hydrophobic trichomes on the abaxial side of leaves) and active responses involving hypersensitivity and the synthesis of specific secondary metabolites contribute to the differentiation between varieties (Gessler et al., 2011; Buonassisi et al., 2017). In experiments conducted by Deglene-Benbrahim et al. (2010), Chardonnay was classified as the most susceptible genotype, while *V. riparia* was extremely resistant. Cabernet Sauvignon, along with Riesling, Pinot Noir, and Pinot Blanc (Boso and Kassemeyer, 2008), was described as a slightly susceptible variety that may be assigned to the OIV class 5, wherein Cabernet Sauvignon belongs based on the results reported here.

Admittedly, this sort of phenotyping is strongly dependent on visual assessment, which takes time, especially in large-scale research. Furthermore, it can cause bias among different experts and experimental repeats. Because of the rapid advancement of high-throughput genotype screening in plant breeding and genomics, there is a growing demand for more effective and trustworthy phenotyping data to assist modern genetic crop improvement (Li et al., 2014). As a result, in this study, the leaf disc test was combined with chlorophyll fluorescence and multispectral imaging to explain differences between separate OIV groups and alterations between non-infected and infected leaf discs. Along with the leaf discs imaging, a detailed analysis of the composition and content of secondary metabolites, namely polyphenolic and volatile organic compounds, in the leaves was carried out to determine possible differences between non-inoculated and inoculated leaf samples, the terms of sampling and the OIV resistance classes. The contribution of each method is described below.

One of the most crucial functions of plant primary metabolism is photosynthesis, so its inhibition is one of the first signs of plant stress. By reducing the nutrients available to the

pathogens, it acts as a plant defence mechanism against biotic stress. Pathogens, on the other hand, have the ability to influence plant metabolism for their own gain (Pérez-Bueno et al., 2019). For this reason, chlorophyll fluorescence and multispectral imaging was applied in this research to determine alterations in photosynthesis until the appearance of *P. viticola* sporulation. F_v/F_m and $F_{q'}/F_{m'}$ are the most sensitive chlorophyll fluorescence parameters of grapevine leaves harbouring *P. viticola* (Cséfalvay et al., 2009). Reduced photosystem II efficiency, specifically increased photoinhibition, is indicated by declines in the F_v/F_m ratio (variable to the maximum value of chlorophyll *a* fluorescence) (Guidi et al., 2019).

For the majority of plant species, an optimal F_v/F_m value is 0.83; values lower than this indicate stress exposure and reduced photosynthetic effectiveness, according to earlier studies (Björkman and Demmig, 1987; Johnson et al., 1993). Such observations are attributable to the overall low F_v/F_m values (<0.71), which were obtained in this study, most probably as a result of *P. viticola* infection, because the experiment was carried out on excised leaf sections, and due to the imaging of their abaxial sides. The leaf excision and transfer from the greenhouse to the laboratory, where leaf discs were placed on wet filter papers, likely caused the lowest values of F_v/F_m to be seen in T_0. Despite this, this parameter could easily discriminate between infected and non-infected leaf discs only 24 hours after inoculation, which is substantially earlier than the prior observation that the earliest change in F_v/F_m pattern on Chardonnay leaves appeared four days after inoculation (Cséfalvay et al., 2009). This study's observation of necrotic areas in the variety Solaris four days after inoculation is consistent with earlier research (Nogueira Júnior et al., 2020), in which a low F_v/F_m value was observed five days after inoculation as a result of the emergence of necrotic spots.

As opposed to infected Solaris, Regent (OIV 7) and *V. riparia* (OIV 9), infected susceptible *V. vinifera* varieties (OIV classes 1, 3, and 5) often had lower $F_{q'}/F_{m'}$ and ETR values, indicating that despite infection, these (partially) resistant genotypes maintain higher photosynthetic rates. Yet, their performance also declined during the later stage of infection (6 and 8 dpi). These alterations can be attributed to the gradual degradation of chlorophyll (Chaerle et al., 2004) and the breakdown of the photosynthetic system brought on by *P. viticola* infection and senescent leaf discs. To supply energy for a defence response or to make up for the loss of green leaf area, ETR can be activated in areas close to infected cells (Rolfe and Scholes, 2010).

NPQ refers to thermal energy dissipation in the PSII antennae (Prokopová et al., 2010). It was previously reported that its levels fell in tomato leaves infected by *B. cinerea* as lesions developed, together with $F_{q'}/F_{m'}$. When powdery mildew interacted with susceptible and

resistant lines of barley, the impact on the compatible interaction was significantly stronger, meaning that the susceptible line showed the highest fall in $F_{q'}/F_{m'}$ and NPQ at the site of infection that spread to neighbouring cells (Swarbrick et al., 2006). Although NPQ responses in the current study were ineffective at differentiating infected from non-infected leaves, they fell at 6 dpi in both treatments when necrotic patches and sporulation had already formed in infected tissues. Furthermore, compared to resistant classes, whose values did not significantly change throughout the experiment, this reduction was more pronounced in susceptible OIV classes (1, 3, and 5).

A change in photochemical quenching (qP) reflects the closure of reaction centres as a result of light saturation of photosynthesis, which is indicated by the proportion of PSII reaction centres that are open. This parameter, along with F_v/F_m, offers details on the underlying mechanisms responsible for the altered photosynthetic efficiency (Maxwell and Johnson, 2000). In line with Gamm et al. (2011), it was found that photochemical quenching was on the decline in this study as well. Since all genotype groups displayed similar and very low qP values after the first appearance of visible changes (4 dpi), this parameter can also be used to distinguish between susceptible and resistant genotypes.

Hue values offer an alternative to photometric analysis of leaf extracts since they are proportional to total chlorophyll. This has been demonstrated using tobacco leaves that have different chlorophyll contents because of senescence, indicating the potential for use in studies of stress conditions that are also accompanied by chlorophyll loss (Sass et al., 2012). Each colour can be expressed in this colour space regardless of its saturation (pale or intense colour) and value (dark or bright colour). This can further be utilised to detect downy mildew symptoms in the field (Abdelghafour et al., 2020). Due to their significantly brighter abaxial sides of leaves (https://www.vivc.de/, the access date: 20 January 2021) and consequently lower Hue values than other analysed genotypes, the Solaris and Regent (OIV 7) varieties stood out in this study's analysis of the Hue parameter. Since the pathogen's mycelium destroys chloroplasts, higher FarRed readings are typically found in genotypes that are more resistant to downy mildew and in leaf discs that are not infected. Under stressful conditions, a decline in reflectance may be an indication of reduced areal interspaces in the mesophyll of leaves (thus lowering carbon dioxide assimilation) (Lenk et al., 2006). Due to this, *V. riparia*, the most resistant evaluated genotype, displayed the highest values in this spectrum. Additionally, it has been found that *V. riparia* has smaller, looser-packed cells with extended intercellular spongy parenchyma (Boso Alonso et al., 2010).

The contents of chlorophyll and anthocyanin were determined by CHI and ARI, respectively. According to these measurements, the OIV classes 3 and 9 have the highest concentrations of chlorophyll and anthocyanin, with no significant changes over the course of the measurement period. However, CHI was able to distinguish between infected and uninfected leaf discs at 6 and 8 dpi. As the disease progressed, Oerke et al. (2016) observed a decrease in chlorophyll content, which they linked to the development of observable symptoms on the adaxial leaf side, such as discolouration and oil spots. In the later stages of infection particularly, NDVI, a plant health indicator, distinguished non-inoculated from inoculated leaf discs with great clarity. *P. viticola* sporulation caused visible changes six or seven days after inoculation, and at 4 dpi, it was possible to distinguish between non-inoculated and inoculated leaf discs using fluorescence (F_v/F_m) and multispectral (CHI and NDVI) channels. These differences are frequently more pronounced among the genotypes from the OIV class 1.

Fluorescence imaging applications in disease and stress resistance screening offer clear potential for quantitative assessment of the plant infection or stress level prior to the emergence of visible symptoms (Lenk et al., 2006). One such example is determining whether the asymptomatic *V. vinifera* Malvasía de Banyalbufar variety is infected with GLRaV-3 (*Grapevine leafroll-associated virus 3*) (Montero et al., 2016).

Whole leaves, detached from the shoots and treated with either ultrapure water (control samples) or *P. viticola* suspension in the laboratory, were used for the analysis of secondary metabolites. Comparing these two approaches, the initial constitutive composition and content of phenolic compounds separated *V. vinifera* varieties into three groups, which correspond to the OIV resistance classes 1, 3 and 5, whereas the absolute peak area (APA) of individual sesquiterpenes (a group of volatile organic compounds) differentiated the partially resistant OIV class 7 and resistant OIV class 9 from other OIV classes.

More precisely, the three susceptible OIV classes (1, 3 and 5) have been found to vary depending on the specific phenolic compounds of each group, as determined by principal component analysis (PCA). The presence of caffeic and vanillic acid, which are hydroxycinnamic and hydroxybenzoic acids, respectively, in high concentrations distinguished the most susceptible OIV class 1 from two other groups. Riesling Weiss, Pinot Noir, Cabernet Sauvignon, and Trincadeira are susceptible *V. vinifera* varieties that have also shown to contain significant amounts of caffeic acid (Maia et al., 2020). Contrastingly, caffeic acid has been linked to constitutive resistance in the partially resistant variety Regent in the past (Figueiredo et al., 2008). Furthermore, this compound was found to participate in enzymatic oxidative mechanisms in response to pathogenic infections of the grapevine

(Mattivi et al., 2011). Among flavan-3-ols, the only discriminator was epigallocatechin-gallate known for its high antioxidant capacity (Kedrina-Okutan et al., 2018).

Flavan-3-ols, i.e., catechin and epicatechin, were more abundant in the presented OIV class 3. A previous study (Maia et al., 2020) hypothesizes that higher levels of catechin/epicatechin may be putative biomarkers of susceptibility. Catechin, together with other phenolic compounds, possesses antioxidant properties and has been previously determined as a part of the grapevine defence mechanism (Kortekamp, 2006). However, there is a presumption that catechin can be degraded by different fungi, used as a carbon source for growth, and finally used for establishing a successful infection (Maia et al., 2020), but the precise potential of *P. viticola* in the degradation of this compound has not yet been fully investigated. Epicatechin has been proposed as a biomarker of resistance in a study by Ciubotaru et al. (2021) due to its higher content in the genotype BC4 possessing resistant locus *Rpv1*. Phenolic acids, namely fertaric, coutaric, and gallic acid, have also contributed to the discrimination. Nevertheless, Ali et al. (2012) identified fertaric acid in the partially resistant variety Regent.

According to a previous study (Maia et al., 2020), where the same flavonol glycoside as well as several others were found in higher concentrations in the resistant/partially resistant genotypes, quercetin-3-*O*-glucoside was a discriminative compound that was more abundant in the OIV class 5. The partially resistant cultivar Bianca was previously found to contain the distinctive flavonol kaempferol-3-*O*-rutinoside at 12 hpi (Chitarrini et al., 2017). Additionally, because they were present in higher concentrations, caftaric acid and quercetin-3-*O*-glucoside allowed researchers to distinguish between Regent and Trincadeira (Ali et al., 2012). According to Latouche et al. (2013), constitutive higher flavonol content inhibited the accumulation of stilbenes in grapevine leaves, delaying the phytoalexin-mediated response of leaves to *P. viticola*. This finding raises the possibility that higher flavonol concentrations could control the spread of the pathogen. Ferulic and *p*-coumaric acids were also discriminative for the OIV 5 class. The highest contents of these acids were previously found in the interspecies hybrid Petra, distinguished by its high level of cold hardiness and decreased susceptibility to *P. viticola* and *Botrytis cinerea* (Cindrić et al., 2003).

Resistance to *P. viticola* can be linked to the synthesis of physical barriers, such as callose and lignin appositions, in addition to constitutive and induced chemical compounds that provide a certain level of tolerance to parasitic microorganisms (Toffolatti et al., 2012). Correspondingly, hydrophobic trichomes on the abaxial leaf sides reduce or repel water droplets, preventing *P. viticola* zoospores from encysting (Kono and Shimizu, 2020), which is necessary for the pathogen to develop inside the leaf tissue and continue fructification

(Rossi and Caffi, 2007; Rossi and Caffi, 2012). The abaxial leaf surfaces of Teran and Ranfol, two native varieties of Croatia, are coated in extremely dense hydrophobic and moderately dense trichomes, respectively. These trichomes undoubtedly prevent *P. viticola* sporangia from reaching the epidermis and stoma at the leaf bottom. Malvazija istarska, on the other hand, has glabrous leaves that are sturdy and robust (Maul et al., 2012, Maletić et al., 2015a), whose thick cuticle possibly protects them from plant pathogens (Serrano et al., 2014). Within the classes of resistance, there are varieties with a comparatively high trichome density level, including Belina starohrvatska, Moslavac, and Plavac mali in class 1, Plavina in class 3, and Ranfol and Teran in class 5. In contrast to this, resistant genotypes (classes 7 and 9) exhibit low trichome density levels. Contrary to several other studies, there was no link found between the density of the trichomes and resistance to *P. viticola*, indicating that this characteristic does not significantly affect the level of resistance of particular genotypes (Kortekamp and Zyprian, 1999; Kono and Shimizu, 2020).

Due to the presence of two resistance genes (*Rpv3-3* and *Rpv10*) (Vezzulli et al., 2019; Possamai et al., 2020), Solaris, one of the control varieties used in this study, has shown strong but not complete resistance to *P. viticola* (OIV 452 = 7) in earlier studies (Vezzulli et al., 2018; Ciubotaru et al., 2021). Such a pyramided resistance offers a greater level of resistance, which is typically described as a feature that is more stable and durable (Merdinoglu et al., 2018). It was discovered, nonetheless, that its reaction to *P. viticola* infection is isolate-specific and highly variable (Heyman et al., 2021). Interestingly, it responded to *P. viticola* inoculation more like *V. vinifera* varieties than *V. riparia* because of its genetic background, which is based on *V. vinifera* [Merzling x (Zarya Severa x Muscat Ottonel)] (Pezet et al., 2004). This is comparable to earlier studies where the metabolic profile of the Regent variety was clustered with *V. vinifera* varieties (Maia et al., 2020).

Native to North America, *Vitis riparia* evolved with *the E. necator* and *P. viticola* fungi/oomycetes and later acquired resistance to mildew diseases (OIV 452 = 9). Previous studies linked this genotype with low or no sporulation values (Boso et al., 2012, Bhattarai et al., 2021). As a result, it has been successfully used in breeding programmes for resistance introgression (Toepfer et al., 2011). Due to the fast constitutive expression of the stilbene synthase genes as well as the extent of their transcriptional activation following *P. viticola* inoculation (Ciaffi et al., 2019), this genotype produced the highest content of resveratrol-3-*O*-glucoside, piceatannol, and total stilbenes observed previously (Boso et al., 2012).

As phytoalexins, stilbenes are toxic to phytopathogenic fungi and may contribute to disease resistance (Ribera and Zuñiga, 2012). Although stilbenes were found in susceptible genotypes as well and helped to distinguish OIV class 1, their significance in identifying the

resistant genotypes is much greater. In addition to stilbenes, the OIV class 9 was distinguished from other OIV classes by epigallocatechin gallate and kaempferol-3-*O*-rutinoside. According to Kedrina-Okutan et al. (2018), *V. riparia* leaves constitutively contain more total polyphenols, total flavonols, and total phenolic acids than *V. rupestris* leaves, which may help to explain why this species has such high resistance to *P. viticola*, as flavonols seem to hinder the development of this pathogen (Ali et al., 2012).

Comparing metabolic compositions associated with disease susceptibility of different *Vitis* species and *V. vinifera* varieties, *V. riparia* clustered together with *V. labrusca*, *V. candicans*, *V. vinifera* subsp. *sylvestris*, and *V. rotundifolia*, whereas the Regent variety was closer to *V. vinifera* varieties, such as Riesling Weiss and Pinot Noir (Maia et al., 2020), confirming the results of the present study. Chardonnay, the susceptible control variety used in this study, was previously included in another study (Toffolatti et al., 2012) where the changes of antifungal compounds upon *P. viticola* infection were described. However, flavonoids were not shown to react to the presence of this pathogen there. Although some compounds (such as protocatechuic acid, gallocatechin, and procyanidins B1, B3, and B4) were higher in Chardonnay than in most other genotypes from OIV class 3 in the present study, the polyphenolic profile of this genotype was generally comparable to that of other genotypes from its class.

As far as volatile organic compounds are concerned, some specificities could be identified among the OIV classes of resistance and the absolute peak area (APA) of sesquiterpenes as the inoculation time progressed. Furthermore, a few compounds, such as geranylacetone, *ß*-ocimene, and (*E*)-2-hexen-1-ol, were found to be potentially responsible for a higher resistance of OIV classes 5, 7, and 9. However, a clear separation of differently resistant genotypes was not achieved.

Terpenes are a class of natural volatile compounds which are predominantly produced by plants. They are responsible for plants' odour and mediate ecological interactions, such as disease resistance and plant-plant communication. They are mostly hydrocarbons whose building block is a five-carbon isoprene unit. Accordingly, terpene hydrocarbons are classified by the number of isoprene units, i.e., monoterpenes consist of two isoprene units and ten carbon atoms, whereas sesquiterpenes consist of three isoprene units and fifteen carbon atoms (Rosenkranz et al., 2021). It is important to emphasize the importance of terpenes in discriminating between the OIV resistance classes as they were identified to be the most discriminative compounds. For example, the high APA of sesquiterpenes *α*-farnesene and *(Z)-ß*-farnesene distinguished OIV classes 7 and 9 from the more susceptible OIV classes 1, 3, and 5, regardless of the treatment and the sampling term. Not only that,

but thanks to its higher APA in inoculated leaves compared to non-inoculated ones, *α*-farnesene was suitable for distinguishing treatments throughout the experiment.

In previous studies, VOCs, particularly sesquiterpenes, produced by downy mildew-resistant genotypes were shown to aid in grapevine defence against *P. viticola*. Namely, higher levels of sesquiterpene emission were found in *in vitro* plantlets of the downy mildew-resistant genotypes SO4 and Kober 5BB, while lower levels of these VOCs were found in the susceptible variety Pinot noir (Algarra Alarcon et al., 2015). A pathogen-dependent transcriptional regulation of terpene biosynthesis was suggested by the increased amount of farnesene that was found in the resistant genotypes of Mgaloblishvili and Bianca after *P. viticola* inoculation along with the up-regulation of terpene synthase genes (Ricciardi et al., 2021). Next to that, according to Ciubotaru et al. (2021), farnesene was expressed in high levels in the mono-locus resistant genotypes BC4 (*Rpv1*), Bianca (*Rpv3-1*) and F12P160 (*Rpv12*), as well as in the pyramided resistant genotype F12P127 (*Rpv3-1*, *Rpv3-3*, and *Rpv10*). To conclude, these findings collectively indicate that certain compounds from the sesquiterpene class could potentially serve as reliable indicators of both plant resistance and infection with *P. viticola*.

It is important to note a contradiction between the findings of Algarra Alarcon et al. (2015) regarding the higher content of monoterpenes in the resistant genotype SO4 and the results of this study concerning total monoterpenes. Contrary to the previous study, this research revealed that the absolute peak area (APA) of total monoterpenes was highest in the OIV class 1, which represents the most susceptible group. However, when examining individual monoterpenes, this research found that a higher APA of *ß*-cyclocitral differentiated *V. vinifera* varieties within OIV classes 1, 3, and 5 from *V. riparia*. Furthermore, linalool exhibited significantly higher levels in OIV class 1 compared to OIV classes 3, 5, and 9, indicating its greater abundance in susceptible varieties. In contrast to the findings of the present study, Lazazzara et al. (2018) detected higher amounts of *ß*-cyclocitral and linalool in the leaves of resistant genotypes (BC4, Kober 5BB, SO4, and Solaris) compared to the susceptible Pinot noir. Moreover, higher contents of linalool and neral were identified in Bianca and F12P60, suggesting their potential antimicrobial activity (Ciubotaru et al., 2021). Additionally, volatile oxides of linalool, namely (*Z*)- and (*E*)-linalool oxides, were found in this study as well. Among them, (*Z*)-linalool oxide exhibited the highest APA in *V. riparia* (OIV 9), distinguishing this genotype from the others evaluated and implying its role in defense-related activity. Furthermore, the presence of a high APA for neral in Solaris (OIV 7), along with its detection in the most susceptible varieties (OIV 1), suggests the potential significance of neral in grapevine resistance. To further understand its discriminative

function, it would be beneficial to conduct additional research encompassing a broader range of genotypes with varying susceptibility.

This study also examines aldehydes, which are organic compounds containing a formyl functional group and known for their antimicrobial effects against bacteria and fungi (Hammerbacher et al., 2019). Among the aldehydes investigated, aledehyde-4-pentenal exhibited a low APA in *V. riparia,* distinguishing it from other genotypes. In contrast, Lazazzara et al. (2018) found a higher APA of (*E*)-2-pentenal in the resistant genotypes BC4 and Kober 5BB compared to Pinot noir. As far as (*E,E*)-2,4-heptadienal and benzeneacetaldehyde are concerned, lower APAs were detected in resistant genotypes in both studies. Interestingly, the same authors found benzaldehyde to be more abundant in resistant varieties, whereas, in the present study, it was most abundant in OIV classes 5 and 9, although a high APA was also observed in OIV class 1. However, previous research by Chitarrini et al. (2017) suggested benzaldehyde as a putative biomarker of resistance to *P. viticola* infection, as it was found in higher concentrations in infected samples of the resistant cultivar Bianca at 48 and 96 hours post-inoculation (hpi). Similarly, Ciubotaru et al. (2021) reported higher levels of benzaldehyde in genotypes such as Bianca, Solaris, and F12P60, which possess at least one resistance locus in their genomes. While the present study does not establish benzaldehyde as a definitive indicator of susceptibility or resistance, previous research by Chitarrini et al. (2017) and Ciubotaru et al. (2021) has suggested a potential association between higher concentrations of benzaldehyde and resistant genotypes, particularly in the context of *P. viticola* infection. In addition to the high absolute peak area (APA) of nonanal observed in OIV class 5, a similar result was found in Solaris (OIV 7), suggesting a potential common feature associated with the lower susceptibility of these two OIV classes. Previous research by Ciubotaru et al. (2021) detected higher levels of nonanal in the leaves of the F12P127 genotype and proposed these volatile organic compounds (VOCs) as biomarkers of resistance, further reinforcing the findings of the present study.

Alcohols, along with aldehydes and esters, are commonly known as green leaf volatiles due to their contribution to the characteristic "fresh green" aroma found in various fruits and vegetables. They are synthesized in green organs as a response to both wounding and pathogen attack (Dudareva et al., 2013; Lazazzara et al., 2018). In the present study, phenylethyl alcohol increased throughout the experiment and was detected in the highest APA in OIV class 9 corroborating the findings of Lazazzara et al. (2018). Another interesting observation was the ascending APAs of 1-hexanol and (*E*)-2-hexen-1-ol, with significantly higher levels in inoculated leaves compared to non-inoculated leaves, suggesting their potential as indicators of *P. viticola infection*. Notably, OIV 5 varieties demonstrated the highest APA of (*E*)-2-hexen-1-ol, indicating its potential involvement in the defence

mechanism of *V. vinifera* varieties with lower susceptibility to *P. viticola*. Additionally, Ciubotaru et al. (2021) identified 1-hexanol, (*E*)-2-hexen-1-ol, 2-ethyl-1-hexanol, and 1-octen-3-ol as potential biomarkers of resistance in Bianca, Solaris, and F12P60 genotypes due to their higher concentrations upon inoculation compared to the susceptible Pinot noir. However, in the present study, the APA of 2-ethyl-1-hexanol decreased over time and was higher in non-inoculated leaves and the most susceptible OIV 1 varieties, whereas 1-octen-3-ol did not show significance for these parameters. Considering that only phenylethyl alcohol yielded similar results in the present study and Lazazzara et al. (2018), further investigation is warranted to explore the individual alcohols and their roles in the defence mechanism against *P. viticola*.

Esters are organic compounds synthesized through esterification, a process involving the reaction of carboxylic acids and alcohols. They exhibit inhibitory effects against fungal growth and can reduce downy mildew development within plants by migrating to distal tissues or communicating between neighbouring plants (Lazazzara et al., 2018). Among esters, (*Z*)-3-hexenyl benzoate was identified as a potential biomarker of resistance in previous research, as it showed higher up-regulation upon *P. viticola* inoculation in the resistant genotype F12P60 compared to Pinot noir (Ciubotaru et al., 2021). Similarly, the present study found a relatively high APA of (*E*)-2-hexenyl benzoate in *V. riparia*. Additionally, methyl salicylate was detected in the highest abundance in the resistant *V. riparia* genotype and the highly resistant Solaris cultivar, indicating its potent antifungal activity in these genotypes. In response to pathogen attack, salicylic acid and methyl salicylate induce systemic acquired resistance and hypersensitive response, triggering cell death (Pedrosa Gomes et al., 2014). Salicylic acid, a precursor of methyl salicylate, functions as a phytohormone that induces host resistance responses against biotrophic pathogens (Lefevere et al., 2020). Methyl salicylate, acting as a volatile defence phytohormone, systemically induces defence responses in distant plant parts and organs, and can also be perceived by neighbouring uninfected plants, triggering a resistance reaction in them as well (Hammerbacher et al., 2019). Previous studies have proposed methyl salicylate as a biomarker of downy mildew infection (Chalal et al., 2015) and as a potential biomarker of resistance to *P. viticola,* as it was found in higher concentrations in Bianca compared to Pinot noir (Ciubotaru et al., 2021). The present study supports these findings by showing a significantly higher APA of methyl salicylate in Solaris and *V. riparia* compared to *V. vinifera* varieties and highlights the importance of esters, particularly methyl salicylate, in the defence response against downy mildew as it could provide valuable insights obtained from further research in this field.

In experiments that included field trials and *in vitro* leaf disc bioassay, three Croatian native grapevine varieties, namely Malvazija istarska, Ranfol, and Teran (OIV class 5), proved to be more resistant to *P. viticola* compared to other evaluated *V. vinifera* varieties. Aiming to define VOCs that could be responsible for the defence mechanism of these three Croatian grapevine varieties, VOCs from OIV class 5 were compared with VOCs from OIV classes 7 and 9. Notably, several compounds, including geranylacetone, *ß*-ocimene, and (*E*)-2-hexen-1-ol, showed a higher absolute peak area (APA) in inoculated leaves compared to non-inoculated leaves across OIV classes 5, 7, and 9. In conclusion, these compounds exhibit the potential to serve as biomarkers of resistance to *P. viticola*. However, further *in vivo* experiments involving a larger number of genotypes are necessary to confirm their direct involvement in the plant-pathogen interaction. Detailed descriptions of each compound will be provided in the following paragraphs.

Geranylacetone belongs to the class of organic compounds known as acyclic monoterpenes. It is a component of essential oils in various plants, including *Nelumbo nucifera,* whose leaf extract possesses strong antioxidant properties (Huang et al., 2010). Up to now, geranylacetone was not recognized as a biomarker of grapevine resistance to *P. viticola*, although it was detected in higher concentrations in the leaves of the resistant pyramided genotype F12P60 compared to Pinot noir (Ciubotaru et al., 2021). In the present study, geranylacetone exhibited an increasing APA throughout the experiment, with significantly higher APAs throughout the sampling terms in the inoculated leaves of the native varieties Malvazija istarska and Ranfol, as well as the highly resistant Solaris cultivar, compared to non-inoculated leaves. These findings strongly suggest the involvement of geranylacetone in the defence mechanism of these cultivars, emphasizing its potential as a key component in the defence response against *P. viticola*.

Another possible indicator of resistance detected in the course of this study is *ß*-ocimene, a volatile organic compound that belongs to the class of monoterpenes. Previously, terpenes have often been recognized as compounds associated with the defence mechanism against downy mildew (Chitarrini et al., 2017; Lazazzara et al., 2018; Ricciardi et al., 2021). For example, allo-ocimene has been found to activate defence genes and induce resistance against *Botrytis cinerea* in *Arabidopsis thaliana* (Kishimoto et al., 2005). The functional properties of terpenes, such as farnesene, nerolidol, valencene, and ocimene have been examined and shown to be effective in counteracting *P. viticola*. Not only were they synthesized in higher amounts in the Mgaloblishvili resistant variety, but their antisporulant activity was also proved in *ad hoc* experimental inoculations in which disease severity and sporangia concentration were inhibited. Among these terpenes, ocimene was found to be the most effective (Ricciardi et al., 2021). In the present study, an induced accumulation of

ß-ocimene was observed in the inoculated leaves of the native variety Malvazija istarska during T_2 (48 hpi), whereas it was not detected in the control leaves. In *V. riparia*, *ß*-ocimene was detected in both inoculated and non-inoculated leaves, although its APAs were higher in inoculated leaves at each sampling term following inoculation. Taking into account the association between terpenes and increased resistance, future studies should prioritize the investigation of individual compounds within this group. By doing so, researchers could uncover further potential biomarkers of resistance, contributing to a deeper understanding of the defence mechanisms against *P. viticola* thus offering new possibilities in disease management.

Now turning to the third compound of interest, (*E*)-2-hexen-1-ol. As previously mentioned, Ciubotaru et al. (2021) proposed (*E*)-2-hexen-1-ol, a volatile alcohol, as a biomarker of resistance. The results of the present study support this proposal since (*E*)-2-hexen-1-ol was found in inoculated leaves of Teran and *V. riparia* in ascending APAs after *P. viticola* inoculation. Another related alcohol, (*Z*)-3-hexenol, was found in higher concentrations in resistant *V. labrusca and V. riparia* genotypes compared to susceptible *V. vinifera* varieties (Gruenwald et al., 2015). Although (*Z*)-3-hexenol has been detected in the leaves of other resistant genotypes, such as Bianca (Chitarrini et al., 2017; Ricciardi et al., 2021), Mgaloblishvili (Ricciardi et al., 2021), and resistant *Vitis* hybrids in previous studies (Chitarrini et al., 2020), its absence in the present study highlights the need for further investigations to specifically detect (*Z*)-3-hexenol, given its substantial role in the defence mechanism against *P. viticol*a.

In a shift of focus from indicators of resistance, this paragraph highlights indicators of *P. viticola* infection among the analysed secondary metabolites (phenolic and volatile organic compounds). These compounds, which are physiologically or metabolically non-essential, can serve as signalling or defence molecules (Pang et al., 2021). Within this context, specific compounds have been identified as indicators of *P. viticola* infection in the evaluated grapevine genotypes. Notably, the content of stilbene resveratrol-3-*O*-glucoside increased throughout the experiment which was more pronounced in inoculated leaves even 24 hours post-inoculation (hpi), regardless of the OIV class. Interestingly, this compound has been previously recognized as an indicator of *P. viticola* infection by other authors as well (Naidenov et al., 2010; Latouche et al., 2013). Similarly, sesquiterpenes, such as humulene, ylangene, and *α*-farnesene, showed an ascending APA throughout the experiment, indicating that their synthesis was upregulated in response to *P. viticola* infection, as observed in previous studies (Algarra Alarcon et al., 2015; Ciubotaru et al., 2021; Ricciardi et al., 2021). In summary, these findings could offer further potential for the development of disease management strategies by utilising these indicators as diagnostic markers.

It is essential to consider the diverse range of experiments conducted in this research, including (1) the preliminary experiment in field conditions, (2) leaf disc bioassay, (3) measurement of chlorophyll fluorescence and multispectral imaging, (4) analysis of phenolic and (5) volatile organic compounds. The findings were found to be consistent since the majority of the procedures yielded similar or identical results. In the field experiments conducted over two consecutive years (2018 and 2019), significant differences in downy mildew development were observed among the native grapevine varieties. Most notably, during the two years, the disease development was slower in Malvazija istarska, Ranfol, and Teran. Furthermore, the leaf disc bioassay classified the native varieties in the OIV resistance classes 1, 3, and 5. The *P. viticola* sporulation developed on the leaf discs of Malvazija istarska, Ranfol, and Teran covered the smallest surface among *V. vinifera* varieties, which placed them in the OIV class 5. Distinctive measurements of chlorophyll fluorescence and multispectral imaging parameters effectively differentiated OIV classes 1, 3, and 5. Moreover, the constitutive content of polyphenolic compounds and phenolic acids separated the OIV resistance classes 1, 3, and 5 into three groups, thus confirming the results obtained by the leaf disc bioassay. While the analysis of volatile organic compounds did not provide definitive results in terms of differentiating native varieties based on OIV classes, the APAs of specific sesquiterpenes effectively distinguished the highly resistant genotypes (OIV classes 7 and 9) from the *V. vinifera* varieties (OIV classes 1, 3, and 5).

The findings and procedures employed in this study offer potential benefits by addressing the challenge of predicting the susceptibility of different genotypes to *P. viticola* without the need for inoculating leaf discs or leaves. The first proposed model involves the use of chlorophyll fluorescence and multispectral imaging on non-inoculated leaf discs, while the second technique focuses on analysing polyphenolic compounds and phenolic acids in non-inoculated leaves. These procedures hold significant promise as they eliminate the requirement for plant material inoculation, thereby potentially expediting and facilitating breeding programs. This is particularly valuable considering that conventional breeding approaches for developing new grapevine varieties typically span a lengthy period of 25 to 30 years (Toepfer et al., 2011).

That being said, in order to ensure reliability and validity of the first model, it is important to conduct more extensive experiments on a broader range of genotypes. In the present study, the imaging was focused on the abaxial surfaces of the leaf discs, which consist of loosely arranged cells of spongy parenchyma. To accomplish a more thorough comprehension of the model, further experiments should include imaging of entire leaves including their adaxial sides, characterised by densely packed palisade parenchyma with high chlorophyll content (Buschmann, 2007). Moreover, other susceptible grapevine tissues should also be analysed

in a similar fashion. Following the validation of the proposed model, the next step would be to establish a large-scale data platform, where genotypes with known responses to downy mildew could be extensively imaged and stored, forming a comprehensive framework for connecting genotypes with their phenotypes. This data platform could then serve as a valuable resource for understanding the intricate relationship between genotypes and their disease response.

The second proposed method of prediction includes the analysis of polyphenolic and phenolic acids in non-inoculated leaves since it was found that their constitutive content affects the level of native varieties' susceptibility to *P. viticola*. More precisely, a higher abundance of certain compounds was detected for each OIV resistance class. For example, the content of flavonol glycosides, such as quercetin-3-*O*-glucoside, myricetin-3-*O*-glucoside, and kaempferol-3-*O*-glucoside, was higher in the leaves of less susceptible native varieties that belong to OIV class 5 compared to OIV classes 1 and 3. Therefore, it could be possible to predict the level of susceptibility of certain genotypes by performing the analysis of phenolic compounds in non-inoculated leaves.

To explore the potential of using these techniques, they could be employed for high-throughput phenotyping (screening) of seedlings resulting from breeding programmes aimed at developing genotypes with robust resistance to mildews. This approach would enable the early classification of seedlings into the appropriate OIV classes, allowing for the timely removal of susceptible ones during the breeding process. Additionally, these techniques hold promise for phenotyping existing grapevine varieties and commercial vineyards where variations in susceptibility to downy mildew have not yet been clearly defined. Such characterization would be particularly valuable in the era of precision viticulture and sustainable agricultural production, as the trends in viticulture lean towards a more individualised approach to different grapevine varieties (Ferro and Catania, 2023). By implementing these techniques, a more targeted and efficient management of downy mildew can be achieved, contributing to the overall vitality and productivity of vineyards.

In conclusion, to achieve more precise and applicable results from the analyses of volatile compounds and to further understand their potential significance in genotype distinctiveness among the OIV resistance classes, additional *in vivo* tests on non-detached leaves should be conducted. Plant volatile organic compounds are highly influenced by external stimuli (Niederbacher et al., 2015), thus reducing the number of procedural steps involved in their analysis is crucial. More precisely, according to Ciubotaru et al. (2021), plants should be artificially inoculated with spores of the pathogen in the greenhouse and kept under controlled and optimal conditions for downy mildew development until sampling. In the

present study, however, the leaves were detached from shoots and transferred from the greenhouse to the laboratory conditions, exposing them to stress even before inoculation with *P. viticola* suspension. With this in mind, to ensure the reliability of future analyses of volatile compounds, a more streamlined and less stress-inducing approach is highly recommended.

Moreover, in addition to the analysis of secondary metabolites, recent studies have utilized noteworthy and novel approaches, indicating a growing interest in investigating grapevine-pathogen interactions and understanding the underlying mechanisms involved in the grapevine's response to *P. viticola*. These approaches include analysing transcriptomic and methylation processes of susceptible and tolerant grapevine genotypes following *P. viticola* infection (Azevedo et al., 2022), defining the differences in epigenetic regulation between the incompatible and compatible interaction (Pereira et al., 2022), describing the stimulation of *P. viticola* effector to the grapevine immunity response (Fu et al., 2023), and identifying the *in-planta* proteome of *P. viticola* during infection of a susceptible and a *Rpv3*-mediated resistance grapevine variety (Figueiredo et al., 2022).

Refocusing on the approaches and techniques employed in this study, the results of the experiments conducted in this research including the preliminary experiment in field conditions, leaf disc bioassay, measurement of chlorophyll fluorescence and multispectral imaging, analysis of phenolic and volatile organic compounds, revealed significant differences between the genotypes evaluated.

Based on the preliminary experiment in field conditions, the leaves of some native varieties, such as Malvazija istarska, Teran, and Ranfol among 51 evaluated varieties, longer remained healthy under disease pressure compared to other ones.

The leaf disc bioassay and the OIV 452-1 descriptor also distinguished native varieties placing them in the resistance classes 1, 3, and 5, which mostly confirmed results gained in the preliminary experiment. This proves that the leaf disc bioassay is a convenient method by which it is possible to predict genotype’s level of susceptibility to *P. viticola* in the vineyard.

Furthermore, to assess stress levels among the evaluated genotypes, measurements of chlorophyll fluorescence and multispectral imaging were conducted. Based on these results, parameters such as F_v/F_m, $F_{q'}/F_{m'}$ and ETR were the most suitable to distinguish non-inoculated and inoculated leaf discs. These results indicate that the impairment of photosynthetic activity of inoculated plant material is one of the initial symptoms of *P. viticola* infection. Moreover, the $F_{q'}/F_{m'}$ and qP parameters differentiated the classes of resistance even before the appearance of visible symptoms of disease, offering potential for early

selection of differently susceptible genotypes. In another study by Oerke et al. (2022), it was found that the resistance of Regent and Solaris was incomplete, and that the characterization and differentiation of the resistance reaction of grapevine varieties can be assessed at the tissue level by hyperspectral imaging, thus confirming the results of the present study.

Regarding secondary metabolites, which function as defence or signalling molecules, the constitutive content and composition of phenolic compounds separated resistance classes 1, 3, and 5, i.e., Croatian native grapevine varieties into three groups. Thus, the analysis of phenolic compounds during preinfectional stage of leaves could serve as an indicator of grapevine varieties' susceptibility to *P. viticola*.

On the other hand, the analysis of volatile organic compounds did not effectively distinguish native varieties into the OIV resistance classes, indicating the need for improvements in this procedure. However, a common feature was found among resistant control genotypes (*V. riparia*, Solaris) and less susceptible native varieties (Malvazija istarska, Teran, Ranfol). Specifically, an increase in the content of geranylacetone, *β*-ocimene, and (*E*)-2-hexen-1-ol was detected upon inoculation, and this increase was more pronounced in inoculated samples, suggesting their potential as resistance indicators to *P. viticol*a.

4. CONCLUSIONS

Based on the appointed hypotheses and conducted research, it is possible to draw the following conclusions:

- According to the sporulation of the downy mildew pathogen on the leaf discs of the native and control genotypes, all native varieties were classified into resistance classes 1, 3 and 5 based on the International Organisation of Vine and Wine descriptor 452-1, thus confirming H1.
- The parameters F_v/F_m and $F_{q'}/F_{m'}$ distinguished non-inoculated and inoculated samples 24 hours after inoculation, while the parameter ETR distinguished them 72 hours after inoculation, i.e., before the development of visible disease symptoms. The parameters $F_{q'}/F_{m'}$ and qP distinguished varieties of different resistance classes, thus confirming H2.
- The classification of native varieties into the corresponding resistance classes depends on the constitutive composition and content of polyphenolic compounds and phenolic acids in the leaves. Primarily, flavonol glycosides were found to be responsible for the lower susceptibility of native varieties. The content of resveratrol-3-*O*-glucoside distinguished the non-inoculated and inoculated leaves in each term after the inoculation. Certain volatile organic compounds, such as humulene, ylangene, and *α*-farnesene helped distinguish the non-inoculated and inoculated leaves. The specific reaction of volatile organic compounds in the leaves of native varieties of different resistance classes was not determined. The response of less susceptible native varieties and resistant control genotypes, on the other hand, was associated with an increase in the absolute peak area of geranylacetone, *β*-ocimene and (*E*)-2-hexen-1-ol. Based on these results, H3 is partially confirmed.

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AUTHOR'S BIOGRAPHY

Petra Štambuk was born in Zagreb on August 7, 1990. She graduated from high school in Petrinja and enrolled in undergraduate studies in Horticulture at the Faculty of Agriculture, University of Zagreb in 2009. She graduated in 2012 with a bachelor's degree in horticulture when she enrolled in graduate studies in Horticulture, taking the Viticulture and Enology course, which she finished in 2015.

She began working at the Department for Viticulture and Enology at the University of Zagreb Faculty of Agriculture in 2015. In 2017, she worked as an expert associate at the Ministry of Agriculture. She has been working as a PhD student since 2018 as part of the Centre of Excellence's Biodiversity and Molecular Plant Breeding project, which is overseen by professor Zlatko Šatović, PhD. At the Faculty of Agriculture, she enrolled in postgraduate doctoral studies in Agricultural Sciences under the supervision of professor Jasminka Karoglan Kontić, PhD, and Ivana Tomaz, PhD. She has been employed at the Department of Viticulture and Enology at the Faculty of Agriculture as an expert associate since 2021.

She participated in expert projects including "The Clonal Selection of Kujundžuša and Trnjak", "The Introduction and Characterization of New Resistant Grapevine Varieties for Organic Cultivation in the Conditions of Zagreb County" and "The Introduction of Grapevine Varieties Resistant to Fungal Diseases Suitable for the Kutjevo Wine-growing Hill". She co-authored or was an author of twelve scientific papers that are listed in the Current Contents database, and she also wrote one of the book's chapters. She actively participated in six international conferences.

List of publications

Scientific and review papers

Štambuk P., Šikuten I., Preiner D., Maletić E., Karoglan Kontić J., Tomaz I. (2023). Croatian Native Grapevine Varieties' VOCs Responses upon *Plasmopara viticola* Inoculation. Plants 12(2), 404.

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APPENDICES

Appendix 1. Scientific paper: Štambuk P., Šikuten I., Preiner D., Nimac A., Lazarević B., Marković Z., Maletić E., Karoglan Kontić J., Tomaz I. (2021). Screening of Croatian Native Grapevine Varieties for Susceptibility to *Plasmopara viticola* Using Leaf Disc Bioassay, Chlorophyll Fluorescence and Multispectral Imaging. Plants 10 (4): 661. doi: 10.3390/plants10040661

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Screening of Croatian Native Grapevine Varieties for Susceptibility to *Plasmopara viticola* Using Leaf Disc Bioassay, Chlorophyll Fluorescence and Multispectral Imaging

Petra Štambuk [1,2], Iva Šikuten [1,2], Darko Preiner [1,2,*], Ana Nimac [2,3], Boris Lazarević [2,4], Zvjezdana Marković [1,2], Edi Maletić [1,2], Jasminka Karoglan Kontić [1,2] and Ivana Tomaz [1,2]

[1] Department of Viticulture and Enology, Faculty of Agriculture, University of Zagreb, Svetošimunska Cesta 25, 10000 Zagreb, Croatia; pstambuk@agr.hr (P.Š.); isikuten@agr.hr (I.Š.); zmarkovic@agr.hr (Z.M.); emaletic@agr.hr (E.M.); jkkontic@agr.hr (J.K.K.); itomaz@agr.hr (I.T.)
[2] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska Cesta 25, 10000 Zagreb, Croatia; animac@agr.hr (A.N.); blazarevic@agr.hr (B.L.)
[3] Department of Seed Science and Technology, Faculty of Agriculture, University of Zagreb, Svetošimunska Cesta 25, 10000 Zagreb, Croatia
[4] Department of Plant Nutrition, Faculty of Agriculture, University of Zagreb, Svetošimunska Cesta 25, 10000 Zagreb, Croatia
* Correspondence: dpreiner@agr.hr

Abstract: In the era of sustainable grapevine production, there is a growing demand to define differences between *Vitis vinifera* varieties in susceptibility to downy mildew. Croatia, as a country with a long tradition of grapevine cultivation, preserves a large number of native grapevine varieties. A leaf disc bioassay has been conducted on 25 of them to define their response to downy mildew, according to the International Organisation of Vine and Wine (OIV) descriptor 452-1, together with the stress response of the leaf discs using chlorophyll fluorescence and multispectral imaging with 11 parameters included. Time points of measurement were as follows: before treatment (T_0), one day post-inoculation (dpi) (T_1), two dpi (T_2), three dpi (T_3), four dpi (T_4), six dpi (T_5), and eight dpi (T_6). Visible changes in form of developed *Plasmopara viticola* (*P. viticola*) sporulation were evaluated on the seventh day upon inoculation. Results show that methods applied here distinguish varieties of different responses to downy mildew. Based on the results obtained, a phenotyping model in the absence of the pathogen is proposed, which is required to confirm by conducting more extensive research.

Keywords: *Vitis vinifera* L.; downy mildew; biotic stress; chlorophyll fluorescence; spectral indices; imaging methodology; phenotyping model

Citation: Štambuk, P.; Šikuten, I.; Preiner, D.; Nimac, A.; Lazarević, B.; Marković, Z.; Maletić, E.; Kontić, J.K.; Tomaz, I. Screening of Croatian Native Grapevine Varieties for Susceptibility to *Plasmopara viticola* Using Leaf Disc Bioassay, Chlorophyll Fluorescence and Multispectral Imaging. *Plants* **2021**, *10*, 661. https://doi.org/10.3390/plants10040661

Academic Editor: Laura Varone

Received: 9 February 2021
Accepted: 25 March 2021
Published: 30 March 2021

1. Introduction

Ever since the troubling 19th century for the European viticulture production when powdery mildew (*Erysiphe necator*), downy mildew (*Plasmopara viticola*), and phylloxera (*Daktulosphaira vitifoliae*) were introduced from the American continent, winegrowers have been seeking an efficient method of their suppression [1]. While phylloxera was solved by grafting the traditional European grapevine (*Vitis vinifera* L.) varieties on the American vine as a rootstock, which is one of the most successful biological control of this pest spreading, mildews have still been causing problems in all grapevine growing regions around the world, especially with temperate-humid climates [2]. After discovering the fungicide activity of sulphur and copper, and later other active substances, their application became widely used in enormous amounts whose impact on the environment, animal, and human health is harmful [3].

Downy mildew is one of the major grapevine diseases which is caused by an obligate biotrophic oomycete *Plasmopara viticola*, meaning that it uses water and nutrients from its living host plant [4]. The nature of this microorganism is polycyclic and demands temperature in the range from 10 to 29 °C (optimum from 20 to 22 °C) and high humidity (>90%). During winter, it survives in decaying leaves and twigs on the vineyard floor in the form of thick-walled oospores [5]. In spring, when the temperature rises and rains more often, a sporangium is produced from the oospores. Essentially in a drop, two-flagellated zoospores are released from sporangium. They encyst near stoma and the germ tube penetrates inside a green tissue. The mycelium is developed intercellularly in the mesophyll of the grapevine leaves with globose haustoria that invade the cells as the source of *P. viticola* nutrients. When the leaf is infected, yellow-brownish lesions ("oil spots") develop on its adaxial surface, while sporangia are produced on its abaxial surface and other green tissues such as inflorescences, berries, and tendrils [6]. Sporangia, looking similar to a white cotton cover, are dispersed by wind or rain splash and, as such, are a source of secondary infection cycles. According to Gobbin et al. 2005 [7], there is a continuous input of new genotypes into an epidemic.

During the last century, a lot of efforts have been made in breeding resistant grape varieties by interspecific hybridisation. As a result, cultivars such as Regent in Germany and Bianca in Hungary are auspiciously introduced into the market, together with few dozen newly bred cultivars [8]. Production of resistant cultivars during the last 20 years has been supported with marker-assisted selection (MAS) and carefully designed phenotyping methods. They allow the creation of varieties with higher and more durable resistance [9].

Resistance is a quantitative trait, and quantitative trait loci (QTLs) of resistance to mildews are generally found in non-vinifera germplasm. Loci of resistance to downy mildew are found in *Muscadinia rotundifolia* (*Rpv1*, *Rpv2*) [10], *Vitis rupestris* (*Rpv3*) [11], *Vitis riparia* (*Rpv5*, *Rpv6* [12], *Rpv9* [13]), and *Vitis amurensis* (*Rpv8* [14], *Rpv10* [15], and *Rpv12* [16]). *Muscadinia rotundifolia* is also resistant to powdery mildew, containing loci *Run1* [17,18] and *Run2* [19]. However, locus containing resistance to powdery mildew, specifically *Ren1*, is found in two cultivars originating from central Asia, Kishmish vatkana, and Dzhandzhal kara (*V. vinifera*) [20]. Cultivars Regent and Solaris are highly resistant to downy mildew, and their typical response to the disease is small brownish spots (necrosis formation). Nevertheless, sporulation emerging from the discoloured tissue indicates that not all cells have undergone programmed cell death [21,22]. The main morphological barrier of *V. riparia* to *P. viticola* attack is the presence of the inner cuticular rim which is a constitutive trait independent of infection [23]. While North American and Asian *Vitis* species develop necrotic spots after *P. viticola* infection, they are not observed in Georgian *Vitis* germplasm, meaning that their defence mechanisms are different [24]. Recently, *V. vinifera* varieties are of great interest to research due to their high genetic variability, local/regional importance, and lacking genetic background with undesirable features. Moreover, differences in susceptibility to downy mildew are found in Spanish [25] and Georgian [26,27] collections between *V. vinifera* varieties.

Since leaves are the pioneers in providing the first visual symptoms of the downy mildew disease, phenotyping methods on leaf discs that are inoculated and maintained in controlled conditions have been widely applied among plant pathologists, breeders, and geneticists who are willing to obtain differences between genotypes regarding their downy mildew susceptibility [28,29]. Leaf disc bioassay is based on the International Organisation of Vine and Wine (OIV) descriptor 452-1 (Leaf: degree of resistance to *Plasmopara* (leaf disc test)) [30]. Leaf disc test is widely accepted and used by many authors [22,31–33] whose aim is to distinguish levels of susceptibility to downy mildew between different varieties. When this method is properly performed, it is reliable and useful for predicting each variety's susceptibility to downy mildew in field conditions [34].

Apart from visible detection of downy mildew infection, there are sophisticated methods that measure plant's stress levels in form of photosynthesis (in)efficiency. Novel

phenotyping methods which include chlorophyll fluorescence and multispectral imaging were previously used for quantification of different plant diseases such as *Blumeria graminis* in barley [35], *Cercospora beticola* in sugar beet [36], and *Puccinia triticina* in wheat [37]. Screening for susceptibility to *P. viticola* among *V. vinifera* varieties was also performed by these methods [38,39]. Recently, alterations of primary metabolism induced by pathogenesis have been the focus of studies. For this purpose, chlorophyll fluorescence imaging, as a non-invasive method, is of principal value since it measures both spatial and temporal changes in photosynthetic processes localised with high precision within plant tissues [40,41]. Generally, downy mildew infection costs energy either for the induction of plant defences or the destruction of carbohydrates. Yellow-brownish lesions (chlorosis) of grapevine photosynthesizing tissues (e.g., leaves) implicate that infection leads to the destruction of chlorophyll and subsequent blockage of CO_2 fixation processes [41].

Chlorophyll fluorescence measurements are based on three possible ways (outcomes) of photon energy transfer—thermal dissipation (heat), photochemistry, and chlorophyll fluorescence emission. When excitations are neither lost as heat nor lead to photochemistry, they are re-emitted as light in a process called chlorophyll α fluorescence [42]. An increase in chlorophyll fluorescence thus implies a decrease in photosynthesis and/or thermal dissipation, and vice versa [43]. It can be used for the early detection of biotic stress, even before the manifestation of visible downy mildew symptoms [38]. As an early answer to downy mildew infection, the plant's primary and secondary metabolism can be affected due to the initiation of plant defence [44].

In plant phenotyping, the application of imaging spectroscopy came from research on the remote sensing of vegetation [45]. Spectral reflectance information of leaves or canopies is used to quantify vegetation indices, which are ratios and differences between spectral reflectance data at given wavelengths (e.g., near-infrared wavelengths (700–1200 nm)) [46]. These indices have been used for fast, non-destructive measurements of green biomass, chlorophyll content, leave and canopy senescence, and plant water status, which can be applied in both field research and breeding programs for large-scale phenotyping [45].

Since Croatia has a long tradition of cultivating grapevine in its geographically and climatically different regions, at least 95 are considered native [47,48] whose susceptibility to main diseases is necessary to define in order to describe their complete biological and economical potential. For this purpose, a study concerning differences in susceptibility to downy mildew was conducted on 25 native grapevine varieties by applying a leaf disc bioassay with chlorophyll fluorescence and multispectral imaging. The aim of this study was (i) to assess the susceptibility among *V. vinifera* varieties to downy mildew by applying leaf disc test, (ii) to examine whether chlorophyll fluorescence and multispectral imaging of leaf discs are suitable methods for distinguishing genotypes of different susceptibility to downy mildew, and (iii) to test the relationship between distinctive OIV classes and their fluorescence and multispectral traits in the absence of the pathogen.

2. Results

2.1. Differences in Chlorophyll Fluorescence and Multispectral Imaging Responses between Infected and Non-Infected Leaf Discs

Chlorophyll fluorescence and multispectral imaging were performed in seven terms, namely, before treatment (T_0), one day post-inoculation (dpi) (T_1), two dpi (T_2), three dpi (T_3), four dpi (T_4), six dpi (T_5) and eight dpi (T_6). The data presented in Figures 1 and 2 are the average of 30 genotypes included in this research. Visible symptoms in the form of sporulation appeared on the sixth and seventh day after inoculation on the most of evaluated *V. vinifera* varieties and control genotypes Solaris and Regent, respectively. Solaris developed necrotic spots on the fourth day after inoculation, while *V. riparia* showed no visible changes. Imaging started with non-infected grapevine leaf discs and terminated after downy mildew sporulation developed. Evaluated fluorescence parameters included

maximum quantum yield of photosystem II (PSII) (F_v/F_m), effective quantum yield of photosystem II (PSII) electron transport ($F_{q'}/F_{m'}$), electron transport rate (ETR), non-photochemical quenching (NPQ), and photochemical quenching (qP), while the focus of multispectral imaging was on colour appearance parameter (Hue), far-red reflectance (FarRed), near-infrared reflectance (NIR), chlorophyll index (CHI), anthocyanin index (ARI) and normalised difference vegetation index (NDVI). Among these 11 parameters, most were significantly different between non-infected and infected leaf discs in at least two terms of measurement (Figures 1 and 2). However, no significant difference was found for NPQ between these two variants of leaf discs (Figure 1d). A detailed description for each parameter follows below.

F_v/F_m was significantly different in all terms of measurement, except in the pre-infection stage (T_0) (Figure 1a). From T_1 to T_6, non-infected leaf discs reached higher values, compared to infected ones, which is expected since decreasing values of this parameter indicate plant stress [49].

The values of $F_{q'}/F_{m'}$ showed to be distinctive in T_1, T_3, T_5, and T_6, with lower values for infected leaf discs (Figure 1b). Similar is observed for ETR values, although this parameter was not significant in T_1 (Figure 1c). Their overall change throughout the period of measurement slightly decreased.

The trend of NPQ (Figure 1d) gradually fell from T_1 to T_6, while total values of qP decreased during the experiment period. Slightly lower qP values were observed for infected leaf discs, compared to non-infected ones in all terms, although this difference was significant only in the later stages of infection (T_5 and T_6) (Figure 1e). In T_0, values for both variants of leaf discs were 0.5, while the values for infected leaf discs were reduced by more than a half during six and eight days after inoculation.

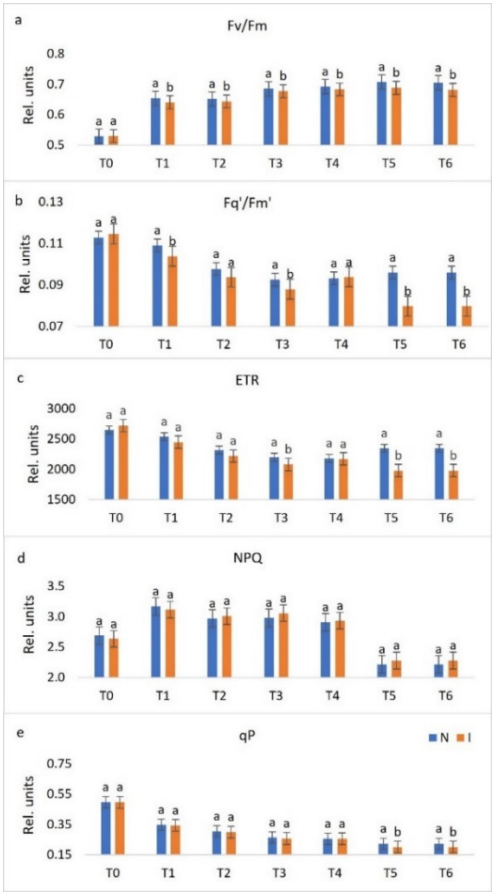

Figure 1. Changes between non-infected (N) and infected (I) leaf discs throughout seven terms (before treatment (T_0), one day post-inoculation (dpi) (T_1), two dpi (T_2), three dpi (T_3), four dpi (T_4), six dpi (T_5) and eight dpi (T_6)) in chlorophyll fluorescence parameters (the average of 30 genotypes).

Differences between the means were evaluated by Duncan's multiple range test at a confidence level of 95% ($p < 0.05$). Means with the same letter are not significantly different. Sub-figures depict parameters as follows: (**a**) Fv/Fm, (**b**) Fq'/Fm', (**c**) ETR, (**d**) NPQ, and (**e**) qP.

Multispectral imaging parameters Hue (Figure 2a) and FarRed (Figure 2b) were not significantly different during the final two measurements between non-infected and infected leaf discs, meaning that by these two parameters, it is not possible to distinguish between non-infected and infected leaf discs from the occurrence of visible symptoms. However, from T_0 until T_4, significantly higher Hue values were observed for infected leaf discs, while the same is true for non-infected leaf discs as far as FarRed values are concerned.

NIR (Figure 2c), ARI (Figure 2e) and NDVI (Figure 2f) values were statistically different for infected and non-infected leaf discs throughout the whole experiment with higher values for non-infected ones. The values of CHI (Figure 2d) were statistically different during the final two terms, while lower values were observed for infected leaf discs, compared to non-infected ones during this final stage of inoculation. The differences between non-infected and infected leaf discs of parameters NIR and ARI remained almost the same throughout the time of the experiment, while NDVI differences fluctuated from T_0 until T_4 and were the highest in the last two terms. Unlike the visible changes that can be observed six or seven days upon inoculation in the form of *P. viticola* sporulation, through fluorescence (F_v/F_m) and multispectral (CHI and NDVI) channels, it is possible to differentiate non-inoculated from inoculated leaf discs at 4 dpi (Figure 3).

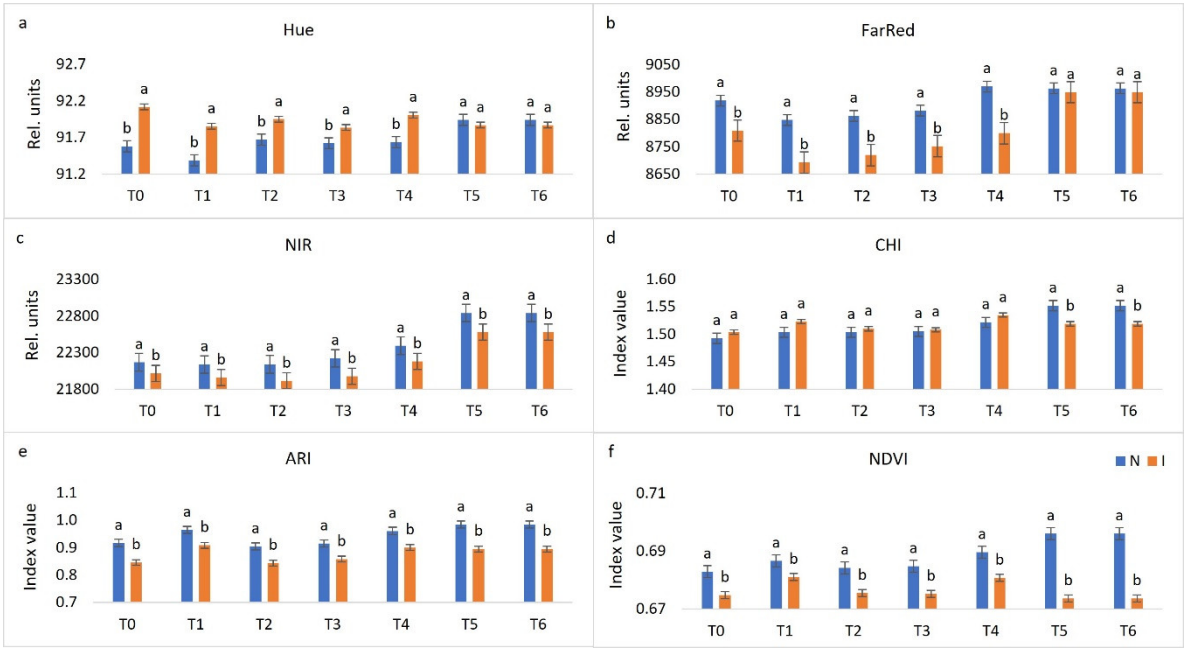

Figure 2. Changes between non-infected (N) and infected (I) leaf discs throughout seven terms (before treatment (T_0), one day post-inoculation (dpi) (T_1), two dpi (T_2), three dpi (T_3), four dpi (T_4), six dpi (T_5) and eight dpi (T_6)) in multispectral parameters (the average of 30 genotypes). Differences between the means were evaluated by Duncan's multiple range test at a confidence level of 95% ($p < 0.05$). Means with the same letter are not significantly different. Sub-figures depict parameters as follows: (**a**) Hue, (**b**) FarRed, (**c**) NIR, (**d**) CHI, (**e**) ARI, and (**f**) NDVI.

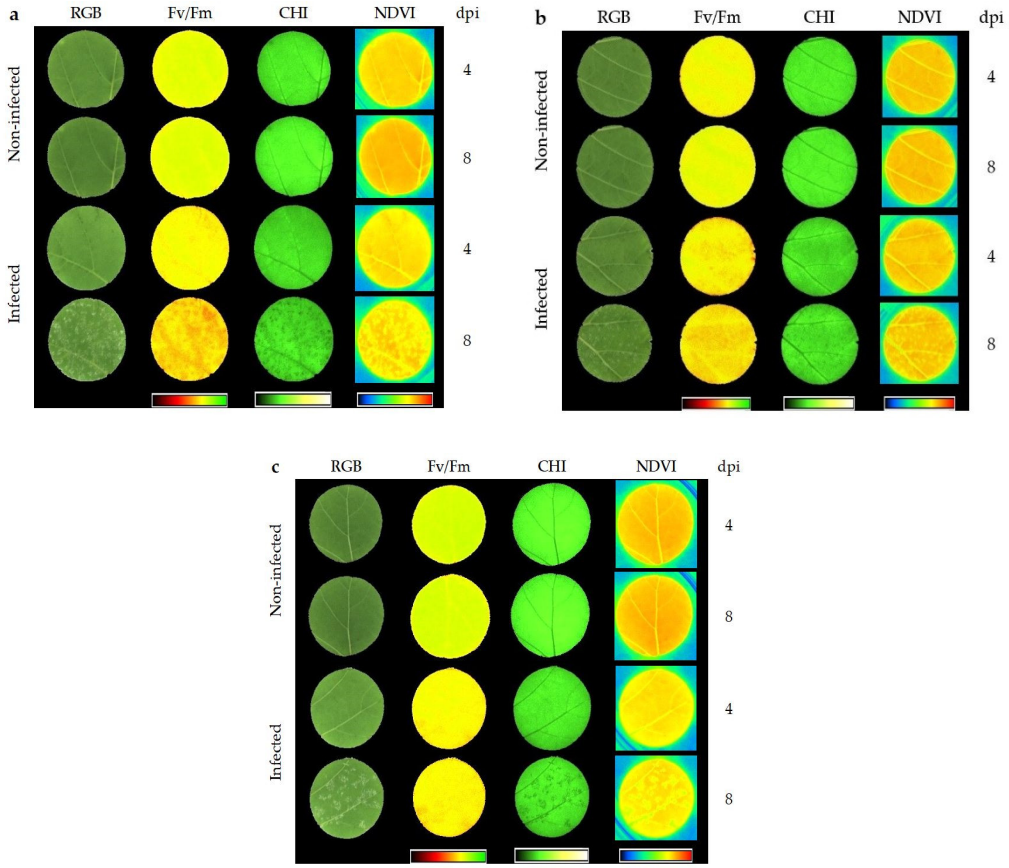

Figure 3. RGB (visual impression), F_v/F_m, chlorophyll index (CHI), and normalised difference vegetation index (NDVI) images of (**a**) Lasina (OIV (International Organisation of Vine and Wine) 1), (**b**) Malvasija dubrovačka (OIV 3), and (**c**) Malvazija istarska (OIV 5) taken at four (T_4) and eight (T_6) dpi.

2.2. Differences in P. viticola Sporulation on Leaf Discs among Genotypes

According to the OIV leaf disc test, on all susceptible *V. vinifera* varieties, *P. viticola* sporulation was developed as expected. However, significant differences in sporulation density and covered surfaces were determined between different varieties. Thus, they were grouped in separated OIV classes, as shown in Table 1, while examples of different visible phenotypic reactions and corresponding OIV classes are presented in Table 2.

Table 1. Genotypes and their corresponding OIV classes 1, 3, 5, 7, and 9 from the most abundant to the totally absent sporulation.

Genotype	OIV Class
Babić	3
Belina starohrvatska	1
Belina svetokriška	5
Cabernet Sauvignon	5
Chardonnay	3
Crljenak viški	3
Debit	1
Divjaka	5
Dišeća ranina	5
Grk	1
Kadarun	5
Kraljevina	3
Lasina	1
Malvasija dubrovačka	3
Malvazija istarska	5

	Mladenka	3
	Moslavac	1
	Ninčuša	3
	Plavac mali	1
	Plavčina	1
	Plavina	1
	Pošip	3
	Regent	7
	Solaris	7
	Škrlet	3
	Teran	5
	Tribidrag	3
	V. riparia	9
	Žlahtina	5
	Žumić	5

Table 2. The OIV 452-1 descriptor with images of visible differences between genotypes.

Representative Leaf Disc					
Genotype	Plavac mali	Babić	Malvazija istarska	Solaris	*V. riparia*
OIV class	1	3	5	7	9
Surface covered with sporulation (%)	61–100	41–60	21–40	1–20	0
Number of genotypes belonging to the class	8	10	9	2	1
Distribution of evaluated genotypes (%)	27	33	30	7	3

2.3. Differences in Chlorophyll Fluorescence and Multispectral Imaging Responses between Diverse OIV Classes

The distinctiveness of OIV classes was shown to be significant in specific terms and by specific parameters. An overall slight increase of F_v/F_m (Figure 4a) values was noticed through the terms. In T_2, T_3, T_4, and T_5 a downward trend is observed from the OIV most susceptible group of genotypes to the resistant group. The highest distinctiveness of the OIV classes was found four days upon inoculation (T_4) when no significant difference was found only between classes 1 and 3.

Another important indicator of a plant's biotic stress is $F_{q'}/F_{m'}$ (Figure 4b). In contrast to F_v/F_m, an overall slight reduction of average $F_{q'}/F_{m'}$ values throughout the measurement period was observed. The OIV classes 9 (*V. riparia*) and 7 (Regent and Solaris) had the highest values in comparison to other OIV classes in T_1, T_2, T_3, and T_4. Similar to F_v/F_m responses in T_4, the separation of different OIV classes was highly distinctive, although classes 3 and 5 were not significantly different in this term. The least distinctiveness was observed in T_5 and T_6 with two and one significantly different OIV classes, respectively.

Similar results were obtained for $F_{q'}/F_{m'}$ (Figure 4b) and ETR (Figure 4c). The highest and significantly different ETR values were observed for the OIV class 9 in each term with the exception of the last term when it was not statistically different from the OIV classes 3 and 7. In T_2 and T_3, the OIV classes 1 and 3 were not statistically different, while in T_1, the same was true for the OIV classes 3 and 5. At later stages (T_5 and T_6) of infection, averaged

values of parameters $F_{q'}/F_{m'}$ (Figure 4b) and ETR (Figure 4c) among all OIV classes declined.

Differences in NPQ among OIV groups through seven terms are depicted in Figure 4d. Significantly the lowest values were ascribed to the OIV class 9, while its neighbouring class 7 had the highest values in each term, except T_1 and T_2. A gradual decline can be observed in average values for each term from one day upon inoculation (T_1) to eight days upon inoculation (T_6). In T_4, there was no statistical difference between susceptible OIV classes 1, 3, and 5. It is interesting to notice the similarity of the bar charts depicting the pre-infection stage (T_0) and the final stage (T_6). In both terms, each OIV class is significantly different from the others. Increasing values can be found from class 1 to class 7, while class 9 had the lowest value as abovementioned.

As an indicator of opened PSII reaction centres [50], qP decreased throughout the period of imaging (Figure 4e). The same trend was noticed in T_1 and T_2 with no significant difference between classes 1 and 3, whereas increasing and significantly different values are observed from classes 5 to 9. In T_6, classes 3, 5, and 9 were not significantly different.

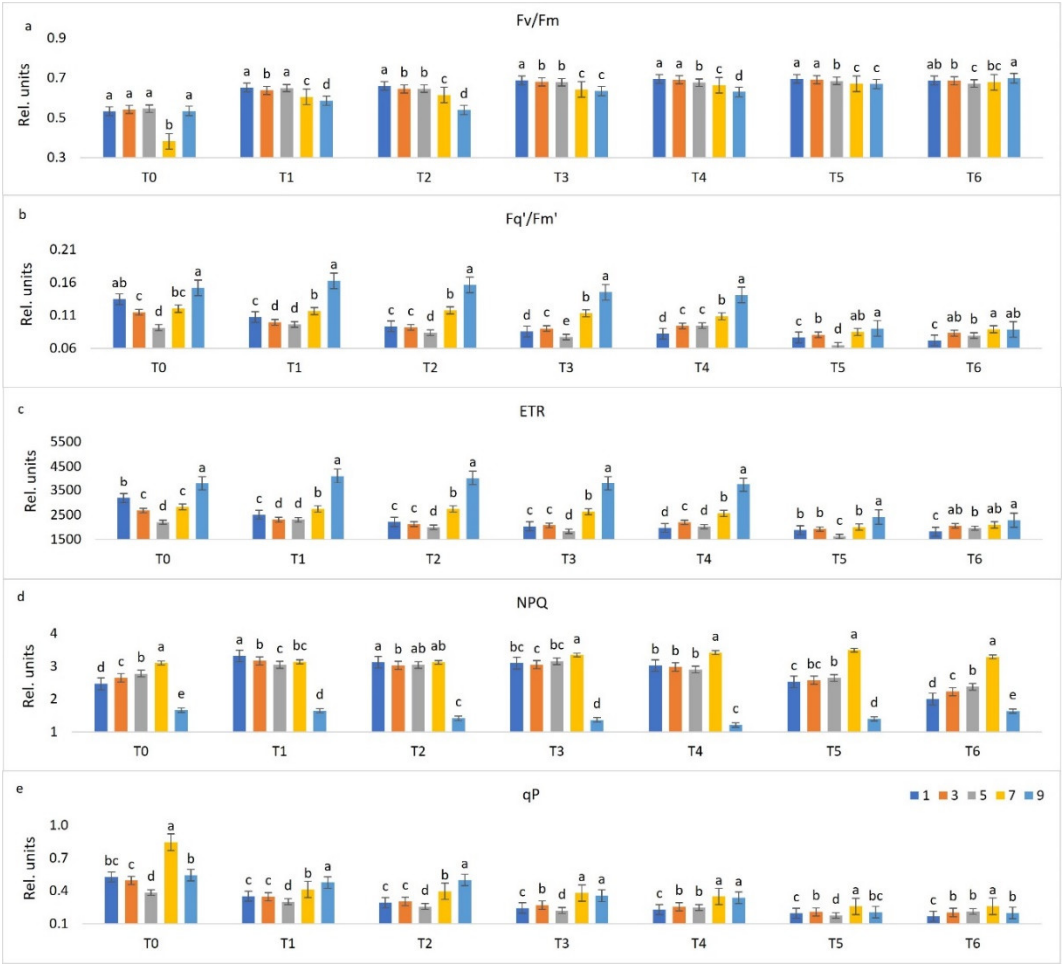

Figure 4. Changes between distinctive OIV classes (1 – most susceptible; 9 – resistant) throughout seven terms (before treatment (T_0), one day post-inoculation (dpi) (T_1), two dpi (T_2), three dpi (T_3), four dpi (T_4), six dpi (T_5) and eight dpi (T_6)) in chlorophyll fluorescence parameters. Differences between the means were evaluated by Duncan's multiple range test at a confidence level of 95% ($p < 0.05$). Means with the same letter are not significantly different. Sub-figures depict parameters as follows: (**a**) Fv/Fm, (**b**) Fq'/Fm', (**c**) ETR, (**d**) NPQ, and (**e**) qP.

Hue values fluctuated between the terms of imaging, while the highest overall value of all OIV classes was observed in T_1 (data not shown). In each term, class 7 had the lowest values, while there were no considerable differences between other classes (Figure 5a).

An upward trend from T_1 to T_6 is observed for far-red fluorescence values of all infected leaf discs (data not shown). Moreover, a slightly increasing trend was observed in T_0, T_1, T_2, and T_6 from class 5 to class 9 (Figure 5b). Significantly the lowest values are measured for class 3 in each term.

The values of NIR (Figure 5c) were higher in the later stages of downy mildew development, compared to the early stages. In all terms, class 9 reached the highest values, while its neighbouring class 7 had the lowest values. Classes susceptible to downy mildew (1, 3, and 5) showed similar values of this parameter.

The values of CHI (Figure 5d) and ARI (Figure 5e) showed no significant differences in all terms between classes 5 and 7, except for ARI in T4. Both indices reached their highest values in T_4 and T_5. Generally, similar bar charts are obtained for these indices, and for NDVI (Figure 5f), with the highest values for classes 3 and 9, and the lowest for 5 and 7 in all terms of imaging. For NDVI, there was no statistical difference between classes 3 and 9 in all terms, except in the final term, in which class 9 reached statistically higher values.

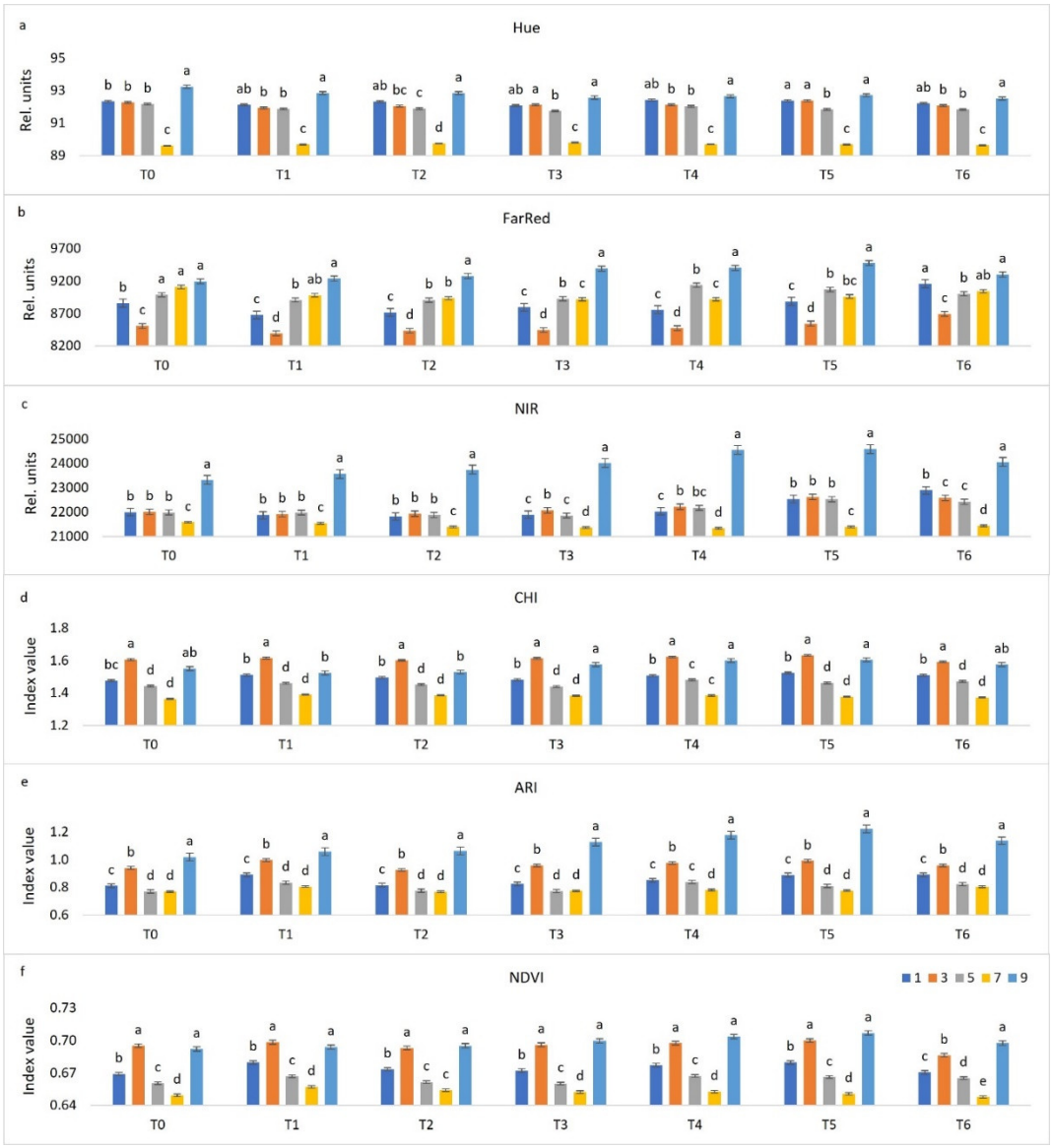

Figure 5. Changes between distinctive OIV classes (1 – most susceptible; 9 – resistant) throughout seven terms (before treatment (T_0), one day post-inoculation (dpi) (T_1), two dpi (T_2), three dpi (T_3), four dpi (T_4), six dpi (T_5) and eight dpi (T_6)) in multispectral parameters. Differences between the means were evaluated by Duncan's multiple range test at a confidence level of 95% ($p < 0.05$). Means with the same letter are not significantly different. Sub-figures depict parameters as follows: (**a**) Hue, (**b**) FarRed, (**c**) NIR, (**d**) CHI, (**e**) ARI, and (**f**) NDVI.

More pronounced differences between OIV classes can be observed by parameters of chlorophyll fluorescence (F_v/F_m, $F_{q'}/F_{m'}$, ETR, NPQ, and qP) during the first five terms of imaging, while at the end of the trial, the values for most of them decreased and became more unified. However, the parameters of multispectral analysis (Hue, FarRed, NIR, CHI, ARI, NDVI) mostly followed the same pattern from T_0 until T_6, which led to the conclusion to propose a phenotyping model for differentiation of OIV classes in the absence of *P. viticola* using the results obtained in T_0.

2.4. Phenotyping Model

One of the objectives of the present study was to find a reliable phenotyping model of grapevines' susceptibility to downy mildew. After comparing the final OIV grouping made by different sporulation on leaf discs with responses of chlorophyll fluorescence and multispectral imaging in the pre-infection term (T_0), and their interactions, a relationship was determined using the logistic regression. The training set of this model consisted of 19 genotypes and 11 measured parameters described in Table 4. This set included 15 Croatian native varieties and two susceptible international *V. vinifera* L. varieties (Cabernet Sauvignon, Chardonnay) to model three susceptible OIV classes (1, 3, and 5), while Solaris and Regent were used to form the OIV class 7 that represents almost completely resistant genotypes. Three genotypes (Cabernet Sauvignon, Divjaka, and Malvazija istarska) were used in two repetitions while the rest of the genotypes were used once. The OIV classes 1, 3, 5, and 7 were represented with 4, 6, 10, and 2 observations, respectively. All of them were classified to the training set with complete accuracy. High R^2 was obtained between the measured parameters and the OIV classes with a value of 0.92 for Cox and Snell's R^2, and values close to 1 for McFadden's and Nagelkerke's R^2. Accordingly, the log-likelihood value for each observation was close to 0.

The prediction set consisted of 10 Croatian native varieties that possess different levels of susceptibility to downy mildew according to the OIV leaf disc test. Interestingly, half of the varieties that were the most susceptible and produced the densest sporulation on the leaf discs (the OIV class 1) belonged to the same class according to the given model. However, the other half was grouped to the neighbouring class 3. A similar observation was obtained for varieties from class 3. The model put half of them to the same class, while the rest varieties were dispersed to the neighbouring classes 1 and 5. Finally, the model was 100% correct for class 5. None of the varieties from the prediction set was put in class 7 provided by the training set, which confirmed the strength of the model (Table 3).

Table 3. Predicted OIV classes for ten native varieties in comparison with visual scoring (1 – variety belongs to this class by prediction; **1** – prediction matches with visual scoring).

		Predicted OIV Classes				
Variety	**OIV Class by Visual Scoring**	**1**	**3**	**5**	**7**	**Prediction Correctness (%)**
Plavčina	1	0	1	0	0	50
Plavina		**1**	0	0	0	
Moslavac		0	1	0	0	
Plavac mali		**1**	0	0	0	
Škrlet	3	0	**1**	0	0	50
Tribidrag		1	0	0	0	
Mladenka		0	0	1	0	
Ninčuša		0	**1**	0	0	
Belina svetokriška	5	0	0	**1**	0	100
Kadarun		0	0	**1**	0	

3. Discussion

Leaf discs test proved to be a simple method to perform and provide results about differences in susceptibility to downy mildew in a short time (usually not longer than seven days) starting from inoculation until the development of *P. viticola* sporulation. To our knowledge, research that includes a high number (25) of Croatian native varieties by using this method was conducted for the first time. Interesting and trustworthy results are gained since genotypes with a known level of susceptibility or resistance were comparatively evaluated. Previously, other authors [21,29,31,51–53] likewise included Solaris, Regent, *V. riparia*, Chardonnay, and Cabernet Sauvignon in their studies with leaf discs, although their scales for determining the level of susceptibility slightly differ one from another. Nonetheless, all these results are similar (or the same) and comparable. More precisely, Regent was characterised as resistant (class > 7) [31] and partially resistant [21,29,51], whereas Solaris was partially resistant [21,29]. Regarding the North American species, *V. riparia* was confirmed as the most resistant species, followed by *V. aestivalis* and *V. rupestris*. *V. riparia* allowed no sporulation and seldom showed necrotic spots [51], which is in agreement with the present research. Coevolving on the same continent with downy mildew, these North American species were subjected to the same stressful stimulus and gained epigenetic modifications responsible for their defence systems [52]. Differences among cultivars in response to the action of *P. viticola* are related to different passive mechanisms (i.e., dense hydrophobic trichomes on the abaxial side of leaves) and active responses involving hypersensitivity and synthesis of specific secondary metabolites [1,5]. Chardonnay was classified as the most susceptible genotype, and *V. riparia* was highly resistant in experiments conducted by [53]. Cabernet Sauvignon was described as a little susceptible cultivar, together with Riesling, Pinot Noir, and Pinot Blanc [54], which could be assigned to the OIV class 5, where Cabernet Sauvignon belongs by here presented results.

However, this type of phenotyping relies largely on visual scoring, which is time-consuming especially for large-scale experiments. Moreover, it can generate bias between different experts and experimental repeats. Due to the rapid development of high-throughput genotype screening in plant breeding and genomics, there is a call for more effective and reliable phenotyping data to support modern genetic crop improvement [45]. For that reason, the leaf disc test was complemented with chlorophyll fluorescence and multispectral imaging in this research to describe differences between distinctive OIV groups and changes between non-infected and infected leaf discs.

Photosynthesis is one of the most important processes of a plant's primary metabolism, meaning that its inhibition is one of the first signals of plant stress. It serves as a plant defence mechanism against biotic stress by limiting the nutrient availability to the pathogens. On the other hand, pathogens are able to manipulate the plant metabolism for their own benefit [55]. The most sensitive chlorophyll fluorescence parameters of grapevine leaves being infected with *P. viticola* are F_v/F_m and $F_{q'}/F_{m'}$ [38]. The decreases in the F_v/F_m ratio (variable to the maximum value of chlorophyll *a* fluorescence) indicate the reduction of photosystem II efficiency, specifically photoinhibition [56]. Photoinhibition is a phenomenon resulting from a reduction of photosynthetic activity predominantly due to light-induced decreases in CO_2 assimilation [57].

According to previous studies [58,59], an optimal value of F_v/F_m is 0.83 for most plant species, while values lower than this mean that the plant is exposed to stress and its photosynthetic performance is impaired. These findings can be ascribed to overall low F_v/F_m values (< 0.71) obtained in the present study, because of *P. viticola* infection and due to conducting the experiment on excised leaf parts and imaging their abaxial sides. The lowest values of F_v/F_m observed in T_0 are probably the result of leaves cutting and their changing environment from the greenhouse to the laboratory, where leaf discs were placed on wet filter papers. Despite these circumstances, only 24 h after inoculation did this parameter clearly distinguish infected from non-infected leaf discs (Figure 1a), which is much earlier than the previous finding where the earliest change of F_v/F_m pattern on

Chardonnay leaves appeared four days upon inoculation [38]. Here, necrotic areas were observed four days after inoculation in cultivar Solaris, which is in accordance with previous research [39], in which low F_v/F_m value was found five days after inoculation due to the development of necrotic spots.

On the contrary, $F_{q'}/F_{m'}$ and ETR values were generally lower for infected susceptible *V. vinifera* varieties (OIV classes 1, 3, and 5) compared to infected Solaris, Regent (OIV 7) and *V. riparia* (OIV 9), suggesting that in spite of being infected, these (partially) resistant genotypes keep higher photosynthetic rate. Yet, their performance also declined during the later stage of infection (6 and 8 dpi) (Figure 4b,c). These changes can be explained by gradual chlorophyll degradation [43] and destruction of the photosynthetic apparatus [49] due to both *P. viticola* infection and leaf discs senescing. ETR can be stimulated in regions adjacent to infected cells to provide energy to fuel defence responses or as a result of compensation for loss of green leaf area [49].

NPQ refers to thermal energy dissipation in the PSII antennae [41]. It was previously reported that its values (together with $F_{q'}/F_{m'}$) decreased in tomato leaves infected by *B. cinerea* in developing lesions. The surrounding areas were also characterised by decreased NPQ, which is indicative of enhanced ATP consumption on CO_2 fixation in the Calvin–Benson cycle [60]. By comparing the interaction of powdery mildew with susceptible and resistant lines of barley, the impact in the compatible interaction was much greater, meaning that the greatest reduction in $F_{q'}/F_{m'}$ and NPQ in the site of infection that extended to neighbouring cells was observed in susceptible line [35]. In the present study, although NPQ responses were not useful for distinguishing infected from non-infected leaves, its values plunged at 6 dpi in both treatments (Figure 4d) when necrotic spots and sporulation had already been developed in infected tissues. Furthermore, this decline was more pronounced for susceptible OIV classes (1, 3, and 5), compared to resistant classes whose values did not change considerably during the experiment (Figure 4d).

Photochemical quenching (qP) indicates the proportion of PSII reaction centres that are open; thus, a change in qP is due to the closure of reaction centres, resulting from a saturation of photosynthesis by light. This parameter, together with F_v/F_m, provides information about the underlying processes which have altered photosynthetic efficiency [50]. A downward trend of photochemical quenching is observed in our study (Figure 1e), in accordance with [61]. This parameter can also be used as a discriminator of susceptible and resistant genotypes until the first appearance of visible changes (4 dpi) because, after that, all groups of genotypes showed similar (and very low) qP values (Figure 4e).

Hue values are proportional to total chlorophyll, offering an alternative to photometric analysis of leaf extracts. This is demonstrated using tobacco leaves with various chlorophyll contents due to senescence and thus shows the possibility of applications in studies of stress conditions accompanied by chlorophyll loss [62]. In this colour space, each colour can be expressed independently from its saturation (pale or intense colour) and value (dark or bright colour). This feature can be used for in-field detection of downy mildew symptoms [63]. In our research, this trait clearly resolved cultivars Solaris and Regent (OIV 7) (Figure 5a) probably due to considerably brighter green colour of their leaves abaxial sides (https://www.vivc.de) and subsequent lower hue values from all other evaluated genotypes. Higher FarRed values are mostly observed in genotypes which are more tolerant to downy mildew (Figure 5b) and in non-infected leaf discs (Figure 2b) since the pathogen's mycelium destroys chloroplasts.

Leaf reflectance is very high in the near-infrared at ~800 nm when leaves are also largely transparent [64]. The absorption by leaf pigments is strongly reduced in this spectrum, and thus, both reflectance and transmittance are much higher than in the visible spectral range. A decrease of the reflectance may be an indicator of reduced areal interspaces (reduced assimilation of CO_2) in the mesophyll of leaves under stress conditions [40]. For that reason, *V. riparia* showed the highest values in this spectrum as the most resistant evaluated genotype (Figure 5c). It has also been reported that *V. riparia* have

smaller, more loosely packed cells with extended intercellular space for the spongy parenchyma [65].

Chlorophyll and anthocyanin contents were calculated by CHI and ARI, respectively. By these measurements, the highest contents of chlorophyll and anthocyanin are observed in the OIV classes 3 and 9 with no considerable changes throughout the measurement period (Figure 5d,e). However, at 6 and 8 dpi, CHI distinguished infected and non-infected leaf discs (Figure 2d). Oerke et al. [21] found decreasing chlorophyll content during disease development which was associated with the appearance of visible symptoms on the adaxial leaf side, such as discolouration and oil spots. NDVI, as an indicator of the plant's health status, clearly separated inoculated from non-inoculated leaf discs, especially in the later stages of infection (Figure 2f). Visible changes were observed six or seven days upon inoculation in the form of *P. viticola* sporulation, while through fluorescence (F_v/F_m) and multispectral (CHI and NDVI) channels was possible to differentiate non-inoculated from inoculated leaf discs at 4 dpi (Figure 3), and these differences are often more pronounced among the genotypes from the OIV class 1 (Figure 3a). The difference between infected and non-infected leaf discs in T0 can be explained by initial differences in plant material, i.e., position and exposure to the light during the development of the leaves. Due to this fact, changes in the difference between infected and non-infected leaf discs throughout seven terms against T0 must also be considered in the case of parameters Hue, FarRed, NIR, ARI, and NDVI.

Applications of fluorescence imaging in screening for disease and stress resistance have a clear potential for quantitative assessment of the plant infection or stress level before the appearance of visible symptoms [40]. An example is detecting whether an asymptomatic *V. vinifera* variety Malvasía de Banyalbufar is infected by GLRaV-3 (*Grapevine leafroll-associated virus 3*) [66]. It was previously reported that logistic regression analysis enabled the determination of probabilistic leaf–cluster relationship in downy mildew natural infection on Cabernet franc [67].

Preliminary results of the proposed model suggest that by chlorophyll fluorescence and multispectral imaging, it is possible to distinguish grapevine genotypes with different susceptibility to downy mildew even before the conditions for the pathogen development are satisfied and before the grapevine inoculation since this model is formed on non-infected leaf discs. However, it is necessary to confirm the model by conducting a more comprehensive experiment with a greater number of genotypes. Imaging of whole leaves and their adaxial sides with high chlorophyll content in densely packed palisade parenchyma, in contrast to spongy parenchyma on the abaxial side [68], and imaging other susceptible tissues (i.e., inflorescence, green berries, and tendrils), will provide more complete information. Once the model is confirmed, the next step is generating a large-scale data platform by imaging the genotypes with known response to downy mildew to create an explanatory background for linking genotypes to phenotypes. This method could be applicable for high-throughput phenotyping (screening) of seedlings that are the result of breeding programs aiming to create genotypes with high resistance to mildews. In this way, the proper OIV classes could be ascribed to many seedlings at the early stage of their development. Another possible application is the phenotyping of existing grapevine collections and commercial vineyards with no defined differences in susceptibility to downy mildew between different genotypes, which is of utterly importance in the era of sustainable agricultural production and precision viticulture.

4. Materials and Methods

4.1. Plant Material

Altogether, 30 genotypes were included in this research—25 Croatian native varieties, two susceptible international *V. vinifera* varieties (Cabernet Sauvignon, Chardonnay), two resistant cultivars (Regent, Solaris), and one *Vitis* species (*Vitis ripara*). One-year cuttings, 20 cm long, containing three to four buds were taken from the Croatian native

grapevine varieties collection, Department of Viticulture and Enology, University of Zagreb Faculty of Agriculture in March 2019. Before planting, a bud from the basal part of each cutting was removed, and the cuttings were soaked overnight in an aqueous solution containing 0,1 mg L^{-1} indole-3-butyric acid (IBA). Each cutting was planted in a 5 L drip-irrigated pot containing standard commercial substrate S2 (Klasmann-Deilmann, Geeste, Germany). The plants were grown in a greenhouse. Fungicide Chromosul® (Chromos Agro, Zagreb, Croatia) was applied in each season to control powdery mildew infection. This fungicide is sulphur based and only has preventive-contact activity on powdery mildew; nevertheless, young leaves sampled at the stage of 10 fully developed leaves were not treated. Each genotype was represented by 12 cuttings. In 2020 shoots' development was uniformed. When they reached a growing stage of 10 fully developed leaves, the fourth and the fifth leaf from the apex were collected since they do not show ontogenic resistance (age-related resistance) [69]. They were washed in distilled water and dried with a paper tissue.

4.2. Suspension Preparation

P. viticola suspension was prepared using naturally infected leaves from the part of the vineyard where chemical protection was not applied. They were soaked in distilled water and gently brushed to detach sporangia from the leaf surface and make a dense suspension. It was adjusted to the concentration of 2×10^5 sporangia mL^{-1} with Neubauer cell counting chamber (hemocytometer).

4.3. Leaf Discs Inoculation and Incubation

A cork borer was used to punch out 3.00 cm diameter leaf lamina parts (discs) from the leaves avoiding main veins. There were 24 leaf discs per genotype, half of which were inoculated with *P. viticola* suspension, while the other half was sprayed with distilled water (mock-inoculated leaf discs). Four leaf discs were placed in a Petri dish with the abaxial side up on a wet filter paper. The Petri dishes were sealed with parafilm and placed in a climate chamber (air temperature 20 °C, air moisture 80%). The samples were kept in dark for the first 24 h, while for the next seven days of incubation, a photoperiod of 16 h was applied. After 24 h drops of suspension and distilled water were collected with filter paper to avoid decaying of the leaf discs [22]. On the seventh day upon inoculation, the leaf discs were evaluated by ascribing to each one a percentage of the area covered by *P. viticola* fructification [70]. Finally, the average percentage of sporulation on the set of 12 inoculated leaf discs per genotype was scored according to the OIV descriptor 452-1 (Leaf: degree of resistance to *Plasmopara* (leaf disc test)) (Tables 1 and 2) [30].

4.4. Chlorophyll Fluorescence and Multispectral Imaging

Chlorophyll fluorescence and multispectral imaging were carried out using the CropReporter™ (PhenoVation B.V., Wageningen, the Netherlands). The measurements were performed seven times starting with no treated leaf discs and terminating with visible downy mildew symptoms (sporulation as white fuzz) on leaf discs' abaxial side. Time points of imaging were as follows: before treatment (T_0), one day post inoculation (dpi) (T_1), two dpi (T_2), three dpi (T_3), four dpi (T_4), six dpi (T_5), and eight dpi (T_6). Obtained parameters are summarised in Table 4. Leaf discs were imaged at a 45 cm distance from the camera always with the abaxial side up. The output is 16-bit RAW format. Automatic analysis of chlorophyll fluorescence, colour, and multispectral images was performed by DA™ software (PhenoVation B.V., Wageningen, the Netherlands). The analysis was performed using regions of interest (the inner part of leaf discs) to avoid information of excised and senescing leaf disc's edge [40].

Table 4. Chlorophyll fluorescence and multispectral imaging parameters.

Parameter	Parameter Explanation
F_v/F_m	Maximum quantum yield of photosystem II (PSII) electron transport (leaf discs preconditioned in the dark)
$F_{q'}/F_{m'}$	Effective quantum yield of photosystem II (PSII) electron transport (leaf discs exposed to actinic light)
ETR	Electron transport rate
NPQ	Non-photochemical quenching (thermal energy dissipation in the PSII antennae)
qP	Photochemical quenching (proportion of open PSII reaction centres)
Hue	Indicator of colour differences (proportional to total chlorophyll content), colour appearance parameter
Far Red	Far-red reflectance
NIR	Near-infrared reflectance
CHI	Chlorophyll index
ARI	Anthocyanin reflection index
NDVI	Normalised difference vegetation index

Leaf discs were imaged with dark-to-light slow fluorescence induction [71], which includes dark adaptation, measurement of the induction curve of the dark-adapted leaf discs, followed by actinic light switching on for light adaptation, and measurement of induction curve of light-adapted leaf discs. For chlorophyll fluorescence measurements of dark-adapted leaf discs (30 min in dark before measurement), saturating light pulse (4500 μmol m^{-2} s^{-1} for 800 ms) was used. Minimum chlorophyll fluorescence (F_0) was measured after 20 μs, and maximum chlorophyll fluorescence (F_m) was measured after saturation. Four dark frames were captured and averaged to one single frame during the time red LEDs were off; overall, 20 frames were captured for the induction curve during 800 ms, and integration time for capturing the chlorophyll fluorescence images was 200 μs.

After the measurement of dark-adapted leaf discs, they were relaxed in the dark for 15 s, and then actinic lights (300 μmol m^{-2} s^{-1}) were switched on enabling leaf discs to adapt to light for 5 min. Steady-state fluorescence yield ($F_{s'}$) was measured at the onset of the saturating pulse, and maximum chlorophyll fluorescence ($F_{m'}$) of light-adapted plants was measured at saturation, using the saturating pulse intensity (4500 μmol m^{-2} s^{-1}). Again, four dark frames were captured and averaged to one single frame during the time red LEDs were off; a total of 20 frames were captured for the induction curve during 800 ms, while integration time for capturing the chlorophyll fluorescence images was 200 μs.

Measured F_0, F_m, $F_{m'}$, $F_{s'}$ were used for calculation of the following fluorescence parameters, which include the following:

Maximum quantum yield of PSII (F_v/F_m): $F_v/F_m = (F_m–F_0)/F_m$ [72];

Effective quantum yield of PSII ($F_{q'}/F_{m'}$): $F_{q'}/F_{m'} = (F_{m'}–F_{s'})/F_{m'}$ [72];

Electron transport rate (ETR) = $F_{q'}/F_{m'} \times PPFD \times (0.5)$ [72];

Non-photochemical quenching (NPQ) = $(F_m–F_{m'})/F_{m'}$ [73].

Colour and spectral reflectance (R) images were captured after chlorophyll fluorescence imaging at 300 μmol m^{-2} s^{-1} produced by broadband white LEDs. Reflectance images were captured at Red – 640 nm, Green – 550 nm, Blue – 475 nm, Chlorophyll (Chl) – 730 nm, Anthocyanin (Anth) – 540 nm, NIR – 769 nm, and FarRed – 710 nm.

From reflectance images, chlorophyll index (CHI) and anthocyanin index (ARI) were calculated using the following equations: $CHI = (Chl)^{-1} - (NIR)^{-1}$ [74], and $ARI = (Anth)^{-1} - (FarRed)^{-1}$ [75]. Hue was calculated after converting reflectance in Red, Green, and Blue into values between 0 and 1.

Hue (0 – 360°) was calculated as follows:

Hue = 60 × (0 + (Green – Blue)/(max–min)), if max = Red;

Hue = 60 × (2 + (Blue – Red)/(max–min)), if max = Green;

Hue = 60 × (4 + (Red – Green)/(max–min)), if max = Blue.
360 was added in the case of Hue < 0.

4.5. Statistical Analyses

Statistical analyses were performed by the XLSTAT statistical and data analysis solution (Addinsoft, 2020, New York, USA) [76]. The number of genotypes used in this study is large, and leaf discs are mostly excised from different leaves that provide heterogeneous samples. Subsequently, observations are contaminated with outliers, which was confirmed using an outlier test (data not shown). Thus, trimmed means are used for a better estimation of the most observations' location. They are robust estimators of central tendency similar to the median [77]. To calculate a trimmed mean, a predetermined amount (25%) of observations of each side of the distribution of each genotype is removed and the remaining observations are averaged.

Trimmed means are used for calculating logistic regression to find a relationship between the ascribed OIV classes and chlorophyll fluorescence parameters of leaf discs before *P. viticola* inoculation. The dependent variable (target) was the OIV classes (1, 3, 5, and 7) that are ascribed to each examined genotype according to the developed sporulation of downy mildew on leaf discs, while explanatory variables were the parameters of chlorophyll fluorescence and multispectral imaging summarised in Table 4. Since there are five categories (OIV classes) with the order, which are described in Table 2, an ordinal logistic regression and logit model with a confidence interval of 95% were used for the statistical analysis. The Newton–Raphson algorithm was used as a method of estimating the regression parameters. The OIV class 9 is considered completely resistant, and as such, was not included in this (modelling) part of the study.

Repeated measures ANOVA was performed to find differences in chlorophyll fluorescence and multispectral imaging parameters between infected and non-infected leaf discs and between infected leaf discs belonging to separated OIV classes throughout seven terms (from T_0 to T_6). The mean values, standard deviations, and significant differences of the data were calculated using XLSTAT (Addinsoft, New York, USA). The results were analysed using one-way ANOVA and the differences between the means were evaluated by Duncan's multiple range test at a confidence level of 95% ($p < 0.05$).

5. Conclusions

The application of the leaf disc test proved to be an appropriate method for distinguishing grapevine genotypes according to their susceptibility to downy mildew. From a physiological point of view, chlorophyll fluorescence and multispectral imaging is a promising tool for precise monitoring of the photosynthesis transmission inside a leaf tissue upon *P. viticola* inoculation, as confirmed previously. Here, this utility is extended in a form of a possible phenotyping method among distinctive classes of grapevine genotypes in susceptibility to downy mildew in the absence of the pathogen. However, it is necessary to conduct more extensive experiments on a large number of genotypes, including the whole leaves and/or other susceptible tissues imaging. Certainly, there are morphological specificities in some cultivars (e.g., dense hydrophobic trichomes on the abaxial leaf sides) that act as a physical barrier and therefore cause lower susceptibility to downy mildew. Further research should also address scrutinised chemical analyses of grapevines' secondary metabolites, such as polyphenolic and volatile compounds since their metabolomic pathways change upon pathogen's attack and that feature could be peculiar to genotypes with a similar response to oomycetes.

Author Contributions: Conceptualisation, I.T. and J.K.K.; methodology, B.L.; formal analysis, P.Š. and A.N.; data curation, D.P.; writing—original draft preparation, P.Š., I.Š., and Z.M.; writing—review and editing, I.T., J.K.K., and D.P.; funding acquisition, E.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the project KK.01.1.1.01.0005 Biodiversity and Molecular Plant Breeding, Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Zagreb, Croatia.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

OIV	International Organisation of Vine and Wine
OIV classes	1, 3, 5, 7, and 9 from the most susceptible to the completely resistant group of genotypes
$T_0 – T_6$	terms of imaging from the pre-infection stage until the appearance of visible symptoms
dpi	day(s) post-inoculation
PSII	photosystem II
F_v/F_m	maximum quantum yield of photosystem II electron transport (variable to maximum value of chlorophyll *a* fluorescence)
$F_{q'}/F_{m'}$	effective quantum yield of photosystem II electron transport
ETR	electron transport rate
NPQ	non-photochemical quenching
qP	photochemical quenching
Hue	colour appearance parameter
Far Red	far red reflectance
NIR	near-infrared reflectance
CHI	chlorophyll index
ARI	anthocyanin reflection index
NDVI	normalised difference vegetation index

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Appendix 2. Scientific paper: Štambuk P., Šikuten I., Karoglan Kontić J., Maletić E., Preiner D., Tomaz I. (2022). Leaf Polyphenolic Profile as a Determinant of Croatian Native Grapevine Varieties' Susceptibility to *Plasmopara viticola*. Frontiers in Plant Science 13: 836318. doi: 10.3389/fpls.2022.836318

frontiers in Plant Science

ORIGINAL RESEARCH
published: 11 March 2022
doi: 10.3389/fpls.2022.836318

Leaf Polyphenolic Profile as a Determinant of Croatian Native Grapevine Varieties' Susceptibility to *Plasmopara viticola*

Petra Štambuk[1,2], Iva Šikuten[1,2], Jasminka Karoglan Kontić[1,2], Edi Maletić[1,2], Darko Preiner[1,2] and Ivana Tomaz[1,2]*

[1] Department of Viticulture and Enology, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia, [2] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Zagreb, Croatia

Since grapevine is highly susceptible to various pathogens, enormous amounts of pesticides are applied each season to achieve profitable production. One of the most destructive grapevine diseases is downy mildew, and their interaction has been in the spotlight for more than a decade. When it comes to a metabolome level, phenolic compounds are relevant to investigate due to their involvement in the plant immune system and known antifungal properties. Croatian grapevine germplasm is highly heterogeneous due to its long history of cultivation in diversified geographical regions. Since it has been found that native varieties react differently to the infection of *Plasmopara viticola*, the intention of this study is to define if the chemical background of the leaves, i.e., polyphenolic composition, is responsible for these dissimilarities. Therefore, the leaves of 17 genotypes, among which 14 were native and 3 were controls, were analyzed using high-performance liquid chromatography (HPLC) in four terms: before inoculation and 24, 48, and 96 h post inoculation (hpi). During this early phase, significant differences were found neither between the terms nor between the non-inoculated and inoculated samples, except for resveratrol-3-*O*-glucoside. By applying principal component analysis (PCA) using initial leaf polyphenolic composition, varieties of *V. vinifera* were clearly separated into three different groups corresponding to their International Organization of Vine and Wine (OIV) classes of susceptibility to *P. viticola*. Results obtained in this research suggest that the initial constitutive polyphenolic composition of the cultivar leaves has a crucial influence on their susceptibility to *P. viticola,* and this finding can be used to improve the success of grapevine breeding programs toward downy mildew resistance.

Keywords: ***Vitis vinifera*** **L., downy mildew, defense mechanism, leaves, chemical composition, HPLC**

OPEN ACCESS

Edited by:
Andreia Figueiredo, University of Lisbon, Portugal

Reviewed by:
Marisa Maia, University of Lisbon, Portugal
Mara Quaglia, University of Perugia, Italy

****Correspondence:***
Darko Preiner
dpreiner@agr.hr

Specialty section:
This article was submitted to Plant Pathogen Interactions, a section of the journal Frontiers in Plant Science

Received: *15 December 2021*
Accepted: *08 February 2022*
Published: *11 March 2022*

Citation:
Štambuk P, Šikuten I, Karoglan Kontić J, Maletić E, Preiner D and Tomaz I (2022) Leaf Polyphenolic Profile as a Determinant of Croatian Native Grapevine Varieties' Susceptibility to Plasmopara viticola. Front. Plant Sci. 13:836318. doi: 10.3389/fpls.2022.836318

INTRODUCTION

About 10,000 years of grapevine evolution and domestication in almost 90 countries (Villano and Aversano, 2020) provided a high number of genotypes possessing various morphological and genetic traits (This et al., 2006). However, the selection process carried out by humans shaped the gene pool of today's varieties with valuable traits in the sense of yield, chemical composition, berry and bunch size, phenology, hermaphrodite flowers etc., while resistance to main pathogens

was unintentionally neglected (Grassi and Arroyo-Garcia, 2020). Nowadays, it is more than ever necessary to produce enough food for the growing human population in a way that achieves a minimal footprint on the environment. That is where breeding programs play a major role and aim to bring about high-quality cultivars that can cope with the difficulties of main diseases. When it comes to the grapevine, downy mildew is one of the most destructive diseases. The causal agent of this disease is *Plasmopara viticola* [(Berk. and Curt.) Berl. and de Toni], which is one of the most damaging pathogens affecting grapevine production in all viticultural regions worldwide (Armijo et al., 2016). Diseases like mildews are allochthonous in Europe and therefore, grapevine (*Vitis vinifera* L.) varieties are susceptible to them unlike the American species [e.g., *Vitis riparia* Michx, *Vitis labrusca* L., *Vitis rupestris* Scheele, *Muscadinia rotundifolia* Small (previously *Vitis rotundifolia* Michaux)] which developed resilience coevolving on the same geographical area (Jürges et al., 2009; Gessler et al., 2011). A considerable level of resistance to downy mildew is observed in the Asian species, such as *Vitis amurensis* Rupr, which coevolved with the species of the pathogen closely related to *P. viticola*, i.e., *Plasmopara cissi* Vienn.-Bourg and *Plasmopara amurensis* Prots (Dick, 2002).

Plasmopara viticola is an obligate biotrophic oomycete meaning that it feeds on the living tissue, through haustoria in order to invade the host cell and obtain plant metabolites (Glazebrook, 2005). Its sporangia have lemon-shaped coenocytic cells that contain four to eight nuclei (Riemann et al., 2002). During the grapevine growing season, when conditions for downy mildew development are favorable, symptoms of infection appear on the green tissues (i.e., leaves, tendrils, inflorescences, shoots, and green berries), always starting with the young leaves. For that reason, *in vitro* experiments on the leaves are often used as an indicator of a variety's susceptibility to *P. viticola* (Jürges et al., 2009; Bove et al., 2019). Visible adaxial symptoms on the leaves, called oil spots, are reported to usually precede the abaxial whitish sporulation (Gessler et al., 2011). When fungicides are not applied during favorable weather conditions for downy mildew development, it can devastate almost the whole yield in one season and weaken the young shoots, causing a considerable economic loss (Buonassisi et al., 2017). Yet, fungicides, both in the organic farming as copper fungicides and in the Integrated Pest Management even with other active substances, act harmfully to the environment, and animal and human health (Wilson and Tisdell, 2001); thus breeding programs aim to produce genotypes with efficient and durable resistance to main diseases, such as mildews and gray mold (Merdinoglu et al., 2018).

The mode of plant-pathogen interaction begins when the initial contact is established between infective propagules (*P. viticola* zoospores) and the plant tissue surfaces (e.g., leaf lamina). To prevent the diseases caused by pathogens, plants use sophisticated defense mechanisms that can be either constitutive or inducible defenses (Muganu and Paolocci, 2013). While the constitutive defense is referred to as a preexisting and continuous resistance (Kono and Shimizu, 2020), the induced defense is triggered by a pathogen attack and recognition and includes the perception of plant tissue signals resulting from pathogen infection (Muganu and Paolocci, 2013). Constitutive defense includes preformed physical barriers present on the plant surface (leaf hairs, wax layers, rigid cell walls, and the number and the activity of stomata) or chemical compounds, such as antimicrobial secondary metabolites (Lattanzio et al., 2006). These compounds are called phytoanticipins, which are defined as compounds that are present in plants before being challenged by microorganisms or are produced after infection solely from preexisting constituents (VanEtten et al., 1994). It has already been emphasized that increasing knowledge about constitutive phytoanticipins, such as leaf polyphenols could be pivotal to explaining the different levels of susceptibility to pathogens displayed by *V. vinifera* genotypes (Kedrina-Okutan et al., 2018). On the other hand, there are compounds that are produced by plants as a response to biotic and abiotic stresses called phytoalexins (Jeandet, 2015). Upon *P. viticola* infection of grapevine leaves, the synthesis of stilbenes is usually induced, among which resveratrol is the most common compound. It can reduce the germination of spores, which proves its strong antimicrobial activity against *P. viticola* (Dercs and Creasy, 1989). Scarce information is available suggesting that specific profiles exist at the transcriptome and metabolome level that can discriminate susceptible and resistant cultivars before being infected with *P. viticola* (Figueiredo et al., 2008).

Up to date, numerous studies have been published considering the composition and content of secondary metabolites, namely polyphenolic and volatile organic compounds, in grapevine leaves before and after *P. viticola* infection aiming to elucidate which compounds are specifically responsible for a certain level of tolerance to this microorganism among different species and varieties (Figueiredo et al., 2008, 2015; Batovska et al., 2009; Chitarrini et al., 2017; Eisenmann et al., 2019; Ciubotaru et al., 2021; Ricciardi et al., 2021). On the other hand, studies focused on the differences among *V. vinifera* varieties with different levels of resistance and their metabolomic discrimination either before or after inoculation with *P. viticola* are deficient. As a part of plants' secondary metabolism, polyphenolic compounds and phenolic acids are not directly involved in their growth, development, and reproduction; yet they eminently participate and influence these processes. They are located in the epidermis of the leaves (cell vacuoles), cuticle, and epicuticular wax—predominantly, in the outer layers of the leaves. This epidermis/cuticle skin forms the first mechanical barrier to invading pathogens by repelling fungal spores due to its self-cleaning surface (Keller, 2020). Moreover, one of the most important roles of polyphenolics is the defense reaction due to their antifungal and antibacterial properties (Lattanzio et al., 2006). Polyphenol accumulation and profiles are influenced by seasonal climatic conditions, biotic and abiotic stressors, soil, cultural practices, and genetics (Kedrina-Okutan et al., 2018). In stressed plants, the level of reactive oxygen species (ROS) is surpassed over the antioxidant compounds. Stressors can induce the activation of the defense mechanism, which increases the biosynthesis of many phenolic compounds (Bouderias et al., 2020).

Until recently, it was thought that genetic variability among *V. vinifera* germplasm is too scarce in a sense of resistance to main fungal diseases. However, in some *V. vinifera* varieties, such

as Kishmish vatkana, Dzandzal kara (Coleman et al., 2009), and Mgaloblishvili (Sargolzaei et al., 2020), resistance genes have been identified. From this kind of research, it can be concluded that varieties of local importance are possible sources of desirable features that can be useful in the upcoming changing climate as some of them are able to cope with abiotic (drought, salinity, iron chlorosis) and biotic stresses (Sargolzaei et al., 2021).

Croatia is a country with a long tradition in grapevine cultivation with many climatically diverse regions that provided to develop a high number of native grapevine varieties. The introduction of phylloxera and mildews at the end of the nineteenth century gradually caused the erosion of this preceding germplasm. Thus, today's native collection counts slightly more than a hundred varieties (Maletić et al., 2015a; Žulj Mihaljević et al., 2020). Due to centuries-old grapevine cultivation and their adjustment to disparate environmental conditions, there is a presumption that diverse responses to diseases exist among the Croatian native varieties. These differences were recently confirmed on a series of studies applying field research, the leaf disc bioassay (OIV, 2009), and by measuring the chlorophyll fluorescence and multispectral imaging traits (Štambuk et al., 2021). In the present study, this research is extended to the metabolomic approach aiming (1) to examine the differences in the content of the polyphenols during the early stage of infection of *V. vinifera* varieties with different degree of resistance to *P. viticola* and (2) to assess the existence of a correlation between the polyphenolic profiles of 14 Croatian native *V. vinifera* genotypes, and their belonging to different classes of resistance to *P. viticola* according to the classification of the International Organization of Vine and Wine (OIV). To the best of our knowledge, up to now, there has been no research that included such a high number (15) of *V. vinifera* varieties considering their constitutive and induced leaf polyphenolic profiles regarding the level of susceptibility to *P. viticola*. Therefore, this study provides an invaluable source of information that could be used for screening other *vinifera* varieties with no defined level of susceptibility to this pathogen and to improve the success of grapevine breeding programs toward downy mildew resistance.

MATERIALS AND METHODS

Preparation of Samples

Plant Material

Overall, 17 genotypes were included in this research, of which 14 were Croatian native grapevine varieties and 3 were controls. Chardonnay was used as a susceptible control variety, while Solaris and *V. riparia* are genotypes with a high and very high degree of resistance to *P. viticola*, respectively (**Table 1**). Chardonnay has also been used previously as a susceptible control (Deglene-Benbrahim et al., 2010; Vezzulli et al., 2018; Possamai et al., 2020). In a previous study (Štambuk et al., 2021), these genotypes were subjected to the leaf disc bioassay of *P. viticola*. According to the OIV descriptor 452-1 [Leaf: degree of resistance to *Plasmopara* (leaf disc test)], each genotype was assigned to an appropriate OIV class of resistance to downy mildew from 1, the most susceptible to 9, the totally resistant varieties (OIV, 2009). The average percentage of the *P. viticola* sporulation developed on the leaf discs of genotypes was obtained by visual scoring according to the guidelines of the European and Mediterranean Plant Protection Organization (OEPP/EPPO, 2001). Data related to OIV 053 descriptor for the young leaf, i.e., density of prostrate hairs between the main veins on the lower side of the blade are also presented in **Table 1**. Hardwood cuttings of the abovementioned genotypes were taken from the Croatian native grapevine varieties collection, Department of Viticulture and Enology, University of Zagreb Faculty of Agriculture in March 2019. Briefly, they were planted in regularly irrigated pots, and the shoots were grown in a greenhouse with air temperature ranging from 15 to 24°C, and relative humidity ranging from 65 to 75% during the cultivation period. In 2020, when the development of the shoots was uniform and reached a growing stage of 10 fully developed leaves (**Supplementary Figure 1**), the fourth and the fifth leaf beneath the apex were sampled since they do not possess age-related resistance (Steimetz et al., 2012). The leaves were transferred from the greenhouse into the laboratory and rinsed with ultrapure water. At the time of sampling in the greenhouse, the leaves were healthy with no evidence of foliar diseases.

Plasmopara viticola Suspension Preparation

Leaves with evident *P. viticola* sporulation were taken from the naturally infected vineyard where chemical protection was not applied. In the laboratory, the leaves were soaked in ultrapure water and *P. viticola* spores were detached with a gentle brush until the water became cloudy. Prepared suspension was sprayed on the abaxial leaf sides of a susceptible variety, Chardonnay to propagate *P. viticola* spores. After 7 days, the leaves with freshly developed sporulation were soaked in ultrapure water and the sporulation was removed using a gentle brush until the suspension became dense or visibly cloudy. Suspension concentration was adjusted to 2×10^5 spores ml^{-1} with Neubauer cell counting chamber (Bellin et al., 2009; Perazzolli et al., 2012; Vezzulli et al., 2018). The freshly prepared suspension was used for the inoculation of the leaves of 17 genotypes.

Inoculation and Incubation of the Leaves

Immediately after sampling, four leaves of each genotype were stored in the freezer at −20°C until analysis (T_0). The remaining plant material (24 leaves per genotype) was separated into two groups, mock-inoculated leaves (treated with ultrapure water) and leaves inoculated with *P. viticola* suspension. Each leaf was placed in a separate Petri dish (150 mm in diameter) on a wet filter paper. The leaves were laid with the abaxial side up and sprayed with ultrapure water or *P. viticola* suspension. The Petri dishes were sealed with parafilm and placed in the climate chamber with optimal conditions for downy mildew development (air temperature 20°C, air moisture 80%). For the first 24 h, the samples were kept in dark, then the drops of water or suspension were removed with sterile filter paper to avoid decaying of the leaves. After that, the photoperiod of 16 h was applied to imitate the outdoor conditions (Bellin et al., 2009; Vezzulli et al., 2018). At certain time points after inoculation [T_1—24 h post inoculation (hpi); T_2—48 hpi; T_3—96 hpi]

(Ali et al., 2012; Chitarrini et al., 2017; Nascimento et al., 2019), the samples were taken from the climate chamber and stored in the freezer (−20°C) until analysis. During this early stage of the infectious process, the following changes in the susceptible cultivar have been previously determined: at 24 hpi, the zoospores germinate and the germ tube penetrates the substomatal cavity; at 48 hpi, the hyphae of *P. viticola* are observed in the intercellular spaces; at 96 hpi, the sporangiophores begin to develop from the stomata (Nascimento-Gavioli et al., 2020). For each genotype, the inoculation was performed on a number of leaves exceeding those necessary for the polyphenols assessment, with the aim to check the success of infection.

Analysis of Polyphenolics

Extraction of Polyphenolics

Before analysis, the leaves were lyophilized (freeze-dried) and then ground using MiniG Mill (SPEX SamplePrep, United States) (1 min, 1,500 rpm) to obtain a powder. The extraction was conducted according to the method described by Sikuten et al. (2021) and Štambuk et al. (2022) with slight modifications. In brief, the solid–liquid extraction technique was performed on the magnetic stirrer (RTC basic, IKA, Staufen, Germany) in the following conditions: extraction temperature of 60°C at 400 rpm for 2 h. The mass of 40 mg of ground grapevine leaves and the volume of 3 ml of extraction solvent was used. The extraction solvent was composed of acetonitrile:water:formic acid (20:79:1, v/v/v). After extraction, each extract was filtered using a Phenex-polytetrafluorethylene (PTFE) 0.20 μm syringe filter (Phenomenex, Torrance, United States), and then analyzed by high-performance liquid chromatography (HPLC). Each sample was analyzed in triplicate.

High-Performance Liquid Chromatography Analysis and Identification of Compounds

The separation, identification, and quantification of polyphenolic compounds was performed on an Agilent 1100 Series system (Agilent, Waldbronn, Germany), equipped with an autosampler, a column thermostat, a diode array detector (DAD), and a fluorescence detector (FLD). The Agilent 1100 Series system is coupled to an Agilent Chem Station data-processing station. The analysis was performed according to the previously described and published method (Tomaz and Maslov, 2016). The separation was performed with a reversed-phase column Luna Phenyl-Hexyl (4.6 × 250 mm; 5 μm particle), with Phenyl guard column (4.0 × 3.0 mm) heated at 50°C. The solvents were water:phosphoric acid (99.5:0.5, v/v, eluent A), and acetonitrile:water:phosphoric acid (50:49.5:0.5, v/v/v, eluent B), and the flow rate was 0.9 ml/min. The linear gradient for eluent B was as follows: 0 min, 0%; 7 min, 20%; 35 min 40%; 40 min, 40%; 45 min 80%; 50 min, 100%; and 60, min 0%. The injection volume for each sample was 20 μl. The DAD was set to an acquisition range of 200–700 nm. Flavonol-glycosides were detected at 360 nm, hydroxycinnamic acids at 320 nm, stilbenes at 308 nm, and hydroxybenzoic acids at 280 nm using the DAD. Flavan-3-ols were detected at λ_{ex} = 225 nm and λ_{em} = 320 nm using FLD. Identification of individual flavonoids was performed

TABLE 1 | Genotypes, additional information on the plant material, the corresponding OIV classes of resistance of the genotypes to *P. viticola* (OIV 452-1), and the density levels of the trichomes on abaxial leaf sides (OIV 053) according to OIV (2009).

Genotype (Accession name)	Holding Institute	Material source ID (EURISCO)	VIVC code	Species	OIV 452-1	OIV 053
Belina starohrvatska	HRV041	VIT00233	5374	*Vitis vinifera* subsp. *vinifera*	1	5
Debit	HRV041	VIT00017	10423	*Vitis vinifera* subsp. *vinifera*	1	1
Grk	HRV041	VIT00030	5066	*Vitis vinifera* subsp. *vinifera*	1	3
Moslavac	HRV041	VIT00052	4292	*Vitis vinifera* subsp. *vinifera*	1	5
Plavac mali	HRV041	VIT00060	9549	*Vitis vinifera* subsp. *vinifera*	1	7
Babić	HRV041	VIT0002	844	*Vitis vinifera* subsp. *vinifera*	3	1
Chardonnay	HRV041	CL-277*	2455	*Vitis vinifera* subsp. *vinifera*	3	3
Kraljevina	HRV041	VIT00035	24904	*Vitis vinifera* subsp. *vinifera*	3	1
Plavina	HRV041	VIT00062	9557	*Vitis vinifera* subsp. *vinifera*	3	9
Pošip	HRV041	VIT00065	16018	*Vitis vinifera* subsp. *vinifera*	3	1
Škrlet	HRV041	VIT00085	22983	*Vitis vinifera* subsp. *vinifera*	3	3
Tribidrag	HRV041	VIT00013	9703	*Vitis vinifera* subsp. *vinifera*	3	3
Malvazija istarska	HRV041	VIT00047	7269	*Vitis vinifera* subsp. *vinifera*	5	1
Ranfol	HRV041	VIT00070	9908	*Vitis vinifera* subsp. *vinifera*	5	5
Teran	HRV041	VIT00087	12374	*Vitis vinifera* subsp. *vinifera*	5	9
Solaris	DEU455	20340**	20340	*Vitis vinifera* subsp. *vinifera*	7	3
Vitis riparia	DEU098	4609**	4609	*Vitis riparia*	9	1

Genotypes used as controls are in bold.
**Plant material from vineyard on Experimental station Jazbina, University of Zagreb, Faculty of Agriculture, Department of Viticulture and Enology, Cv. Chardonnay, clone CL-277.*
*** According to VIVC.*
VIVC—Vitis International Variety Catalog (https://www.vivc.de).
OIV 452-1—Descriptor for leaf: degree of resistance to Plasmopara (leaf disc test).
OIV 053—Descriptor for young leaf: density of prostrate hairs between the main veins on the lower side of blade.

by matching the retention time of each chromatographic peak with external standards and the DAD spectrum. Individual flavonoid peaks were quantified using a calibration curve of the corresponding standard compound which was based on the peak area. When reference compounds were not available, the calibration of structurally related substances was used, including a molecular weight correction factor (Kammerer et al., 2004). The results are expressed in mg/kg or g/kg of dry weight (DW) of grapevine leaves.

Statistical Analysis

In order to define the effects of treatment (non-inoculated vs. inoculated samples), the classes of resistance and terms (time period) of sampling after inoculation, on the content of polyphenolic compounds, a factorial ANOVA was performed and the differences between the means of specific factors and their interactions were evaluated by Duncan's multiple range test at a confidence level of 95% ($p < 0.05$) (**Supplementary Table 1**). However, since there was no treatment involved in the sampling term 0 (before inoculation), it was excluded from the second factorial ANOVA that was used to define the exact effects of all factors (**Supplementary Table 2**).

Principal component analysis (PCA) was performed using average polyphenolic profiles of grapevine leaves for treatment (non-inoculated and inoculated), which were sampled in different terms before and upon inoculation (0, 24, 48, and 96 hpi) belonging to all genotypes used in the research (**Supplementary Figure 2**). Additional PCA was performed using only the average polyphenolic content of leaves within the sampling term and treatment belonging to OIV classes 1, 3, and 5 (*V. vinifera* varieties only) to focus on differences among them (**Figure 1**). The correlation was calculated between the data of resistance level of OIV descriptor 452-1 and the level of density of prostrate hair between the main veins on the lower side of the blade on the young leaves (OIV descriptor 053) of genotypes. Additional correlations were performed between the content of phenolic compounds and the terms of sampling separately for inoculated and non-inoculated samples (**Supplementary Table 2**). Correlations were calculated using Spearman's coefficient and were tested for significance. The XLSTAT statistical and data analysis solution (Addinsoft, 2020, New York, NY, United States) was used for statistical analyses.

RESULTS

Among the phenolic compounds, 10 flavan-3-ols, nine flavonol glycosides, eight hydroxycinnamic acids, four hydroxybenzoic acids, and two stilbenes were detected (**Supplementary Table 1**). The most abundant class of phenolic compounds were hydroxycinnamic acids with an average value of 25.19 g/kg among which caftaric acid (4.99 g/kg) contributed the most. Hydroxycinnamic acids were followed by flavonol glycosides (20.2 g/kg), flavan-3-ols (5.4 g/kg), hydroxybenzoic acids (227.32 mg/kg), and stilbenes (151.72 mg/kg). As far as individual compounds are concerned, the highest amount was detected for quercetin-3-*O*-glucoside (26.39 g/kg) in the samples representing the inoculated leaves of the OIV class 3 at 48 hpi (T_2) (**Supplementary Table 1**). Correlation between the content of the phenolic compounds and the terms of sampling was significant only in the case of resveratrol-3-*O*-glucoside and total stilbenes in both the inoculated and non-inoculated samples (**Supplementary Table 2**). There was no significant correlation found between OIV resistance classes and the density of prostrate hairs between the main veins on the lower side of the young leaves.

Polyphenolic Profiles of Cultivars Belonging to Different International Organization of Vine and Wine Resistance Classes

A significant effect ($p < 0.05$) of OIV resistance class was observed for all 33 phenolic compounds detected in the leaves of 17 genotypes used in this research (**Table 2**). The effect of artificial inoculation using *P. viticola* was significant ($p < 0.05$) only in the case of compounds belonging to the group of stilbenes (piceatannol and resveratrol-3-*O*-glucoside) same as in the case of sampling term upon inoculation where one additional compound (epicatechin) was affected. There was no significant interaction of OIV classes neither with the terms of sampling nor with treatment, as well as between the terms of sampling and inoculation (**Supplementary Table 2**).

Comparing the mean values of individual phenolic compounds within the different OIV classes of resistance, especially classes 1, 3, and 5 involving *V. vinifera* cultivars (**Table 2**), the most abundant compounds detected in class 5 were the following: myricetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, caftaric acid, gallocatechin, procyanidin B1, and piceatannol. The content of myricetin-3-*O*-glucoside and gallocatechin were significantly the highest in OIV 5, whereas the contents of the remaining mentioned compounds were not statistically different from OIV 7. Three varieties belonging to OIV 5 showed variations in the content of these compounds, especially the variety, Teran which showed the highest concentration of procyanidin A1 and caftaric acid (**Supplementary Table 3**).

Class 1 represents the most susceptible group of varieties (**Table 1**). By comparing the OIV class 1 with classes 3 and 5, significantly higher contents were detected for isorhamnetin-3-*O*-glucoside, aesculin, resveratrol-3-*O*-glucoside, and the total content of stilbenes, whereas the least detected were procyanidin B1, procyanidin B4, and the content of total flavan-3-ols (**Table 2**).

Varieties belonging to the OIV class 3, including the control variety, Chardonnay, had a significantly higher content of taxifolin, coutaric acid, gallic acid, catechin, procyanidins B2 and B3 when compared to OIV classes 1 and 5. No significant differences between these three classes were detected for quercetin-3-*O*-glucoside, caffeic acid, fertaric acid, protocatechuic acid, vanillic acid, syringic acid, epigallocatechin, and procyanidin A1, total flavonol glycosides, hydroxycinnamic, and hydroxybenzoic acids (**Table 2**). Varieties belonging to OIV 3 have similar profiles of polyphenolic compounds except for the

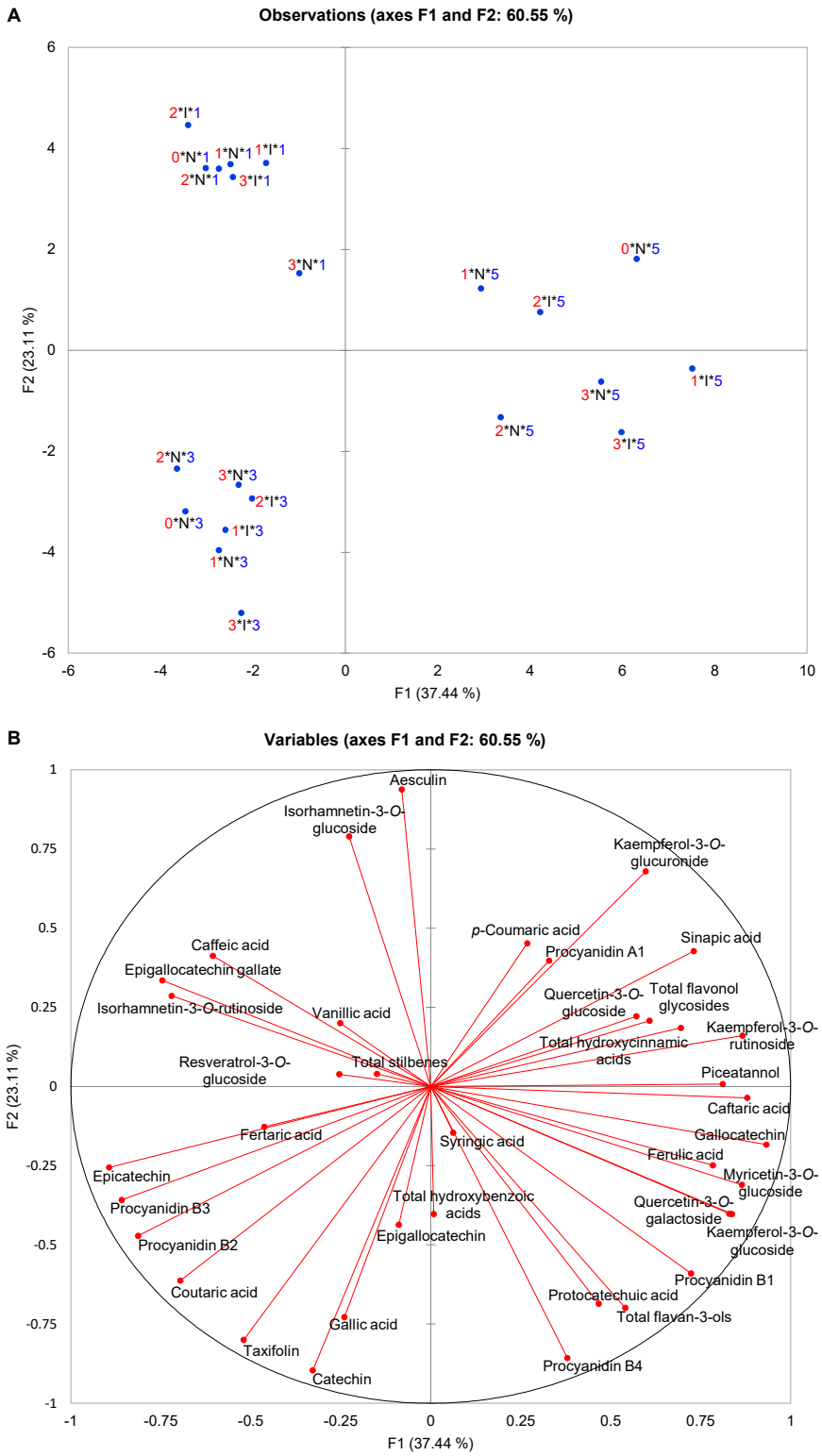

FIGURE 1 | Principal component analysis (PCA) scatter plot depicting **(A)** three OIV classes of susceptibility (1, 3, and 5—*V. vinifera* varieties) based on the polyphenolic composition of their leaves before and after artificial *P. viticola* inoculation at 0, 24, 48, and 96 hpi in the space defined by the first two principal components explaining 60.55% of the variability; **(B)** the vector diagram of correlation among the content of polyphenolic compounds and the first two principal components. 0, 1, 2, 3, Terms of sampling (0, 24, 48, and 96 hpi); N, I, Non-inoculated and inoculated observations; 1, 3, 5, OIV classes of resistance.

TABLE 2 | The differences between OIV classes of resistance to *P. viticola* in the content of polyphenolic compounds (mg/kg dw) in the young leaves.

Polyphenolic compound (mg/kg dw)	OIV class of resistance					Polyphenolic compound (mg/kg dw)	OIV class of resistance				
	1	3	5	7	9		1	3	5	7	9
Myricetin 3-*O*-glucoside	284.76 bc*	341.57 b	450.71 a	246.94 bc	142.38 c	Gallic acid	0.14 b	4.56 a	1.00 b	0.00 b	0.00 b
Quercetin 3-*O*-galactoside	10.98 bc	31.32 b	67.71 a	79.83 a	0.00 c	Protocatechuic acid	120.17 ab	131.65 a	134.73 a	99.79 b	67.06 c
Quercetin 3-*O*-glucoside	21476.02 a	20459.28 a	22230.20 a	19939.34 ab	12930.30 b	Vanillic acid	37.62 c	34.33 c	31.03 c	150.65 a	85.56 b
Kaempferol 3-*O*-rutinoside	107.08 c	82.88 c	187.92 b	186.31 b	356.74 a	Syringic acid	48.08 b	47.61 b	45.10 b	6.72 c	96.87 a
Isorhamnetin 3-*O*-rutinoside	80.80 a	35.87 ab	0.00 b	0.00 b	0.00 b	*Total hydroxybenzoic acids*	206.01 a	218.15 a	211.86 a	257.16 a	249.49 a
Kaempferol 3-O-glucoside	97.56 b	119.99 ab	152.31 a	143.20 a	116.43 ab	Epigallocatechin gallate	96.61 b	69.74 bc	27.70 c	79.61 bc	510.13 a
Kaempferol 3-*O*-glucuronide	24.03 a	11.89 b	25.05 a	0.00 c	2.74 bc	Gallocatechin	602.10 b	675.72 b	1365.01 a	652.79 b	31.69 c
Izorhamnetin 3-O-glucoside	6.74 a	0.26 b	1.92 b	0.00 b	0.00 b	Epigallocatechin	1389.55 a	1607.88 a	1337.18 a	1429.44 a	313.19 b
Taxifolin	0.56 b	10.94 a	0.00 b	0.00 b	0.00 b	Procyanidin B1	2209.83 c	3019.73 b	3683.26 a	3193.26 ab	1213.24 d
Total flavonol glycosides	22088.52 a	21094.01 a	23115.82 a	20595.62 ab	13548.60 b	Procyanidin B3	36.81 b	48.72 a	19.65 c	40.82 ab	3.93 d
Caftaric acid	5362.67 b	5424.84 b	6101.63 a	5544.56 ab	2511.22 c	Catechin	31.36 b	60.74 a	37.21 b	54.00 a	10.87 c
Aesculin	686.79 a	352.53 b	481.52 b	227.66 b	139.39 b	Procyanidin B4	111.97 b	151.69 a	150.75 a	124.66 ab	23.30 c
Coutaric acid	120.23 b	269.23 a	69.08 b	330.28 a	217.99 ab	Procyanidin B2	133.44 b	193.71 a	65.65 c	147.83 b	49.24 c
Caffeic acid	888.08 b	840.94 b	769.88 b	1443.86 a	280.46 c	Epicatechin	391.86 b	474.72 ab	107.81 d	485.20 a	225.81 c
Fertaric acid	15.28 bc	16.19 b	14.16 bc	26.31 a	11.04 c	Procyanidin A1	82.02 a	74.12 a	82.18 a	65.61 a	26.47 b
p-Coumaric acid	26.71 b	20.71 c	25.66 b	12.27 d	33.83 a	*Total flavan-3-ols*	5085.54 b	6376.75 a	6876.40 a	6273.21 ab	2407.87 c
Ferulic acid	31.28 b	34.12 ab	40.94 a	41.42 a	5.44 c	Piceatannol	13.44 c	13.42 c	27.97 b	37.79 b	63.97 a
Sinapic acid	3633.00 a	3213.18 b	3801.47 a	3372.13 ab	2154.91 c	Resveratrol 3-*O*-glucoside	183.21 a	143.12 b	101.44 c	70.09 c	208.62 a
Total hydroxycinnamic acids	27451.19 a	26518.85 a	29217.45 a	26140.19 a	16059.81 b	*Total stilbenes*	196.64 b	156.54 c	129.41 cd	107.88 d	272.59 a

**Means were evaluated by Duncan's multiple range test at a confidence level of 95% ($p < 0.05$). Different letters show statistical significance.*

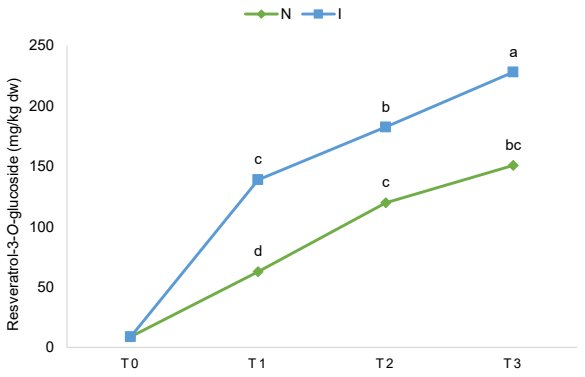

FIGURE 2 | The ascending content of resveratrol-3-*O*-glucoside throughout the experiment [before inoculation (T_0), 24 hpi (T_1), 48 hpi (T_2), and 96 hpi (T_3)] for non-inoculated (N) and inoculated (I) samples regardless of the International Organization of Vine and Wine (OIV) class. The values for each time point and treatment were obtained by the mean of the values of 17 genotypes. The differences between the means were evaluated by Duncan's multiple range test at a confidence level of 95% ($p < 0.05$). Different letters show statistical significance.

varieties, Pošip and Plavina. Pošip has a high content of caftaric acid, and resveratrol 3-*O*-glucoside, whereas in both of them, high content of procyanidin A1 is detected (**Supplementary Table 3** and **Supplementary Figure 2**).

Significant effect of inoculation on the content of piceatannol and resveratrol-3-*O*-glucoside was detected. Differences between the inoculated and non-inoculated samples were already significant in T_1 (24 h after inoculation) and continued through T_2 and T_3 for resveratrol-3-*O*-glucoside (**Figure 2**), while for piceatannol, besides the overall significant effect in factorial ANOVA, differences within the terms were not significant. Consequently, the total content of stilbenes was also significantly different since this group of polyphenolics is comprised of piceatannol and resveratrol-3-*O*-glucoside only (**Supplementary Table 2**). The ascending content of resveratrol-3-*O*-glucoside throughout the experiment is depicted in **Figure 2** for non-inoculated (N) and inoculated (I) samples regardless of the OIV class. Upon inoculation, the ascending content between the terms of sampling was significant for flavan-3-ol epicatechin (**Supplementary Table 2**).

Discrimination of *Vitis vinifera* Varieties

The focus of this study was on the variability of leaf polyphenolic compounds related to the difference of *V. vinifera* varieties. Therefore, a PCA was performed to analyze the total variability of the polyphenolic composition of the leaves before and after artificial *P. viticola* inoculation at 0, 24, 48, and 96 hpi that belong to OIV classes 1, 3, or 5 (*V. vinifera* varieties). Mock-inoculated (control, non-inoculated) leaves were sampled throughout the experiment simultaneously with inoculated ones. The PCA scatter plot of the first two components explained 60.55% of the variability (**Figure 1**) with the first principal component (PC1) accounting for 37.44% and the second (PC2) for 23.11%. Projection on these two axes separated the samples into three groups corresponding to three OIV classes (1, 3, and 5), whereas the infection status of the samples (non-inoculated (N) or inoculated (I)), and the terms of sampling were not separated (**Figure 1A**).

Based on the vector diagram (**Figure 1B**), it is possible to define the phenolic compounds that contributed to such distribution and grouping of samples belonging to different OIV classes in the space defined by the first two principal components. A group containing all the samples belonging to OIV class 5 regardless of the treatment and the sampling term was separated from the other two groups mainly based on the higher content of quercetin-3-*O*-glucoside, myricetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, piceatannol, caftaric acid, ferulic acid, and gallocatechin together with the contents of total flavonol glycosides and hydroxycinnamic acids. As for the group containing all the samples belonging to OIV class 3, all the observations are in the third quadrant and almost diametrically opposed to OIV class 5. The position of this group was defined mainly by a higher content of catechin, epicatechin, epigallocatechin, taxifolin, coutaric and gallic acid, and procyanidins, B2 and B3. Class 1 is distinguished by a higher content of isorhamnetin-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, caffeic and vanillic acid, epigallocatechin-gallate, resveratrol-3-*O*-glucoside, and by the content of total stilbenes.

DISCUSSION

Studies considering metabolomic changes of the grapevines and profiling regarding the different levels of susceptibility to *P. viticola* have been intriguing for more than a decade and this trend does not seem to fade. For this purpose, HPLC proved to be a reliable and scrutinized analytical technique by which it is possible to quantify phenolic acids and polyphenolic compounds (Tomaz and Maslov, 2016). As possible progress of breeding programs that are oriented toward improved resistance, *V. vinifera* varieties are in the spotlight to research and use as progenitors since most of them do not contain undesirable viticultural and oenological features like American species (Toepfer et al., 2011).

Plant metabolites can be either included in the primary metabolism, such as lipid compounds, amino acids, and sugars, or secondary metabolism, such as phenolic compounds arising biogenetically from the shikimate-phenylpropanoids-flavonoids pathways (Lattanzio et al., 2006). Some of the important physiological roles of phenolic compounds are the formation of the cell wall polymers, lignin (Paone et al., 2020) and suberin (Bernards and Razem, 2001), floral and fruit pigment synthesis (Tanaka et al., 2008), ultraviolet sunscreen protection (Cefali et al., 2016), formation of flavor compounds (Kielhorn and Thorngate, 1999), and defense against pathogens (Zaynab et al., 2018).

Gómez-Zeledón and Kaiser (2016); Buonassisi et al. (2018), and Vezzulli et al. (2018) have proposed OIV descriptor 452-1 (OIV, 2009), and therefore it was also applied in the research aiming to distinguish groups of resistance among Croatian native grapevine varieties as this method is reliable with included

control genotypes (Štambuk et al., 2021). Nonetheless, for comprehensive research purposes, in the present work, the screening is extended and dedicated to the analysis of secondary metabolites, namely polyphenolic compounds and phenolic acids aiming to define their possible relation with differences in the resistance level among them.

The most abundant group of polyphenolic compounds detected in the leaves of genotypes included in this study were hydroxycinnamic acids (25.19 g/kg DW) followed by flavonol glycosides, whose content was slightly lower (20.2 g/kg DW). Flavonol glycosides have photoprotective roles by filtering the UV-B light while allowing to pass photosynthetically active visible light (Agati et al., 2013) together with an antioxidant function during plant response to environmental stress (Hernández et al., 2009). Quercetin-3-*O*-glucoside was the most abundant flavonol in the research performed by Anđelković et al. (2015) in the grapevine leaf extracts from Vranac and Merlot (*V. vinifera*). Previous studies confirmed the higher susceptibility of shaded leaves grown in the greenhouse that contains less flavonols (Agati et al., 2008; Latouche et al., 2013) thus supporting the results of the present study where hydroxycinnamic acids are the most abundant polyphenolic group, probably due to reduced ultraviolet radiation conditions in the greenhouse. According to Meyer et al. (2021), supplemental UV-B light has a positive effect on disease resistance in many plant-pathogen combinations, mainly through the induction of the production of specialized metabolites like flavonoids.

No significant differences were found between the control and the inoculated leaves regardless of the OIV class and the term of sampling, with the exception of stilbenes and resveratrol-3-*O*-glucoside specifically. That is in accordance with the previous study where non-destructive optical methods were used (Latouche et al., 2013) for monitoring flavonols, hydroxycinnamic acids, and stilbenes throughout 6 days upon *P. viticola* inoculation in the Cabernet Sauvignon leaves grown in the greenhouse. Resveratrol-3-*O*-glucoside also showed a significant positive correlation in terms of sampling both in the case of inoculated and non-inoculated samples. Stilbenes are the most important class of phytoalexins in the *Vitaceae* family, which are dynamically accumulated in response to various abiotic and biotic stresses, including pathogen attacks (Ciaffi et al., 2019). Thus, their ascending content in the non-inoculated leaves could be explained by picking them as well as changing the environment from the greenhouse to the laboratory conditions. Moreover, inoculation with *P. viticola* suspension caused additional stress and thus in these leaves, the contents of resveratrol-3-*O*-glucoside and total stilbenes were even higher throughout the experiment. Previous studies have shown that the accumulation of higher stilbenes is usually associated with the response of the resistant genotypes to *P. viticola* infection (Boso et al., 2012; Chitarrini et al., 2017). In our study, huge variability was detected among the genotypes belonging to different resistance classes confirming that the accumulation of stilbene resveratrol-3-*O*-glucoside is related to infection. However, the obtained results also suggest that increased content of resveratrol-3-*O*-glucoside is not sufficient to achieve a high level of resistance to *P. viticola*. In response to the presence of *P. viticola*, stilbenes are synthesized in grapevine leaves (Chalal et al., 2014). They possess antimicrobial activity that may be strong enough to inhibit the infection in resistant genotypes (Chong et al., 2009), which is not accurate for susceptible genotypes. Based on the previous studies, the content of stilbenes increases in accordance with *P. viticola* development in susceptible varieties suggesting that the accumulation of stilbenes can be used as an indicator of *P. viticola* infection (Naidenov et al., 2010; Latouche et al., 2013).

In the present work, three susceptible OIV classes (1, 3, and 5) are distinguished by each group characteristic of polyphenolic compounds provided by PCA. More specifically, the most susceptible OIV class 1 was separated from two other groups by being abundant in caffeic and vanillic acid, which are hydroxycinnamic and hydroxybenzoic acids, respectively. Caffeic acid has also been found in high amounts in susceptible *V. vinifera* varieties (Riesling Weiss, Pinot Noir, Cabernet Sauvignon, and Trincadeira) (Maia et al., 2020). On the contrary, caffeic acid has been previously related to constitutive resistance in the partially resistant cultivar Regent (Figueiredo et al., 2008). This compound participates in enzymatic oxidative mechanisms in response to the pathogenic infection of the grapevine (Mattivi et al., 2011). Among flavan-3-ols, the only discriminator was epigallocatechin-gallate known for its high antioxidant capacity (Kedrina-Okutan et al., 2018).

Flavan-3-ols, i.e., catechin and epicatechin, were more abundant in the presented OIV class 3. A previous study (Maia et al., 2020) hypothesizes that higher levels of catechin/epicatechin and over-expression of *LAR2* gene (involved in the conversion of leucocyanidin into catechin and epicatechin) may be putative biomarkers of susceptibility. Catechin, together with other phenolic compounds, possesses antioxidant properties and has been previously determined as a part of the grapevine defense mechanism (Kortekamp, 2006). However, there is a presumption that catechin can be degraded by different fungi, used as a carbon source for growth, and finally used for establishing a successful infection (Maia et al., 2020), but the precise potential of *P. viticola* in the degradation of this compound is not investigated. Epicatechin has been proposed as a biomarker of resistance in a study by Ciubotaru et al. (2021) due to its higher content in the genotype, BC4 possessing resistant locus *Rpv1*. Phenolic acids, namely fertaric, coutaric, and gallic acid, have also contributed to the discrimination. Nevertheless, Ali et al. (2012) identified fertaric acid in the partially resistant cultivar Regent.

Quercetin-3-*O*-glucoside was a discriminative compound that was more abundant in the OIV class 5, which is in accordance with a previous study (Maia et al., 2020) where the same flavonol glycoside together with several others was found in higher concentrations in the resistant/partially resistant genotypes. Another flavonol, namely kaempferol-3-*O*-rutinoside that distinguished this class, was also detected previously in the partially resistant cultivar, Bianca at 12 hpi (Chitarrini et al., 2017). Furthermore, quercetin-3-*O*-glucoside and caftaric acid were found at higher concentrations and therefore were responsible for distinguishing Regent from Trincadeira (Ali et al., 2012). Latouche et al. (2013) observed that constitutive higher content of flavonols slowed down the accumulation of stilbenes in

the grapevine leaves, and thus the phytoalexin-mediated response of leaves to *P. viticola* was delayed, suggesting that constitutive higher amounts of flavonols could confine the spreading of the pathogen. *Trans*-caftaric acid was the most abundant phenolic acid in the leaf extracts of Vranac and Merlot, with lower content in infected ones (Anđelković et al., 2015). Ferulic and *p*-coumaric acids were also discriminative for the OIV 5. The highest contents of these acids were previously found in the interspecies hybrid Petra, with 12.5% of *Vitis amurensis* and 87.5% of *Vitis vinifera* in its genetic background, among other pure *V. vinifera* varieties (Pantelić et al., 2017). Petra is known for high cold hardiness and reduced susceptibility to *P. viticola* and *Botrytis cinerea* (Cindric et al., 2003).

Apart from constitutive and induced chemical compounds that provide a certain level of tolerance to parasitic microorganisms, resistance to *P. viticola* can be associated with the synthesis of physical barriers, such as callose and lignin appositions (Toffolatti et al., 2012). Moreover, hydrophobic trichomes on the abaxial leaf sides reduce the retention or repel water drops, thus preventing the encystment of *P. viticola* zoospores (Kono and Shimizu, 2020), a step that is essential for the pathogen development inside a leaf tissue and further fructification (Rossi and Caffi, 2007, 2012). This morphological feature is an example of passive resistance, whereas active responses involve hypersensitivity and synthesis of specific secondary metabolites (Buonassisi et al., 2017). The morphological characteristic of Croatian native varieties, i.e., Teran and Ranfol, is abaxial leaf sides covered by extremely dense hydrophobic and moderately dense trichomes, respectively, that certainly obstruct *P. viticola* sporangia to reach the epidermis and stoma at the leaf bottom. On the other hand, the leaves of Malvazija istarska are glabrous; yet they are firm and robust (Maul et al., 2012; Maletić et al., 2015a) whose possibly thick cuticle protect them from plant pathogens (Serrano et al., 2014). There are varieties with a relatively high density level of trichomes within the classes of resistance, such as Belina starohrvatska, Moslavac, and Plavac mali in class 1, Plavina in class 3, and Ranfol and Teran in class 5. Opposed to this, in the case of resistant genotypes (class 7 and 9), low density levels of the trichomes are present. Subsequently, no correlation between the density of the trichomes and resistance to *P. viticola* was determined suggesting that this feature does not have a major effect on the resistance level of specific genotypes, in contrast to some previous studies (Kortekamp and Zyprian, 1999; Kono and Shimizu, 2020).

Solaris, one of the control varieties used in this research, proved its high yet not complete resistance to *P. viticola* (OIV 452 $\approx$ 7) in previous studies (Vezzulli et al., 2018; Ciubotaru et al., 2021) since this variety contains two resistance genes (*Rpv3-3* and *Rpv10*) (Vezzulli et al., 2019; Possamai et al., 2020). Such a pyramided resistance provides a higher level of resistance generally expressed as a more stable and durable feature (Merdinoglu et al., 2018). However, it was found that its response to *P. viticola* infection is isolate-specific and highly variable (Heyman et al., 2021). Due to its genetic background based on *V. vinifera* [Merzling × (Zarya Severa × Muscat Ottonel)] (Pezet et al., 2004), it reacted more similarly to *V. vinifera* varieties, when compared to *V. riparia* upon *P. viticola* inoculation. This is comparable with previous research where Regent's (on which backcrosses were made with *V. vinifera*) metabolic profile clustered together with *V. vinifera* varieties (Maia et al., 2020). In Bianca, which has an *Rpv3* locus in its genome, the content of the secondary metabolites increased at later stages after the infection (96 hpi). These were phenylpropanoids, flavonols, and stilbenes, whereas the earliest modifications included primary metabolites, i.e., lipids, amino acids, acids, and sugars at 24–48 hpi (Chitarrini et al., 2017).

Vitis riparia is an indigenous species to North America where it evolved with fungi/oomycete, *E. necator* and *P. viticola,* and subsequently developed resistance to mildew diseases (OIV 452 = 9). Low or no sporulation values were associated with this genotype in the previous studies (Boso et al., 2012; Bhattarai et al., 2021). Thus, it has been effectively used in breeding programs for resistance introgression (Toepfer et al., 2011). Upon *P. viticola* infection, this genotype produced the highest content of resveratrol-3-*O*-glucoside, piceatannol, and total stilbenes which have been observed previously (Boso et al., 2012) due to the fast constitutive expression of the stilbene synthase genes as well as the extent of their transcriptional activation following *P. viticola* inoculation (Ciaffi et al., 2019). Stilbenes are toxic to phytopathogenic fungi and may contribute to disease resistance as phytoalexins (Ribera and Zuñiga, 2012). Although stilbenes were also identified in susceptible genotypes, they contributed to the differentiation of the OIV class 1; their importance is much greater in discriminating the resistant genotype. Along with stilbenes, epigallocatechin-gallate and kaempferol-3-*O*-rutinoside discriminated the OIV class 9 from all other OIV classes. Kedrina-Okutan et al. (2018) stated that *V. riparia* leaves constitutively contain a higher amount of total polyphenols, total flavonols, and total phenolic acids compared to *V. rupestris*, which could explain its specifically high resistance to *P. viticola*, as the flavonols limit this pathogen development (Ali et al., 2012). Comparing metabolic compositions associated with disease susceptibility of different *Vitis* species and *V. vinifera* varieties, *V. riparia* clustered together with *V. labrusca*, *V. candicans*, *V. vinifera* subsp. *sylvestris,* and *V. rotundifolia*, whereas the Regent was closer to *V. vinifera* varieties, such as Riesling Weiss and Pinot Noir (Maia et al., 2020), confirming the results of the present study. The susceptible control variety, Chardonnay was previously included in a study (Toffolatti et al., 2012) where the changes of antifungal compounds upon *P. viticola* infection are described and flavonoids showed no specific reaction to the presence of this pathogen. In our study, the polyphenolic profile of Chardonnay was similar to most of the other genotypes belonging to OIV class 3, although some compounds (i.e., protocatechuic acid, gallocatechin, procyanidins B1, B3, and B4) were higher than in the case of other genotypes from this class. Chardonnay and the native variety, Kraljevina were specific for the highest content of gallic acid.

There were no previous studies on the polyphenolic composition of the leaves of Croatian native varieties used in this study and our previous study (Štambuk et al., 2021) was the first one on the susceptibility of Croatian

grapevine germplasm. Results from both of these studies, confirm the high level of variability within the Croatian native varieties previously defined for other important characteristics (Maletić et al., 2015b).

CONCLUSION

This work has demonstrated the importance of secondary metabolites in grapevine defense responses against *P. viticola* with particular emphasis on Croatian native varieties. The research is based on a detailed analysis of phenolic compounds responsible for the discrimination of varieties among the OIV classes of resistance. The performed polyphenolic analysis confirmed and fulfilled the previous studies suggesting that constitutive polyphenolic profile contributes to the separation of susceptible OIV classes (1, 3, and 5) into three groups. The high variability in the content of resveratrol-3-*O*-glucoside and total stilbenes was determined, and discrimination among non-infected and infected samples was detected. However, the content of this compound did not show a clear difference between the resistant and susceptible genotypes. The content of piceatannol and total stilbenes discriminated completely resistant OIV class 9 (*V. riparia*) and the remaining OIV classes, thus confirming their strong antimicrobial properties. Considering the polyphenolic profiles of *V. vinifera* varieties, mostly flavonol glycosides were found to be responsible for lower susceptibility. Multivariate analysis shows complex relations among phenolic profiles and resistance levels suggesting that the preinfectional phenolic profile of leaves could be a determinant for different susceptibility to *P. viticola*.

Less susceptible grapevine varieties that belong to OIV class 5 (Malvazija istarska, Ranfol, Teran) could be interesting to use in breeding programs aiming to produce high-quality genotypes resistant to main fungal diseases. A further intention is directed toward analyzing constitutive and induced volatile organic compounds since their profile should distinguish grapevine classes of susceptibility to *P. viticola* likewise, whereas potential early metabolomic changes should elucidate additional bioactive molecules.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

PŠ, JK, and IT contributed to the conceptualization of the study. PŠ and IT contributed to the methodology. PŠ, IŠ, and DP conducted the formal analysis. PŠ and IŠ performed the investigations. DP performed data curation. PŠ wrote the original draft preparation. IŠ, DP, JK, EM, and IT contributed to the writing, reviewing, and editing. JK and IT supervised the work. EM contributed to funding acquisition. All authors contributed to the manuscript revision, read, and approved the submitted version.

FUNDING

This research was funded by the project KK.01.1.1.01.0005 Biodiversity and Molecular Plant Breeding, Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-Bio-Div), Zagreb, Croatia.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.836318/full#supplementary-material

Supplementary Figure 1 | Genotypes in the greenhouse.

Supplementary Figure 2 | PCA_Single genotypes and polyphenolic compounds.

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Appendix 2A. Supplement material related to scientific paper Štambuk P., Šikuten I., Karoglan Kontić J., Maletić E., Preiner D., Tomaz I. (2022). Leaf Polyphenolic Profile as a Determinant of Croatian Native Grapevine Varieties' Susceptibility to *Plasmopara viticola*. Frontiers in Plant Science 13: 836318. doi: 10.3389/fpls.2022.836318

Supp. Table 1 - Factorial ANOVA was performed to test the interaction of Term of sampling (including T0 (before inoculation)), Treatment and OIV class based on the polyphenolic content (mg/kg) in the leaves of 17 genotypes. The differences between the means (the interaction of Term of sampling, Treatment and OIV class) were evaluated by Duncan's multiple range test at a confidence level of 95 % ($p < 0.05$). Different letters show statistical significance.

Term*Treatment*OIV class	Myricetin 3-*O*-glucoside	Quercetin 3-*O*-galactoside	Quercetin 3-*O*-glucoside	Kaempferol 3-*O*-rutinoside	Isorhamnetin 3-*O*-rutinoside	Kaempferol 3-O-glucoside	Kaempferol 3-*O*-glucuronide	Izorhamnetin 3-O-glucoside	Taxifolin	Total flavonol glycosides	Caftaric acid	Aesculin	Coutaric acid	Caffeic acid	Fertaric acid	*p*-Coumaric acid	Ferulic acid	Sinapic acid	Total hydroxycinnamic acids
0*N*1	255.19 a	8.29 d	20338.53 a	84.99 hi	80.28 a	68.78 ab	22.31 ab	8.14 a	0.38 a	20866.89 a	5261.47 a	616.66 a	119.90 a	840.40 cdefgh	12.68 efgh	20.14 defghij	27.82 bcdef	3422.69 abcdefg	26128.35 ab
0*N*3	357.61 a	37.35 cd	18421.28 a	37.31 i	151.28 a	105.64 ab	13.00 ab	1.73 a	8.35 a	19133.54 a	4948.74 a	343.07 a	246.66 a	792.03 cdefghi	13.11 defg	17.71 defghij	29.05 abcdef	3285.01 abcdefg	24082.28 ab
0*N*5	497.54 a	59.09 abcd	27083.62 a	180.87 efghi	0.00 a	154.56 a	36.79 a	1.69 a	0.00 a	28014.16 a	6417.45 a	499.34 a	57.88 a	820.61 cdefghi	9.97 efgh	19.49 defghij	37.20 abcde	4573.94 a	34431.60 a
0*N*7	277.86 a	122.32 abc	21446.45 a	327.85 cde	0.00 a	142.71 a	1.15 b	0.00 a	0.00 a	22318.34 a	5930.17 a	245.64 a	213.36 a	1309.56 abc	23.17 abcde	10.79 ij	51.79 abc	3623.18 abcd	28248.51 ab
0*N*9	137.20 a	0.00 d	13700.25 a	68.92 hi	0.00 a	0.00 b	0.00 b	0.00 a	0.00 a	13906.36 a	2358.51 b	120.34 a	190.06 a	320.79 efghi	5.14 gh	38.50 a	6.98 fgh	2309.29 defgh	16264.87 ab
1*I*1	270.01 a	23.01 d	21492.00 a	139.81 fghi	101.95 a	102.88 ab	29.80 ab	7.47 a	0.94 a	22167.87 a	5450.32 a	677.21 a	114.06 a	909.17 bcde	15.37 cdefg	26.75 abcdefg	33.73 abcde	3616.60 abcd	27618.19 ab
1*I*3	335.62 a	26.62 d	18989.21 a	53.65 i	30.43 a	126.57 a	12.65 ab	0.77 a	11.30 a	19586.81 a	5161.52 a	365.22 a	331.91 a	792.98 cdefghi	13.59 defg	20.01 defghij	31.41 abcdef	3200.39 bcdefgh	24748.34 ab
1*I*5	491.75 a	72.94 abcd	24487.77 a	282.56 cdef	0.00 a	152.99 a	27.40 ab	2.37 a	0.00 a	25517.78 a	6297.88 a	465.16 a	48.92 a	683.78 defghi	12.37 efgh	23.20 bcdefghij	45.13 abc	4130.73 ab	31815.65 ab
1*I*7	205.38 a	58.90 abcd	19248.35 a	191.53 efghi	0.00 a	143.55 a	0.00 b	0.00 a	0.00 a	19847.70 a	5121.72 a	243.77 a	334.45 a	1245.35 abcd	23.71 abcde	13.44 fghij	36.64 abcde	3253.81 abcdefgh	24969.42 ab
1*I*9	188.24 a	0.00 d	13277.95 a	369.52 bcd	0.00 a	139.33 a	4.27 b	0.00 a	0.00 a	13979.31 a	2504.48 b	125.68 a	222.57 a	306.43 fghi	19.46 abcdef	29.40 abcde	0.00 h	2235.37 efgh	16483.79 ab
1*N*1	319.80 a	9.09 d	21741.34 a	108.17 fghi	53.19 a	72.64 ab	26.14 ab	10.87 a	0.00 a	22341.24 a	5170.80 a	694.51 a	151.45 a	909.24 bcde	17.84 abcdefg	24.49 abcdefghij	32.85 abcde	3660.27 abcd	27512.04 ab
1*N*3	398.70 a	48.61 bcd	18871.19 a	98.47 ghi	32.97 a	106.92 ab	10.80 ab	0.00 a	13.34 a	19581.00 a	5347.15 a	323.43 a	250.66 a	850.66 bcdefgh	16.12 bcdefg	16.94 defghij	39.23 abcd	3179.86 bcdefgh	24928.14 ab
1*N*5	471.18 a	46.76 bcd	19375.92 a	200.38 efghi	0.00 a	151.17 a	26.04 ab	3.33 a	0.00 a	20274.78 a	5659.33 a	605.42 a	123.68 a	892.37 bcdefg	18.87 abcdefg	25.41 abcdefghij	42.68 abc	3579.33 abcde	25934.11 ab
1*N*7	179.99 a	44.12 bcd	21703.40 a	144.52 fghi	0.00 a	141.87 a	0.00 b	0.00 a	0.00 a	22213.90 a	5757.47 a	225.30 a	420.69 a	1537.72 a	27.24 abc	10.99 hij	33.02 abcde	3669.22 abc	27971.36 ab
1*N*9	85.30 a	0.00 d	12711.85 a	444.00 abc	0.00 a	0.00 b	2.37 b	0.00 a	0.00 a	13243.52 a	2234.42 b	144.35 a	227.67 a	236.00 i	7.52 fgh	26.26 abcdefgh	3.70 gh	2145.89 fgh	15477.94 b
2*I*1	251.23 a	6.82 d	20043.22 a	99.56 ghi	83.47 a	88.61 ab	27.20 ab	6.75 a	0.00 a	20606.86 a	5283.57 a	669.01 a	138.49 a	902.80 bcdef	14.68 cdefg	28.83 abcde	30.05 abcdef	3375.65 abcdefg	25890.43 ab
2*I*3	316.48 a	28.75 d	26393.94 a	91.45 ghi	54.75 a	125.96 a	10.35 ab	0.00 a	9.00 a	27030.68 a	5128.84 a	316.83 a	262.91 a	821.12 cdefghi	18.75 abcdefg	22.43 cdefghij	33.32 abcde	3115.54 bcdefgh	32159.52 ab
2*I*5	439.72 a	59.04 abcd	23106.73 a	116.56 fghi	0.00 a	152.54 a	28.23 ab	0.00 a	0.00 a	23902.83 a	6235.36 a	468.91 a	56.70 a	716.25 cdefghi	13.25 defg	25.88 abcdefghi	35.80 abcde	3898.18 ab	30138.19 ab
2*I*7	309.14 a	129.05 ab	20311.85 a	237.95 defgh	0.00 a	142.20 a	0.00 b	0.00 a	0.00 a	21130.18 a	5620.31 a	237.27 a	419.10 a	1502.71 a	29.57 a	15.57 efghij	53.26 ab	3440.31 abcdef	26750.49 ab
2*I*9	105.40 a	0.00 d	13118.45 a	270.78 defg	0.00 a	139.08 a	0.46 b	0.00 a	0.00 a	13634.17 a	2547.35 b	127.54 a	229.49 a	297.72 ghi	0.00 h	36.64 abc	0.00 h	2210.39 fgh	16181.51 ab
2*N*1	247.60 a	1.79 d	22347.11 a	73.52 hi	77.81 a	98.59 ab	20.54 ab	6.23 a	2.07 a	22875.26 a	5572.73 a	677.74 a	115.67 a	893.30 bcdefg	13.90 cdefg	24.89 abcdefghij	26.11 cdefg	3767.24 ab	28447.99 ab
2*N*3	284.29 a	26.03 d	19029.13 a	76.96 hi	43.71 a	105.67 ab	11.38 ab	0.00 a	8.45 a	19585.62 a	5646.00 a	366.74 a	225.02 a	890.73 bcdefg	16.79 abcdefg	19.10 defghij	32.60 abcde	3209.76 bcdefgh	25231.62 ab
2*N*5	373.17 a	84.48 abcd	18512.60 a	161.35 efghi	0.00 a	151.32 a	19.57 ab	0.00 a	0.00 a	19302.49 a	6261.21 a	438.83 a	57.90 a	721.50 cdefghi	14.23 cdefg	19.85 defghij	41.55 abc	3124.13 bcdefgh	25563.70 ab
2*N*7	235.73 a	68.62 abcd	19200.65 a	149.71 fghi	0.00 a	144.76 a	0.00 b	0.00 a	0.00 a	19799.48 a	5541.77 a	229.97 a	309.76 a	1480.58 a	26.54 abcd	12.52 ghij	38.88 abcde	3248.24 abcdefgh	25341.24 ab
2*N*9	118.50 a	0.00 d	12347.30 a	548.83 a	0.00 a	139.34 a	4.75 b	0.00 a	0.00 a	13158.71 a	2431.35 b	150.95 a	180.31 a	262.77 hi	6.71 fgh	36.15 abc	2.75 gh	1933.27 h	15590.06 ab
3*I*1	295.59 a	8.30 d	21350.73 a	113.89 fghi	72.94 a	101.52 ab	15.14 ab	6.18 a	0.00 a	21964.29 a	5308.46 a	699.09 a	122.36 a	916.38 bcde	15.54 cdefg	25.63 abcdefghi	34.13 abcde	3694.64 ab	27272.75 ab
3*I*3	338.05 a	22.18 d	19272.32 a	96.14 ghi	12.51 a	126.67 a	12.43 ab	0.00 a	13.16 a	19893.45 a	5691.96 a	345.43 a	274.13 a	803.71 cdefghi	15.86 cdefg	23.61 abcdefghij	33.22 abcde	3172.70 bcdefgh	25585.41 ab
3*I*5	466.72 a	80.25 abcd	24362.05 a	170.56 efghi	0.00 a	153.73 a	21.05 ab	5.84 a	0.00 a	25260.19 a	6196.49 a	441.41 a	53.71 a	777.20 cdefghi	14.05 cdefg	27.86 abcdef	40.03 abc	4108.55 ab	31456.68 ab
3*I*7	352.11 a	138.94 a	19036.15 a	284.58 cdef	0.00 a	142.98 a	0.00 b	0.00 a	0.00 a	19954.76 a	5613.56 a	217.80 a	244.35 a	1423.98 ab	29.27 ab	10.94 ij	55.06 a	3218.30 abcdefgh	25568.32 ab
3*I*9	141.92 a	0.00 d	12364.25 a	487.61 ab	0.00 a	141.37 a	4.61 b	0.00 a	0.00 a	13139.76 a	3038.21 b	152.77 a	278.26 a	272.18 hi	17.98 abcdefg	36.29 abc	12.85 efgh	2080.63 gh	16177.97 ab
3*N*1	324.30 a	16.89 d	21881.73 a	107.54 fghi	95.43 a	121.10 a	25.34 ab	2.92 a	0.33 a	22575.57 a	5390.17 a	703.20 a	79.37 a	797.62 cdefghi	14.37 cdefg	29.69 abcde	30.79 abcdef	3683.61 ab	27965.74 ab
3*N*3	376.28 a	35.73 cd	20199.89 a	80.62 hi	40.81 a	128.18 a	13.75 ab	0.81 a	10.42 a	20886.48 a	5573.58 a	397.55 a	270.78 a	886.43 bcdefg	16.04 bcdefg	22.15 cdefghij	34.96 abcde	3400.85 abcdefg	26460.06 ab
3*N*5	461.73 a	62.79 abcd	23536.13 a	196.09 efghi	0.00 a	152.09 a	28.02 ab	0.00 a	0.00 a	24436.85 a	5959.54 a	469.42 a	73.56 a	828.17 cdefgh	12.22 efgh	31.75 abcd	40.46 abc	3967.89 ab	30396.39 ab
3*N*7	199.30 a	39.38 cd	20135.65 a	109.57 fghi	0.00 a	143.85 a	0.00 b	0.00 a	0.00 a	20627.74 a	5612.55 a	211.89 a	253.33 a	1472.86 a	21.57 abcde	10.19 j	31.65 abcdef	3402.94 abcdefg	26240.28 ab
3*N*9	214.93 a	0.00 d	13762.00 a	19.73 i	0.00 a	139.45 a	0.00 b	0.00 a	0.00 a	14136.11 a	2311.49 b	135.05 a	169.66 a	307.69 fghi	14.55 cdefg	38.23 ab	13.35 defgh	2323.90 cdefgh	16447.60 ab
Pr > F(Model)	0.54	0.00	0.76	<0.0001	0.28	0.13	0.00	0.00	0.09	0.75	<0.0001	0.82	0.81	<0.0001	0.01	<0.0001	<0.0001	<0.0001	0.34
Pr > F(Term*Treatment*OIV class)	1.00	0.80	0.95	0.00	1.00	0.93	0.96	0.88	1.00	0.94	1.00	1.00	1.00	1.00	0.68	0.98	0.98	0.96	0.95
Significant	No	Yes	No	Yes	No	No	Yes	Yes	No	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No
Significant	No	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No

Supp. Table 1 continued

Term*Treatment*OIV class	Gallic acid	Protocatechuic acid	Vanillic acid	Syringic acid	Total hydroxybenzoic acids	Epigallocatechin gallate	Gallocatechin	Epigallocatechin	Procyanidin B1	Procyanidin B3	Catechin	Procyanidin B4	Procyanidin B2	Epicatechin	Procyanidin A1	Total flavan-3-ols	Piceatannol	Resveratrol 3-*O*-glucoside	Total stilbenes
0*N*1	0.00 a	115.29 abcd	50.48 abcdef	33.99 fgh	199.76 a	109.06 ef	500.92 cd	1123.56 ab	2401.12 abcdefg	33.69 abcd	28.72 abcdefg	109.68 a	114.50 abcdefghi	318.07 bcdefg	77.60 ab	4816.92 abcde	9.64 gh	0.00 g	9.64 lm
0*N*3	1.75 a	131.92 abcd	40.99 bcdef	30.51 fgh	205.16 a	61.62 ef	594.61 bcd	1628.57 ab	3653.74 abc	44.52 abc	65.84 a	160.81 a	182.58 a	488.82 abc	70.60 abc	6951.69 ab	4.42 gh	21.50 fg	25.92 klm
0*N*5	0.00 a	133.07 abcd	29.09 cdef	38.61 efgh	200.77 a	16.09 f	1028.26 abc	1200.40 ab	3401.38 abc	22.05 abcde	24.82 abcdefg	131.30 a	59.93 defghi	91.53 gh	80.89 ab	6056.65 abcd	26.08 efgh	0.00 g	26.08 klm
0*N*7	0.00 a	96.20 abcd	170.45 ab	0.00 h	266.64 a	79.62 ef	459.04 cd	1576.81 ab	2735.09 abcdef	41.74 abc	57.46 abc	124.91 a	175.92 ab	414.24 bcd	69.26 abc	5734.07 abcde	66.22 bcd	0.00 g	66.21 jklm
0*N*9	0.00 a	55.09 d	65.24 abcdef	107.62 ab	227.94 a	757.38 a	59.70 d	346.52 b	899.65 fg	0.00 e	7.59 efg	25.66 bc	29.43 ghi	7.74 h	27.55 d	2161.23 cde	3.31 gh	0.00 g	3.30 m
1*I*1	0.00 a	119.06 abcd	52.71 abcdef	30.33 fgh	202.11 a	55.97 ef	583.38 bcd	1064.48 ab	2174.20 abcdefg	36.18 abcd	33.83 abcdefg	121.09 a	152.60 abcd	387.12 bcde	83.10 ab	4691.94 abcde	18.79 efgh	151.70 cde	170.50 defghij
1*I*3	0.74 a	133.58 abcd	23.35 def	55.09 cdefg	212.75 a	74.60 ef	685.55 bcd	1653.38 ab	3283.42 abcde	47.52 a	60.12 abc	154.22 a	177.71 ab	436.04 bcd	69.10 abc	6641.67 abcd	21.14 efgh	139.92 cde	161.07 efghij
1*I*5	0.00 a	133.63 abcd	30.28 cdef	47.41 defgh	211.32 a	34.04 ef	1442.99 ab	1348.06 ab	4127.06 a	21.09 abcde	33.77 abcdefg	163.21 a	64.13 cdefghi	103.56 fgh	86.29 ab	7424.19 a	48.23 cde	103.22 defg	151.45 fghij
1*I*7	0.00 a	117.44 abcd	146.59 abcd	0.00 h	264.03 a	67.85 ef	524.53 bcd	1178.15 ab	3393.05 abcd	46.88 a	47.99 abcde	116.82 a	131.83 abcdefg	354.48 bcdef	77.64 ab	5939.20 abcd	36.71 defg	132.58 cdef	169.29 defghij
1*I*9	0.00 a	63.98 bcd	101.64 abcdef	118.42 a	284.04 a	687.81 ab	50.63 d	314.63 b	838.17 fg	0.00 e	6.41 fg	24.86 bc	27.02 hi	26.13 h	24.75 d	2000.42 de	65.27 bcd	180.87 bcde	246.14 bcdef
1*N*1	0.00 a	119.43 abcd	49.91 abcdef	46.02 defgh	215.36 a	96.74 ef	526.10 bcd	1187.46 ab	2341.20 abcdefg	36.97 abcd	30.88 abcdefg	118.06 a	130.58 abcdefg	358.93 bcdef	78.73 ab	4905.66 abcde	15.02 fgh	110.66 defg	125.68 fghijkl
1*N*3	3.40 a	125.99 abcd	71.71 abcdef	29.51 fgh	230.61 a	62.21 ef	632.03 bcd	1651.27 ab	3012.14 abcdef	49.26 a	61.48 ab	153.98 a	190.55 a	441.33 bcd	63.22 abcd	6317.47 abcd	14.01 fgh	73.21 efg	87.22 hijklm
1*N*5	0.00 a	123.66 abcd	26.84 cdef	23.12 gh	173.61 a	2.89 f	1156.80 abc	1297.50 ab	3161.32 abcde	27.32 abcde	27.91 abcdefg	136.39 a	56.61 defghi	88.99 gh	76.21 abc	6031.94 abcd	31.33 efgh	0.00 g	31.33 klm
1*N*7	0.00 a	91.30 abcd	135.94 abcde	40.31 efgh	267.55 a	76.71 ef	634.58 bcd	1471.59 ab	3295.55 abcde	51.89 a	47.33 abcdef	115.03 a	120.46 abcdefghi	358.17 bcdef	60.27 abcd	6231.56 abcd	28.42 efgh	0.00 g	28.42 klm
1*N*9	0.00 a	60.27 cd	56.79 abcdef	76.83 abcdef	193.89 a	433.99 cd	20.11 d	228.23 b	493.83 g	0.00 e	0.00 g	19.02 c	19.42 i	6.05 h	24.18 d	1244.83 e	80.74 ab	0.00 g	80.74 ijklm
2*I*1	0.00 a	112.64 abcd	62.71 abcdef	41.46 efgh	216.80 a	111.20 ef	530.59 bcd	1056.09 ab	2029.15 abcdefg	33.91 abcd	25.63 abcdefg	101.17 ab	117.59 abcdefghi	366.42 bcde	81.11 ab	4452.84 abcde	10.73 gh	219.85 abcd	230.57 cdefg
2*I*3	4.88 a	133.16 abcd	17.63 def	43.21 efgh	198.89 a	64.42 ef	684.79 bcd	1624.08 ab	2936.81 abcdef	50.50 a	59.62 abc	147.66 a	196.99 a	477.11 abcd	69.25 abc	6311.23 abcd	10.44 gh	180.46 bcde	190.89 cdefghi
2*I*5	1.90 a	129.88 abcd	30.24 cdef	45.34 defgh	207.36 a	70.35 ef	1265.88 abc	1146.21 ab	3463.48 abc	13.91 cde	32.09 abcdefg	129.05 a	59.94 defghi	102.03 fgh	88.10 ab	6371.04 abcd	14.80 fgh	118.77 defg	133.56 fghijk
2*I*7	0.00 a	97.35 abcd	164.89 ab	0.00 h	262.23 a	74.75 ef	543.52 bcd	1384.59 ab	2994.88 abcdef	28.37 abcde	56.23 abc	129.88 a	165.83 abc	521.70 ab	58.77 abcd	5958.50 abcd	48.30 cde	140.00 cde	188.30 cdefghij
2*I*9	0.00 a	69.03 bcd	81.61 abcdef	109.99 ab	260.64 a	549.19 bc	32.36 d	311.58 b	1188.33 defg	0.00 e	13.89 defg	24.84 bc	47.33 efghi	210.04 defgh	27.31 d	2404.90 bcde	44.95 cdef	244.05 abc	288.99 abcd
2*N*1	0.00 a	123.54 abcd	52.64 abcdef	52.38 cdefg	228.56 a	136.61 e	624.22 bcd	1098.81 ab	2208.09 abcdefg	38.39 abcd	27.92 abcdefg	109.23 a	124.52 abcdefgh	353.79 bcdef	82.90 ab	4804.48 abcde	8.03 gh	142.36 cde	150.39 fghij
2*N*3	4.63 a	122.59 abcd	47.43 abcdef	42.61 efgh	217.25 a	64.77 ef	560.39 bcd	1438.35 ab	2795.15 abcdef	45.18 ab	58.37 abc	138.79 a	192.37 a	480.56 abc	70.61 abc	5844.54 abcd	9.33 gh	111.42 defg	120.76 ghijklm
2*N*5	0.00 a	145.96 ab	32.06 cdef	45.54 defgh	223.56 a	18.75 ef	1278.78 abc	1157.03 ab	3159.99 abcde	27.36 abcde	33.30 abcdefg	140.38 a	59.72 defghi	87.81 gh	81.79 ab	6044.91 abcd	19.19 efgh	98.54 defg	117.73 ghijklm
2*N*7	0.00 a	91.79 abcd	174.96 a	0.00 h	266.75 a	74.15 ef	681.21 bcd	1631.79 ab	3352.42 abcde	41.11 abc	56.48 abc	125.74 a	140.96 abcdef	484.97 abc	69.86 abc	6658.68 abcd	26.70 efgh	0.00 g	26.70 klm
2*N*9	0.00 a	77.65 abcd	95.08 abcdef	93.62 abcd	266.34 a	412.99 cd	22.62 d	271.52 b	1149.70 efg	0.00 e	2.62 g	20.68 c	42.03 fghi	244.16 cdefgh	22.70 d	2189.02 cde	102.36 a	249.08 abc	351.43 ab
3*I*1	0.82 a	121.09 abcd	7.76 ef	52.81 cdefg	182.48 a	83.15 ef	639.35 bcd	1065.20 ab	1972.61 abcdefg	38.44 abcd	31.68 abcdefg	106.05 a	128.02 abcdefgh	398.25 bcd	81.52 ab	4544.26 abcde	15.89 efgh	287.27 ab	303.49 abc
3*I*3	8.34 a	148.62 a	14.25 def	59.86 cdefg	231.07 a	77.68 ef	820.40 abcd	1727.52 ab	3178.85 abcde	47.67 a	63.43 ab	164.27 a	204.36 a	509.70 abc	72.77 abc	6866.65 abc	13.03 fgh	208.00 abcd	221.23 cdefg
3*I*5	3.89 a	145.32 abc	33.36 cdef	49.51 cdefgh	232.08 a	22.87 ef	1683.98 a	1573.28 ab	4089.25 ab	13.89 cde	49.77 abcd	169.64 a	76.92 bcdefghi	128.64 efgh	83.07 ab	7891.31 a	26.13 efgh	178.97 bcde	205.27 cdefgh
3*I*7	0.00 a	110.49 abcd	158.20 abc	0.00 h	268.69 a	106.10 ef	783.82 bcd	1553.82 ab	3299.28 abcde	37.22 abcd	64.97 ab	138.49 a	183.78 a	710.61 a	77.26 ab	6955.33 ab	67.59 bc	147.98 cde	215.57 cdefg
3*I*9	0.00 a	63.93 bcd	94.05 abcdef	85.17 abcde	243.14 a	329.69 d	20.32 d	350.43 b	1896.33 bcdefg	8.45 de	18.73 cdefg	21.98 c	79.77 bcdefghi	434.54 bcd	25.41 d	3185.66 abcde	89.72 ab	299.14 a	388.86 a
3*N*1	0.00 a	125.26 abcd	0.00 f	65.49 bcdefg	190.75 a	95.96 ef	708.96 bcd	2865.26 a	2533.71 abcdefg	36.96 abcd	38.23 abcdefg	116.23 a	147.32 abcde	486.65 abc	84.74 ab	7114.03 ab	12.16 fgh	187.39 abcde	199.55 cdefghi
3*N*3	5.34 a	125.99 abcd	31.62 cdef	55.39 cdefg	218.34 a	74.75 ef	671.14 bcd	1552.67 ab	2912.00 abcdef	52.19 a	61.39 ab	151.22 a	200.27 a	503.56 abc	99.74 a	6278.92 abcd	12.54 fgh	145.72 cde	158.27 efghij
3*N*5	0.21 a	129.94 abcd	33.40 cdef	59.69 cdefg	223.24 a	17.29 f	1361.64 abc	1501.01 ab	4098.46 ab	14.34 bcde	46.43 abcdef	165.80 a	76.59 bcdefghi	135.83 efgh	77.62 ab	7495.01 a	28.17 efgh	109.14 defg	137.31 fghijk
3*N*7	0.00 a	90.38 abcd	123.36 abcdef	0.00 h	213.74 a	78.08 ef	749.11 bcd	1356.74 ab	2824.38 abcdef	39.44 abc	50.97 abcd	121.99 a	144.16 abcdef	481.30 abc	49.87 bcd	5896.01 abcd	19.02 efgh	0.00 g	19.02 klm
3*N*9	0.00 a	67.53 bcd	84.20 abcdef	97.19 abc	248.91 a	647.09 ab	44.12 d	402.75 b	1713.10 cdefg	15.11 bcde	23.53 bcdefg	28.43 bc	79.87 bcdefghi	433.95 bcd	34.44 cd	3422.39 abcde	0.79 h	278.59 ab	279.37 abcde
Pr > F(Model)	0.20	0.10	0.03	<0.0001	1.00	<0.0001	<0.0001	0.44	0.00	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.02	<0.0001	<0.0001	<0.0001
Pr > F(Term*Treatment*OIV class)	1.00	1.00	1.00	0.63	0.99	0.01	1.00	0.70	1.00	0.95	1.00	0.99	0.98	0.93	0.75	0.96	<0.0001	0.44	0.26
Significant	No	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No	No	Yes	No	No

Term - before inoculation (**0**), 24 hours post-inoculation (hpi) (**1**), 48 hpi (**2**) and 96 hpi (**3**)
Treatment - **N** - Non-inoculated leaves; **I** - Inoculated leaves
OIV class - **1**, **3**, **5**, **7** and **9** from the most susceptible to the completely resistant group

Supp. Table 2 - Factorial ANOVA was performed to test the effects of Term of sampling (without T_0 (before inoculation)), Treatment, OIV class and their interaction on the polyphenolic content (mg/kg) in the leaves of 17 genotypes.

	Myricetin 3-*O*-glucoside	Quercetin 3-*O*-galactoside	Quercetin 3-*O*-glucoside	Kaempferol 3-*O*-rutinoside	Isorhamnetin 3-*O*-rutinoside	Kaempferol 3-O-glucoside	Kaempferol 3-*O*-glucuronide	Izorhamnetin 3-O-glucoside	Taxifolin	Total flavonol glycosides	Caftaric acid	Aesculin	Coutaric acid	Caffeic acid	Fertaric acid	*p*-Coumaric acid	Ferulic acid	Sinapic acid	Total hydroxycinnamic acids
R^2	0.13	0.23	0.09	0.35	0.15	0.13	0.23	0.23	0.19	0.09	0.32	0.11	0.11	0.31	0.21	0.29	0.28	0.26	0.12
F	1.29	2.57	0.86	4.70	1.47	1.32	2.58	2.59	2.04	0.86	4.00	1.11	1.07	3.82	2.24	3.54	3.35	3.12	1.20
Pr > F	0.19	0.00	0.64	<0.0001	0.09	0.17	0.00	0.00	0.01	0.64	<0.0001	0.34	0.38	<0.0001	0.00	<0.0001	<0.0001	<0.0001	0.26
Term	0.43	0.28	0.02	1.03	0.03	1.08	0.15	0.39	0.03	0.02	0.28	0.01	0.20	0.02	0.69	2.01	0.55	0.57	0.04
	0.65	0.76	0.98	0.36	0.97	0.34	0.86	0.68	0.97	0.98	0.76	0.99	0.82	0.98	0.50	0.14	0.58	0.57	0.96
Treatment	0.11	1.45	0.20	2.41	0.00	1.01	0.01	0.09	0.00	0.23	0.04	0.02	0.08	0.40	0.19	0.54	0.53	0.05	0.25
	0.74	0.23	0.66	0.12	0.96	0.32	0.92	0.76	1.00	0.63	0.83	0.88	0.78	0.53	0.67	0.46	0.47	0.83	0.62
OIV class	5.53	11.25	2.57	20.33	7.00	4.50	12.33	11.17	10.26	2.62	19.88	5.71	5.15	19.18	7.97	14.30	14.88	14.02	4.56
	0.00	**<0.0001**	**0.04**	**<0.0001**	**<0.0001**	**0.00**	**<0.0001**	**<0.0001**	**<0.0001**	**0.04**	**<0.0001**	**0.00**	**0.00**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.00**
Term*Treatment	0.32	0.00	0.77	0.86	0.66	1.05	1.03	0.73	0.09	0.77	0.59	0.00	0.04	0.16	1.22	1.56	0.41	0.22	0.58
	0.73	1.00	0.46	0.42	0.52	0.35	0.36	0.48	0.91	0.46	0.55	1.00	0.96	0.85	0.30	0.21	0.67	0.81	0.56
Term*OIV class	0.11	0.45	0.36	1.28	0.09	0.39	0.24	0.66	0.14	0.36	0.11	0.03	0.09	0.13	1.39	0.63	0.51	0.23	0.32
	1.00	0.89	0.94	0.26	1.00	0.93	0.98	0.73	1.00	0.94	1.00	1.00	1.00	1.00	0.20	0.75	0.84	0.98	0.96
Treatment*OIV class	0.23	1.32	0.48	0.72	0.09	0.40	0.01	0.13	0.01	0.46	0.34	0.01	0.12	0.38	0.35	0.20	1.06	1.25	0.46
	0.92	0.26	0.75	0.58	0.98	0.81	1.00	0.97	1.00	0.76	0.85	1.00	0.97	0.82	0.85	0.94	0.38	0.29	0.77

	Gallic acid	Protocatechuic acid	Vanillic acid	Syringic acid	Total hydroxybenzoic acids	Epigallocatechin gallate	Gallocatechin	Epigallocatechin	Procyanidin B1	Procyanidin B3	Catechin	Procyanidin B4	Procyanidin B2	Epicatechin	Procyanidin A1	Total flavan-3-ols	Piceatannol	Resveratrol 3-*O*-glucoside	Total stilbenes
R^2	0.16	0.17	0.21	0.34	0.06	0.57	0.32	0.12	0.27	0.40	0.36	0.36	0.49	0.54	0.28	0.20	0.38	0.48	0.48
F	1.67	1.81	2.28	4.44	0.51	11.69	4.06	1.14	3.27	5.84	4.86	4.78	8.34	10.04	3.43	2.17	5.20	7.97	7.97
Pr > F	0.04	0.02	0.00	<0.0001	0.97	<0.0001	<0.0001	0.31	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.00	<0.0001	<0.0001	<0.0001
Term	0.50	0.09	0.60	0.50	0.15	0.04	0.70	0.57	0.74	0.46	2.03	0.57	1.90	12.16	0.31	1.29	1.46	16.57	13.60
	0.61	0.91	0.55	0.61	0.86	0.96	0.50	0.57	0.48	0.63	0.13	0.57	0.15	**<0.0001**	0.74	0.28	0.24	**<0.0001**	**<0.0001**
Treatment	0.16	0.44	0.00	0.02	0.16	0.18	0.18	0.24	0.29	1.07	0.23	0.15	0.30	0.28	0.16	0.02	4.54	35.84	42.26
	0.69	0.51	0.99	0.89	0.69	0.68	0.68	0.62	0.59	0.30	0.63	0.70	0.58	0.59	0.69	0.88	**0.03**	**<0.0001**	**<0.0001**
OIV class	5.72	8.08	8.51	16.45	1.35	59.32	19.85	2.81	14.91	28.91	23.48	23.46	41.95	45.06	13.59	8.81	21.06	12.96	13.40
	0.00	**<0.0001**	**<0.0001**	**<0.0001**	**0.25**	**<0.0001**	**<0.0001**	**0.03**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Term*Treatment	1.14	0.47	0.15	1.40	0.28	0.53	0.13	0.71	0.25	0.00	0.03	0.08	0.04	0.10	1.87	0.36	1.26	0.21	0.60
	0.32	0.63	0.86	0.25	0.75	0.59	0.88	0.49	0.78	1.00	0.97	0.92	0.96	0.91	0.16	0.70	0.29	0.81	0.55
Term*OIV class	0.46	0.10	0.59	0.78	0.46	0.55	0.08	0.46	0.44	0.58	0.44	0.29	0.46	1.86	0.78	0.22	1.18	1.28	1.10
	0.88	1.00	0.79	0.62	0.88	0.82	1.00	0.88	0.90	0.79	0.90	0.97	0.88	0.07	0.62	0.99	0.31	0.26	0.37
Treatment*OIV class	0.13	0.54	0.90	2.04	0.22	0.53	0.38	0.90	0.65	0.27	0.11	0.17	0.16	0.32	0.78	0.93	0.88	0.73	1.21
	0.97	0.70	0.46	0.09	0.93	0.71	0.82	0.46	0.63	0.90	0.98	0.95	0.96	0.86	0.54	0.45	0.48	0.57	0.31

Supp. Table 2 continued - The differences between the means of treatment (inoculated vs. non-inoculated leaves) were evaluated by Duncan's multiple range test at a confidence level of 95 % ($p < 0.05$). Different letters show statistical significance.

Phenolic compound	Treatment		Phenolic compound	Treatment	
	Inoculated leaves (I)	Non-inoculated leaves (N)		Inoculated leaves (I)	Non-inoculated leaves (N)
Myricetin 3-*O*-glucoside	323.76 a	329.91 a	Gallic acid	2.34 a	1.85 a
Quercetin 3-*O*-galactoside	33.28 a	32.27 a	Protocatechuic acid	125.89 a	120.41 a
Quercetin 3-*O*-glucoside	21181.27 a	20013.47 a	Vanillic acid	39.83 a	49.32 a
Kaempferol 3-*O*-rutinoside	137.37 a	124.08 a	Syringic acid	48.44 a	47.16 a
Isorhamnetin 3-*O*-rutinoside	38.74 a	38.33 a	Total hydroxybenzoic acids	216.49 a	218.73 a
Kaempferol 3-O-glucoside	124.43 a	116.08 a	Epigallocatechin gallate	97.37 a	96.05 a
Kaempferol 3-*O*-glucuronide	16.63 a	16.46 a	Gallocatechin	769.32 a	703.62 a
Izorhamnetin 3-O-glucoside	2.59 a	2.27 a	Epigallocatechin	1338.42 a	1480.06 a
Taxifolin	4.69 a	4.66 a	Procyanidin B1	2849.47 a	2755.65 a
Total flavonol glycosides	21862.74 a	20677.53 a	Procyanidin B3	35.88 a	38.09 a
Caftaric acid	5347.51 a	5375.78 a	Catechin	44.96 a	44.27 a
Aesculin	444.12 a	463.33 a	Procyanidin B4	132.30 a	129.10 a
Coutaric acid	199.28 a	182.05 a	Procyanidin B2	142.80 a	141.57 a
Caffeic acid	826.40 a	863.12 a	Epicatechin	372.14 a	371.01 a
Fertaric acid	15.77 a	15.94 a	Procyanidin A1	73.92 a	75.20 a
p-Coumaric acid	24.34 a	22.90 a	Total flavan-3-ols	5856.58 a	5834.62 a
Ferulic acid	33.25 a	33.21 a	Piceatannol	22.73 a	18.07 b
Sinapic acid	3386.39 a	3388.75 a	Resveratrol 3-*O*-glucoside	183.14 a	111.08 b
Total hydroxycinnamic acids	27210.25 a	26053.31 a	Total stilbenes	205.87 a	129.15 b

Supp. Table 2 continued - The differences between the terms of sampling were evaluated by Duncan's multiple range test at a confidence level of 95 % ($p < 0.05$). Different letters show statistical significance.

Phenolic compound	Term of sampling			Phenolic compound	Term of sampling		
	1 (24 hpi)	2 (48 hpi)	3 (96 hpi)		1 (24 hpi)	2 (48 hpi)	3 (96 hpi)
Myricetin 3-*O*-glucoside	342.26 a	291.38 a	346.86 a	Gallic acid	0.85 b	2.13 ab	3.30 a
Quercetin 3-*O*-galactoside	33.80 a	31.02 a	33.49 a	Protocatechuic acid	121.01 a	121.60 a	126.83 a
Quercetin 3-*O*-glucoside	19991.83 a	21169.10 a	20631.19 a	Vanillic acid	52.67 a	51.05 a	30.00 a
Kaempferol 3-*O*-rutinoside	144.21 a	120.16 a	127.82 a	Syringic acid	41.80 b	45.48 b	56.12 a
Isorhamnetin 3-*O*-rutinoside	35.87 a	43.99 a	35.74 a	Total hydroxybenzoic acids	216.33 a	220.25 a	216.25 a
Kaempferol 3-O-glucoside	113.21 a	118.66 a	128.89 a	Epigallocatechin gallate	91.13 a	103.58 a	95.41 a
Kaempferol 3-*O*-glucuronide	17.96 a	15.86 a	15.81 a	Gallocatechin	699.99 a	688.35 a	821.07 a
Izorhamnetin 3-O-glucoside	3.36 a	1.91 a	2.02 a	Epigallocatechin	1338.87 a	1256.49 a	1632.36 a
Taxifolin	5.21 a	3.90 a	4.90 a	Procyanidin B1	2839.17 a	2643.11 a	2925.41 a
Total flavonol glycosides	20687.71 a	21795.98 a	21326.71 a	Procyanidin B3	37.86 a	36.02 a	37.08 a
Caftaric acid	5239.88 a	5392.23 a	5452.82 a	Catechin	42.99 a	41.74 a	49.12 a
Aesculin	459.71 a	440.81 a	460.66 a	Procyanidin B4	133.17 a	122.55 a	136.38 a
Coutaric acid	209.67 a	181.43 a	180.90 a	Procyanidin B2	136.90 a	137.97 a	151.68 a
Caffeic acid	842.69 a	847.66 a	843.93 a	Epicatechin	329.25 b	362.80 ab	422.68 a
Fertaric acid	16.05 a	15.79 a	15.73 a	Procyanidin A1	70.87 a	73.16 a	79.64 a
p-Coumaric acid	21.79 b	23.45 ab	25.63 a	Total flavan-3-ols	5720.19 a	5465.76 a	6350.85 a
Ferulic acid	34.24 a	31.45 a	34.01 a	Piceatannol	25.44 a	16.37 b	19.39 ab
Sinapic acid	3396.49 a	3290.90 a	3475.33 a	Resveratrol 3-*O*-glucoside	100.79 c	151.15 b	189.39 a
Total hydroxycinnamic acids	25927.60 a	27188.21 a	26779.53 a	Total stilbenes	126.23 c	167.52 b	208.78 a

Supp. Table 2 continued - Correlation (Spearman) between Terms of sampling after inoculation and the polyphenolic content in the leaves of 17 genotypes.

Variables	Term (Inoculated leaves)	Term (Non-inoculated leaves)	Variables	Term (Inoculated leaves)	Term (Non-inoculated leaves)
Term	**1**	**1**	Gallic acid	0.19	-0.03
Myricetin 3-*O*-glucoside	0.00	0.03	Protocatechuic acid	0.05	0.06
Quercetin 3-*O*-galactoside	0.07	-0.03	Vanillic acid	-0.09	-0.04
Quercetin 3-*O*-glucoside	0.00	0.12	Syringic acid	0.14	**0.30**
Kaempferol 3-*O*-rutinoside	0.01	-0.09	Total hydroxybenzoic acids	0.01	0.07
Isorhamnetin 3-*O*-rutinoside	-0.07	0.03	Epigallocatechin gallate	0.04	0.01
Kaempferol 3-O-glucoside	0.05	0.15	Gallocatechin	0.08	0.10
Kaempferol 3-*O*-glucuronide	-0.05	0.07	Epigallocatechin	0.05	0.03
Izorhamnetin 3-O-glucoside	-0.04	-0.14	Procyanidin B1	-0.01	0.06
Taxifolin	-0.05	0.02	Procyanidin B3	-0.04	-0.02
Total flavonol glycosides	-0.01	0.11	Catechin	0.06	0.15
Caftaric acid	0.03	0.06	Procyanidin B4	-0.02	0.06
Aesculin	0.04	0.01	Procyanidin B2	0.07	0.12
Coutaric acid	-0.01	-0.09	Epicatechin	0.15	**0.22**
Caffeic acid	0.04	-0.03	Procyanidin A1	0.03	0.12
Fertaric acid	0.06	-0.09	Total flavan-3-ols	0.04	0.10
p-Coumaric acid	0.11	**0.20**	Piceatannol	-0.07	-0.08
Ferulic acid	0.02	-0.12	Resveratrol 3-*O*-glucoside	**0.42**	**0.43**
Sinapic acid	0.00	0.09	Total stilbenes	**0.32**	**0.40**
Total hydroxycinnamic acids	0.02	0.11			

Values in bold are different from 0 with a significance level alpha=0.05

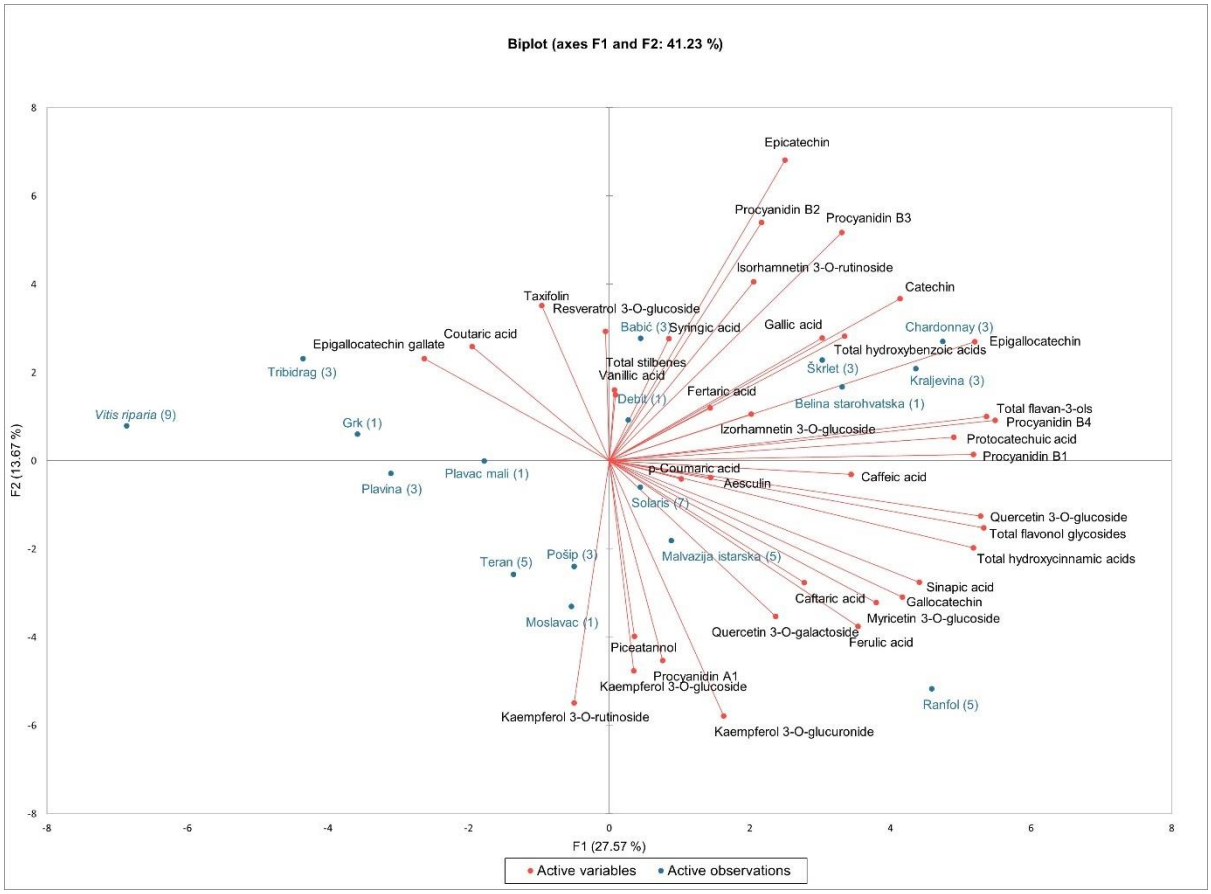
Supp. Table 3 - The differences between the means of genotypes were evaluated by Duncan's multiple range test at a confidence level of 95 % ($p < 0.05$). Different letters show statistical significance.

Genotype (OIV class)	Myricetin 3-*O*-glucoside	Quercetin 3-*O*-galactoside	Quercetin 3-*O*-glucoside	Kaempferol 3-*O*-rutinoside	Isorhamnetin 3-*O*-rutinoside	Kaempferol 3-O-glucoside	Kaempferol 3-*O*-glucuronide	Izorhamnetin 3-O-glucoside	Taxifolin	Total flavonol glycosides	Caftaric acid	Aesculin	Coutaric acid	Caffeic acid	Fertaric acid	*p*-Coumaric acid	Ferulic acid	Sinapic acid	Total hydroxycinnamic acids
Belina starohvatska (1)	332.97 e	21.60 efg	24703.03 ab	0.00 h	41.70 d	65.06 e	28.74 c	22.45 a	0.00 de	25215.56 ab	5251.11 e	2115.57 a	184.51 c	1346.54 a	22.30 b	32.04 bc	26.35 fg	4143.42 bc	30466.66 ab
Debit (1)	292.44 ef	4.50 hi	23849.10 ab	54.54 fg	288.41 a	6.38 fg	9.89 f	7.43 b	0.00 de	24512.69 ab	6482.35 b	304.91 g	26.51 f	997.82 c	1.07 i	33.70 ab	23.59 gh	4017.51 c	30995.05 ab
Grk (1)	159.07 g	27.61 ef	15381.31 def	72.22 ef	79.88 c	23.53 f	2.73 g	4.25 c	0.00 de	15750.61 de	4074.12 f	223.37 h	23.34 f	339.64 gh	9.11 g	16.91 f	29.46 f	2675.58 jk	19824.73 def
Moslavac (1)	429.96 d	0.00 i	21231.42 abcde	252.94 bc	0.00 e	208.48 a	62.96 a	1.39 cd	0.00 de	22187.13 abcd	5555.04 de	555.92 e	204.05 c	856.50 d	19.16 cd	31.54 bc	47.64 c	3579.64 ef	27742.17 abc
Plavac mali (1)	156.78 g	0.00 i	20150.84 abcdef	141.42 d	0.00 e	148.51 b	13.69 f	0.00 d	2.25 d	20613.49 bcde	5305.21 e	140.26 i	168.26 cd	867.03 d	21.41 bc	11.59 h	26.71 fg	3397.59 fg	25918.71 bcde
Babić (3)	423.89 d	79.91 c	16459.17 cdef	0.00 h	107.26 c	0.00 g	0.64 g	0.00 d	5.55 c	17076.42 cde	5552.45 de	133.18 i	282.92 b	676.89 e	17.45 de	13.96 gh	41.45 d	2774.78 ij	22628.87 cdef
Chardonnay (3)	185.46 g	12.65 ghi	23332.46 abc	0.00 h	163.23 b	147.65 b	21.61 de	0.00 d	21.42 b	23884.47 abc	5624.03 de	151.48 i	73.69 ef	1265.48 b	20.12 bc	21.27 de	24.89 gh	3925.08 cd	29508.50 abc
Kraljevina (3)	819.35 a	34.59 e	27625.23 a	129.95 d	176.76 b	108.89 cd	9.35 f	3.92 c	0.00 de	28908.06 a	6231.68 bc	128.10 i	56.69 f	583.14 f	16.03 e	30.00 c	55.71 b	4676.05 a	35139.73 a
Plavina (3)	51.92 h	12.03 ghi	15861.16 def	69.02 efg	0.00 e	150.61 b	18.65 e	0.47 d	0.00 de	16163.86 de	3292.09 g	832.80 c	21.01 f	405.24 g	8.91 g	16.83 f	22.92 gh	2975.48 hi	19455.94 ef
Pošip (3)	617.66 c	55.04 d	18534.26 bcdef	137.73 d	0.00 e	146.98 b	21.06 de	0.00 d	0.00 de	19512.73 bcde	7837.75 a	122.91 i	67.17 ef	1201.00 b	5.74 h	20.33 e	35.63 e	3208.24 gh	27350.48 bc
Škrlet (3)	258.00 f	9.40 ghi	25466.88 ab	109.37 de	0.00 e	119.59 c	2.33 g	0.00 d	0.00 e	25965.58 ab	4217.93 f	382.01 f	202.92 c	989.36 c	13.02 f	23.79 d	22.88 gh	2571.38 jk	30183.51 ab
Tribidrag (3)	43.93 h	17.89 fgh	14499.15 ef	51.46 fg	0.00 e	152.50 b	11.55 f	0.00 d	45.52 a	14821.99 e	4269.09 f	690.87 d	1171.46 a	624.79 ef	26.51 a	15.73 fg	24.40 gh	2434.79 kl	19091.08 ef
Malvazija istarska (5)	440.89 d	0.00 i	22033.58 abcd	213.31 c	0.00 e	143.02 b	13.71 f	2.02 cd	0.00 de	22846.52 abcd	5949.54 cd	377.86 f	30.63 f	556.07 f	8.97 g	32.33 bc	30.36 f	3715.33 de	28796.06 abc
Ranfol (5)	703.82 b	193.87 a	25342.38 ab	327.44 a	0.00 e	163.13 b	46.17 b	4.19 c	0.00 de	26780.99 ab	5220.06 e	951.20 b	119.95 de	803.23 d	20.40 bc	22.99 d	68.42 a	4348.65 b	32001.05 ab
Teran (5)	254.06 f	5.07 hi	24271.19 ab	19.20 gh	0.00 e	153.04 b	24.46 cd	0.00 d	0.00 e	24727.02 ab	7478.37 a	111.59 i	36.27 f	955.28 c	9.26 g	17.02 f	20.77 h	4103.04 bc	32205.39 ab
Solaris (7)	265.15 f	97.74 b	20214.31 abcdef	234.62 c	0.00 e	143.00 b	0.29 g	0.00 d	0.00 e	20955.12 bcde	5617.79 de	233.48 h	301.64 b	1396.99 a	25.83 a	12.16 h	45.74 c	3417.90 fg	26572.90 bcd
Vitis riparia (9)	141.79 g	0.00 i	13120.27 f	289.59 ab	0.00 e	93.20 d	2.15 g	0.00 d	0.00 de	13646.99 e	2519.41 h	133.61 i	217.37 c	293.46 h	9.93 g	35.06 a	5.54 i	2198.64 l	16166.40 f
Pr > F(Model)	<0.0001	<0.0001	0.00	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.00	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Genotype (OIV class)	Gallic acid	Protocatechuic acid	Vanillic acid	Syringic acid	Total hydroxybenzoic acids	Epigallocatechin gallate	Gallocatechin	Epigallocatechin	Procyanidin B1	Procyanidin B3	Catechin	Procyanidin B4	Procyanidin B2	Epicatechin	Procyanidin A1	Total flavan-3-ols	Piceatannol	Resveratrol 3-*O*-glucoside	Total stilbenes
Belina starohvatska (1)	0.00 d	146.49 d	190.70 a	63.97 c	401.17 a	27.56 fgh	722.87 f	2194.79 ab	3097.14 e	46.72 c	35.28 fg	164.22 d	138.06 e	475.96 d	77.05 bc	6979.63 cd	6.72 efgh	199.31 a	206.03 ab
Debit (1)	0.00 d	120.87 fg	12.54 h	57.03 c	190.44 fgh	355.02 b	787.11 ef	1126.27 cdef	2546.56 g	36.31 def	30.18 gh	111.30 f	104.89 fg	363.33 ef	72.92 bcd	5533.89 e	2.49 fgh	128.78 cd	131.26 cde
Grk (1)	0.00 d	92.20 ij	0.00 h	19.86 f	112.06 k	21.17 gh	493.63 g	915.52 def	1726.16 i	33.38 efg	24.48 i	89.46 g	121.53 f	325.63 fg	78.25 bc	3829.21 gh	5.41 efgh	125.37 cd	130.77 cde
Moslavac (1)	0.68 d	110.86 gh	0.00 h	33.53 de	145.08 ij	14.45 gh	568.46 g	1027.90 cdef	2020.88 h	27.21 g	27.62 hi	94.90 g	110.23 f	286.15 g	100.76 a	4278.56 fg	35.54 b	150.46 bc	185.99 b
Plavac mali (1)	0.00 d	121.11 fg	5.24 h	40.22 d	166.57 hij	63.93 de	289.87 h	941.25 def	1708.56 i	35.74 def	34.72 fg	93.94 g	167.94 d	406.07 e	75.44 bcd	3817.46 gh	14.43 def	128.61 cd	143.04 cd
Babić (3)	0.77 d	77.80 k	124.99 c	18.24 f	221.80 def	120.89 c	239.51 hi	1947.70 abc	4089.16 d	44.02 c	101.64 a	189.70 b	244.67 b	610.71 b	66.50 cde	7654.50 c	19.91 d	66.76 e	86.66 fg
Chardonnay (3)	8.85 b	214.49 a	0.00 h	79.92 b	303.26 b	64.75 de	1654.25 b	2413.45 ab	5438.07 a	66.67 b	77.09 c	206.30 a	156.18 d	531.07 c	62.57 def	10670.40 a	5.79 efgh	143.78 bc	149.58 c
Kraljevina (3)	17.43 a	142.88 de	0.00 h	63.02 c	223.33 de	87.63 d	233.72 hi	1499.17 bcde	2844.04 f	41.43 cd	89.81 b	181.16 bc	333.23 a	733.45 a	51.18 f	6094.82 de	22.38 cd	200.60 a	222.98 a
Plavina (3)	0.10 d	112.11 gh	0.00 h	25.45 ef	137.66 jk	60.74 de	314.78 h	1016.41 cdef	1993.25 h	33.63 efg	37.98 f	112.21 f	160.03 d	339.06 f	101.37 a	4169.46 fg	3.40 efgh	58.90 e	62.30 gh
Pošip (3)	0.00 d	97.71 i	21.24 gh	21.36 ef	140.31 jk	0.00 h	872.34 e	1183.27 cdef	2605.73 g	31.30 fg	30.98 gh	140.19 e	73.10 h	117.78 i	86.55 b	5141.23 ef	15.05 de	168.35 b	183.40 b
Škrlet (3)	0.00 d	177.00 c	0.00 h	87.88 b	264.88 c	58.67 def	1211.34 d	2560.46 a	4356.09 c	87.14 a	70.31 d	169.20 cd	187.18 c	628.74 b	79.42 bc	9408.55 b	12.93 defg	106.31 d	119.24 de
Tribidrag (3)	0.00 d	111.12 gh	70.64 de	17.54 f	199.31 efg	89.07 d	159.82 i	779.85 ef	1125.22 j	29.71 fg	28.15 hi	86.88 g	187.23 c	401.92 e	58.27 ef	2946.14 hi	1.55 gh	112.68 d	114.24 e
Malvazija istarska (5)	2.95 c	132.47 ef	0.00 h	38.27 d	173.69 ghi	40.55 efg	1477.61 c	1512.77 bcde	4069.93 d	26.72 g	37.38 f	169.80 cd	67.51 h	120.86 i	62.28 def	7585.40 c	31.25 bc	147.79 bc	179.04 b
Ranfol (5)	0.00 d	189.04 b	51.08 ef	28.12 def	268.24 c	44.88 efg	1924.21 a	1759.13 abcd	4877.30 b	12.63 h	56.55 e	204.88 a	90.18 g	127.06 i	82.82 b	9179.64 b	52.15 a	0.00 f	52.15 h
Teran (5)	0.00 d	82.60 jk	40.75 fg	65.78 c	189.13 fgh	0.00 h	515.10 g	651.07 ef	2048.64 h	18.89 h	9.41 j	65.38 h	35.98 i	66.00 j	103.19 a	3513.68 gh	0.41 h	104.61 d	105.02 ef
Solaris (7)	0.00 d	101.05 hi	157.08 b	3.36 g	261.49 c	80.43 d	595.48 g	1451.97 bcde	3087.67 e	40.22 cde	55.46 e	125.65 f	158.02 d	478.39 d	67.92 cde	6141.21 de	48.16 a	70.09 e	118.26 de
Vitis riparia (9)	0.00 d	63.72 l	82.20 d	101.47 a	247.38 cd	574.96 a	39.38 j	324.61 f	1158.44 j	2.67 i	10.58 j	24.04 i	44.82 i	170.74 h	26.58 g	2376.82 i	49.47 a	164.65 b	214.12 a
Pr > F(Model)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.00	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Supp. Figure 1 - Genotypes in the greenhouse.

Supp. Figure 2 - PCA Single genotypes and polyphenolic compounds.



Appendix 3. Scientific paper: Štambuk P., Šikuten I., Preiner D., Maletić E., Karoglan Kontić J., Tomaz I. (2023). Croatian Native Grapevine Varieties' VOCs Responses upon *Plasmopara viticola* Inoculation. Plants 12(2): 404. doi: 10.3390/plants12020404

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Croatian Native Grapevine Varieties' VOCs Responses upon *Plasmopara viticola* Inoculation

Petra Štambuk [1,2], Iva Šikuten [1,2], Darko Preiner [1,2], Edi Maletić [1,2], Jasminka Karoglan Kontić [1,2,*] and Ivana Tomaz [1,2]

[1] Department of Viticulture and Enology, Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia
[2] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska cesta 25, 10000 Zagreb, Croatia
* Correspondence: jkkontic@agr.hr

Abstract: The *Plasmopara viticola* pathogen causes one of the most severe grapevine diseases, namely downy mildew. The response to *P. viticola* involves both visible symptoms and intricate metabolomic alterations, particularly in relation to volatile organic compounds, and depends on the degree of resistance of a particular variety. There are numerous native grapevine varieties in Croatia, and they vary in susceptibility to this oomycete. As previously reported, in vitro leaf disc bioassay and polyphenolic compound analysis are complementary methods that can be used to separate native varieties into various resistance classes. This research used the Solid Phase Microextraction-Arrow Gas Chromatography-Mass Spectrometry method to identify the early alterations in the VOCs in the leaves after *P. viticola* inoculation. Based on the absolute peak area of sesquiterpenes, some discrepancies between the sampling terms were noticed. The presence of certain chemical compounds such as humulene, ylangene, and α-farnesene helped distinguish the non-inoculated and inoculated samples. Although specific VOC responses to *P. viticola* infection of native varieties from various resistance classes could not be identified, the response of less susceptible native varieties and resistant controls was associated with an increase in the absolute peak area of several compounds, including geranylacetone, β-ocimene, and (*E*)-2-hexen-1-ol.

Keywords: *Vitis vinifera* L.; downy mildew; secondary metabolites; SPME-*Arrow*-GC/MS

Citation: Štambuk, P.; Šikuten, I.; Preiner, D.; Maletić, E.; Karoglan Kontić, J.; Tomaz, I. Croatian Native Grapevine Varieties' VOCs Responses upon *Plasmopara viticola* Inoculation. *Plants* **2023**, *12*, 404. https://doi.org/10.3390/plants12020404

Academic Editors: Ines Mihaljević and Marija Viljevac Vuletić

Received: 14 December 2022
Revised: 11 January 2023
Accepted: 12 January 2023
Published: 15 January 2023

1. Introduction

In addition to insects and weeds, pathogenic microorganisms cause structural and/or functional damage to plants, resulting in biotic stress. Plant-pathogen interactions can be viewed as a two-way communication process in which not only is the plant able to recognise a foreign organism and defend itself against it, but the pathogen must also be able to manipulate the plant's biology to create an optimal environment for its own growth and development while avoiding the plant's response [1]. The first line of plant defence can be triggered through the pathogen-associated molecular pattern (PAMP) system, which recognizes the pathogen. This is followed by a series of signal changes that finally impart a defence strategy, also known as the "zigzag" model [2]. Photosynthesis-related alterations, pathogenesis-related protein synthesis, restructuring of the cytoskeleton, generation of reactive oxygen species, and activation of programmed cell death constitute the fundamental level of plant pathogen recognition modifications [3]. Due to the plasticity of the plants in response to the pathogen and the establishment of either a compatible or incompatible interaction, the modulation of several classes of primary and secondary metabolites alters. During the initial phases of the infection, plant reactions to oomycetes are identical, albeit less effective and slower in susceptible plants [4].

Unlike primary metabolites, such as carbohydrates, lipids, and proteins, which are directly involved in plant development and growth, secondary metabolites are multifunctional compounds that are typically involved in the plant's defence system or they act indirectly, mediating the signals between different parts of the same plant, from plant to plant and between plants and other organisms. Due to their sessile nature, plants synthesize these compounds to repel herbivores, build barriers against pathogen invasion and mitigate oxidative stress [5]. On the basis of the accumulation and concentration of secondary metabolites in grapevine berries, such as polyphenolic and volatile organic compounds, it has been discovered that it is possible to classify *V. vinifera* varieties into genetic-geographic groups [6,7].

Terpenes, alkanes, alkenes, alcohols, esters, and acids belong to the class of volatile organic compounds. Terpenes are the largest and most researched class of these compounds. Their building block is a five-carbon isoprene unit. Through the condensation of two or more isoprene units, mono-(C_{10}), sesqui-(C_{15}), and diterpene (C_{20}) precursors are formed [8]. VOCs perform essential functions in how plants interact with other organisms and how they respond to biotic stress. They constitute about 1% of the secondary metabolites found in plants. Due to their low molecular weight and high vapour pressure, these lipophilic molecules freely diffuse into the environment and pass through biological membranes. Typically, pathogen-induced volatile organic compounds (VOCs) are composed of methyl salicylate (MeSA), mono- and sesquiterpenes, heterocyclic compounds, green leaf volatiles (GLVs), and ketones [9–11]. Plants continuously produce GLVs, such as C_6 aldehydes, alcohols, and esters, and do so to a greater extent under stress conditions [12].

In grapevine, both the defensive role and accumulation of volatile organic compounds, following an attack by *Plasmopara viticola* [(Berk. et Curt.) Berl. et de Toni], have been demonstrated. Some volatile organic compounds, such as 2-phenylethanol, 2-ethylfuran, (*E*)-2-pentenal, *ß*-cyclocitral, *ß*-caryophyllene, and *ß*-selinene, inhibited *P. viticola* infection in leaf tissues. The abundance of the studied VOCs was greater in resistant (BC4, Kober 5BB, SO4, and Solaris) genotypes than in susceptible genotypes (Pinot noir) [11]. Monoterpenes and sesquiterpenes are found in higher quantities in SO4 and Kober 5BB plants compared to Pinot noir plants after inoculation [13]. The direct antibacterial action of four selected compounds (farnesene, nerolidol, ocimene, and valencene) as well as the role of leaf terpenes in the resistance mechanism of two resistant cultivars (Mgaloblishvili, a pure *V. vinifera* cultivar, and Bianca, an interspecific hybrid) were determined [8]. Benzaldehyde has been suggested as a putative biomarker of resistance because it acts as a stimulator of salicylic acid (SA)-mediated defence [14]. It was also discovered in greater amounts in mono-locus (Solaris) and pyramided (F12P60) resistant genotypes. Farnesene was abundantly expressed in three mono-locus resistant genotypes (BC4, Bianca, F12P160). Linalool was substantially more abundant in Bianca, whereas (*E*)-nerolidol and neral distinguished the pyramidal genotype F12P60 [15].

P. viticola, the causal agent of grapevine downy mildew, is an obligate biotrophic oomycete native to North America. Therefore, European grapevine (*V. vinifera*) varieties are generally susceptible to this pathogen since it was introduced in this area in the late 19th century [16]. Biotrophic microorganisms have an important function in the ecosystem because they decompose organic matter [17]. However, when *P. viticola* sporangia develop in large quantities under favourable weather conditions (temperatures between 20 and 25 °C and leaf wetness) [18], they cause disastrous consequences such as defoliation, reduced and/or complete loss of grape quality and quantity [16,19]. In conventional vineyards, downy mildew epidemics cause severe economic losses when fungicides are not administered. Due to the detrimental effects of chemical pesticides on the environment, and human and animal health, as well as the emergence of pathogens resistant to these treatments [20–22], the European Union has restricted their usage through the Farm to Fork Strategy [23]. Today, the focus lies on the development of alternative tools, such as the breeding of resistant cultivars, the development of new active substances, and the search

for natural compounds and biocontrol agents that can be applied individually or in combination to eradicate the pathogen or mitigate its effects [4].

The genetic diversity of traditional grapevine varieties is an inexhaustible source of traits potentially useful in the upcoming challenging environmental conditions. Therefore, they should not be neglected since each variety has a unique characteristic that differentiates it from all other varieties. There is a possibility that these characteristics will become desirable or valuable in the future, despite the fact that they may seem mundane at present. Their preservation and continuous research are of utmost importance for maintaining biodiversity and expanding the scope of future breeding programs [24]. Quantitative trait loci (QTL) named *Rpv*, an acronym for resistance to *P. viticola*, are responsible for grapevine's resistance response. The Table of Loci for Traits in Grapevine Relevant for Breeding and Genetics (https://www.vivc.de/, the access date: 15 November 2022) lists and describes the 31 QTLs discovered in *Vitis* species to date. Most of them have been found in the *Muscadinia* subgenus, along with several wild North American and Asian *Vitis* species. However, the last three QTLs named *Rpv29*, *Rpv30*, and *Rpv31*, have been identified in the Georgian *V. vinifera* variety of Mgaloblishvili [25] which confirms the importance of preserving and studying rare varieties that are cultivated in limited areas.

The 4th century BCE saw the beginning of viticulture production in Croatia, both in the continental Pannonia region with the arrival of Celts and along the eastern Adriatic coast where Greeks founded cities [26]. Due to turbulent historical events and the introduction of mildews (*P. viticola* and *Erysiphe necator*) and phylloxera (*Daktulosphaera vitifoliae*) from the North American continent during the 19th century, the number of grapevine varieties and vineyard areas have changed significantly since then. Consequently, today's Croatian grapevine biodiversity consists of about one hundred varieties [27]. According to previous research conducted on some of these varieties, their susceptibility to the downy mildew disease under field conditions varies. Moreover, this was confirmed in controlled laboratory conditions using the leaf disc bioassay according to the OIV 452-1 descriptor [Leaf: degree of resistance to *Plasmopara* (leaf disc test)]. After measuring chlorophyll fluorescence and multispectral imaging traits, it is possible to distinguish non-infected and infected leaf discs 24 h after inoculation, whereas, on the fourth day upon inoculation, the differences between varieties belonging to various OIV classes are observed [28]. As far as secondary metabolites are considered, it was found that the constitutive polyphenolic profile of leaves is responsible for genotype differentiation among the OIV classes of resistance to *P. viticola* [29].

This study's primary objective was to expand the analysis of secondary metabolites in leaves before and during the early stage of inoculation, with a particular emphasis on volatile organic compounds. The research was performed using Solid Phase Microextraction (SPME)–Arrow Gas Chromatography–Mass Spectrometry (GC–MS). Similar methods have been used previously [8,11,14,15], but this one has proven to be particularly effective for the detection of VOCs [30]. Defining the differences between the metabolomic profiles of susceptible and resistant grapevines and detecting the resistance–related metabolites could broaden and introduce new concepts in plant protection strategies. Moreover, the detection of VOC emission patterns could be used to screen hybrids with varying resistance levels or to diagnose diseased plants [31].

2. Results

Eighty-six VOCs were identified through SPME-*Arrow*-GC/MS analysis of 17 genotype leaf samples collected at 0, 24, 48, and 94 h after *P. viticola* inoculation. There were 19 alcohols, 18 carbonyls (aldehydes and ketones), 17 monoterpenes, 10 sesquiterpenes, 10 esters, 9 acids, and 3 compounds that belong to some other groups of compounds, such as C_{13}-norisoprenoids and lactones, were detected (Tables S2 and S3). As far as individual compounds are concerned, the highest absolute peak areas were detected for 2-hexenal, benzyl alcohol, 3-hexen-1-ol, nonanal, and 4-hexen-1-ol acetate that were calculated as average values of all analysed samples. The interactions of all three factors (sampling term,

treatment, and OIV resistance class), including T_0, were significant for 17 identified VOCs, the majority of which were carbonyls (5), alcohols (4) and acids (3). The mean values of the interactions are presented in Table S2.

2.1. Changes throughout the Terms of Sampling

Of the 86 identified VOCs, 49 compounds significantly contributed to the ability to distinguish between the terms of sampling upon inoculation regardless of treatment and OIV class of resistance (Table S3). A slightly higher number of compounds increased significantly upon inoculation in at least one term (28) compared to the number of compounds that decreased (18). The most numerous increasing compounds were those that belong to monoterpenes (7), alcohols (6), and sesquiterpenes (5).

The alcohols with the highest APA in T_1 were: 1-nonanol, 1-butoxy-2-propanol, 1-methoxy-2-propanol, and *α*,*α*-dimethylbenzyl alcohol, whereas the alcohols with the lowest APA in T_3 were 3,7-dimethyl-1,7-octanediol and benzyl alcohol. The ascending significant change between all three terms of sampling was obtained for 1-hexanol, (*E*)-2-hexen-1-ol, 2-ethyl-2-hexen-1-ol, and 3-hexen-1-ol. However, the 2-ethyl-1-hexanol APA plunged significantly from T_1 to T_3. Significant differences between T_1 and T_3 were observed for 1-octen-3-ol with higher APA in T_1, whereas phenylethyl alcohol was more abundant in T_3.

In T_1, the 2-hexenal APA was significantly the lowest, whereas the (*E*)-2-nonenal, acetophenone, nonanal, and octanal APAs were the highest. The APA of benzaldehyde decreased significantly throughout the terms following the inoculation, whereas a significant increment was observed for 6-methyl-5-hepten-2-one from T_1 to T_3. The APA of (*E*,*Z*)-2,6-nonadienal and (*E*,*E*)-3,5-octadien-2-one distinguished between T_1 and T_3 with the highest APA being observed in T_3 and T_1, respectively. The APA of hexanal did not change significantly in the first two terms following the inoculation, although it increased significantly in the third term.

Markedly, the APA of (*E*)-3-hexenyl butanoate and (*Z*)-2-hexenyl acetate was lowest in T_3. The lowest APA of hexyl acetate, phenylmethyl formate, and methyl salicylate was observed in T_1. A higher APA of ethyl octanoate in T_1 distinguished it significantly from T_3.

In terms of monoterpenes, citronellol, neral, and *α*-terpineol had significantly lower APAs in T_1, whereas *p*-cymene had a significantly lower APA in T_3.

Significantly, the highest APAs in T_1 and T_2 were observed for geranyl vinyl ether and (*E*)-linalool oxide, respectively. The APAs of (*Z*)-linalool oxide and *ß*-ocimene increased significantly from T_1 to T_2, whereas geranylacetone increased significantly from T_1 to T_3.

The *α*-farnesene sesquiterpene increased significantly from T_1 to T_3, whereas from T_1 to T_2 the same is true for humulene and *ß*-guaiene. Finally, compounds belonging to another group of VOCs, such as (*E*)-*ß*-ionone, dihydroactinidiolide, and 5-ethyl-2(5H)-furanone, also increased from T_1 to T_2.

2.2. Differences between Non-Inoculated and Inoculated Leaves

Compounds that belong to sesquiterpenes and alcohols contributed the most to the discrimination between non-inoculated and inoculated leaves, regardless of the sampling term following the inoculation and the OIV resistance class. More precisely, the infected samples contained significantly elevated levels of humulene, ylangene, and *α*-farnesene, as well as 1-hexanol, (*E*)-2-hexen-1-ol, and 2,4-dimethyl-3-pentanol. In contrast, non-infected samples contained significantly higher APAs of 2-ethyl-1-hexanol, 1-nonanol, and 1-butoxy-2-propanol alcohols.

Among the remaining compounds, inoculated leaves measured significantly higher monoterpene geranylacetone, aldehyde octanal, ketone 6-methyl-5-hepten-2-one, and 2-hexonoic acid APAs (Table S3).

2.3. Differences between the OIV Resistance Classes

As previously explained, each genotype included in this research was assigned to the appropriate OIV class of resistance based on the severity of *P. viticola* sporulation and the OIV 452-1 descriptor. Although only seven out of eighty-six identified compounds failed to significantly differentiate the OIV resistance classes, no clear separation of genotypes into the OIV resistance classes was obtained. Thus, the contribution of individual compounds is described below.

The lowest APA of 2-hexenoic acid distinguished the completely resistant OIV class 9 from all other OIV classes, whereas the low APA of pentanoic acid distinguished OIV classes 1 and 9 from classes 3, 5, and 7. The lowest APAs of (*E*)-3-hexenoic acid and decanoic acid were observed in OIV class 3 compared to other OIV classes. Benzoic acid was significantly the most abundant in OIV class 5, whereas heptanoic acid was the most abundant in OIV classes 1 and 5. Decanoic acid distinguished OIV class 3 by its low APA compared to other classes.

The highest APA of 2-ethyl-1-hexanol distinguished the most susceptible OIV class 1 from the highly resistant OIV class 7, and the resistant OIV class 9. Similarly, the APA of 1-nonanol distinguished OIV class 1 and OIV class 9, in which the lowest APA of this alcohol was observed. OIV class 3 had the lowest APA of 1-octanol. Alcohols 2-ethyl-2-hexen-1-ol and (*E*)-2-hexen-1-ol were found in the highest APA in OIV classes 7 and 5, respectively. OIV classes 3 and 9 were specified by the lowest APA of 3-hexen-1-ol. The highest APAs of 3,7-dimethyl-3-octanol, 2,4-dimethyl-3-pentanol, benzyl alcohol, and phenylethyl alcohol distinguished OIV class 9 from all other classes.

As far as carbonyls are concerned, a higher APA of (*E*,*E*)-2,4-heptadienal differentiated OIV classes 1, 3, and 5 (pure *V. vinifera* varieties) from OIV classes 7 (interspecific hybrid Solaris) and 9 (*V. riparia*). Similarly, (*E*,*Z*)-2,6-nonadienal was observed in significantly higher APA in pure *V. vinifera* varieties compared to *V. riparia*. Aldehydes 2-hexenal and (*E*)-2-nonenal and their higher APAs discriminated OIV classes 1 and 3 from other classes, whereas a higher APA of (*E*,*E*)-3,5-octadien-2-one distinguished the susceptible OIV classes 1 and 3 from the partially resistant and resistant OIV classes 5, 7 and 9. OIV class 9 was specified by the highest APA of 4-pentenal compared to all other classes. The APA of 6-methyl-5-hepten-2-one was the highest in OIV class 7 followed by OIV class 9 and then OIV classes 5 and 1. Finally, the lowest APA of this ketone separated OIV class 3 from others. The highest APA of 2,5-dimethyl-benzaldehyde and heptanal distinguished OIV classes 9 and 5 from others, respectively. A higher APA of hexanal separated OIV class 5 from classes 1 and 3. Nonanal and octanal were highest in OIV classes 5 and 7 and lowest in OIV class 3.

The APA of phenylmethyl acetate was the highest in OIV class 9, followed by OIV classes 1 and 5, and the lowest in OIV classes 3 and 7. Significantly the highest APA of ethyl octanoate discriminated OIV class 1 from others. Similarly, 3-hexenyl butanoate distinguished OIV classes 1 and 5 from others. Significant differences were observed for (*E*)-2-hexenyl benzoate and its higher APA in OIV classes 3 and 5 compared to OIV class 7. In contrast, phenylmethyl formate had a higher APA in OIV class 7 compared to classes 1, 3, and 5. The highest APA of ethyl octanoate distinguished OIV class 1 from others, whereas the lowest APA of (*Z*)-2-hexenyl acetate was obtained in OIV class 9.

Among monoterpenes, the APA of ß-cyclocitral did not distinguish OIV classes 7 and 9, although it separated OIV class 9 from classes 1, 3, and 5 by being less abundant in OIV class 9. The highest APA of citronellol and nerol differentiated OIV class 7 from others. OIV class 9 was specific by the highest APA of (*Z*)-linalool oxide, eucalyptol, and *p*-cymene. A higher APA of geranylacetone differentiated OIV classes 7 and 9 from others. The highest APA of limonene was obtained in the most susceptible OIV class 1. In the same way, menthol distinguished OIV class 1 from classes 7 and 9, whereas linalool separated OIV class 1 from classes 3, 5, and 9. Low APA of α-terpineol distinguished OIV classes 3 and 5 from others. Higher APA of ß-ocimene differentiated OIV classes 1 and 3

from class 5. Geranylacetone was the most abundant in OIV class 9 followed by OIV class 7, whereas it did not distinguish OIV classes 1 and 5.

Higher APAs of (*Z*)-*ß*-farnesene and *α*-farnesene differentiated OIV classes 7 and 9 from others. In comparison, the APAs of humulene and *ß*-guaiene were the highest in OIV classes 1 and 5. OIV class 9 was specific due to the lowest APA of 5-ethyl-2(5H)-furanone, whereas a higher APA of dihydroactinidiolide was detected in OIV class 5 compared to OIV classes 1 and 3 (Table S3).

2.4. Compounds Related to Higher Resistance

Aiming to identify VOCs that could be responsible for higher resistance to *P. viticola*, highly positive correlations between the infected samples and genotypes that belong to OIV classes 5, 7, and 9 were sought throughout the experiment. At the same time, negative or low correlations were sought for the non-infected (control) samples to verify that the ascending APA of a VOC in the infected sample is associated with a defence mechanism against infection. Moreover, when the same trend was observed for VOCs in varieties that are most susceptible to *P. viticola* (OIV class 1), these compounds were excluded from consideration.

Throughout the experiment, highly positive correlations were obtained for 2-hexenoic acid in samples from all three OIV class 5 (Malvazija istarska, Ranfol, and Teran) infected varieties, whereas negative or low correlations were obtained for non-infected (control) samples of the same resistance class. The same is true for the following VOCs in Malvazija istarska specifically: 2-ethyl-2-hexen-1-ol, *α*,*α*-dimethylbenzyl alcohol, phenylmethyl acetate, phenylmethyl formate, limonene, *α*-terpineol, *ß*-myrcene, *ß*-ocimene, geranylactone, copaene, *α*-farnesene, and *γ*-muurolene. The infected samples of Teran exhibited highly positive correlation for (*E*)-2-hexen 1-ol, (*E*)-2-hexenyl benzoate, *ß*-cyclocitral, citronellol, linalool, and *p*-cymene, whereas Malvazija istarska and Ranfol exhibited the same for benzeneacetaldehyde, ethyl benzoate, citronellol, menthol, caryophyllene, humulene, and *α*-muurolene. However, the same trend was observed for the majority of the above-mentioned compounds in varieties that are the most susceptible to *P. viticola* (OIV class 1) (Table S4). Therefore, they cannot be considered the cause of resistance reactions.

Nevertheless, a few compounds detected in OIV 5 were also detected in OIV 7 or 9 but not in OIV 1, having a high positive correlation in infected samples and a low or negative correlation in non-infected ones. In particular, VOCs detected in OIV 9 were ocimene, (*E*)-2-hexen-1-ol, whereas geranylacetone was found in OIV 7. Figure 1 depicts the APA changes of specific VOCs in response to inoculation, which may have contributed to the increased resistance of genotypes belonging to OIV classes 5, 7, or 9.

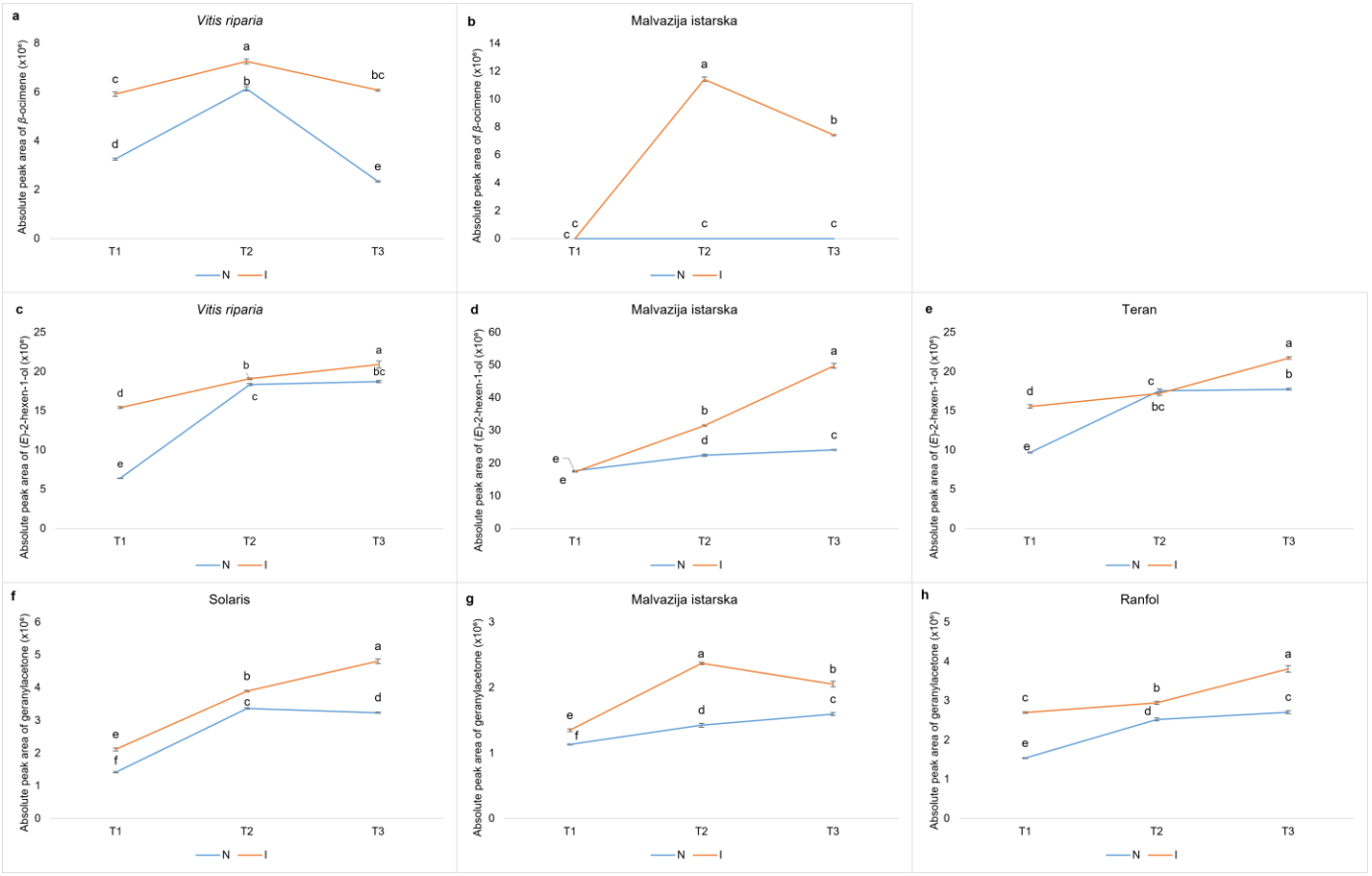


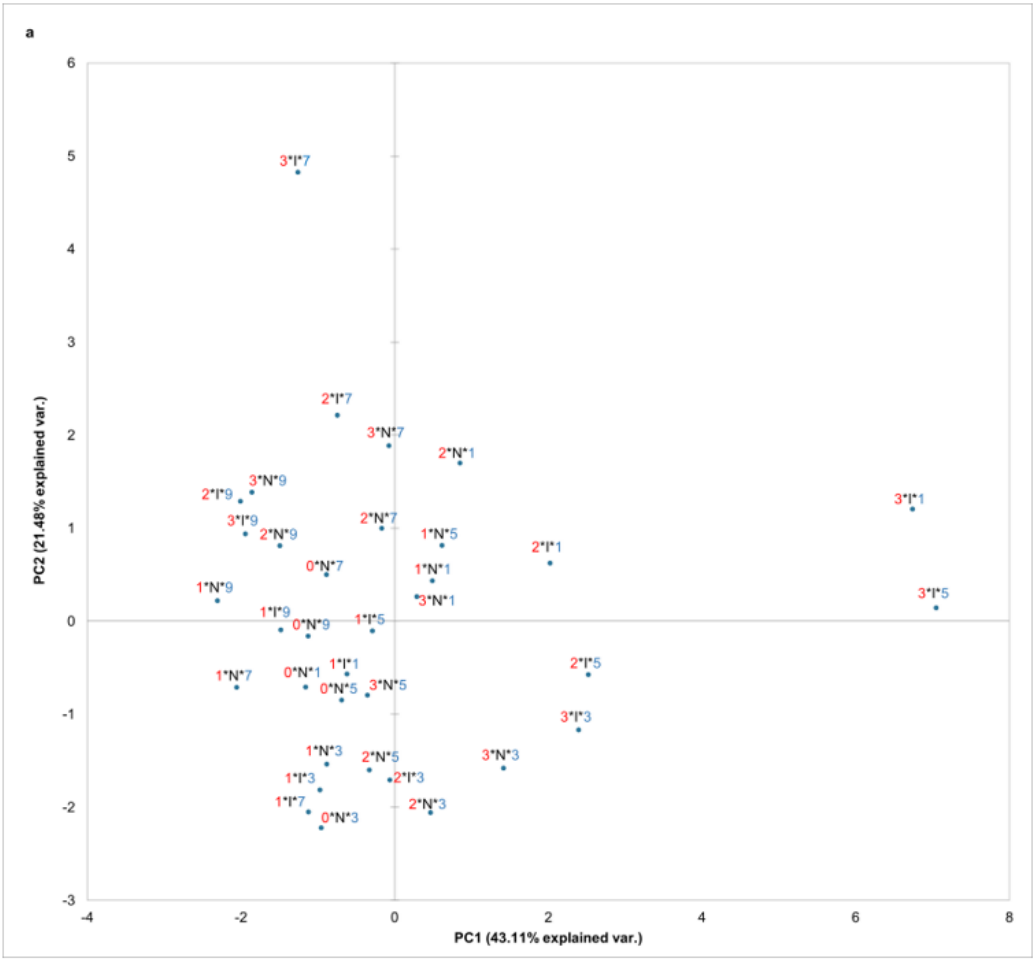
Figure 1. The absolute peak areas of *β*-ocimene, (*E*)-2-hexen-1-ol, and geranylacetone in the sampling terms upon *P. viticola* inoculation (24 hpi (T1), 48 hpi (T2) and 96 hpi (T3)) for inoculated (I) and non-inoculated (N) samples of genotypes that belong to resistance classes 5 (Malvazija istrska, Ranfol, and Teran), 7 (Solaris), or 9 (*Vitis riparia*). The differences between the means were evaluated by Duncan's multiple range test at a confidence level of 95% ($p < 0.05$). Different letters indicate statistical significance. Sub-figures depict the absolute peak areas of volatile organic compounds as follows: (**a**) *β*-ocimene in *Vitis riparia*, (**b**) *β*-ocimene in Malvazija istarska, (**c**) (*E*)-2-hexen-1-ol in *Vitis riparia*, (**d**) (*E*)-2-hexen-1-ol in Malvazija istarska, (**e**) (*E*)-2-hexen-1-ol in Teran, (**f**) geranylacetone in Solaris, (**g**) geranylacetone in Malvazija istarska, and (**h**) geranylacetone in Ranfol.

2.5. Sesquiterpenes and the OIV Resistance Classes

To analyse the total variability of the volatile compounds' absolute peak area related to the division of the OIV resistance classes (1, 3, 5, 7, and 9), terms of sampling (0, 24, 48, and 96 hpi), and treatments (considering non-inoculated and inoculated samples), a principal component analysis (PCA) was performed using all detected VOCs and using each group of detected volatile compounds separately. Most groups of compounds did not contribute to a distinct separation of the samples by any of the aforementioned factors (data not shown). However, the PCA based on the APA of individual sesquiterpenes contributed the most to distinguishing the OIV classes of resistance. In particular, the PCA scatter plot of the first two components explained 64.59% of the variability (Figure 2) with the first principal component (PC1) accounting for 43.11% and the second (PC2) for 21.48%. The projection on these two axes distinguished the two highly resistant genotypes (OIV classes 7 and 9) from *V. vinifera* genotypes (OIV classes 1, 3, and 5). However, in OIV classes 1, 3, and 5, terms and treatments were not clearly separated (Figure 2a).

Based on the related vector diagram (Figure 2b), it is possible to define the sesquiterpenes that contributed to such distribution and grouping of samples that belong to either OIV classes 7 and 9 or OIV classes 1, 3, and 5 in the space defined by the first two principal

components. One group containing all the samples belonging to OIV classes 7 and 9 regardless of the treatment and sampling term was separated from the other group due to the higher APA of *α*-farnesene and *(Z)-ß*-farnesene. Most of these observations are located in the second quadrant and a few of them are in the third quadrant.



0, 1, 2, 3—Terms of sampling (0, 24, 48, 96 hpi)
N, I—Non-inoculated and Inoculated observations
1, 3, 5, 7, 9—OIV classes of resistance

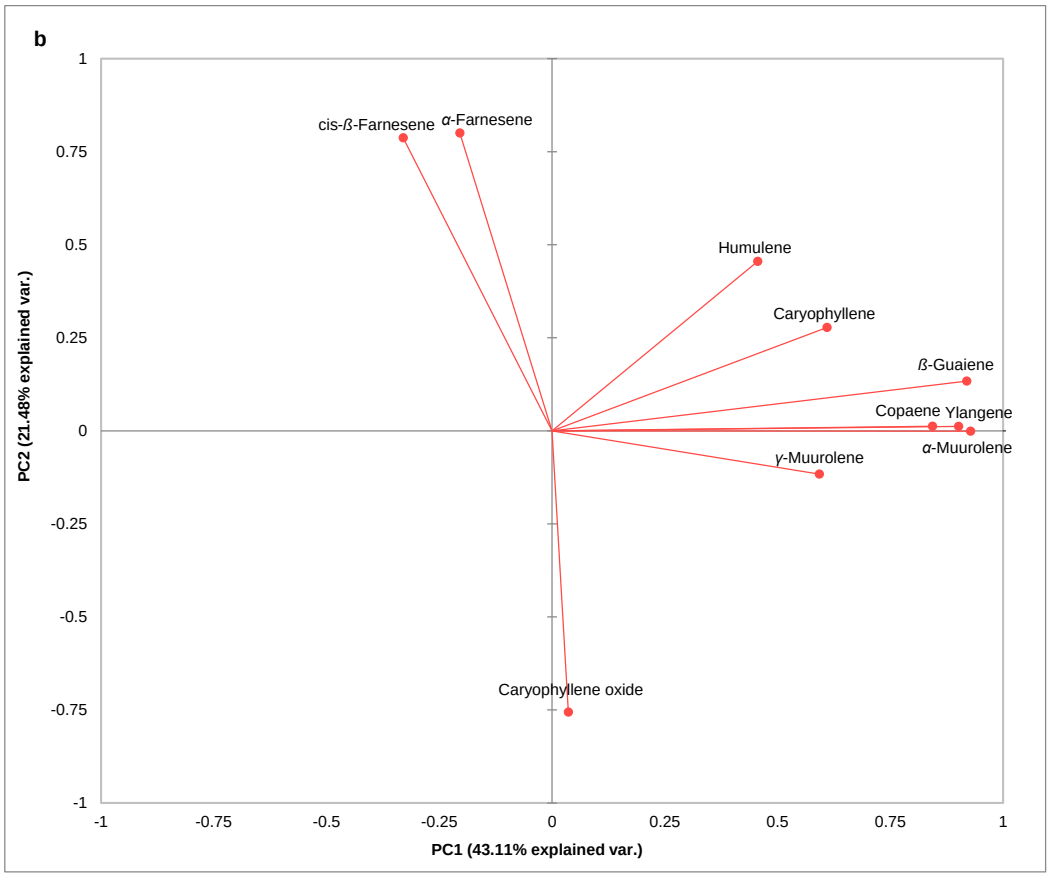


Figure 2. PCA scatter plot depicting (**a**) OIV classes of resistance (1, 3, 5, 7, and 9 from the most susceptible to totally resistant) based on their leaves' sesquiterpenes absolute peak area before and after artificial *P. viticola* inoculation at 0, 24, 48 and 96 hpi in the space defined by the first two principal components explaining 64.59% of the variability; (**b**) the vector diagram of correlation among the absolute peak area of sesquiterpenes and the first two principal components.

3. Discussion

P. viticola maintains its life cycle in living tissue as its tubular mycelium grows intercellularly and obtains nutrients by parasitizing the host cells through haustoria [32]. Leaves are typically the first to show symptoms of downy mildew, especially young leaves that have not yet developed ontogenic resistance to the disease [33]. Biosynthesis of VOCs occurs in the leaf mesophyll tissues, specifically in the palisade mesophyll cells [34]. Consequently, since phytopathogens alter plant VOCs emission, decreasing or increasing the amount of some pre-existing VOCs, and inducing the appearance of newly synthesized VOCs, this research was conducted on young leaves and their volatile organic compounds (VOCs) [35]. It is known that VOCs can directly inhibit pathogen growth, induce plant resistance mechanisms in neighbouring plants, and mediate associational resistance by adsorption to the cuticle of receiver tissues [36]. During the early stages of infection, the susceptible cultivar undergoes the following changes: at 24 hpi, the zoospores germinate and the germ tube penetrates the substomatal cavity; at 48 hpi, the *P. viticola* hyphae are observed in the intercellular spaces; at 96 hpi, the sporangiophores begin to develop from the stomata [37]. A novel SPME-Arrow GC/MS technique proved to be efficient for this kind of analysis by processing a large number of samples and providing a whole range of VOCs [30].

As mentioned previously, phenotypic differences among Croatian native varieties have been investigated, and some specificities corresponding to OIV resistance classes have been observed. Based on the content and composition of polyphenolic compounds, it was found that their constitutive profiles in leaves are responsible for diverse levels of

resistance to *P. viticola* [29]. The analysis of 86 volatile organic compounds (VOCs) in the leaves of 14 native Croatian varieties, including Chardonnay, Solaris, and *V. riparia*, was performed with the intention of expanding the current findings based on secondary metabolites. To the best of our knowledge, this is the first time such extensive research on this topic has been conducted. Although a clear separation of differently resistant genotypes was not accomplished, as the inoculation time progressed, some specificities were defined among the OIV classes of resistance and the APA of sesquiterpenes. Moreover, a few compounds, such as geranylacetone, *ß*-ocimene, and (*E*)-2-hexen-1-ol could be responsible for a higher resistance of OIV classes 5, 7, and 9.

Considering the sampling terms upon *P. viticola* inoculation, a slightly higher number of VOCs was detected in increased APAs over time, based on the average values of all 17 analysed genotypes. This has already been observed for the resistant genotypes of BC4, Kober 5BB, SO4, and Solaris whose leaf VOCs were analysed at 6 dpi and compared to 0 dpi, whereas the APA of VOCs in Pinot noir leaves decreased [11]. Although benzaldehyde was not an indicator of *P. viticola* inoculation, its APA decreased throughout the experiment which is in accordance with Ricciardi et al., (2021) [8] whose samples were frozen likewise. At the same time, benzaldehyde content increased in the Bianca cultivar when fresh leaves were analysed [14], meaning that VOC emission patterns could be related to the sample preparation and the term of sampling. The most numerous increasing compounds were alcohols (e.g., 3-hexen-1-ol and 2-ethyl-2-hexen-1-ol), monoterpenes (e.g., citronellol and neral), and carbonyls (e.g., hexanal and (*E,Z*)-2,6-nonadienal). These groups of VOCs increased in the leaves of Bianca and Mgaloblishvili [8].

Terpenes were found to be the most discriminative among the OIV classes of resistance. The high APA of sesquiterpenes *α*-farnesene and *(Z)-ß*-farnesene distinguished OIV classes 7 and 9 from the more susceptible OIV classes 1, 3, and 5 regardless of the treatment and the sampling term. Moreover, *α*-farnesene was suitable for distinguishing treatments throughout the experiment due to its higher APA in inoculated leaves compared to non-inoculated ones. Similarly, increased emission of sesquiterpenes were detected in in vitro plantlets of the downy mildew–resistant genotypes of SO4 and Kober 5BB, whereas the increment of these VOCs was lower in the Pinot noir susceptible variety [13]. Likewise, an increased amount of farnesene was detected in the resistant genotypes of Mgaloblishvili and Bianca upon *P. viticola* inoculation together with the up-regulation terpene synthase genes, suggesting a pathogen-dependent transcriptional regulation of terpene biosynthesis [8]. In a study conducted by Ciubotaru et al., (2021), farnesene was expressed in high concentrations in the genotypes with mono-locus resistance, namely BC4 (*Rpv1*), Bianca (*Rpv3-1*) and F12P160 (*Rpv12*), and in the pyramided resistant genotype F12P127 (*Rpv3-1*, *Rpv3-3*, *Rpv10*) [15]. These findings indicate that VOCs, especially sesquiterpenes, produced by downy mildew-resistant genotypes contribute to grapevine defence against *P. viticola*.

Algarra Alarcon et al., (2015) [13] detected a higher content of monoterpenes in SO4 which contradicts our findings as far as total monoterpenes are concerned since their APA was the highest in the most susceptible OIV class 1. Considering individual monoterpenes, a higher APA of *ß*-cyclocitral differentiated pure *V. vinifera* varieties (OIV classes 1, 3, and 5) from *V. riparia*, whereas linalool was significantly higher in OIV class 1 compared to OIV classes 3, 5, and 9. In contrast, these monoterpenes were detected in higher amounts in the leaves of resistant genotypes (i.e., BC4, Kober 5BB, SO4, and Solaris) compared to the susceptible Pinot noir [11]. Higher contents of linalool and neral were detected in Bianca and F12P60 suggesting their antimicrobial activity [15]. In addition to linalool, our research identified its volatile oxides, namely *(Z)-* and *(E)*-linalool oxides. Defence–related activity could be ascribed to *(Z)*-linalool oxide since its highest APA was detected in *V. riparia* (OIV 9) distinguishing this genotype from the others evaluated. Neral was detected in Solaris (OIV 7) with a high APA, but it was also found in the most susceptible varieties (OIV 1).

Aldehyde 4-pentenal distinguished *V. riparia* from other genotypes by its low APA, whereas Lazazzara et al., (2018) [11] detected a higher APA of (*E*)-2-pentenal in the resistant genotypes of BC4 and Kober 5BB compared to Pinot noir. As far as (*E*,*E*)-2,4-heptadienal and benzeneacetaldehyde are concerned, lower APAs were detected in resistant genotypes in both of these studies. The same authors found benzaldehyde to be more abundant in resistant varieties, whereas, in our study, it was most abundant in OIV classes 5 and 9, although a high APA was also detected in OIV class 1. Nevertheless, Chitarrini et al., (2017) [14] suggested benzaldehyde as a putative biomarker of resistance to *P. viticola* infection since it was detected in higher concentrations in infected samples of the resistant cultivar Bianca at 48 and 96 hpi. Bianca, Solaris, and F12P60, which possess at least one locus of resistance in their genomes, had a higher level of benzaldehyde [15]. Moreover, it was found that benzaldehyde acts as a promoter of salicylic acid (SA)–mediated defence, as it accumulated early and in high concentration in the plant metabolome with *Rpv12*–mediated resistance [38]. Salicylic acid acts as a phytohormone precursor of the volatile compound methyl salicylate, which is known for activating induced resistance upon attack by biotrophic microorganisms, such as *P. viticola* [12]. Aldehyde 2-hexenal was found in increasing APAs throughout the sampling terms and its high APAs were detected in OIV classes 1 and 5. In our experiment, however, the APA of nonanal decreased. Furthermore, the high APA of nonanal in OIV class 5, the same was detected in Solaris (OIV 7), which could be one of the possible commonalities related to lower susceptibility among these two OIV classes. Previously, a higher content of 2-hexenal was associated with the Solaris cultivar resistance, whereas a higher content of nonanal was detected in the leaves of the F12P127 genotype, and based on that, these VOCs were proposed as biomarkers of resistance [15].

Phenylethyl alcohol increased throughout the experiment and was detected in the highest APA in OIV class 9 corroborating the findings of Lazazzara et al., (2018) [11]. Throughout the experiment, the alcohols 1-hexanol and (*E*)-2-hexen-1-ol were found in ascending APAs, while their higher APAs in inoculated leaves distinguished them from non-inoculated leaves. OIV 5 varieties have the highest APA of (*E*)-2-hexen-1-ol, which suggests that it may play a role in the defence mechanism of *V. vinifera* varieties that are less susceptible to *P. viticola*. Both alcohols, together with 2-ethyl-1-hexanol and 1-octen-3-ol, have been identified as potential biomarkers of resistance in Bianca, Solaris, and F12P60 genotypes due to their higher concentration upon inoculation compared to Pinot noir, which is susceptible [15]. In our study, the APA of 2-ethyl-1-hexanol decreased with time and was higher in non-inoculated leaves and in varieties that are the most susceptible to *P. viticola* (OIV 1), whereas 1-octen-3-ol was not significant for any of these parameters.

Among esters, *(Z)*-3-hexenyl benzoate was proposed as a potential biomarker of resistance in previous research due to its higher up-regulation upon *P. viticola* inoculation in the resistant genotype of F12P60 compared to Pinot noir [15]. Similarly, we identified a relatively high APA of (*E*)-2-hexenyl benzoate in *V. riparia*. Methyl salicylate is synthesized from salicylic acid by salicylate methyl transferase and is widespread in plants as a volatile odorous compound associated with mint-like and green pepper aromas [39]. It was demonstrated that a higher concentration of methyl salicylate in stems, grapes, and consequently in red and white wines was related to vine diseases (downy mildew, grape black rot, Esca) suggesting a host plant-induced defence mechanism against fungal infection. Thus, methyl salicylate can serve as a volatile indicator of the vineyard's infection status [40]. In our study, it was detected in the highest abundance in the resistant genotype of *V. riparia* as well as the highly resistant Solaris cultivar confirming its potent antifungal activity in these genotypes. Salicylic acid and methyl salicylate induce systemic acquired resistance and hypersensitive response (cell death) as a reaction to a pathogen attack [41]. Although methyl salicylate did not help distinguish the non-inoculated and inoculated samples, its higher APA was observed 48 h upon inoculation corroborating that the synthesis of this VOC is induced by a pathogen attack. Acting as a volatile form of a defence phytohormone, methyl salicylate systemically induces defence responses in plant parts

and organs that are distant from the initial infection site. Moreover, these airborne signals can be perceived by uninfected neighbouring plants and induce a resistance reaction in them as well [12]. Previously, methyl salicylate has been proposed as a biomarker of downy mildew infection [42] and as a potential biomarker of resistance to *P. viticola* since it was detected in higher concentrations in Bianca compared to Pinot noir [15].

In previous studies that included field trials and in vitro leaf disc bioassay, three Croatian native grapevine varieties, namely Malvazija istarska, Ranfol, and Teran (OIV class 5), proved to be more resistant to *P. viticola* compared to other evaluated *V. vinifera* varieties [28]. Moreover, their constitutive polyphenolic profile of leaves, i.e., higher content of flavonol glycosides mostly, distinguish them from the more susceptible varieties [29]. Aiming to define VOCs that could be responsible for the defence mechanism of these three Croatian grapevine varieties, VOCs from OIV class 5 were compared with VOCs from OIV classes 7 and 9. Therefore, a few compounds with a higher APA upon inoculation in inoculated leaves compared to non-inoculated ones in OIV classes 5, 7, and 9 were observed, namely geranylacetone, *ß*-ocimene, and (*E*)-2-hexen-1-ol.

Geranylacetone belongs to the class of organic compounds known as acyclic monoterpenes. It is a component of essential oils in various plants including *Nelumbo nucifera* whose leaf extract has strong antioxidant properties [43]. Up to now, geranylacetone was not recognized as a biomarker of grapevine resistance to *P. viticola*, although it was detected in higher concentrations in the leaves of the resistant pyramided genotype F12P60 compared to Pinot noir [15]. Ricciardi et al., (2021) [8] did not find significant changes in the quantity of this compound upon inoculation in either Bianca or Mgaloblishvili. In this study, geranylacetone was found to have an ascending APA throughout the experiment and a significantly higher APA in each sampling term in the inoculated leaves of the native varieties Malvazija istarska and Ranfol, as well as the highly resistant Solaris cultivar, compared to non-inoculated leaves, indicating that geranylacetone may be involved in the defence mechanism of these cultivars.

Another possible indicator of resistance is *ß*-ocimene, a volatile organic compound that belongs to the class of monoterpenes. During T_2 (48 hpi), its induced accumulation was observed in the inoculated leaves of the native variety Malvazija istarska, whereas it was never detected in the control leaves. *ß*-ocimene was detected in both inoculated and non-inoculated leaves of the *V. riparia* resistant genotype, although its APAs were higher in inoculated leaves in each term following inoculation. Previously, terpenes were often recognized as compounds associated with the defence mechanism against downy mildew [8,11,13]. Specifically, allo-ocimene was found to activate defence genes and induce resistance against *Botrytis cinerea* in *Arabidopsis thaliana* [44]. Functional properties of terpenes, such as farnesene, nerolidol, valencene, and ocimene were examined and found efficient in counteracting *P. viticola*. Not only were they synthesized in higher amounts in the resistant variety of Mgaloblishvili, but their antisporulant activity was also proved in ad hoc experimental inoculations in which disease severity and sporangia concentration were inhibited. Among these terpenes, ocimene was found to be the most effective [8].

As previously mentioned, the volatile alcohol (*E*)-2-hexen-1-ol was proposed as a biomarker of resistance in the study conducted by Ciubotaru et al., (2021) [15]. In our research, the same compound was detected in inoculated leaves of Teran and *V. riparia* in ascending APAs upon *P. viticola* inoculation. (*Z*)-3-hexenol, an alcohol with a similar structure, was detected in higher concentrations in the resistant *V. labrusca* and *V. riparia* genotypes compared to the susceptible *V. vinifera* varieties [45]. (*Z*)-3-hexenol is known to induce up-regulation of defence genes during the *Botrytis cinerea* infection of *Arabidopsis thaliana,* acting in the same way as allo-ocimene [44].

4. Materials and Methods

4.1. Preparation of samples

4.1.1. Plant Material

The plant material needed for this experiment was prepared in the same way as in our previous research [29]. In short, plant material of 17 grapevine genotypes was used in this research. Out of these genotypes, 14 were Croatian native grapevine varieties. Chardonnay served as the susceptible control variety, while Solaris and *Vitis riparia* served as the partially resistant and resistant control genotypes, respectively (Table 1). Previously, they were distributed into the OIV classes of resistance by applying the leaf disc bioassay [28] according to the OIV 452-1 descriptor [Leaf: degree of resistance to *Plasmopara* (leaf disc test)] [46]. Therefore, each genotype was assigned to the relevant OIV class of resistance (Tables 1 and 2). The leaf discs used for assigning each genotype to the appropriate OIV class of resistance were excised from the exact same leaves used for the analysis of volatile organic compounds. Moreover, the leaf discs were inoculated with the same *P. viticola* suspension as the leaves. The sporulation was evaluated on the seventh day upon inoculation.

Table 1. Genotypes, additional information on the plant material, and genotypes' corresponding OIV classes of resistance to *P. viticola* (OIV 452-1) according to OIV [46]. Genotypes in bold were used as controls.

Genotype (Accession Name)	Holding Institute	Material Source ID (EURISCO)	VIVC Code	Species	OIV 452-1
Belina starohrvatska	HRV041	VIT00233	5374	*Vitis vinifera* subsp. *vinifera*	1
Debit	HRV041	VIT00017	10423	*Vitis vinifera* subsp. *vinifera*	1
Grk	HRV041	VIT00030	5066	*Vitis vinifera* subsp. *vinifera*	1
Moslavac	HRV041	VIT00052	4292	*Vitis vinifera* subsp. *vinifera*	1
Plavac mali	HRV041	VIT00060	9549	*Vitis vinifera* subsp. *vinifera*	1
Babić	HRV041	VIT0002	844	*Vitis vinifera* subsp. *vinifera*	3
Chardonnay	HRV041	CL-277*	2455	*Vitis vinifera* subsp. *vinifera*	3
Kraljevina	HRV041	VIT00035	24904	*Vitis vinifera* subsp. *vinifera*	3
Plavina	HRV041	VIT00062	9557	*Vitis vinifera* subsp. *vinifera*	3
Pošip	HRV041	VIT00065	16018	*Vitis vinifera* subsp. *vinifera*	3
Škrlet	HRV041	VIT00085	22983	*Vitis vinifera* subsp. *vinifera*	3
Tribidrag	HRV041	VIT00013	9703	*Vitis vinifera* subsp. *vinifera*	3
Malvazija istarska	HRV041	VIT00047	7269	*Vitis vinifera* subsp. *vinifera*	5
Ranfol	HRV041	VIT00070	9908	*Vitis vinifera* subsp. *vinifera*	5

Teran	HRV041	VIT00087	12374	*Vitis vinifera* subsp. *vinifera*	5
Solaris	DEU455	20340 *	20340	*Vitis vinifera* subsp. *vinifera*	7
Vitis riparia	DEU098	4609 *	4609	*Vitis riparia*	9

Plant material from the vineyard on Experimental station Jazbina, University of Zagreb, Faculty of Agriculture, Department of Viticulture and Enology, Cv. Chardonnay, clone CL-277. * According to VIVC. VIVC—Vitis International Variety Catalogue (https://www.vivc.de, the access date: 15 November 2022). OIV 452-1—Descriptor for leaf: degree of resistance to *Plasmopara* (leaf disc test).

Table 2. Phenotypes of representative inoculated leaf discs at the time of the *Plasmopara viticola* sporulation evaluation.

Representative leaf disc					
Genotype	Plavac mali	Babić	Malvazija istarska	Solaris	*Vitis riparia*
OIV resistance class	1	3	5	7	9
Surface covered with sporulation (%)	61–100	41–60	21–40	1–20	0

In order to produce healthy leaves, hardwood cuttings were planted in regularly drop-irrigated pots under greenhouse conditions of 15 to 24 °C air temperature and 65 to 75% relative humidity during the cultivation period. The fourth and the fifth leaves beneath the apex were used since they are not mature enough to resist or tolerate the downy mildew disease with the exception of resistant genotypes. The leaves were transferred from the greenhouse to the laboratory, where they were rinsed with ultrapure water. There was no evidence of foliar diseases on the leaves at the time of sampling in the greenhouse.

4.1.2. *Plasmopara viticola* Suspension Preparation

For the preparation of a dense and cloudy *P. viticola* suspension, naturally infected leaves were used that were taken from the vineyard where no chemical protection was applied. This suspension was applied to the abaxial leaf surfaces of the susceptible Chardonnay variety in order to produce fresh sporulation. The leaves were placed in in vitro laboratory conditions optimal for *P. viticola* propagation. The leaves with freshly developed sporulation were soaked in ultrapure water and the sporulation was removed using a soft brush until the water became cloudy. The suspension concentration was adjusted to 2×10^5 spores ml^{-1} with a Neubauer cell counting chamber [47,48]. The freshly prepared suspension was used to inoculate the leaves of 17 genotypes.

4.1.3. Inoculation and Incubation of the Leaves

Four leaves from each genotype were sampled and frozen at −20 °C until analysis (T_0). The remaining 24 leaves per genotype were separated into two groups: mock-inoculated leaves (treated with ultrapure water) and leaves inoculated with *P. viticola* suspension. Each leaf was placed in a separate Petri dish (150 mm in diameter) on wet filter paper. The leaves were laid with the abaxial side up and sprayed with either ultrapure water or the *P. viticola* suspension. The Petri dishes were sealed with parafilm and placed in the

climate chamber with optimal conditions for downy mildew development (air temperature of 20°C, air humidity of 80%). The samples were kept in dark for the first 24 h. Then, water or suspension droplets were removed with a sterile filter paper to prevent leaf decay. After that, a 16-h photoperiod was applied to simulate outdoor conditions [47,48]. At certain time points after inoculation [T_1—24 h post-inoculation (hpi); T_2—48 hpi; T_3—96 hpi] [14,49,50], the samples were taken from the climate chamber and stored in the freezer (−20 °C) until freeze drying (lyophilization). For each genotype, additional leaves were inoculated beyond those required for volatile organic compound analysis to ensure that infection was successful.

4.2. Analysis of Volatile Organic Compounds

4.2.1. SPME-Arrow Extraction of VOCs

Before the analysis, the frozen leaves were lyophilized and ground into a powder using a MiniG Mill (SPEX Sample Prep, Metuchen, NJ, USA) (1 min, 1500 rpm). The SPME-Arrow extraction was carried out following the method described by Šikuten et al., (2021) [30]. In short, the SPME-Arrow extraction was conducted using the RSH TriPlus autosampler (Thermo Fisher Scientific Inc., Brookfield, WI, USA). A 100 mg sample was placed in 20 mL headspace screw-top vials with PTFE/silicone septum caps.

The sorption conditions were as follows: the sample was incubated at 60 °C for 20 min, and then SPME-Arrow fiber DVB/CWR/PDMS (120 µm × 20 mm; Thermo Fisher Scientific Inc., Brookfield, WI, USA) was exposed for 49 min. Then, the fiber was inserted into the GC injector port operating in the splitless mode and desorbed at 250 °C for 10 min. All leaf samples were analysed in triplicate.

4.2.2. GC–MS Analysis

The analytes were separated and detected using a TRACE™ 1300 Gas Chromatographer coupled with an ISQ 7000 TriPlus quadrupole mass spectrometer (Thermo Fisher Scientific Inc., Bartlesville, OK, USA) equipped with a TG-WAXMS A capillary column (60 m × 0.25 mm × 0.25 µm film thickness; Thermo Fisher Scientific Inc., Bartlesville, OK, USA). The volatile compounds injected into the inlet were delivered to the column in the splitless mode. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The oven temperature program was as follows: an initial temperature of 40 °C was maintained for 5 min, increased by 2 °C every minute until the temperature reached 210 °C, and then it was maintained for 10 min. The MS spectra were recorded in the electron impact ionization mode (EI) with an ionization energy of 70 eV. The mass spectrometry was performed in the full scan mode between 30 and 300 *m*/*z*. Chromeleon™ Data System was utilised to process the obtained data (Thermo Fisher Scientific Inc., Bartlesville, OK, USA). The volatile compounds were identified by comparing the recorded mass spectrum to the information contained in the Wiley Registry 12th Edition/NIST Spectral Library. The Retention Index (RI) was calculated using alkane standards C_8-C_{20} (Sigma Aldrich, St. Louis, MO, USA) in accordance with the equation in Song et al., (2019) [51] and compared to previously published results [52,53]. The retention indices are presented in Table S1. All results are expressed as absolute peak areas (APA).

4.3. Statistical Analysis

Factorial ANOVA was performed on the absolute peak area (APA) of the volatile organic compounds to define the effects of treatment (non-inoculated vs. inoculated samples), the classes of resistance, and the terms of sampling after inoculation. The differences between the means of specific factors were evaluated by Duncan's multiple range test at a confidence level of 95% ($p < 0.05$). Since there was no treatment in T_0 (the sampling period preceding inoculation), it was excluded from the factorial ANOVA used to define the exact effect of each individual factor (Table S3).

To compare the differences in VOCs response between inoculated and non-inoculated leaves, correlations were calculated between VOCs APA and the sampling time from T_0 to T_3 (time in days after the T_0) separately for each genotype and treatment (Table S4) using Pearson's coefficient. Principal component analysis (PCA) was performed using the average values of APA of different VOC groups for treatment (non-inoculated and inoculated), which were sampled in different terms before and upon inoculation (0, 24, 48, and 96 hpi) belonging to different OIV classes of resistance. The XLSTAT statistical and data analysis solution (Addinsoft, 2021, New York, NY, USA) was used for statistical analyses.

5. Conclusions

This work provides an insight into the profiles of leaf volatile organic compounds (VOCs) before and after *P. viticola* inoculation in 15 *V. vinifera* varieties, 14 of which are native to Croatia, as well as the resistant Solaris and *V. riparia* genotypes. Sesquiterpenes proved to be the most appropriate for distinguishing highly resistant genotypes (OIV classes 7 and 9) from *V. vinifera* varieties (OIV classes 1, 3, and 5), as well as non-inoculated from inoculated leaves. Moreover, their ascending APA throughout the experiment was more pronounced in inoculated samples confirming that the synthesis of sesquiterpenes was upregulated by the *P. viticola* infection. However, neither sesquiterpenes nor other groups of VOCs separated the OIV resistance classes into different groups. Nevertheless, a few compounds, namely geranylacetone, *ß*-ocimene, and (*E*)-2-hexen-1-ol were identified in the Croatian native grapevine varieties of lower susceptibility to *P. viticola* (Malvazija istarska, Ranfol, and Teran) that could be involved in their defence mechanism since the same was detected in Solaris or *V. riparia*. However, due to the fact that plant volatilomics is highly dependent on numerous stressors, additional research is necessary to determine the direct role of these VOCs by conducting experiments on non-detached, in vivo leaves.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants12020404/s1, Table S1: Retention indices, Table S2: ANOVA Interactions, Table S3: ANOVA_Term_Treatment_OIV class, Table S4: Correlations.

Author Contributions: Conceptualisation, I.T., and J.K.K.; methodology, P.Š., I.Š. and I.T.; formal analysis, P.Š., and I.Š.; data curation, D.P.; writing—original draft preparation, P.Š.; writing—review and editing, J.K.K., D.P., and E.M.; funding acquisition, J.K.K., and E.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the project KK.01.1.1.01.0005 Biodiversity and Molecular Plant Breeding, Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Zagreb, Croatia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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Appendix 3A. Supplement material related to scientific paper Štambuk P., Šikuten I., Preiner D., Maletić E., Karoglan Kontić J., Tomaz I. (2023). Croatian Native Grapevine Varieties' VOCs Responses upon *Plasmopara viticola* Inoculation. Plants 12(2): 404. doi: 10.3390/plants12020404

Table S1 - Retention time and retention indices calculated and obtained from the literature

Compound	RT/min	RI (cal)*	RI (lit)**	Compound	RT/min	RI (cal)*	RI (lit)**
2-Hexenoic acid	71.254	1973	1933-2002	Hexanal	17.354	1076	1056-1106
(*E*)-3-Hexenoic acid	70.422	1957	1929-1957	Nonanal	38.114	1398	1370-1414
Benzoic acid	92.923	2428	2387-2444	Octanal	31.084	1292	1267-1312
Heptanoic acid	70.37	1956	1916-1993	Hexyl acetate	29.79	1273	1262-1305
2-Ethyl-hexanoic acid	70.153	1951	1910-1969	Phenylmethyl acetate	58.63	1737	1688-1771
Decanoic acid	85.42	2257	2227-2316	Ethyl benzoate	55.13	1676	1641-1683
Nonanoic acid	80.73	2153	2110-2196	(*E*)-3-Hexenyl butanoate	42.26	1463	1431-1454
Octanoic acid	75.56	2057	2013-2091	(*E*)-2-Hexenyl benzoate	52.55	1631	2081-2127
Pentanoic acid	58.976	1744	1685-1780	Phenylmethyl formate	56.37	1698	1671-1687
1-Heptanol	41.787	1456	1419-1467	Ethyl octanoate	40.645	1438	1414-1458
1-Hexanol	35.063	1352	1316-1377	(*Z*)-2-Hexenyl acetate	34.05	1337	1319-1327
2-Ethyl-1-hexanol	43.86	1488	1441-1502	4-Hexenyl acetate	32.86	1319	1326
1-Nonanol	54.3	1662	1624-1674	Methyl salicylate	61.47	1787	1715-1813
1-Octanol	48.209	1556	1519-1574	ß-Cyclocitral	52.368	1628	1548-1638
1-Octen-3-ol	41.488	1451	1411-1465	(*Z*)-Linalool oxide	40.88	1442	1409-1480
(*E*)-2-Hexen-1-ol	39.284	1417	1380-1441	(*E*)-Linalool oxide	42.72	1470	1432-1490
2-Ethyl-2-hexen-1-ol	47.79	1549	1518	Citronellol	60.29	1766	1755-1782
1-Butoxy-2-propanol	35.847	1364	1364	Eucalyptol	25.05	1201	1186-1223
1-Methoxy-2-propanol	19.84	1122	1117-1160	Geraniol	64.7	1845	1797-1879
3-Hexen-1-ol	35.829	1364	1344-1399	Geranyl vinyl ether	53.51	1648	1476-1519
(*E*)-3-Nonen-1-ol	55.66	1685	1682-1693	Menthol	53.11	1641	1619-1642
3,7-Dimethyl-3-octanol	40.16	1430	1414-1420	Limonene	24.72	1196	1180-1217
2,4-Dimethyl-3-pentanol	22.174	1156	1157-1187	Linalool	47.651	1547	1507-1564
6-Methyl-5-hepten-2-ol	42.21	1462	1446-1468	Neral	56.834	1706	1641-1706
α,α-Dimethylbenzyl alcohol	60.1	1763	1759-1779	*p*-Cymene	29.734	1272	1246-1291
Benzyl alcohol	66.727	1885	1821-1905	α-Terpineol	56.52	1700	1650-1739
Eugenol	81.142	2162	2100-2198	ß-Myrcene	22.47	1161	1137-1173
Phenylethyl alcohol	68.52	1919	1859-1944	ß-Ocimene	28.013	1246	1211-1251
(*E,E*)-2,4-Heptadienal	42.954	1474	1455-1514	Geranylacetone	65.32	1858	1811-1865
(*E,E*)-2,4-Hexadienal	39.2	1415	1371-1438	Nerol	62.25	1801	1760-1816
(*E,Z*)-2,6-Nonadienal	50.334	1593	1555-1601	Caryophyllene	50.742	1601	1570-1685
2-Hexenal	26.367	1221	1196-1238	Caryophyllene oxide	68.96	1926	1963-2014
(*E*)-2-Nonenal	47.239	1539	1509-1569	(*Z*)-ß-Farnesene	53.565	1649	1643-1684
(*E*)-2-Octenal	40.512	1436	1407-1463	Copaene	44.178	1493	1462-1522
(*E,E*)-3,5-Octadien-2-one	46.311	1523	1516-1569	Humulene	55.016	1674	1637-1689
4-Pentenal	20.435	1128	1123	Ylangene	43.603	1484	1459-1500
6-Methyl-5-hepten-2-one	34.366	1342	1317-1357	α-Farnesene	59.507	1753	1714-1763
Acetophenone	54.29	1661	1609-1669	α-Muurolene	56.07	1693	1684-1750
Benzaldehyde	46.749	1531	1481-1555	ß-Guaiene	57.37	1715	1648-1702
2,5-Dimethyl-benzaldehyde	55.632	1685	1683-1705	γ-Muurolene	54.47	1666	1655-1714
4-Ethyl-benzaldehyde	57.701	1721	1719-1730	(*E*)-ß-Ionone	69.84	1945	1892-1958
Benzeneacetaldehyde	54	1656	1529-1650	5-Ethyl-2(5H)-furanone	59.14	1746	1700-1755
Heptanal	24.01	1185	1163-1208	Dihydroactinidiolide	89.18	2343	2280-2359

*RI (cal) - retention index calculated

**RI (lit) - retention index obtained from the literature

Table S2 - Factorial ANOVA was performed to test the interaction of Term of sampling (including T_0 (before inoculation)), Treatment and OIV class based on the absolute peak area of volatile organic compounds in the leaves of 17 genotypes. The differences between the means (the interaction of Term of sampling, Treatment and OIV class) were evaluated by Duncan's multiple range test at a confidence level of 95% ($p < 0.05$). Different letters show statistical significance. Results are expressed as absolute peak areas.

Term*Treatment*OIV class	Acids								
	2-Hexenoic acid	(*E*)-3-Hexenoic acid	Benzoic acid	Heptanoic acid	2-Ethyl-hexanoic acid	Decanoic acid	Nonanoic acid	Octanoic acid	Pentanoic acid
0*N*1	526836.650 efg	11851324.830 bcdefg	1925100.506 a	1223787.740 abcd	1123059.198 a	2079343.560 cdefghij	7976648.316 abcde	1833447.546 abcd	421587.490 a
0*N*3	2230095.746 defg	2161583.810 g	2501726.954 a	365694.884 bcd	474482.819 a	1093713.696 fghij	3788949.304 cde	746995.217 cd	585689.017 a
0*N*5	6914519.267 cdefg	5210855.920 fg	4921735.847 a	1499271.957 abc	1240800.073 a	1979031.550 cdefghij	9768814.835 abcde	2426085.442 abcd	839825.277 a
0*N*7	4349718.520 cdefg	2067759.870 g	2401231.570 a	333532.300 cd	651563.120 a	873232.870 ij	1707363.590 e	586293.890 cd	1123845.600 a
0*N*9	1885298.580 efg	3229191.900 g	1480419.270 a	349634.340 bcd	673262.970 a	2134536.020 cdefghij	8326845.010 abcde	1234794.690 cd	363636.360 a
1*I*1	1258547.398 efg	10175014.280 bcdefg	2586626.396 a	2036704.199 a	1681280.112 a	2205748.207 bcdefghij	12937276.483 ab	4479427.578 a	584431.876 a
1*I*3	3021145.557 cdefg	2606318.817 g	1949206.168 a	531897.235 bcd	476048.094 a	931915.562 ghij	4193205.389 cde	1012293.554 cd	816347.699 a
1*I*5	5325500.587 cdefg	10850564.440 bcdefg	5912239.673 a	1538725.172 abc	1670630.333 a	3049701.485 abcd	11244260.952 abcd	3372636.628 abc	618174.362 a
1*I*7	51692.810 g	7305791.570 cdefg	2841949.110 a	498865.395 bcd	986962.655 a	2561033.790 abcdefghi	6077189.685 abcde	1160692.085 cd	1001732.170 a
1*I*9	171813.120 fg	8499740.850 cdefg	2247893.370 a	270373.970 cd	423888.920 a	2247729.750 bcdefghij	4301366.790 cde	833336.860 cd	641140.440 a
1*N*1	3538366.510 cdefg	14072675.590 bcdefg	2354879.994 a	1137496.736 abcd	966353.523 a	2863674.295 abcde	11529654.610 abc	2278783.704 abcd	587320.113 a
1*N*3	5875678.427 cdefg	2772175.425 g	2145436.762 a	1022291.349 abcd	1163272.795 a	710883.132 j	2669730.202 de	1156895.138 cd	1017024.424 a
1*N*5	9124242.880 bcdef	6379252.573 efg	4795233.607 a	808982.873 abcd	556684.393 a	2266568.943 bcdefghij	6795553.710 abcde	1187151.980 cd	1002863.915 a
1*N*7	4590925.020 cdefg	2984088.690 g	2113054.020 a	0.000 d	409588.330 a	1033263.330 fghij	1943358.170 e	326715.810 d	997508.320 a
1*N*9	3871375.450 cdefg	5054149.080 fg	1975112.570 a	322164.750 cd	620006.680 a	2567316.980 abcdefghi	7864778.090 abcde	959667.660 cd	523707.755 a
2*I*1	6906952.266 cdefg	18863440.942 abcde	2826996.262 a	1639998.415 abc	1612364.357 a	3074588.806 abcd	14205331.150 a	4164281.915 ab	462222.462 a
2*I*3	4445633.802 cdefg	4187265.936 fg	2455543.568 a	907398.796 abcd	559857.014 a	1200394.051 efghij	6805281.143 abcde	1953845.932 abcd	986049.458 a
2*I*5	8275458.050 bcdefg	19057767.560 abcd	5850652.300 a	1008021.825 abcd	1086149.128 a	3313598.152 abcd	7887950.500 abcde	1501217.103 bcd	772653.042 a
2*I*7	9386625.890 bcde	19185085.340 abcd	3896527.480 a	610243.010 abcd	1431691.160 a	1760263.350 cdefghij	4447077.470 bcde	1337683.390 cd	903969.960 a
2*I*9	1746602.550 efg	4978725.840 fg	2340465.930 a	319728.420 cd	684610.320 a	1753926.610 cdefghij	3374013.070 cde	917064.850 cd	262445.470 a
2*N*1	3397095.640 cdefg	16489280.272 abcdef	2725517.980 a	637718.244 abcd	1020108.990 a	2119947.443 cdefghij	4722691.641 bcde	960691.674 cd	228682.733 a
2*N*3	4789005.304 cdefg	4103120.327 fg	2395382.484 a	554669.981 bcd	525285.393 a	805559.395 ij	2696918.101 de	775892.764 cd	859591.465 a
2*N*5	4797956.727 cdefg	22443923.557 ab	4941779.540 a	1827789.738 ab	1118845.103 a	2675156.473 abcdefg	10364325.418 abcde	2373345.972 abcd	972109.388 a
2*N*7	2590385.380 defg	10266672.220 bcdefg	3592674.030 a	490267.130 bcd	713650.850 a	4109096.120 a	8254183.590 abcde	1452069.930 bcd	935607.175 a
2*N*9	212145.450 fg	7250733.440 cdefg	2526850.320 a	389791.320 bcd	868709.080 a	1715706.190 cdefghij	3555836.300 cde	780458.310 cd	299049.940 a
3*I*1	19369636.680 a	21590496.416 ab	2426122.254 a	647439.858 abcd	786380.950 a	2246939.526 bcdefghij	6710090.944 abcde	1432860.942 bcd	186627.194 a
3*I*3	10997872.394 bcd	6887742.473 defg	2564540.523 a	1010018.649 abcd	832175.113 a	1641471.767 defghij	8394841.114 abcde	2065786.824 abcd	649689.951 a
3*I*5	15864089.893 ab	18596553.197 abcde	5194759.197 a	1000364.625 abcd	786962.762 a	2635891.773 abcdefgh	8007662.528 abcde	1466540.152 bcd	681634.835 a
3*I*7	11515057.290 bc	10786511.340 bcdefg	2128974.210 a	255375.450 cd	737601.990 a	1040712.080 fghij	2696108.140 de	970289.830 cd	743829.650 a
3*I*9	6114299.620 cdefg	19611255.850 abc	2455492.810 a	281917.260 cd	448290.520 a	3873884.525 ab	9660662.410 abcde	1163837.570 cd	145294.205 a
3*N*1	4347400.678 cdefg	19486721.140 abcd	2477441.620 a	588812.682 abcd	586019.776 a	2564027.006 abcdefghi	7214361.926 abcde	1288204.298 cd	265506.367 a
3*N*3	4492141.074 cdefg	3995856.273 fg	2322712.160 a	557974.309 bcd	435349.389 a	879396.756 hij	3261836.496 cde	766474.643 cd	878872.302 a
3*N*5	6778998.600 cdefg	26955304.530 a	4900672.260 a	959697.090 abcd	768135.973 a	2699793.630 abcdef	8592144.740 abcde	1295440.477 cd	867741.163 a
3*N*7	4563255.750 cdefg	14586771.480 bcdefg	3277295.470 a	294171.590 cd	513882.950 a	3413828.280 abc	8673344.700 abcde	1312741.440 cd	1089776.420 a
3*N*9	426749.240 efg	16501017.410 abcdef	2265725.930 a	1241817.220 abcd	1741863.170 a	2428285.430 abcdefghij	8955450.830 abcde	4119359.740 ab	376658.090 a
Pr > F(Model)	<0,0001	<0,0001	0.052	0.000	0.013	<0,0001	<0,0001	<0,0001	0.138
Pr > F(Term*Treatment*OIV)	0.628	0.254	1.000	0.057	0.104	0.008	0.021	0.019	0.999
Significant	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No
Significant	No	No	No	No	No	Yes	Yes	Yes	No

Table S2 continued

Term*Treatment*OIV class	Alcohols									
	1-Heptanol	1-Hexanol	2-Ethyl-1-hexanol	1-Nonanol	1-Octanol	1-Octen-3-ol	(*E*)-2-Hexen-1-ol	2-Ethyl-2-hexen-1-ol	1-Butoxy-2-propanol	1-Methoxy-2-propanol
0*N*1	1449150.912 abcd	10143283.723 ef	44389631.747 bcdef	3104289.780 bcde	5580018.509 bcdefg	5126190.594 bcd	9366286.998 lm	1799919.849 ijkl	3246474.285 defghi	25976029.104 a
0*N*3	1018793.912 bcdefgh	7502088.879 f	42026948.049 cdef	2674998.720 cdefg	3491867.499 efg	5163461.775 bcd	7082629.940 m	1324530.104 kl	3749936.629 cdefg	14206265.911 b
0*N*5	1310442.345 abcde	12023438.598 ef	65475275.157 ab	4785532.710 b	5298651.898 bcdefg	7775532.137 a	14375128.850 ghijklm	1856473.185 ijkl	5923946.907 ab	10437655.542 bc
0*N*7	983570.765 bcdefgh	8857285.470 f	56661305.580 abcd	4087345.680 bcd	8571899.130 a	3403154.720 de	14744592.570 ghijklm	1536576.510 jkl	5885329.230 ab	8667685.620 bcd
0*N*9	601796.585 efgh	6981231.825 f	68814271.925 a	6786926.785 a	5242329.570 bcdefg	4262960.815 cde	11489901.690 jklm	561607.065 l	4079819.610 bcde	7296137.190 cde
1*I*1	1070445.068 bcdefgh	14314544.591 cdef	52813110.154 abcde	3527314.998 bcd	6047865.475 abcde	5170581.345 bcd	11702620.255 jklm	2553815.484 defghijk	3868130.123 cdef	3639876.732 de
1*I*3	707137.468 defgh	8766141.306 f	37455166.893 def	2493493.454 defghi	3531822.568 defg	3995904.790 cde	9191897.767 lm	1949629.448 hijkl	3067912.308 defghij	1952807.350 de
1*I*5	1197129.153 bcdef	15864704.708 cdef	41815796.890 cdef	2536129.668 defgh	5574395.703 bcdefg	4979781.043 bcd	19716452.832 cdefghijk	2636785.313 defghijk	3854676.928 cdef	3178868.162 de
1*I*7	890783.700 bcdefgh	10020774.350 ef	22969893.400 fgh	3042680.850 bcdef	6576657.120 abc	3998552.580 cde	17308520.460 efghijkl	2816530.200 defghijk	814892.470 k	910390.190 e
1*I*9	481994.975 fgh	10089249.390 ef	31045174.380 efg	1316246.580 efghij	5627713.035 bcdef	4651272.770 cde	15423412.415 fghijklm	1801231.585 ijkl	717478.740 k	1684823.580 e
1*N*1	850529.053 cdefgh	13257043.292 def	46825180.575 abcde	4532412.675 bc	6056755.726 abcde	4906501.948 cde	16191424.288 fghijklm	3222106.387 defghi	3590078.156 cdefgh	3674046.116 de
1*N*3	889144.100 bcdefgh	7990431.190 f	42136268.621 cdef	3818907.328 bcd	4271667.374 bcdefg	4288036.239 cde	9682297.037 klm	2454408.012 fghijk	3284097.142 defghi	2241817.917 de
1*N*5	1592912.778 ab	10801594.697 ef	64204758.900 abc	3906652.867 bcd	5832493.897 bcde	6815187.777 ab	14884623.637 ghijklm	2159093.198 ghijk	7022063.620 a	4380837.220 cde
1*N*7	715968.920 defgh	8145219.130 f	52465474.385 abcde	4120200.825 bcd	4835122.690 bcdefg	3622893.040 cde	13228619.025 hijklm	2452160.805 fghijk	4783537.695 bcd	1721018.280 e
1*N*9	489497.720 fgh	7490824.110 f	54848942.280 abcd	3405685.140 bcd	3998350.785 cdefg	5042273.940 bcd	6403004.100 m	1728437.385 ijkl	5470953.840 abc	1894930.290 de
2*I*1	739179.408 cdefgh	21536071.007 cde	15129392.715 gh	1333195.380 efghij	4838663.156 bcdefg	4505490.792 cde	22044223.737 bcdefghi	2306993.293 ghijk	1086639.842 jk	403187.346 e
2*I*3	596359.360 efgh	13291312.134 def	10670654.648 gh	925129.586 ghij	4003084.318 cdefg	4214329.971 cde	16209093.382 fghijklm	2475573.550 efghijk	1024852.901 jk	714927.039 e
2*I*5	1463633.405 abc	25370268.462 abc	7168615.450 h	1021116.107 ghij	5342334.208 bcdefg	5170907.610 bcd	27905627.562 bcd	3009448.180 defghij	2391372.272 efghijk	477575.895 e
2*I*7	1106557.865 bcdefg	17554697.820 cdef	6377404.770 h	139552.380 j	5692670.615 bcde	2964970.650 e	26527224.365 bcde	5139420.765 b	1847711.250 fghijk	475371.270 e
2*I*9	669910.320 efgh	10136109.010 ef	7511257.370 h	903376.005 ghij	4662608.805 bcdefg	4244098.065 cde	19073317.125 defghijkl	4007285.745 bcde	1864283.040 fghijk	339837.735 e
2*N*1	1439024.393 abcd	21786439.908 cde	43803155.456 bcdef	3029797.837 bcdef	5094111.400 bcdefg	5600503.157 bc	18640474.245 defghijkl	2276528.890 ghijk	1625609.396 ghijk	528322.012 e
2*N*3	1032556.939 bcdefgh	11413447.891 ef	14852311.971 gh	1242888.769 efghij	3963622.179 cdefg	3723281.690 cde	14742512.852 ghijklm	2336664.047 ghijk	1199208.096 ijk	439765.184 e
2*N*5	806653.873 cdefgh	16331453.432 cdef	8091433.260 h	678162.715 ghij	5857960.792 bcde	3698891.567 cde	22671023.120 bcdefgh	3167757.530 defghi	1882444.753 fghijk	1001362.440 e
2*N*7	1928663.490 a	14386376.070 cdef	6712230.690 h	383227.020 j	6975608.690 ab	4519459.150 cde	12216666.715 ijklm	4833272.200 bc	2010869.190 efghijk	591185.430 e
2*N*9	325050.000 h	12627427.230 def	11148353.370 gh	449523.360 j	3934642.925 cdefg	4235574.705 cde	18331334.740 defghijkl	2612262.055 defghijk	3163656.870 defghij	410123.340 e
3*I*1	721311.166 cdefgh	34836154.778 a	5944120.229 h	942865.972 ghij	4980149.268 bcdefg	3909311.538 cde	30905094.907 ab	4044836.097 bcd	1761053.964 fghijk	1128504.678 e
3*I*3	634281.967 efgh	21467568.160 cde	5344962.172 h	479789.518 ij	2844332.305 g	3828136.866 cde	25126941.894 bcdef	3708572.021 bcdefg	1540521.299 hijk	437557.231 e
3*I*5	1085040.480 bcdefg	32761328.015 ab	4748688.853 h	676281.567 ghij	5253758.720 bcdefg	4155515.368 cde	37201667.635 a	3028869.380 defghij	1861149.365 fghijk	712929.867 e
3*I*7	1443666.235 abcd	17639305.140 cdef	8218627.240 h	553951.195 hij	5529813.640 bcdefg	3732392.535 cde	26906010.750 bcde	6843018.870 a	1740333.375 fghijk	560522.670 e
3*I*9	582298.200 efgh	17896029.030 cdef	1499530.230 h	261277.830 j	6294365.550 abcd	3958995.150 cde	20898231.815 cdefghij	3461899.715 cdefgh	2144509.495 efghijk	715683.270 e
3*N*1	788243.295 cdefgh	25118721.724 abc	7774590.770 h	505558.637 ij	5390993.492 bcdefg	5041337.399 bcd	23703090.909 bcdefg	3949025.879 bcdef	1916370.738 fghijk	233232.237 e
3*N*3	663777.488 efgh	14120836.235 cdef	5275082.241 h	491269.741 ij	2878588.394 fg	3204864.201 de	20641641.741 cdefghij	3657658.356 cdefg	1463352.860 hijk	549185.631 e
3*N*5	850361.663 cdefgh	23932009.817 bcd	6720065.642 h	524553.213 ij	6172152.168 abcde	3674546.768 cde	29171418.770 abc	3264418.555 defghi	1223705.420 ijk	320976.565 e
3*N*7	998935.780 bcdefgh	17188408.710 cdef	8290257.030 h	1141077.960 fghij	6313186.445 abcd	3351498.300 de	25090438.395 bcdef	5130823.965 b	1675495.800 ghijk	604608.840 e
3*N*9	430546.455 gh	12797899.290 def	5012762.040 h	359403.660 j	4935297.510 bcdefg	5164910.675 bcd	18719472.300 defghijkl	4078784.595 bcd	1887501.330 fghijk	904784.760 e
Pr > F(Model)	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Pr > F(Term*Treatment*OIV)	0.002	0.885	0.003	0.368	0.865	0.004	0.260	0.455	0.001	1.000
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	Yes	No	Yes	No	No	Yes	No	No	Yes	No

Table S2 continued

Term*Treatment*OIV class	Alcohols								
	3-Hexen-1-ol	(*E*)-3-Nonen-1-ol	3,7-Dimethyl-3-octanol	2,4-Dimethyl-3-pentanol	6-Methyl-5-hepten-2-ol	*α,α*-Dimethylbenzyl alcohol	Benzyl alcohol	Eugenol	Phenylethyl alcohol
0*N*1	59473711.641 ghijk	656735.446 bcd	2165314.350 ab	7071503.375 efg	2529351.792 abcd	1346308.321 ab	23809657.462 k	55922.237 b	1067736.189 c
0*N*3	30721558.191 ijk	482434.812 cd	789007.379 cdefg	16150249.078 bcdef	605628.936 d	1097265.015 b	30048223.521 k	43479.779 b	3289696.849 c
0*N*5	35628306.150 hijk	450756.972 cd	1007811.257 cdefg	9634164.892 defg	3824319.213 abc	1896585.743 ab	32481032.717 k	0.000 b	695174.792 c
0*N*7	19440890.370 k	315523.890 d	0.000 g	8553603.240 defg	4912174.680 a	1658166.585 ab	46328419.650 jk	0.000 b	693579.950 c
0*N*9	14126716.170 k	280325.500 d	2111696.890 ab	33235569.040 a	2045518.660 bcd	3177951.870 a	120665014.110 bcdefghi	0.000 b	3154883.315 c
1*I*1	66462808.377 ghijk	784943.389 bcd	702728.616 efg	9660495.955 defg	0.000 d	1822625.224 ab	138234641.337 bcdefg	56697.562 b	967613.645 c
1*I*3	53642035.407 ghijk	789536.036 bcd	622887.701 efg	11115109.413 defg	1279153.023 cd	1245391.432 ab	108902537.426 cdefghi	54153.714 b	2248938.019 c
1*I*5	97600538.095 cdefghij	594684.188 bcd	884024.120 cdefg	11211159.038 defg	1027114.113 cd	1792860.032 ab	150127941.373 bcd	0.000 b	743657.337 c
1*I*7	68913344.210 fghijk	847918.535 bcd	523010.375 efg	7461225.280 efg	1506244.310 cd	1105029.890 b	108614485.950 cdefghi	204814.870 ab	1171422.240 c
1*I*9	57899115.450 ghijk	890777.250 bcd	1798294.740 abcd	21540157.965 bc	1870657.755 bcd	1683512.685 ab	164302380.780 b	0.000 b	8175033.300 bc
1*N*1	79080861.838 defghijk	954345.513 bcd	550483.881 efg	7693672.933 efg	675113.976 d	1753450.860 ab	161490035.768 bc	105794.668 ab	1202349.812 c
1*N*3	42903190.029 ghijk	683106.492 bcd	721292.667 defg	10089802.474 defg	1129438.055 cd	1369418.698 ab	123338752.334 bcdefghi	123713.722 ab	3279377.486 c
1*N*5	51493481.995 ghijk	575764.000 bcd	560018.743 efg	10084638.802 defg	4546191.682 ab	2031755.008 ab	122613708.550 bcdefghi	0.000 b	599247.363 c
1*N*7	25895561.640 jk	328768.130 d	0.000 g	7166587.310 efg	0.000 d	1865054.890 ab	125181856.320 bcdefgh	0.000 b	644517.220 c
1*N*9	30387019.410 ijk	350558.010 d	2299773.660 a	17641596.135 bcd	1867586.475 bcd	2155056.675 ab	245190725.355 a	0.000 b	6038222.820 bc
2*I*1	147682834.077 abcd	1153590.737 abcd	984093.196 cdefg	12674553.440 defg	184816.149 d	1179874.068 b	111045778.762 cdefghi	181189.071 ab	1337499.020 c
2*I*3	73509339.576 efghijk	879245.504 bcd	807176.904 cdefg	12382477.071 defg	420348.859 d	626997.341 b	104620588.787 defghi	236702.472 ab	4930891.422 bc
2*I*5	173117213.858 ab	1229013.273 abcd	1286245.355 bcdef	16559688.643 bcde	0.000 d	1042018.780 b	149684129.610 bcde	45229.483 b	1212272.858 c
2*I*7	161296699.410 abc	1529236.170 abcd	540562.365 efg	7813607.670 efg	0.000 d	1025174.370 b	100148239.950 defghi	297163.580 ab	2175534.760 c
2*I*9	41952234.585 ghijk	792531.850 bcd	1204939.090 bcdef	24389813.300 b	0.000 d	687251.470 b	239995240.500 a	0.000 b	7294787.775 bc
2*N*1	151277767.398 abcd	2315393.553 a	1560213.791 abcde	11159202.483 defg	1586620.312 cd	1306766.136 ab	142198524.472 bcdefg	207198.066 ab	1819395.196 c
2*N*3	84608773.183 defghijk	1063249.775 abcd	975456.084 cdefg	11301528.075 defg	765723.137 d	504913.319 b	91300217.169 ghij	117219.879 ab	5189230.647 bc
2*N*5	110412946.708 bcdefg	859145.823 bcd	428238.912 fg	11400412.677 defg	861407.790 cd	337840.623 b	125881107.263 bcdefgh	0.000 b	670106.603 c
2*N*7	96230163.150 cdefghij	1805406.570 abc	935963.535 cdefg	7085356.530 efg	2358244.560 abcd	708924.990 b	121006094.370 bcdefghi	383099.570 ab	2438421.790 c
2*N*9	65113102.890 ghijk	1187990.100 abcd	1819354.515 abc	14099428.410 cdef	0.000 d	825066.810 b	237952143.510 a	0.000 b	7473161.815 bc
3*I*1	168291621.903 abc	1560434.966 abcd	930898.740 cdefg	11416404.787 defg	0.000 d	2167953.959 ab	128408404.420 bcdefgh	130030.838 ab	1530819.836 c
3*I*3	114093816.321 bcdefg	1072167.581 abcd	706589.962 efg	11568367.327 defg	381490.274 d	1364039.629 ab	96456502.303 efghi	176526.646 ab	4996853.145 bc
3*I*5	204632202.953 a	914500.060 bcd	724588.983 defg	11529136.258 defg	955134.443 cd	1943641.307 ab	126213647.070 bcdefgh	0.000 b	1049157.800 c
3*I*7	107075980.320 bcdefgh	1506188.760 abcd	909323.200 cdefg	3411969.880 g	1733685.200 bcd	1564418.290 ab	80977438.820 hij	331127.490 ab	1785993.160 c
3*I*9	102482547.325 bcdefghi	1170052.340 abcd	1024804.230 cdefg	21804454.875 bc	1811548.740 bcd	219942.760 b	93811137.250 fghij	0.000 b	19703247.105 a
3*N*1	144883439.520 abcde	1578320.428 abcd	787797.988 cdefg	9794454.012 defg	1115213.511 cd	406474.929 b	144712572.122 bcdef	145115.386 ab	1831457.255 c
3*N*3	103289239.596 bcdefghi	975650.900 bcd	601660.525 efg	13556988.832 cdef	1377303.829 cd	1084898.289 b	91113723.139 ghij	95624.636 b	4394014.076 c
3*N*5	167199259.445 abc	934504.857 bcd	586802.257 efg	11874359.920 defg	682633.750 d	167180.250 b	111832227.443 bcdefghi	0.000 b	805502.582 c
3*N*7	140460090.210 abcdef	1904730.300 ab	611997.765 efg	6942807.330 fg	2036182.260 bcd	992726.940 b	121326357.720 bcdefghi	548221.800 a	3044156.535 c
3*N*9	107117934.660 bcdefgh	946987.470 bcd	866026.590 cdefg	8638178.010 defg	0.000 d	747311.970 b	70978508.910 ijk	0.000 b	12303159.085 b
Pr > F(Model)	<0,0001	0.000	<0,0001	<0,0001	<0,0001	0.006	<0,0001	0.591	0.000
Pr > F(Term*Treatment*OIV)	0.485	0.673	0.440	0.871	0.102	0.612	0.330	0.937	0.989
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Significant	No	No	No	No	No	No	No	No	No

Table S2 continued

Term*Treatment*OIV class	Carbonyls								
	(*E*,*E*)-2,4-Heptadienal	(*E*,*E*)-2,4-Hexadienal	(*E*,*Z*)-2,6-Nonadienal	2-Hexenal	(*E*)-2-Nonenal	(*E*)-2-Octenal	(*E*,*E*)-3,5-Octadien-2-one	4-Pentenal	6-Methyl-5-hepten-2-one
0*N*1	10158211.426 abcde	10785526.675 abcdefgh	1858193.393 bcd	559422319.094 ijkl	2356647.878 abcd	5819554.092 cdefgh	3153276.766 abcde	1866165.947 bcdef	13480679.226 k
0*N*3	12242243.127 ab	9360806.309 fghi	2067866.464 abcd	467200660.941 klm	1940900.411 bcdefg	7395537.571 bcd	3229930.321 abcde	3991932.151 a	14273482.699 k
0*N*5	10484347.505 abcd	10704939.205 abcdefgh	1725265.470 bcd	606712361.652 ghijkl	2965666.892 a	6156532.590 cdefg	3517207.265 abc	3288412.300 abc	17982909.137 ijk
0*N*7	6313526.345 cdef	11201666.875 abcdefgh	1485152.060 cd	553464494.680 ijkl	1564856.695 defgh	2205912.425 fgh	1905980.910 efg	2301900.675 abcdef	21782707.160 ghijk
0*N*9	4616705.475 f	6789166.585 i	1508692.630 cd	269919690.645 m	2447554.730 abc	2594096.010 defgh	3140874.990 abcde	2138445.540 abcdef	17073771.660 jk
1*I*1	14974351.425 a	10523113.295 bcdefgh	2106942.468 abcd	688004336.793 defghijk	2356358.434 abcd	3893194.238 defgh	3879265.618 a	3421002.927 ab	18743120.561 hijk
1*I*3	12423528.828 ab	9976369.554 cdefghi	2483756.832 abcd	547935574.096 ijkl	1641188.247 defgh	4765092.397 defgh	3607033.660 ab	1964906.978 bcdef	15569166.266 jk
1*I*5	10002849.863 bcde	13008504.645 abcdef	1823606.358 bcd	845812451.967 abcdef	1960173.067 bcdefg	5878433.382 cdefgh	3011362.520 abcdef	3145138.910 abc	21270194.640 hijk
1*I*7	8693582.070 bcdef	12941306.610 abcdef	1888488.465 bcd	682329991.575 efghijk	1472474.745 efgh	2141806.755 fgh	2857700.415 abcdef	2292441.445 abcdef	34172422.165 fghi
1*I*9	6089403.180 def	9413553.555 efghi	1162794.150 cd	549867991.760 ijkl	2108057.600 bcdef	3434481.255 defgh	2353435.875 bcdefg	1279634.185 cdef	29611851.725 ghijk
1*N*1	11335857.326 ab	13342189.137 abcd	2280807.110 abcd	817360307.608 abcdefgh	2279214.637 abcd	2277074.011 efgh	2863906.468 abcdef	2061482.946 abcdef	21421039.807 hijk
1*N*3	12079661.291 ab	10234798.430 bcdefghi	2268208.367 abcd	560455938.233 ijkl	1649099.012 defgh	4895622.743 defgh	3200886.658 abcde	2143362.932 abcdef	16846787.123 jk
1*N*5	9868781.217 bcde	12293214.457 abcdefgh	1833138.753 bcd	680046147.380 efghijk	2625163.473 ab	9741906.327 abc	2512216.975 bcdefg	1985132.555 bcdef	21844038.950 ghijk
1*N*7	8650059.365 bcdef	10108472.655 bcdefghi	1275388.015 cd	489157989.650 jkl	1576944.655 defgh	1000557.360 h	2864953.080 abcdef	1813728.510 bcdef	23585075.910 ghijk
1*N*9	8765663.830 bcdef	8898303.015 hi	1034268.760 d	440358688.130 lm	1439348.980 efgh	2811684.470 defgh	3634855.865 ab	415152.875 f	21958540.575 ghijk
2*I*1	11467276.294 ab	12583429.659 abcdefgh	2684432.220 abcd	864260396.438 abcde	1720608.687 cdefgh	2087456.713 fgh	2743474.114 abcdef	2909884.165 abc	29694189.934 ghijk
2*I*3	10833268.675 abcd	10424314.829 bcdefgh	2746314.885 abcd	592052371.786 hijkl	1242372.698 gh	5009448.324 defgh	3417880.185 abcd	2194860.624 abcdef	21980288.319 ghijk
2*I*5	11272147.512 ab	13130889.783 abcde	2858562.625 abc	946504761.157 abc	2200750.035 bcde	6599220.448 cdefg	2629013.640 abcdefg	2564316.947 abcde	29629007.572 ghijk
2*I*7	9320616.095 bcdef	11836642.755 abcdefgh	1796938.745 bcd	624703179.610 fghijkl	1144951.195 gh	1693652.340 gh	2379018.295 bcdefg	1945022.370 bcdef	109351460.175 b
2*I*9	5495061.625 ef	10701312.560 abcdefgh	1328180.725 cd	643432702.835 efghijkl	1616757.590 defgh	11963893.590 a	2940902.910 abcdef	692151.570 def	61740605.520 e
2*N*1	10455664.713 abcd	13438292.965 abc	2279035.887 abcd	913676432.527 abcd	1614132.690 defgh	4287894.381 defgh	2813231.921 abcdef	3493590.433 ab	27565317.623 ghijk
2*N*3	11156853.958 abc	11491609.744 abcdefgh	2817418.649 abcd	733150419.225 bcdefghi	900616.759 h	7003606.681 bcdef	2933127.211 abcdef	3298746.733 abc	22422179.162 ghijk
2*N*5	11479256.772 ab	13813722.947 ab	1929320.795 bcd	945355198.457 abc	1290272.248 gh	5300827.095 cdefgh	2415100.955 bcdefg	3163727.097 abc	26531180.060 ghijk
2*N*7	8257898.915 bcdef	13055746.060 abcdef	1973396.495 abcd	720250492.390 cdefghij	1303554.910 fgh	939744.175 h	2159831.170 cdefg	2028087.420 abcdef	60984282.320 e
2*N*9	8457335.250 bcdef	10680974.920 bcdefgh	1042125.875 d	653805491.200 efghijkl	1030974.875 h	3270704.320 defgh	2953521.515 abcdef	1614037.745 bcdef	47489596.810 f
3*I*1	10247731.767 abcde	11507505.413 abcdefgh	3347096.538 ab	834366231.993 abcdefg	1434567.624 efgh	3723596.398 defgh	2673896.571 abcdef	2546563.664 abcde	38311357.871 fg
3*I*3	10153182.681 abcde	9978335.000 cdefghi	2640703.026 abcd	697458243.596 defghijk	1141625.020 gh	5342853.154 cdefgh	3284759.968 abcd	1878160.166 bcdef	31867826.360 ghij
3*I*5	10736052.735 abcd	11905761.788 abcdefgh	3711191.307 a	938992786.557 abc	1625419.717 defgh	1787701.745 gh	2722916.350 abcdef	2646518.530 abcde	32229813.878 ghij
3*I*7	9334344.745 bcdef	9642195.755 defghi	2296823.175 abcd	429294192.750 lm	1199007.835 gh	6009522.750 cdefg	2127035.790 defg	1868866.560 bcdef	216601327.800 a
3*I*9	4671596.775 f	9184876.905 ghi	1339054.970 cd	552930469.100 ijkl	1187130.770 gh	11113497.630 ab	1354024.180 g	659977.470 ef	76799361.375 d
3*N*1	10569585.065 abcd	14385673.432 a	2399929.936 abcd	1040740869.944 a	1879497.626 bcdefg	2394241.173 efgh	3090113.028 abcdef	2659259.645 abcde	35347863.914 fgh
3*N*3	10836752.701 abcd	11256826.413 abcdefgh	2543605.033 abcd	727204473.776 cdefghi	1251016.368 gh	4618161.419 defgh	3250290.133 abcde	2424970.534 abcde	27968240.510 ghijk
3*N*5	11681208.792 ab	12932402.718 abcdef	1885493.823 bcd	956279233.715 ab	1264627.422 gh	4793289.270 defgh	2207285.850 cdefg	2781001.080 abc	32127536.100 ghij
3*N*7	9502722.320 bcde	12893238.470 abcdefg	2199082.760 abcd	710490520.405 defghij	1346851.570 fgh	1850458.430 gh	2364165.480 bcdefg	2681478.305 abcd	91332312.655 c
3*N*9	7658609.420 bcdef	8890714.875 hi	1599366.965 bcd	467358119.280 klm	1584879.775 defgh	7167552.455 bcde	1761336.615 fg	1919734.465 bcdef	76392732.920 d
Pr > F(Model)	<0,0001	<0,0001	0.002	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Pr > F(Term*Treatment*OIV)	0.959	0.521	0.608	0.118	0.021	0.017	0.348	0.925	<0,0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	No	No	No	No	Yes	Yes	No	No	Yes

Table S2 continued

Term*Treatment*OIV class	Carbonyls								
	Acetophenone	Benzaldehyde	2,5-Dimethyl-benzaldehyde	4-Ethyl-benzaldehyde	Benzeneacetaldehyde	Heptanal	Hexanal	Nonanal	Octanal
0*N*1	27394591.735 bcd	31901209.415 bcdef	9834730.245 i	861994.465 a	643142.381 a	7106890.845 b	16179561.366 bcd	112872247.156 bcde	15512172.427 defghijkl
0*N*3	20021078.119 cde	28899590.752 cdefghi	13852520.807 fghi	1776608.576 a	723796.074 a	7146980.029 b	19037817.467 abc	82447944.531 efghijkl	17670677.904 cdefg
0*N*5	37745165.280 ab	45896961.637 a	18335310.465 cdefghi	2310050.373 a	1994815.468 a	22975398.403 a	19494113.405 abc	116608183.850 bcd	21140921.248 bcd
0*N*7	23076181.290 cd	30886665.625 bcdefgh	14620753.875 efghi	846158.340 a	0.000 a	6998629.880 b	21498625.760 ab	172349371.285 a	28126753.800 a
0*N*9	28314174.715 bcd	33321051.530 bcde	23563716.365 bcd	412959.825 a	0.000 a	3468820.740 b	20620598.340 ab	142912905.260 b	19636259.410 bcde
1*I*1	38056103.878 ab	42330932.519 ab	14761232.732 efghi	630942.361 a	736158.093 a	6669970.193 b	13962631.988 bcd	113147964.784 bcde	19188876.502 cdef
1*I*3	24757213.923 bcd	31126681.761 bcdefg	14423306.815 fghi	966683.182 a	672073.775 a	4612148.618 b	13819095.278 bcd	68115834.512 ghijklmn	16115893.446 cdefghijk
1*I*5	34552399.387 abc	39132932.688 abc	15806005.900 defghi	1936744.738 a	1906212.987 a	21672186.193 a	18381221.892 abc	102501291.905 cdef	17234295.937 cdefgh
1*I*7	14098986.405 def	23595462.415 defghij	14099080.800 fghi	1346503.650 a	0.000 a	4750901.125 b	16978694.925 bc	117623089.200 bc	25118625.750 ab
1*I*9	23349588.385 cd	35357870.260 abcd	24153945.390 bc	640953.290 a	263783.985 a	2529062.360 b	17764934.010 abc	103397504.710 cdef	21578141.855 bc
1*N*1	29573230.438 bc	32906188.740 bcde	17075734.448 cdefghi	890719.441 a	1363382.773 a	4703321.676 b	14100202.939 bcd	98830098.907 cdefgh	16620848.641 cdefghi
1*N*3	22674433.727 cd	29099519.925 cdefghi	15429248.715 defghi	1113733.731 a	790698.222 a	4355472.390 b	14259212.551 bcd	84281137.793 defghijkl	13414022.130 fghijkl
1*N*5	46092833.057 a	47143158.373 a	16578921.503 cdefghi	2380131.662 a	1764296.972 a	17941855.530 a	17475862.360 abc	112879114.833 bcde	21834336.410 bc
1*N*7	24161397.940 bcd	30764301.520 bcdefgh	12753235.390 ghi	663430.180 a	0.000 a	3997138.875 b	16858813.950 bc	110373311.060 cde	19806450.470 bcde
1*N*9	29945545.870 bc	38043309.590 abc	22790476.990 bcde	401681.030 a	377926.770 a	2045721.875 b	10665054.010 cd	89075903.165 cdefghij	14279172.670 efghijkl
2*I*1	4181762.732 f	20342838.471 efghij	15703507.262 defghi	913352.892 a	923948.247 a	5153475.170 b	17454925.653 abc	65594137.553 ijklmn	12518134.809 ghijkl
2*I*3	5221840.523 f	20480749.334 efghij	14349804.172 fghi	1224649.090 a	1106211.561 a	7142177.059 b	14933873.025 bcd	57102763.495 jklmn	14124126.238 efghijkl
2*I*5	4750096.977 f	24677588.215 defghij	18568403.288 cdefgh	2041350.935 a	1664789.923 a	21672818.507 a	22796775.262 ab	80747622.982 efghijkl	12746844.872 ghijkl
2*I*7	2526192.565 f	16305876.920 ij	14355116.735 fghi	1465444.585 a	1057220.200 a	3964060.595 b	17930783.385 abc	77414771.845 fghijklm	11361564.255 hijkl
2*I*9	5814211.855 f	31872140.490 bcdef	31766742.130 a	183118.805 a	492196.650 a	2175988.385 b	17464162.500 abc	86934043.100 cdefghijk	16361058.680 cdefghij
2*N*1	5541796.200 f	25048994.626 defghij	16105615.939 cdefghi	852988.679 a	1957478.907 a	5702516.065 b	14196730.498 bcd	81914609.230 efghijkl	12860920.943 ghijkl
2*N*3	3313784.258 f	18318636.939 ghij	12084730.946 hi	1287198.804 a	414543.488 a	3611151.372 b	14868818.271 bcd	56683106.029 jklmn	10039843.838 kl
2*N*5	4381340.998 f	23297043.812 defghij	13982058.752 fghi	2153451.967 a	1437239.690 a	23636715.867 a	21264831.957 ab	84893379.538 defghijkl	13256226.195 ghijkl
2*N*7	3905832.700 f	19889333.600 fghij	11836237.420 hi	1572508.175 a	0.000 a	3550139.955 b	15530061.600 bcd	100440642.075 cdefg	12119134.650 ghijkl
2*N*9	8878669.570 ef	41348135.425 abc	27243824.360 ab	202841.050 a	650431.945 a	1462692.495 b	7729004.425 d	52587279.485 lmn	12534725.850 ghijkl
3*I*1	3801396.107 f	19089128.929 fghij	13254751.608 ghi	1037777.796 a	1046524.644 a	7840108.005 b	22785110.131 ab	54506778.454 klmn	10347996.915 jkl
3*I*3	3332611.150 f	18962195.004 fghij	14189382.876 fghi	1097184.510 a	1645586.720 a	6098380.301 b	17189087.707 abc	41108404.732 n	9836501.935 l
3*I*5	3887757.193 f	23136412.452 defghij	17595675.160 cdefghi	2000472.043 a	1323508.205 a	22125374.547 a	18072971.597 abc	90679069.970 cdefghi	12574252.690 ghijkl
3*I*7	1743639.990 f	13722807.170 j	1260961.510 j	1175617.375 a	1310723.450 a	6060801.710 b	19397784.270 abc	71800812.130 fghijklmn	17188426.440 cdefgh
3*I*9	5346271.365 f	23905419.435 defghij	27635066.895 ab	100386.435 a	306608.940 a	2700920.970 b	26194454.550 a	47024522.820 mn	10689121.080 ijkl
3*N*1	4616249.367 f	24655611.853 defghij	20658437.158 bcdefg	586399.324 a	1371148.021 a	6340046.349 b	18256635.834 abc	66326168.923 hijklmn	13273219.055 ghijkl
3*N*3	3391760.663 f	18840487.274 ghij	15394642.886 defghi	1236637.200 a	2048169.506 a	4803906.294 b	17091957.141 bc	45221206.184 n	10480592.966 jkl
3*N*5	3932613.788 f	20822260.525 efghij	13143841.213 ghi	2208275.803 a	1493821.692 a	21034108.297 a	20839930.277 ab	88971340.697 cdefghij	12214904.313 ghijkl
3*N*7	3136485.765 f	21822748.945 efghij	15819926.880 defghi	1559836.150 a	1630792.595 a	3643896.195 b	15845612.260 bcd	91575816.420 cdefghi	12741736.405 ghijkl
3*N*9	4643383.415 f	17894468.050 hij	22140234.540 bcdef	173753.015 a	282665.450 a	1321813.855 b	22379223.640 ab	69072248.350 ghijklmn	10690980.195 ijkl
Pr > F(Model)	<0,0001	<0,0001	<0,0001	0.776	0.052	<0,0001	0.000	<0,0001	<0,0001
Pr > F(Term*Treatment*OIV)	0.319	0.022	0.183	0.999	0.870	0.982	0.882	0.032	0.060
Significant	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes
Significant	No	Yes	No	No	No	No	No	Yes	No

Table S2 continued

Term*Treatment*OIV class	Esters									
	Hexyl acetate	Phenylmethyl acetate	Ethyl benzoate	(*E*)-3-Hexenyl butanoate	(*E*)-2-Hexenyl benzoate	Phenylmethyl formate	Ethyl octanoate	(*Z*)-2-Hexenyl acetate	4-Hexenyl acetate	Methyl salicylate
0*N*1	2322750.258 abcde	575406.644 l	1624185.949 a	15245515.159 a	1173184.253 a	293062.199 fgh	3732107.254 abcdef	811013.478 abcde	55749691.711 ab	266240.160 ghi
0*N*3	1283601.648 de	886993.771 kl	994563.879 ab	3549169.981 b	531718.635 abcd	279416.519 fgh	2191923.739 cdef	516778.374 cdef	18236961.056 de	286322.005 ghi
0*N*5	3586815.770 ab	905630.732 kl	110329.098 b	6871471.870 b	787030.822 abc	436128.795 defgh	8108514.023 abc	1445794.050 a	39203985.833 abcde	132176.493 ghi
0*N*7	2414967.840 abcde	1311377.780 jkl	0.000 b	2325033.400 b	386599.380 cd	299693.010 fgh	5416753.020 abcdef	0.000 f	33073478.670 abcde	0.000 i
0*N*9	1372915.220 de	2762506.550 defghi	272390.940 ab	0.000 b	532585.725 abcd	502402.670 cdefgh	7471833.180 abcd	1258369.545 ab	9906508.240 e	457562.000 ghi
1*I*1	1515740.561 de	2537831.313 defghi	222116.471 ab	2480732.923 b	728918.164 abc	441911.408 cdefgh	7676393.907 abcd	938710.473 abcde	27534838.266 bcde	491410.829 ghi
1*I*3	1040478.175 e	2065887.715 fghij	730958.015 ab	1482447.539 b	904625.260 abc	359221.898 efgh	5184981.933 abcdef	420491.617 cdef	18520943.450 de	286543.685 ghi
1*I*5	2422204.213 abcde	2723281.600 defghi	0.000 b	2598943.202 b	668393.968 abcd	375255.725 efgh	5523200.868 abcdef	989375.518 abcd	55200771.472 ab	322363.248 ghi
1*I*7	2091743.725 abcde	1785003.135 ijk	361657.625 ab	0.000 b	617372.910 abcd	566177.040 bcdefgh	7174099.350 abcde	554661.360 bcdef	30123299.550 abcde	1391812.880 e
1*I*9	2381312.360 abcde	4444340.685 bc	428386.350 ab	0.000 b	743999.490 abc	312680.625 efgh	7620198.435 abcd	0.000 f	28714724.325 bcde	2922344.355 c
1*N*1	1553369.258 de	3050594.115 defgh	71159.348 b	3434903.958 b	629454.798 abcd	458761.297 cdefgh	8244148.481 ab	336707.421 def	31441925.174 abcde	722302.399 fgh
1*N*3	1242538.493 de	2231659.508 efghij	655699.918 ab	2034895.207 b	899111.654 abc	366505.492 efgh	4695570.537 abcdef	599510.595 bcdef	18136307.359 de	250818.917 ghi
1*N*5	2199172.527 abcde	2354772.357 efghij	0.000 b	961752.030 b	952242.175 abc	397467.433 efgh	3082727.020 abcdef	919629.940 abcde	30244348.557 abcde	82636.575 hi
1*N*7	1711373.290 bcde	1756054.680 ijk	0.000 b	1614469.850 b	430382.330 bcd	291996.215 fgh	6245585.290 abcde	766348.680 abcde	15710918.650 de	465363.250 ghi
1*N*9	2231460.370 abcde	4609441.460 b	577184.390 ab	0.000 b	615622.800 abcd	408841.035 efgh	7860858.750 abc	0.000 f	27713969.445 bcde	1378208.615 e
2*I*1	2791946.658 abcde	2598188.152 defghi	27814.627 b	2817482.299 b	985873.290 abc	587826.420 bcdefgh	7644230.523 abcd	630254.707 bcdef	46749889.540 abcd	581511.501 ghi
2*I*3	1575793.620 cde	1944244.878 hijk	497388.742 ab	846445.580 b	936764.106 abc	367772.920 efgh	4558325.810 abcdef	427714.237 cdef	20449150.293 cde	420261.256 ghi
2*I*5	2719629.190 abcde	3351650.858 de	140737.827 b	3608126.320 b	1115161.473 ab	564730.753 bcdefgh	6166179.207 abcde	692077.867 bcdef	45581607.763 abcd	363180.335 ghi
2*I*7	2662422.495 abcde	1924538.310 hijk	72240.885 b	2441132.395 b	509679.720 abcd	874150.005 abcd	5806111.125 abcdef	749961.350 abcdef	63022081.695 a	1824984.210 de
2*I*9	1739717.930 bcde	9037124.480 a	474391.950 ab	495991.810 b	1166354.770 a	723134.720 abcdef	2629738.125 bcdef	789956.130 abcde	12580240.840 de	1571903.095 de
2*N*1	3758840.918 a	3152445.852 def	0.000 b	2662860.356 b	752943.808 abc	489540.513 cdefgh	7765094.725 abcd	1139731.828 abc	42054565.604 abcde	741864.228 fg
2*N*3	1585661.618 cde	1719467.481 ijk	480503.306 ab	1197564.230 b	908780.056 abc	345528.189 efgh	1856655.544 def	361496.335 def	27295446.276 bcde	471573.736 ghi
2*N*5	3546857.037 ab	2232963.518 efghij	24576.500 b	1631486.960 b	711435.587 abc	235446.892 gh	3730035.357 abcdef	406294.450 cdef	55309864.360 ab	322192.105 ghi
2*N*7	2425121.220 abcde	2422416.410 efghij	68458.485 b	0.000 b	772138.485 abc	763855.275 abcde	7037566.770 abcde	476691.565 cdef	53329770.240 abc	2123522.100 d
2*N*9	3470466.260 abc	8426300.700 a	311751.515 ab	0.000 b	794332.905 abc	979371.495 ab	7397785.905 abcd	0.000 f	31406670.090 abcde	3506271.825 b
3*I*1	2748629.684 abcde	2619934.331 defghi	454049.338 ab	1629619.087 b	646545.087 abcd	630188.431 abcdefg	5128922.607 abcdef	261412.158 def	26817925.514 bcde	332453.270 ghi
3*I*3	3051358.856 abcd	1976447.270 ghijk	621239.905 ab	809546.279 b	844008.501 abc	477107.564 cdefgh	3591598.814 abcdef	573454.239 bcdef	25441531.311 bcde	466155.694 ghi
3*I*5	2788416.747 abcde	3088455.763 defg	884391.157 ab	1035678.763 b	909311.933 abc	379630.020 efgh	7192956.618 abcde	388519.560 def	41956869.943 abcde	263128.032 ghi
3*I*7	2486989.660 abcde	1315446.220 jkl	231604.110 ab	0.000 b	0.000 d	1037118.880 a	0.000 f	298098.075 def	28169815.060 bcde	1261611.505 ef
3*I*9	2149943.400 abcde	3556999.710 cd	572881.320 ab	0.000 b	853420.455 abc	889948.895 abc	1867766.240 def	0.000 f	20272400.640 cde	4842266.590 a
3*N*1	2941565.625 abcde	2795072.014 defghi	0.000 b	1514338.939 b	685595.579 abc	282991.159 fgh	9034008.567 a	293321.877 def	26764299.196 bcde	222582.361 ghi
3*N*3	1959613.696 abcde	2115136.797 fghij	648960.361 ab	1062656.374 b	838061.619 abc	373406.309 efgh	3154625.275 abcdef	214962.684 ef	23635770.741 bcde	298256.880 ghi
3*N*5	3732163.110 a	2233605.910 efghij	0.000 b	1240243.303 b	663981.535 abcd	147534.648 h	4941545.770 abcdef	514177.095 cdef	46115649.590 abcd	227389.773 ghi
3*N*7	2580024.290 abcde	2039692.690 fghij	81446.695 b	0.000 b	1095140.460 ab	876263.520 abcd	6236250.120 abcde	582822.245 bcdef	43433876.940 abcde	2087968.525 d
3*N*9	1582200.575 cde	2736666.870 defghi	300343.245 ab	272232.330 b	540466.890 abcd	265733.055 fgh	1351871.730 ef	0.000 f	22014032.550 bcde	2949249.570 c
Pr > F(Model)	<0,0001	<0,0001	0.023	0.001	0.022	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Pr > F(Term*Treatment*OIV)	0.394	0.761	0.978	1.000	0.248	0.374	0.573	0.001	0.462	<0,0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	No	No	No	No	No	No	No	Yes	No	Yes

Table S2 continued

Term*Treatment*OIV class	Monoterpenes							
	ß-Cyclocitral	(*Z*)-Linalool oxide	(*E*)-Linalool oxide	Citronellol	Eucalyptol	Geraniol	Geranyl vinyl ether	Menthol
0*N*1	7211366.311 abcdef	6044394.387 a	292675.777 bcdefgh	229502.437 efgh	13233642.968 cd	9425496.866 ab	728600.668 cdef	747488.880 ab
0*N*3	7764000.234 abcde	799028.691 b	51624.741 hi	130744.634 fgh	11561572.731 cd	9743182.950 ab	658250.763 cdefgh	389840.521 ab
0*N*5	8630858.327 abc	1644964.092 b	175616.045 fghi	0.000 h	30062636.533 bc	5887729.350 ab	1155062.597 ab	781709.363 ab
0*N*7	4718771.475 ef	624763.275 b	0.000 i	0.000 h	54612836.655 a	4086643.560 ab	816363.810 bcd	607802.850 ab
0*N*9	4908899.310 def	914156.705 b	0.000 i	0.000 h	46722798.970 ab	1934582.280 b	909741.340 bc	1158326.580 ab
1*I*1	7373439.198 abcdef	1487677.801 b	300830.758 bcdefgh	9440.066 h	9278267.513 cd	8102580.873 ab	1021102.122 abc	826062.638 ab
1*I*3	8147582.177 abcde	837363.582 b	117751.112 ghi	206468.774 efgh	6228557.274 cd	13745582.073 ab	766090.889 bcde	640654.343 ab
1*I*5	8321546.772 abcd	1363059.822 b	260913.848 cdefghi	0.000 h	5854844.580 cd	10022969.583 ab	1070084.227 abc	706221.963 ab
1*I*7	9107952.480 ab	955734.120 b	282068.820 bcdefgh	743672.160 defgh	0.000 d	7652045.830 ab	429857.010 defghi	512428.550 ab
1*I*9	6713201.010 abcdef	1531106.445 b	187611.390 efghi	0.000 h	57250156.650 a	3076255.755 b	660998.540 cdefgh	549935.910 ab
1*N*1	7692761.505 abcde	1247916.327 b	396042.554 bcdefg	280592.992 defgh	9558844.038 cd	18861801.517 ab	762856.838 bcde	904424.902 ab
1*N*3	8607493.704 abc	1049409.553 b	130461.707 ghi	726537.690 defgh	0.000 d	14671083.726 ab	680367.247 cdefg	619530.833 ab
1*N*5	7931896.992 abcde	1351148.397 b	346408.308 bcdefg	0.000 h	0.000 d	5736568.790 ab	1363137.410 a	847629.370 ab
1*N*7	5280635.955 cdef	733200.475 b	349460.440 bcdefg	88337.630 gh	36592872.670 ab	4125180.370 ab	807672.760 bcde	600555.090 ab
1*N*9	4849844.580 def	1747791.480 b	0.000 i	0.000 h	0.000 d	2578006.905 b	996999.280 abc	842504.630 ab
2*I*1	7893859.764 abcde	1247410.173 b	388646.073 bcdefg	731086.314 defgh	0.000 d	16529390.112 ab	200648.418 i	955945.810 ab
2*I*3	8093952.043 abcde	871677.340 b	131686.756 ghi	704367.654 defgh	7294629.188 cd	14370797.066 ab	226683.390 i	585861.033 ab
2*I*5	8839163.502 ab	1336370.972 b	457715.320 abcde	1174158.638 cdef	6375926.317 cd	23692332.240 ab	267211.323 ghi	760971.033 ab
2*I*7	8491453.035 abc	1155169.110 b	444270.300 abcdef	1792113.990 abc	0.000 d	16019295.990 ab	165808.670 i	262727.260 b
2*I*9	5208724.220 cdef	2783189.885 b	496638.485 abc	0.000 h	0.000 d	3585688.870 b	292446.510 ghi	275607.790 b
2*N*1	8680409.749 abc	1679167.487 b	680101.274 a	1141224.155 cdefg	8082879.106 cd	28408848.961 a	384188.446 efghi	1365177.408 a
2*N*3	9702176.041 a	953185.893 b	258475.062 cdefghi	739093.209 defgh	1870236.046 d	14212339.653 ab	176813.962 i	680756.894 ab
2*N*5	8956399.875 ab	1049761.448 b	222196.808 cdefghi	196991.850 fgh	0.000 d	7411205.943 ab	222545.420 i	420162.357 ab
2*N*7	7626263.610 abcde	1049153.670 b	495478.065 abc	2484535.875 a	9275571.120 cd	21382399.095 ab	192599.930 i	397734.970 ab
2*N*9	7309586.100 abcdef	2498376.735 b	478476.480 abcd	0.000 h	55553159.880 a	3327403.245 b	323799.940 fghi	278070.170 b
3*I*1	7279064.902 abcdef	1085702.358 b	338472.899 bcdefg	1325052.623 bcd	2245857.816 d	21931020.542 ab	51949.552 i	1229204.744 ab
3*I*3	7365124.920 abcdef	901469.346 b	204423.494 defghi	798839.443 defgh	9968038.594 cd	22667928.507 ab	116614.167 i	814107.470 ab
3*I*5	7612850.095 abcde	1001020.062 b	297364.638 bcdefgh	1270004.237 bcde	1557346.943 d	16807862.480 ab	246655.803 hi	1133727.020 ab
3*I*7	5718285.685 bcdef	1504151.845 b	548276.610 ab	2229086.160 ab	0.000 d	18679719.720 ab	119566.830 i	283658.500 b
3*I*9	3951380.815 f	2166193.605 b	245887.385 cdefghi	0.000 h	10493707.350 cd	3968571.895 ab	157133.110 i	541336.300 ab
3*N*1	7568427.261 abcde	1166089.583 b	322203.356 bcdefgh	574854.193 defgh	14854984.347 cd	23255819.406 ab	128823.884 i	389492.562 ab
3*N*3	7800138.846 abcde	903742.822 b	249564.860 cdefghi	620237.575 defgh	6752229.611 cd	15040533.197 ab	159449.277 i	687281.193 ab
3*N*5	8508052.770 abc	1149304.123 b	199760.120 defghi	0.000 h	0.000 d	8189388.050 ab	217207.907 i	481136.730 ab
3*N*7	5922067.020 bcdef	1183127.205 b	438732.750 abcdef	2051109.525 abc	0.000 d	16425356.190 ab	241320.310 hi	354214.070 b
3*N*9	6540897.780 abcdef	1789325.115 b	234082.590 cdefghi	541274.910 defgh	0.000 d	4536972.000 ab	437731.980 defghi	663348.810 ab
Pr > F(Model)	0.005	0.007	<0,0001	<0,0001	<0,0001	0.022	<0,0001	0.029
Pr > F(Term*Treatment*OIV)	0.743	1.000	0.052	0.134	<0,0001	0.957	0.082	0.350
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	No	No	No	No	Yes	No	No	No

Table S2 continued

Term*Treatment*OIV class	Monoterpenes								
	Limonene	Linalool	Neral	*p*-Cymene	*α*-Terpineol	ß-Myrcene	ß-Ocimene	Geranylacetone	Nerol
0*N*1	48938933.092 abcd	2757862.974 ab	591152.192 abcd	1707053.924 cd	402200.382 efghij	1200587.606 a	3520634.770 b	1277105.700 i	799331.384 a
0*N*3	15331142.057 defg	1655568.481 b	385310.094 bcd	2307238.517 bcd	90505.523 ij	2265518.509 a	3707030.701 ab	1340257.929 i	131398.814 b
0*N*5	30064629.937 bcdefg	1493503.497 b	151158.283 cd	1669885.183 cd	238864.663 ghij	568736.050 a	4187679.817 ab	1454997.833 i	109955.010 b
0*N*7	40939831.870 abcdef	610091.510 b	0.000 d	2223321.080 cd	0.000 j	0.000 a	4220352.670 ab	1659875.000 hi	0.000 b
0*N*9	0.000 g	832281.410 b	0.000 d	2760185.570 bc	263494.860 ghij	0.000 a	2566765.520 b	1564599.000 hi	146884.770 b
1*I*1	46257063.730 abcde	2088199.240 b	154025.606 cd	2467463.128 bcd	181109.160 ghij	0.000 a	4012130.093 ab	1452856.800 i	61093.000 b
1*I*3	16157300.192 defg	2581566.371 b	318916.512 bcd	2879632.092 bc	170705.383 ghij	4658819.075 a	6748516.495 ab	1192890.308 i	135750.828 b
1*I*5	19516200.037 defg	1693089.597 b	352095.427 bcd	2264845.547 bcd	205234.020 ghij	0.000 a	3900079.630 ab	1872866.167 hi	169925.103 b
1*I*7	27101331.010 bcdefg	2453432.410 b	349140.840 bcd	2020804.970 cd	225211.820 ghij	0.000 a	2664020.835 b	2114446.000 ghi	242714.340 ab
1*I*9	4038379.960 fg	1408226.840 b	0.000 d	2834311.490 bc	288847.880 ghij	0.000 a	5915233.670 ab	2853530.500 efg	298270.155 ab
1*N*1	50893324.906 abcd	3769398.780 ab	807495.606 abcd	2514701.030 bc	359904.208 fghij	438863.786 a	5648346.259 ab	1680447.200 hi	270710.866 ab
1*N*3	16499609.132 defg	1917803.550 b	337672.900 bcd	2539253.933 bc	143472.183 ghij	470458.150 a	5258070.059 ab	1328043.833 i	207335.087 ab
1*N*5	16223372.113 defg	1618313.237 b	0.000 d	1887845.877 cd	283502.287 ghij	626760.887 a	2639777.747 b	1374201.333 i	0.000 b
1*N*7	42303028.870 abcdef	1629354.220 b	0.000 d	2037467.950 cd	0.000 j	0.000 a	4464865.590 ab	1414853.500 i	258921.165 ab
1*N*9	0.000 g	895287.230 b	0.000 d	2871817.840 bc	0.000 j	0.000 a	3247853.970 b	2056285.000 ghi	0.000 b
2*I*1	60254021.874 abc	3328218.458 ab	808112.918 abcd	1776278.516 cd	565132.370 cdefg	1902627.472 a	5188255.020 ab	1833826.100 hi	269779.834 ab
2*I*3	15584410.667 defg	1826471.375 b	641920.794 abcd	2356247.901 bcd	227349.413 ghij	1368987.999 a	6190707.759 ab	1471921.786 i	252554.427 ab
2*I*5	33912440.740 abcdefg	3263781.670 ab	1258485.250 abcd	2510894.003 bc	436147.290 defghij	2664530.453 a	6783961.407 ab	2514191.500 fgh	263062.892 ab
2*I*7	26777126.060 bcdefg	2840043.240 ab	1445720.060 abc	1959417.170 cd	830205.860 abcd	0.000 a	6841698.590 ab	3895642.000 cd	434977.185 ab
2*I*9	30263308.720 bcdefg	1596921.100 b	0.000 d	3629675.380 ab	802773.250 bcde	0.000 a	7253117.040 ab	4034878.500 bcd	0.000 b
2*N*1	63457961.448 ab	6228214.490 a	1912381.268 a	2382891.990 bcd	886302.068 abc	2233285.134 a	9208332.581 a	2038222.700 ghi	415547.264 ab
2*N*3	16698046.623 defg	2399304.415 b	476219.883 bcd	2271833.775 bcd	265458.589 ghij	973683.518 a	5646288.239 ab	1610330.857 hi	233618.169 ab
2*N*5	8951577.143 efg	1493260.087 b	292194.347 bcd	1887842.847 cd	108516.420 hij	0.000 a	2866169.075 b	1964512.333 ghi	63508.965 b
2*N*7	23922979.180 cdefg	2990948.350 ab	1327238.980 abcd	1929328.260 cd	562264.980 cdefgh	2576912.990 a	7372478.840 ab	3363229.500 def	406355.015 ab
2*N*9	27523142.360 bcdefg	1485595.870 b	0.000 d	4222152.490 a	874101.470 abc	0.000 a	6131412.050 ab	3673853.500 de	0.000 b
3*I*1	68755393.470 a	2852768.432 ab	1154353.846 abcd	1836672.880 cd	350593.018 fghij	3699449.614 a	5745267.840 ab	1755135.900 hi	316961.946 ab
3*I*3	23888456.514 cdefg	3124013.541 ab	1298959.124 abcd	1971588.103 cd	378999.326 efghij	5014816.335 a	7577620.065 ab	1878264.071 hi	279429.178 ab
3*I*5	22002319.413 cdefg	1993932.573 b	253528.853 bcd	2270936.520 bcd	321895.753 ghij	1193053.255 a	6145302.465 ab	2470368.500 fgh	189296.910 b
3*I*7	8609994.470 efg	3412346.610 ab	1569869.260 ab	1057980.050 d	1176984.310 ab	4394554.440 a	6460915.460 ab	4807683.500 ab	577265.970 ab
3*I*9	4228687.740 fg	1348013.870 b	0.000 d	2607554.055 bc	545284.860 cdefgh	0.000 a	6075598.860 ab	4626081.500 abc	0.000 b
3*N*1	62672790.276 ab	3880423.636 ab	1517034.746 abc	2591953.708 bc	511692.260 cdefghi	1127507.036 a	7777030.300 ab	2032309.300 ghi	281053.991 ab
3*N*3	20272603.417 defg	2033389.859 b	580054.687 abcd	1940554.699 cd	248685.086 ghij	1807952.953 a	6061032.324 ab	1541873.714 hi	188665.266 b
3*N*5	14108848.233 defg	1734831.213 b	397922.493 bcd	1917033.193 cd	118042.740 ghij	0.000 a	3257048.337 b	1937633.333 ghi	60369.345 b
3*N*7	35917919.970 abcdefg	2740665.300 ab	1581855.940 ab	1941821.960 cd	776038.550 bcdef	0.000 a	7018578.880 ab	3234620.500 def	421328.770 ab
3*N*9	0.000 g	1480367.100 b	432196.170 bcd	2116038.880 cd	1221788.920 a	0.000 a	2341168.890 b	5192503.000 a	307282.865 ab
Pr > F(Model)	<0,0001	0.002	<0,0001	0.003	<0,0001	0.296	0.002	<0,0001	0.023
Pr > F(Term*Treatment*OIV)	0.938	0.853	0.406	0.933	0.086	0.670	0.940	0.490	0.915
Significant	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Significant	No	No	No	No	No	No	No	No	No

Table S2 continued

Term*Treatment*OIV class	Sesquiterpenes									
	Caryophyllene	Caryophyllene oxide	(*Z*)-*ß*-Farnesene	Copaene	Humulene	Ylangene	*α*-Farnesene	*α*-Muurolene	*ß*-Guaiene	*γ*-Muurolene
0*N*1	955820.497 bc	0.000 a	456169.797 cdefgh	0.000 a	1509235.092 ghij	0.000 c	268234.503 h	121969.075 b	936018.936 bc	298646.682 bc
0*N*3	977436.643 bc	117840.114 a	95863.861 h	289887.625 a	1162514.736 ij	159737.710 c	767636.831 h	69441.805 b	624915.004 bc	106499.639 c
0*N*5	601855.532 bc	69199.945 a	566675.412 bcdefgh	0.000 a	2423762.185 cdefghi	0.000 c	991122.677 h	90464.233 b	1215882.123 bc	520848.390 bc
0*N*7	881767.040 bc	0.000 a	700644.350 bcdefg	0.000 a	4694294.815 ab	0.000 c	1506064.105 h	0.000 b	1035567.960 bc	173943.595 c
0*N*9	122948.310 c	0.000 a	994644.970 bc	0.000 a	1384410.615 hij	0.000 c	1578486.165 h	0.000 b	573320.340 bc	1098330.330 ab
1*I*1	10564026.831 abc	92738.104 a	495113.739 cdefgh	0.000 a	3019541.252 abcdefghi	35726.376 c	2407594.002 h	116859.378 b	636586.810 bc	162114.595 c
1*I*3	4019286.128 bc	158129.741 a	377188.093 fgh	0.000 a	1792853.902 fghij	27048.188 c	1841743.578 h	171931.943 b	828653.652 bc	283026.035 bc
1*I*5	8711300.592 abc	73487.610 a	550379.577 bcdefgh	0.000 a	3968456.087 abcdef	263534.115 c	2113037.923 h	97336.470 b	1105031.177 bc	112702.555 c
1*I*7	5557018.860 bc	209198.790 a	637650.390 bcdefgh	0.000 a	0.000 j	0.000 c	7191662.730 gh	0.000 b	1175916.070 bc	280766.255 bc
1*I*9	884855.685 bc	0.000 a	706383.160 bcdefg	0.000 a	1858186.890 efghij	277665.430 c	7364373.100 gh	77825.125 b	938366.485 bc	0.000 c
1*N*1	12925245.068 abc	12208.623 a	366193.463 fgh	0.000 a	4697706.121 ab	53144.385 c	7979033.212 gh	306932.448 ab	1200048.154 bc	239798.495 bc
1*N*3	3982935.756 bc	141481.147 a	411711.481 efgh	0.000 a	1946169.773 efghij	174988.432 c	2144271.855 h	165753.110 b	829984.879 bc	304218.713 bc
1*N*5	10651739.103 abc	0.000 a	576045.350 bcdefgh	0.000 a	5252066.100 a	0.000 c	2069957.475 h	151067.915 b	1432504.178 bc	519270.157 bc
1*N*7	6180924.270 bc	0.000 a	603973.940 bcdefgh	0.000 a	0.000 j	0.000 c	1806906.585 h	0.000 b	0.000 c	155295.615 c
1*N*9	764939.525 bc	0.000 a	1063853.980 b	0.000 a	1718618.300 fghij	0.000 c	3498783.155 h	0.000 b	0.000 c	175472.185 c
2*I*1	13369898.223 ab	33543.885 a	450955.053 defgh	328872.525 a	4148812.688 abcde	1852461.478 bc	27394907.449 efgh	818376.636 ab	1866129.874 ab	35903.595 c
2*I*3	5047336.232 bc	146391.910 a	240680.771 gh	127173.992 a	2033348.702 defghij	618410.005 c	6559445.042 gh	603151.228 ab	1039881.436 bc	48452.765 c
2*I*5	8423890.335 abc	155043.747 a	500522.340 cdefgh	352204.323 a	4407806.545 abc	3102811.170 bc	8726448.030 gh	772061.280 ab	1998424.578 ab	456559.857 bc
2*I*7	7915323.220 abc	0.000 a	966245.490 bcd	0.000 a	3964370.760 abcdef	0.000 c	133260247.260 c	99313.690 b	1331118.705 bc	0.000 c
2*I*9	1169133.580 bc	0.000 a	956856.830 bcd	0.000 a	2118749.040 defghij	0.000 c	121680191.085 c	0.000 b	694260.030 bc	0.000 c
2*N*1	11576403.043 abc	0.000 a	925748.677 bcde	0.000 a	4264790.915 abcd	266153.106 c	44759104.232 ef	636838.515 ab	2009184.369 ab	156041.656 c
2*N*3	5690292.614 bc	191089.056 a	200467.934 gh	338312.294 a	2068648.020 defghij	857841.170 bc	5368951.484 gh	579689.221 ab	1135555.670 bc	60502.265 c
2*N*5	9469291.887 abc	186363.515 a	184995.375 gh	0.000 a	3407764.385 abcdefghi	156421.650 c	3813256.802 h	104235.605 b	1006911.990 bc	0.000 c
2*N*7	9580005.435 abc	0.000 a	604560.390 bcdefgh	0.000 a	3480665.405 abcdefgh	174832.735 c	62030632.860 de	380455.495 ab	1316917.840 bc	0.000 c
2*N*9	974471.050 bc	0.000 a	849273.845 bcdef	0.000 a	2200463.160 cdefghij	0.000 c	81988227.685 d	397612.040 ab	758145.115 bc	0.000 c
3*I*1	19093452.587 a	0.000 a	480214.717 cdefgh	974689.401 a	3775451.093 abcdefg	6133600.391 ab	41739429.912 efg	2203296.826 a	2851746.300 a	661986.264 bc
3*I*3	4594737.379 bc	113281.214 a	265783.057 gh	645953.556 a	2315057.269 cdefghi	2255557.509 bc	19786693.071 fgh	1222797.067 ab	1841381.190 ab	233842.622 bc
3*I*5	7969124.102 abc	0.000 a	362009.375 fgh	806349.690 a	3911933.020 abcdef	10670339.875 a	8498498.942 gh	1566324.317 ab	2933203.282 a	1456910.023 a
3*I*7	7762721.630 abc	0.000 a	1913645.990 a	426436.575 a	3075785.415 abcdefghi	277958.880 c	300483194.265 a	66972.195 b	801114.645 bc	0.000 c
3*I*9	1389416.520 bc	0.000 a	648297.360 bcdefg	0.000 a	1514334.000 ghij	0.000 c	153712709.850 bc	0.000 b	776176.575 bc	0.000 c
3*N*1	9317553.206 abc	0.000 a	243549.638 gh	124343.625 a	3550032.893 abcdefgh	341740.187 c	37112850.640 efgh	226180.921 b	1537974.913 abc	0.000 c
3*N*3	4227222.206 bc	134683.017 a	245790.654 gh	513142.663 a	1934206.186 efghij	1549774.208 bc	14785116.531 fgh	1028239.572 ab	1347462.906 bc	207855.392 bc
3*N*5	6047335.273 bc	109942.203 a	370767.970 fgh	132353.475 a	3569649.450 abcdefgh	237732.750 c	6180540.960 gh	46979.730 b	1143169.075 bc	0.000 c
3*N*7	11897363.100 abc	0.000 a	703277.260 bcdefg	0.000 a	3666213.495 abcdefgh	0.000 c	131351603.705 c	401138.150 ab	1428917.000 bc	0.000 c
3*N*9	1017526.335 bc	0.000 a	680082.480 bcdefg	0.000 a	2763391.295 bcdefghi	0.000 c	168676198.705 b	0.000 b	684056.205 bc	0.000 c
Pr > F(Model)	<0,0001	0.001	<0,0001	0.016	<0,0001	<0,0001	<0,0001	0.014	<0,0001	0.003
Pr > F(Term*Treatment*OIV)	0.763	0.957	0.002	0.909	0.545	0.202	0.000	0.519	0.054	0.058
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	No	No	Yes	No	No	No	Yes	No	No	No

Table S2 continued

Term*Treatment*OIV class	Other VOCs		
	(*E*)-ß -Ionone	5-Ethyl-2(5H)-furanone	Dihydroactinidiolide
0*N*1	1386749.076 bcdef	2562422.080 defghijkl	851962.852 c
0*N*3	1392224.622 bcdef	1641761.851 jkl	967186.829 bc
0*N*5	1459356.168 bcdef	2216519.878 hijkl	1039055.975 abc
0*N*7	745150.070 f	2120391.210 hijkl	852338.130 c
0*N*9	975856.200 ef	1026728.325 l	1210189.845 abc
1*I*1	1520883.037 bcdef	2891787.996 bcdefghijk	1117244.230 abc
1*I*3	1586552.653 bcdef	2326629.598 ghijkl	1225397.006 abc
1*I*5	1658336.997 bcdef	3369087.767 abcdefghi	1442463.603 abc
1*I*7	1879318.110 abcde	3022394.760 bcdefghijk	1403111.640 abc
1*I*9	1262409.295 def	2178495.285 hijkl	1540726.355 abc
1*N*1	2129619.648 abcd	3940834.148 abcdefg	1272035.955 abc
1*N*3	1701567.534 bcdef	3389558.521 abcdefghi	1074952.972 abc
1*N*5	1506410.787 bcdef	3236206.210 abcdefghij	1026293.940 abc
1*N*7	960506.335 ef	2477067.915 efghijkl	958102.680 bc
1*N*9	965372.590 ef	1545377.445 kl	1261833.790 abc
2*I*1	2415571.499 abc	4105809.960 abcde	1509557.938 abc
2*I*3	1518869.434 bcdef	3072795.949 abcdefghijk	1225149.656 abc
2*I*5	2047800.673 abcde	4280488.680 abc	1582617.445 abc
2*I*7	2229293.220 abcd	4684620.570 a	1962729.860 a
2*I*9	1413462.175 bcdef	2727613.560 cdefghijk	1283772.930 abc
2*N*1	2853214.273 a	3714693.734 abcdefgh	1222726.823 abc
2*N*3	2048274.537 abcde	2896271.934 bcdefghijk	1336133.208 abc
2*N*5	2302162.640 abcd	4114730.003 abcde	1670775.410 abc
2*N*7	2454617.130 ab	4131715.800 abcd	1966113.870 a
2*N*9	2040023.125 abcde	1984536.315 ijkl	1777052.865 abc
3*I*1	2028111.323 abcde	4309926.200 abc	1334041.148 abc
3*I*3	1815568.360 abcdef	3484381.198 abcdefghi	1402084.590 abc
3*I*5	1859388.798 abcde	3686140.272 abcdefgh	1933125.365 a
3*I*7	1749783.120 bcdef	4503558.045 ab	1157232.375 abc
3*I*9	1342587.540 cdef	2018512.350 ijkl	1240955.910 abc
3*N*1	2418164.109 abc	3974921.709 abcdef	1402421.008 abc
3*N*3	1796228.608 abcdef	3076428.768 abcdefghijk	1424889.054 abc
3*N*5	2215777.108 abcd	4053843.010 abcde	1815142.560 ab
3*N*7	2086302.050 abcd	4163673.795 abcd	1789453.120 abc
3*N*9	1995216.965 abcde	2402710.785 fghijkl	1747541.740 abc
Pr > F(Model)	<0,0001	<0,0001	0.009
Pr > F(Term*Treatment*OIV)	0.595	0.360	0.560
Significant	Yes	Yes	Yes
Significant	No	No	No

Term - before inoculation (**0**), 24 hours post-inoculation (hpi) (**1**), 48 hpi (**2**) and 96 hpi (**3**)
Treatment - **N** - Non-inoculated leaves; **I** - Inoculated leaves
OIV class - **1**, **3**, **5**, **7** and **9** from the most susceptible to the completely resistant group

Table S3 - Factorial ANOVA was performed to test the effects of Term of sampling (without T_0 (before inoculation)), Treatment, OIV class and their interaction on the absolute peak area of volatile organic compounds in the leaves of 17 genotypes.

	Acids									Alcohols									
	2-Hexenoic acid	(*E*)-3-Hexenoic acid	Benzoic acid	Heptanoic acid	2-Ethyl-hexanoic acid	Decanoic acid	Nonanoic acid	Octanoic acid	Pentanoic acid	1-Heptanol	1-Hexanol	2-Ethyl-1-hexanol	1-Nonanol	1-Octanol	1-Octen-3-ol	(*E*)-2-Hexen-1-ol	2-Ethyl-2-hexen-1-ol	1-Butoxy-2-propanol	1-Methoxy-2-propanol
R^2	0.440	0.559	0.157	0.270	0.217	0.467	0.353	0.327	0.172	0.379	0.552	0.678	0.600	0.341	0.373	0.623	0.514	0.574	0.593
F	4.694	7.559	1.109	2.206	1.656	5.235	3.253	2.903	1.242	3.636	7.343	12.543	8.966	3.085	3.556	9.843	6.318	8.026	8.695
Pr > F	0.000	0.000	0.332	0.001	0.026	0.000	0.000	0.000	0.198	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Term	7.319	13.979	0.183	0.345	0.533	0.675	0.119	0.024	0.738	1.870	21.067	77.074	50.996	0.203	4.244	38.589	32.379	28.565	40.513
	0.001	**0.000**	0.833	0.709	0.588	0.511	0.888	0.977	0.480	0.157	**0.000**	**0.000**	**0.000**	0.817	**0.016**	**0.000**	**0.000**	**0.000**	**0.000**
Treatment	7.008	0.213	0.120	0.574	0.954	0.028	0.803	1.938	0.608	0.117	7.067	8.618	5.818	0.021	1.189	13.438	0.917	12.377	0.443
	0.009	0.645	0.729	0.450	0.330	0.867	0.371	0.166	0.436	0.733	**0.009**	**0.004**	**0.017**	0.885	0.277	**0.000**	0.340	**0.001**	0.507
OIV	3.303	37.680	7.380	5.014	3.233	28.525	9.892	4.760	6.578	9.993	18.399	4.242	4.622	17.102	9.458	17.334	10.547	5.404	3.914
	0.012	**0.000**	**0.000**	**0.001**	**0.014**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	**0.003**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.005**
Term*Treatment	11.222	0.288	0.077	0.581	0.613	0.433	0.329	1.474	0.300	1.431	0.893	2.055	3.199	0.645	0.155	0.405	0.460	10.763	0.884
	0.000	0.750	0.926	0.561	0.543	0.649	0.720	0.232	0.741	0.242	0.411	0.131	**0.043**	0.526	0.857	0.667	0.632	**0.000**	0.415
Term*OIV	1.419	2.535	0.117	1.598	0.969	0.979	2.043	2.061	0.293	1.447	1.140	2.343	1.066	1.187	1.469	0.403	4.508	2.766	3.455
	0.192	**0.012**	0.999	0.128	0.462	0.454	**0.044**	0.042	0.967	0.180	0.339	**0.020**	0.389	0.310	0.172	0.918	**0.000**	**0.007**	**0.001**
Treatment*OIV	1.536	0.516	0.140	1.666	1.864	1.780	1.576	2.367	0.354	1.756	0.779	0.419	0.208	0.635	1.661	1.120	0.864	2.067	0.639
	0.194	0.724	0.967	0.160	0.119	0.135	0.183	0.055	0.841	0.140	0.541	0.795	0.934	0.638	0.161	0.349	0.487	0.087	0.635
Term*Treatment*OIV	0.665	1.336	0.071	1.893	1.474	2.543	2.214	2.122	0.099	3.198	0.393	2.729	0.991	0.330	3.063	1.186	0.841	3.336	0.508
	0.722	0.229	1.000	0.064	0.170	**0.012**	**0.029**	**0.036**	0.999	**0.002**	0.923	**0.007**	0.445	0.953	**0.003**	0.310	0.568	**0.001**	0.849

	Carbonyls									Esters									
	Acetophenone	Benzaldehyde	2,5-Dimethyl-benzaldehyde	4-Ethyl-benzaldehyde	Benzeneacetaldehyde	Heptanal	Hexanal	Nonanal	Octanal	Hexyl acetate	Phenylmethyl acetate	Ethyl benzoate	(*E*)-3-Hexenyl butanoate	(*E*)-2-Hexenyl benzoate	Phenylmethyl formate	Ethyl octanoate	(*Z*)-2-Hexenyl acetate	4-Hexenyl acetate	Methyl salicylate
R^2	0.729	0.585	0.410	0.116	0.175	0.468	0.308	0.589	0.532	0.414	0.735	0.256	0.322	0.229	0.280	0.339	0.346	0.438	0.821
F	16.066	8.401	4.152	0.783	1.269	5.238	2.654	8.547	6.781	4.217	16.532	2.054	2.836	1.774	2.325	3.055	3.162	4.645	27.590
Pr > F	0.000	0.000	0.000	0.779	0.177	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.013	0.000	0.000	0.000	0.000	0.000
Term	100.445	39.160	0.963	0.024	1.090	0.113	7.614	29.103	41.953	6.711	31.910	0.968	3.023	2.829	4.984	3.819	4.116	6.838	16.812
	0.000	**0.000**	0.384	0.977	0.339	0.893	**0.001**	**0.000**	**0.000**	**0.002**	**0.000**	0.382	0.051	0.062	**0.008**	**0.024**	**0.018**	**0.001**	**0.000**
Treatment	0.895	1.576	0.003	0.018	0.084	0.659	5.987	1.012	4.298	0.610	0.340	2.973	0.326	0.137	5.741	0.240	0.863	0.019	2.175
	0.345	0.211	0.954	0.893	0.772	0.418	0.015	0.316	0.040	0.436	0.560	0.086	0.569	0.712	0.018	0.625	0.354	0.892	0.142
OIV	3.915	8.074	17.959	5.011	2.494	36.444	5.380	24.322	5.436	9.566	66.426	8.948	10.122	3.556	6.617	11.329	5.145	19.796	154.903
	0.005	**0.000**	**0.000**	**0.001**	**0.045**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.008**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**
Term*Treatment	0.418	0.137	3.043	0.004	0.227	0.067	0.351	0.902	1.008	1.666	0.416	0.780	2.014	1.511	2.408	0.869	0.541	1.902	19.149
	0.659	0.872	0.050	0.996	0.797	0.935	0.705	0.407	0.367	0.192	0.660	0.460	0.137	0.224	0.093	0.421	0.583	0.152	**0.000**
Term*OIV	3.004	2.048	1.221	0.111	1.506	0.129	2.121	1.187	1.344	2.389	16.602	0.442	1.095	0.276	2.020	1.637	2.741	2.502	9.621
	0.004	**0.044**	0.289	0.999	0.158	0.998	**0.036**	0.310	0.225	**0.018**	**0.000**	0.894	0.369	0.973	**0.047**	0.118	**0.007**	**0.014**	**0.000**
Treatment*OIV	1.003	0.748	3.716	0.090	0.899	0.040	1.636	0.504	2.828	1.569	5.007	0.629	1.957	2.013	0.625	3.308	0.299	0.468	1.891
	0.408	0.561	**0.006**	0.985	0.466	0.997	0.167	0.733	0.026	0.185	0.001	0.643	0.103	0.095	0.646	**0.012**	0.879	0.759	0.114
Term*Treatment*OIV	1.135	2.183	1.502	0.093	0.434	0.232	0.501	1.987	1.908	1.139	0.554	0.726	0.930	1.890	0.859	0.781	3.736	1.836	9.388
	0.342	**0.031**	0.160	0.999	0.899	0.985	0.854	0.051	0.061	0.339	0.814	0.669	0.493	0.064	0.553	0.620	0.000	0.073	**0.000**

Table S3 continued

	Alcohols									Carbonyls								
	3-Hexen-1-ol	(*E*)-3-Nonen-1-ol	3,7-Dimethyl-3-octanol	2,4-Dimethyl-3-pentanol	6-Methyl-5-hepten-2-ol	*α,α*-Dimethylbenzyl alcohol	Benzyl alcohol	Eugenol	Phenylethyl alcohol	(*E,E*)-2,4-Heptadienal	(*E,E*)-2,4-Hexadienal	(*E,Z*)-2,6-Nonadienal	2-Hexenal	(*E*)-2-Nonenal	(*E*)-2-Octenal	(*E,E*)-3,5-Octadien-2-one	4-Pentenal	6-Methyl-5-hepten-2-one
R^2	0.561	0.234	0.368	0.264	0.268	0.209	0.508	0.126	0.241	0.301	0.355	0.225	0.586	0.554	0.373	0.273	0.342	0.870
F	7.616	1.821	3.469	2.145	2.181	1.575	6.158	0.858	1.895	2.568	3.281	1.737	8.439	7.403	3.548	2.242	3.095	39.801
Pr > F	0.000	0.010	0.000	0.001	0.001	0.040	0.000	0.677	0.007	0.000	0.000	0.016	0.000	0.000	0.000	0.001	0.000	0.000
Term	30.514	6.451	3.210	1.320	2.410	5.404	15.300	1.442	2.932	0.845	2.620	2.810	9.227	17.991	1.011	4.789	1.194	165.379
	0.000	**0.002**	**0.043**	0.270	0.093	**0.005**	**0.000**	0.239	0.056	0.431	0.076	0.063	**0.000**	**0.000**	0.366	**0.009**	0.306	**0.000**
Treatment	3.608	0.093	0.057	6.148	2.895	0.893	2.084	0.000	0.340	0.462	2.533	2.547	1.176	0.662	2.558	0.195	0.968	54.609
	0.059	0.760	0.812	0.014	0.091	0.346	0.151	0.999	0.561	0.497	0.113	0.112	0.280	0.417	0.112	0.660	0.327	0.000
OIV	17.640	4.257	7.566	8.359	1.539	1.075	19.291	3.426	11.423	9.159	14.123	4.561	38.313	21.085	10.170	7.283	9.650	133.807
	0.000	**0.003**	**0.000**	**0.000**	0.193	0.371	**0.000**	**0.010**	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Term*Treatment	0.308	1.018	1.331	0.527	0.802	1.824	1.066	0.083	0.218	0.522	1.741	0.297	3.072	3.429	0.876	0.306	6.077	12.637
	0.735	0.363	0.267	0.592	0.450	0.164	0.347	0.921	0.805	0.594	0.178	0.744	**0.049**	**0.035**	0.418	0.737	**0.003**	**0.000**
Term*OIV	1.782	0.636	2.620	0.711	2.334	0.564	5.067	0.370	0.988	1.643	0.307	0.435	1.020	0.960	3.548	1.105	0.451	32.228
	0.084	0.747	**0.010**	0.682	**0.021**	0.806	**0.000**	0.935	0.447	0.116	0.963	0.899	0.423	0.469	**0.001**	0.362	0.889	**0.000**
Treatment*OIV	2.156	1.011	2.624	2.218	1.460	0.944	3.561	0.210	0.332	1.987	1.675	1.081	2.840	1.056	3.188	0.971	2.480	26.916
	0.076	0.403	**0.036**	0.069	0.217	0.440	**0.008**	0.933	0.856	0.099	0.158	0.367	**0.026**	0.380	**0.015**	0.425	**0.046**	**0.000**
Term*Treatment*OIV	0.865	0.627	1.701	0.465	2.084	0.682	0.946	0.307	0.157	0.358	0.843	0.715	1.495	2.647	2.321	1.003	0.711	7.967
	0.547	0.754	0.101	0.879	**0.040**	0.707	0.480	0.962	0.996	0.941	0.566	0.678	0.162	**0.009**	**0.022**	0.436	0.681	**0.000**

	Monoterpenes																
	ß-Cyclocitral	(*Z*)-Linalool oxide	(*E*)-Linalool oxide	Citronellol	Eucalyptol	Geraniol	Geranyl vinyl ether	Menthol	Limonene	Linalool	Neral	*p*-Cymene	*α*-Terpineol	*ß*-Myrcene	*ß*-Ocimene	Geranylacetone	Nerol
R^2	0.214	0.470	0.480	0.414	0.381	0.189	0.681	0.194	0.497	0.235	0.344	0.243	0.501	0.159	0.229	0.647	0.311
F	1.625	5.293	5.511	4.213	3.667	1.392	12.742	1.440	5.885	1.828	3.122	1.919	5.978	1.127	1.768	10.957	2.694
Pr > F	0.031	0.000	0.000	0.000	0.000	0.101	0.000	0.080	0.000	0.010	0.000	0.006	0.000	0.310	0.014	0.000	0.000
Term	3.578	3.845	10.713	15.089	3.855	2.421	75.750	0.270	1.052	1.210	7.537	3.368	25.372	0.709	3.961	42.105	2.990
	0.030	**0.023**	**0.000**	**0.000**	**0.023**	0.092	**0.000**	0.763	0.352	0.301	**0.001**	**0.037**	**0.000**	0.494	**0.021**	**0.000**	0.053
Treatment	0.286	0.347	0.035	0.739	0.380	0.107	3.060	0.105	0.021	0.006	0.000	0.061	0.214	1.537	0.829	6.307	0.568
	0.593	0.556	0.852	0.391	0.538	0.744	0.082	0.746	0.885	0.940	0.988	0.806	0.644	0.217	0.364	0.013	0.452
OIV	5.028	26.564	20.212	8.874	4.565	3.576	4.420	3.669	37.368	5.973	6.761	3.884	14.555	1.623	2.790	50.565	7.518
	0.001	**0.000**	**0.000**	**0.000**	**0.002**	**0.008**	**0.002**	**0.007**	**0.000**	**0.000**	**0.000**	**0.005**	**0.000**	0.171	**0.028**	**0.000**	**0.000**
Term*Treatment	2.683	0.119	0.558	0.992	6.034	0.182	0.407	1.181	0.323	0.150	0.021	0.287	0.204	0.746	0.195	0.077	0.029
	0.071	0.888	0.573	0.373	**0.003**	0.834	0.666	0.309	0.725	0.861	0.979	0.751	0.816	0.476	0.823	0.926	0.971
Term*OIV	0.208	2.905	2.875	1.852	1.796	0.429	2.484	0.974	1.084	0.769	1.371	1.648	5.316	0.692	0.620	5.056	0.973
	0.989	**0.005**	**0.005**	0.070	0.081	0.903	**0.014**	0.458	0.376	0.631	0.212	0.114	**0.000**	0.698	0.760	**0.000**	0.459
Treatment*OIV	1.096	0.814	2.763	2.907	4.069	2.381	0.926	0.517	0.916	3.134	4.356	2.352	4.754	0.665	5.210	4.287	3.325
	0.360	0.518	**0.029**	**0.023**	**0.004**	0.053	0.450	0.724	0.456	**0.016**	**0.002**	0.056	**0.001**	0.617	**0.001**	**0.002**	0.012
Term*Treatment*OIV	0.637	1.459	1.887	1.287	5.718	0.279	1.655	0.966	0.382	0.442	0.950	0.345	1.418	0.614	0.344	0.838	1.188
	0.746	0.176	0.065	0.253	**0.000**	0.972	0.113	0.464	0.929	0.894	0.477	0.947	0.192	0.766	0.947	0.570	0.309

Table S3 continued

	Sesquiterpenes										Other VOCs		
	Caryophyllene	Caryophyllene oxide	(*Z*)-ß-Farnesene	Copaene	Humulene	Ylangene	α-Farnesene	α-Muurolene	ß-Guaiene	γ-Muurolene	(*E*)-ß-Ionone	5-Ethyl-2(5H)-furanone	Dihydroactinidiolide
R^2	0.267	0.245	0.428	0.233	0.452	0.308	0.787	0.207	0.365	0.222	0.301	0.351	0.182
F	2.174	1.940	4.471	1.812	4.916	2.652	22.054	1.555	3.431	1.699	2.571	3.224	1.326
Pr > F	0.001	0.005	0.000	0.011	0.000	0.000	0.000	0.045	0.000	0.020	0.000	0.000	0.137
Term	0.187	0.687	0.044	3.574	3.418	2.999	114.308	2.078	6.151	1.040	9.134	7.289	4.224
	0.830	0.504	0.957	**0.030**	**0.035**	0.052	**0.000**	0.128	**0.003**	0.356	**0.000**	**0.001**	**0.016**
Treatment	0.011	0.308	2.768	2.370	0.524	4.396	15.738	0.990	3.611	1.398	2.782	0.341	0.077
	0.917	0.579	0.098	0.126	0.470	0.037	0.000	0.321	0.059	0.239	0.097	0.560	0.782
OIV	12.392	8.671	15.918	0.771	26.034	1.817	73.657	1.189	5.860	1.831	6.402	12.727	2.691
	0.000	**0.000**	**0.000**	0.546	**0.000**	0.128	**0.000**	0.317	**0.000**	0.125	**0.000**	**0.000**	**0.033**
Term*Treatment	0.257	1.573	2.313	1.237	1.020	2.094	4.275	0.906	0.639	2.053	1.916	0.770	1.549
	0.773	0.210	0.102	0.293	0.363	0.126	**0.015**	0.406	0.529	0.132	0.150	0.465	0.215
Term*OIV	0.312	1.063	3.393	0.276	2.774	0.866	23.439	0.250	0.807	0.523	0.849	0.817	0.786
	0.961	0.391	**0.001**	0.973	**0.007**	0.546	**0.000**	0.980	0.597	0.838	0.561	0.588	0.615
Treatment*OIV	0.555	0.612	2.223	1.469	0.402	2.998	9.255	1.017	0.999	1.394	0.788	0.376	0.314
	0.696	0.655	0.068	0.214	0.807	**0.020**	**0.000**	0.400	0.410	0.238	0.535	0.826	0.868
Term*Treatment*OIV	0.545	0.274	2.750	0.410	0.670	1.188	3.330	0.782	1.694	1.705	0.699	0.918	0.766
	0.821	0.974	**0.007**	0.914	0.717	0.309	**0.001**	0.620	0.103	0.100	0.692	0.503	0.633

Table S3 continued - The differences between the means of Term (1 - 24 hpi; 2 - 48 hpi; 3 - 96 hpi) were evaluated by Duncan's multiple range test at a confidence level of 95 % ($p < 0.05$). Different letters show statistical significance. Results are expressed as absolute peak areas.

Term of sampling	2-Hexenoic acid	(*E*)-3-Hexenoic acid	Benzoic acid	Heptanoic acid	2-Ethyl-hexanoic acid	Decanoic acid	Nonanoic acid	Octanoic acid	Pentanoic acid	1-Heptanol	1-Hexanol	2-Ethyl-1-hexanol	1-Nonanol	1-Octanol	1-Octen-3-ol	(*E*)-2-Hexen-1-ol	2-Ethyl-2-hexen-1-ol	1-Butoxy-2-propanol	1-Methoxy-2-propanol
1 (24 hpi)	3773498.991 b	7145868.853 b	3045568.942 a	808396.928 a	884988.319 a	2067349.948 a	7002221.535 a	1673109.462 a	769820.577 a	892237.174 a	10737434.203 c	44966176.057 a	3275955.974 a	5259040.311 a	4755284.454 a	13375515.940 c	2369647.488 c	3645265.472 a	2545693.275 a
2 (48 hpi)	4654786.106 b	12682601.543 a	3355238.989 a	838562.688 a	962127.140 a	2252823.659 a	6631360.838 a	1621655.184 a	668238.109 a	1010758.905 a	16443360.296 b	13146480.970 b	1010596.916 b	5036530.709 a	4287750.736 ab	19836149.784 b	3216520.626 b	1809664.761 b	538165.769 b
3 (96 hpi)	8440896.360 a	15880405.926 a	2998942.462 a	683479.747 a	763222.407 a	2336698.125 a	7202373.542 a	1586433.634 a	588348.297 a	820434.453 a	21793902.887 a	5884383.322 c	593866.846 b	5065621.694 a	4006149.865 b	25868225.630 a	4122062.672 a	1724665.115 b	617888.448 b
Pr > F(Model)	<0,0001	<0,0001	0.332	0.001	0.026	<0,0001	<0,0001	<0,0001	0.198	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Pr > F(Term)	0.001	<0,0001	0.833	0.709	0.588	0.511	0.888	0.977	0.480	0.157	<0,0001	<0,0001	<0,0001	0.817	0.016	<0,0001	<0,0001	<0,0001	<0,0001
Significant	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	Yes	Yes	No	No	No	No	No	No	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes

Term of sampling	3-Hexen-1-ol	(*E*)-3-Nonen-1-ol	3,7-Dimethyl-3-octanol	2,4-Dimethyl-3-pentanol	6-Methyl-5-hepten-2-ol	α,α-Dimethylbenzyl alcohol	Benzyl alcohol	Eugenol	Phenylethyl alcohol	(*E*,*E*)-2,4-Heptadienal	(*E*,*E*)-2,4-Hexadienal	(*E*,*Z*)-2,6-Nonadienal	2-Hexenal	(*E*)-2-Nonenal	(*E*)-2-Octenal	(*E*,*E*)-3,5-Octadien-2-one	4-Pentenal	6-Methyl-5-hepten-2-one
1 (24 hpi)	57353636.350 c	676262.395 b	878764.629 ab	11480965.336 a	1394970.529 a	1686609.869 a	145304042.789 a	52363.302 a	2471278.284 b	10340653.374 a	10992058.939 b	1804042.270 b	632685271.785 b	1910130.785 a	4041322.933 a	3061745.071 a	2058177.275 a	22610922.065 c
2 (48 hpi)	110520107.484 b	1281480.336 a	1054224.375 a	12886606.830 a	617716.081 b	824482.791 b	142383206.439 a	146780.212 a	3454130.189 ab	9819537.981 a	12115693.622 a	2145572.690 ab	763719144.562 a	1406499.169 b	4815644.807 a	2738510.192 ab	2390442.510 a	43738810.749 b
3 (96 hpi)	136004111.993 a	1256941.732 a	775564.001 b	11064669.136 a	1010229.527 ab	1065969.356 b	106630193.195 b	142664.680 a	5134633.447 a	9536854.523 a	11253183.486 ab	2394908.956 a	734964058.202 a	1390286.996 b	4869083.979 a	2482241.778 b	2206324.695 a	65859628.701 a
Pr > F(Model)	<0,0001	0.010	<0,0001	0.001	0.001	0.040	<0,0001	0.677	0.007	<0,0001	<0,0001	0.016	<0,0001	<0,0001	<0,0001	0.001	<0,0001	<0,0001
Pr > F(Term)	<0,0001	0.002	0.043	0.270	0.093	0.005	<0,0001	0.239	0.056	0.431	0.076	0.063	0.000	<0,0001	0.366	0.009	0.306	<0,0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	Yes	Yes	Yes	No	No	Yes	Yes	No	No	No	No	No	Yes	Yes	No	Yes	No	Yes

Term of sampling	Acetophenone	Benzaldehyde	2,5-Dimethyl-benzaldehyde	4-Ethyl-benzaldehyde	Benzeneacetaldehyde	Heptanal	Hexanal	Nonanal	Octanal	Hexyl acetate	Phenylmethyl acetate	Ethyl benzoate	(*E*)-3-Hexenyl butanoate	(*E*)-2-Hexenyl benzoate	Phenylmethyl formate	Ethyl octanoate	(*Z*)-2-Hexenyl acetate	4-Hexenyl acetate	Methyl salicylate
1 (24 hpi)	28949679.872 a	35306001.813 a	16746366.674 a	1189296.590 a	791640.814 a	7360012.006 a	15410137.692 b	100698634.713 a	18712712.384 a	1857440.105 b	2771448.436 b	290127.941 a	1457495.226 a	714237.299 b	399391.427 b	6399002.283 a	547595.751 a	28642765.445 b	834447.917 b
2 (48 hpi)	4851552.838 b	24158133.783 b	17599604.100 a	1189690.498 a	970406.061 a	7807173.547 a	16416996.657 b	74431235.533 b	12792258.033 b	2627645.695 a	3680934.064 a	209786.384 a	1570108.995 a	865346.420 a	593135.718 a	5459172.309 ab	567417.847 a	39777928.670 a	1192726.439 a
3 (96 hpi)	3780557.044 b	20273260.720 c	16095543.283 a	1117584.022 a	1246264.628 a	8199663.855 a	19831735.786 a	66676136.386 b	12014570.291 b	2604262.224 a	2451338.687 b	380070.281 a	756431.507 b	708082.060 b	536439.459 a	4250893.150 b	312676.793 b	30472404.285 b	1295106.220 a
Pr > F(Model)	<0,0001	<0,0001	<0,0001	0.779	0.177	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	0.002	<0,0001	0.013	0.000	<0,0001	<0,0001	<0,0001	<0,0001
Pr > F(Term)	<0,0001	<0,0001	0.384	0.977	0.339	0.893	0.001	<0,0001	<0,0001	0.002	<0,0001	0.382	0.051	0.062	0.008	0.024	0.018	0.001	<0,0001
Significant	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes

Term of sampling	ß-Cyclocitral	(*Z*)-Linalool oxide	(*E*)-Linalool oxide	Citronellol	Eucalyptol	Geraniol	Geranyl vinyl ether	Menthol	Limonene	Linalool	Neral	*p*-Cymene	α-Terpineol	ß-Myrcene	ß-Ocimene	Geranylacetone	Nerol
1 (24 hpi)	7390217.367 ab	1220330.881 b	234450.076 b	193651.044 b	12848135.233 a	8640040.440 a	860856.537 a	696265.051 a	23548375.406 a	1978440.053 a	224989.371 b	2429047.360 a	182529.767 b	593124.817 a	4371930.423 b	1737188.868 c	160540.476 b
2 (48 hpi)	8080198.794 a	1462346.271 a	405368.462 a	896357.168 a	8845240.166 ab	14893970.118 a	245274.601 b	598301.472 a	30734501.482 a	2745275.905 a	816227.350 a	2492656.233 a	555825.171 a	1172002.757 a	6348242.060 a	2640060.845 b	233940.375 ab
3 (96 hpi)	6828614.628 b	1286101.146 ab	308000.432 b	941045.867 a	4603196.731 b	15156360.709 a	187884.571 b	658575.110 a	26052140.976 a	2462128.026 a	878577.512 a	2029184.299 b	564729.196 a	1723733.363 a	5842933.656 ab	2945345.772 a	262165.424 a
Pr > F(Model)	0.031	<0,0001	<0,0001	<0,0001	<0,0001	0.101	<0,0001	0.080	<0,0001	0.010	<0,0001	0.006	<0,0001	0.310	0.014	<0,0001	<0,0001
Pr > F(Term)	0.030	0.023	<0,0001	<0,0001	0.023	0.092	<0,0001	0.763	0.352	0.301	0.001	0.037	<0,0001	0.494	0.021	<0,0001	0.053
Significant	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Significant	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	Yes	Yes	Yes	No	Yes	Yes	No

Term of sampling	Caryophyllene	Caryophyllene oxide	(*Z*)-ß-Farnesene	Copaene	Humulene	Ylangene	α-Farnesene	α-Muurolene	ß-Guaiene	γ-Muurolene	(*E*)-ß-Ionone	5-Ethyl-2(5H)-furanone	Dihydroactinidiolide
1 (24 hpi)	6375886.649 a	65573.744 a	570273.524 a	0.000 b	2421978.061 b	87166.957 b	3804527.652 c	105174.652 a	804062.138 b	218494.754 a	1504132.670 b	2822338.700 b	1224032.449 b
2 (48 hpi)	7321604.562 a	71243.211 a	588030.670 a	114656.313 ab	3209541.962 a	702893.131 ab	49558141.193 b	439173.371 a	1315652.961 a	75746.014 a	2132328.871 a	3571327.651 a	1553663.000 a
3 (96 hpi)	7330953.982 a	35790.643 a	591019.314 a	362326.899 a	3006852.012 ab	2146670.380 a	88156209.673 a	676192.878 a	1534134.052 a	256059.430 a	1930044.844 a	3566405.378 a	1524071.296 a
Pr > F(Model)	0.001	0.005	<0,0001	0.011	<0,0001	<0,0001	<0,0001	0.045	<0,0001	0.020	<0,0001	<0,0001	0.137
Pr > F(Term)	0.830	0.504	0.957	0.030	0.035	0.052	<0,0001	0.128	0.003	0.356	0.000	0.001	0.016
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Significant	No	No	No	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes

Table S3 continued - The differences between the means of treatment (Inoculated vs. Non-inoculated leaves) were evaluated by Duncan's multiple range test at a confidence level of 95 % ($p < 0.05$). Different letters show statistical significance. Results are expressed as absolute peak areas.

Treatment	2-Hexenoic acid	(*E*)-3-Hexenoic acid	Benzoic acid	Heptanoic acid	2-Ethyl-hexanoic acid	Decanoic acid	Nonanoic acid	Octanoic acid	Pentanoic acid	1-Heptanol	1-Hexanol	2-Ethyl-1-hexanol	1-Nonanol	1-Octanol	1-Octen-3-ol	(*E*)-2-Hexen-1-ol	2-Ethyl-2-hexen-1-ol	1-Butoxy-2-propanol	1-Methoxy-2-propanol
Inoculated leaves (I)	6987352.825 a	12216231.506 a	3224646.629 a	836451.743 a	946114.246 a	2235210.064 a	7378164.103 a	1852846.365 a	628236.004 a	894002.871 a	18151408.949 a	17292134.618 b	1342948.339 b	5143401.139 a	4234699.237 a	21796283.216 a	3320031.542 a	1974909.586 b	1164053.965 a
Non-inoculated leaves (N)	4258768.146 b	11589686.042 a	3041853.634 a	717174.500 a	794110.998 a	2202704.424 a	6512473.174 a	1401285.822 a	722701.985 a	921617.484 a	14498389.308 b	25372558.948 a	1910664.819 a	5097394.004 a	4464757.466 a	17590311.020 b	3152122.315 a	2811487.313 a	1303777.696 a
Pr > F(Model)	<0,0001	<0,0001	0.332	0.001	0.026	<0,0001	<0,0001	<0,0001	0.198	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Pr > F(Treatment)	0.009	0.645	0.729	0.450	0.330	0.867	0.371	0.166	0.436	0.733	0.009	0.004	0.017	0.885	0.277	0.000	0.340	0.001	0.507
Significant	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	Yes	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes	No	No	Yes	No	Yes	No

Treatment	3-Hexen-1-ol	(*E*)-3-Nonen-1-ol	3,7-Dimethyl-3-octanol	2,4-Dimethyl-3-pentanol	6-Methyl-5-hepten-2-ol	*α,α*-Dimethylbenzyl alcohol	Benzyl alcohol	Eugenol	Phenylethyl alcohol	(*E,E*)-2,4-Heptadienal	(*E,E*)-2,4-Hexadienal	(*E,Z*)-2,6-Nonadienal	2-Hexenal	(*E*)-2-Nonenal	(*E*)-2-Octenal	(*E,E*)-3,5-Octadien-2-one	4-Pentenal	6-Methyl-5-hepten-2-one
Inoculated leaves (I)	109244942.892 a	1047352.553 a	913637.356 a	13005353.087 a	754182.736 a	1299598.034 a	126977855.920 a	113984.507 a	3946123.081 a	9725533.504 a	11111256.558 a	2276554.497 a	695904092.084 a	1602183.754 a	5006516.174 a	2794309.109 a	2129155.506 a	51145981.852 a
Non-inoculated leaves (N)	93340294.326 a	1095770.423 a	892064.647 a	10616141.114 b	1261094.689 a	1085109.977 a	135900439.028 a	113887.622 a	3427238.199 a	10072497.081 a	11796034.140 a	1953128.114 a	725008224.282 a	1535760.879 a	4144184.973 a	2727355.585 a	2307474.148 a	36993592.492 b
Pr > F(Model)	<0,0001	0.010	<0,0001	0.001	0.001	0.040	<0,0001	0.677	0.007	<0,0001	<0,0001	0.016	<0,0001	<0,0001	<0,0001	0.001	<0,0001	<0,0001
Pr > F(Treatment)	0.059	0.760	0.812	0.014	0.091	0.346	0.151	0.999	0.561	0.497	0.113	0.112	0.280	0.417	0.112	0.660	0.327	<0,0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	No	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes

Treatment	Acetophenone	Benzaldehyde	2,5-Dimethyl-benzaldehyde	4-Ethyl-benzaldehyde	Benzeneacetaldehyde	Heptanal	Hexanal	Nonanal	Octanal	Hexyl acetate	Phenylmethyl acetate	Ethyl benzoate	(*E*)-3-Hexenyl butanoate	(*E*)-2-Hexenyl benzoate	Phenylmethyl formate	Ethyl octanoate	(*Z*)-2-Hexenyl acetate	4-Hexenyl acetate	Methyl salicylate
Inoculated leaves (I)	11740266.059 a	25673164.283 a	16788455.508 a	1145101.960 a	966783.145 a	8368343.919 a	18376169.651 a	78673829.242 a	15190706.995 a	2285544.030 a	3006023.668 a	378228.914 a	1342683.806 a	774030.860 a	573080.977 a	5216405.965 a	512310.145 a	32780708.401 a	1157663.3 a
Non-inoculated leaves (N)	13314260.443 a	27485099.927 a	16839220.531 a	1185945.446 a	1038757.857 a	7209555.686 a	16063077.105 b	82530175.179 a	13822320.144 b	2440687.985 a	2929790.456 a	208427.490 a	1180006.680 a	751079.660 a	446230.092 b	5522972.530 a	439483.449 a	33148023.865 a	1057190.4 a
Pr > F(Model)	<0,0001	<0,0001	<0,0001	0.779	0.177	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	0.002	<0,0001	0.013	0.000	<0,0001	<0,0001	<0,0001	<0,0001
Pr > F(Treatment)	0.345	0.211	0.954	0.893	0.772	0.418	0.015	0.316	0.040	0.436	0.560	0.086	0.569	0.712	0.018	0.625	0.354	0.892	0.1
Significant	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	No	No	No	No	No	No	Yes	No	Yes	No	No	No	No	No	Yes	No	No	No	No

Treatment	*ß*-Cyclocitral	(*Z*)-Linalool oxide	(*E*)-Linalool oxide	Citronellol	Eucalyptol	Geraniol	Geranyl vinyl ether	Menthol	Limonene	Linalool	Neral	*p*-Cymene	*α*-Terpineol	*ß*-Myrcene	*ß*-Ocimene	Geranylacetone	Nerol
Inoculated leaves (I)	7331684.906 a	1345224.678 a	313025.514 a	731302.819 a	8028329.632 a	13350220.955 a	387513.624 a	669395.536 a	27083782.532 a	2379619.754 a	638823.249 a	2298007.058 a	446104.507 a	1637604.390 a	5799344.098 a	2581620.603 a	232092.352 a
Non-inoculated leaves (N)	7534335.620 a	1300627.520 a	318853.799 a	622733.234 a	9502718.454 a	12443359.889 a	475163.515 a	632698.887 a	26472896.044 a	2410942.903 a	641039.573 a	2335918.203 a	422618.249 a	688302.901 a	5242726.661 a	2300109.721 b	205671.831 a
Pr > F(Model)	0.031	<0,0001	<0,0001	<0,0001	<0,0001	0.101	<0,0001	0.080	<0,0001	0.010	<0,0001	0.006	<0,0001	0.310	0.014	<0,0001	<0,0001
Pr > F(Treatment)	0.593	0.556	0.852	0.391	0.538	0.744	0.082	0.746	0.885	0.940	0.988	0.806	0.644	0.217	0.364	0.013	0.452
Significant	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Significant	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	No

Treatment	Caryophyllene	Caryophyllene oxide	(*Z*)-*ß*-Farnesene	Copaene	Humulene	Ylangene	*α*-Farnesene	*α*-Muurolene	*ß*-Guaiene	*γ*-Muurolene	(*E*)-*ß*-Ionone	5-Ethyl-2(5H)-furanone	Dihydroactinidiolide
Inoculated leaves (I)	7086933.925 a	64701.335 a	634783.905 a	244112.004 a	2784606.111 a	1700878.760 a	56124258.885 a	520264.353 a	1383662.646 a	247469.895 a	1750426.827 a	3374720.261 a	1419415.352 a
Non-inoculated leaves (N)	6932029.537 a	50370.398 a	531431.767 a	73876.804 a	2974308.578 a	256941.552 b	38221660.127 b	293429.580 a	1052236.787 a	119396.904 a	1960577.430 a	3265327.558 a	1448429.144 a
Pr > F(Model)	0.001	0.005	<0,0001	0.011	<0,0001	<0,0001	<0,0001	0.045	<0,0001	0.020	<0,0001	<0,0001	0.137
Pr > F(Treatment)	0.917	0.579	0.098	0.126	0.470	0.037	0.000	0.321	0.059	0.239	0.097	0.560	0.782
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Significant	No	No	No	No	No	Yes	Yes	No	No	No	No	No	No

Table S3 continued - The differences between the means of OIV classes (1, 3, 5, 7 and 9) were evaluated by Duncan's multiple range test at a confidence level of 95 % ($p < 0.05$). Different letters show statistical significance. Results are expressed as absolute peak areas.

OIV class of resistance	2-Hexenoic acid	(*E*)-3-Hexenoic acid	Benzoic acid	Heptanoic acid	2-Ethyl-hexanoic acid	Decanoic acid	Nonanoic acid	Octanoic acid	Pentanoic acid	1-Heptanol	1-Hexanol	2-Ethyl-1-hexanol	1-Nonanol	1-Octanol	1-Octen-3-ol	(*E*)-2-Hexen-1-ol	2-Ethyl-2-hexen-1-ol	1-Butoxy-2-propanol	1-Methoxy-2-propanol
1	6469666.529 a	16779604.773 a	2566264.084 b	1114695.022 a	1108751.285 a	2512487.547 a	9553234.459 a	2434041.685 a	385798.458 b	934788.731 ab	21808162.550 a	28714924.983 a	2311857.583 a	5401423.086 a	4855621.030 a	20531154.724 b	3058884.338 b	2307980.370 b	1601194.854 a
3	5754529.785 a	4218566.078 c	2561146.570 b	750119.654 b	647859.192 b	1067547.444 b	4747942.285 b	1282447.079 b	852588.332 a	760014.355 b	12947258.697 b	19802740.106 ab	1585218.959 ab	3621779.312 b	3889402.138 b	15936112.043 c	2750797.083 b	1926463.051 b	1085596.210 ab
5	8361041.123 a	17380560.976 a	5265889.429 a	1190596.887 a	997901.282 a	2773451.743 a	8815316.308 a	1866055.385 ab	819196.118 a	1165955.226 a	20843559.855 a	22124893.166 ab	1557149.356 ab	5672182.581 a	4749138.356 a	25258468.926 a	2877728.693 b	3039235.393 a	1678758.358 a
7	5449657.023 a	10852486.773 b	2975079.053 b	358153.763 b	798896.323 ab	2319699.492 a	5348543.626 b	1093365.414 b	945403.949 a	1180762.665 a	14155796.870 b	17505647.919 b	1563448.372 ab	5987176.533 a	3698294.376 b	20212913.285 b	4535871.134 a	2145473.297 b	810516.113 b
9	2080407.968 b	10283575.270 b	2297871.520 b	470500.280 b	797155.028 ab	2421599.993 a	6261556.513 ab	1459420.903 ab	374358.116 b	497529.912 c	11869717.672 b	18513527.740 b	1116358.624 b	4919426.343 a	4556185.859 ab	16527836.613 bc	2957103.394 b	2546840.137 ab	993513.618 ab
Pr > F(Model)	<0,0001	<0,0001	0.332	0.001	0.026	<0,0001	<0,0001	<0,0001	0.198	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Pr > F(OIV)	0.012	<0,0001	<0,0001	0.001	0.014	<0,0001	<0,0001	0.001	<0,0001	<0,0001	<0,0001	0.003	0.001	<0,0001	<0,0001	<0,0001	<0,0001	0.000	0.005
Significant	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

OIV class of resistance	3-Hexen-1-ol	(*E*)-3-Nonen-1-ol	3,7-Dimethyl-3-octanol	2,4-Dimethyl-3-pentanol	6-Methyl-5-hepten-2-ol	*α,α*-Dimethylbenzyl alcohol	Benzyl alcohol	Eugenol	Phenylethyl alcohol	(*E,E*)-2,4-Heptadienal	(*E,E*)-2,4-Hexadienal	(*E,Z*)-2,6-Nonadienal	2-Hexenal	(*E*)-2-Nonenal	(*E*)-2-Octenal	(*E,E*)-3,5-Octadien-2-one	4-Pentenal	6-Methyl-5-hepten-2-one
1	126279888.852 ab	1391171.431 a	919369.369 b	10399797.268 b	593627.325 b	1439524.196 a	137681659.480 b	137670.932 a	1448189.127 c	11508411.098 a	12630033.984 a	2516374.027 a	859734762.551 a	1880729.950 a	3110576.152 b	3010647.953 a	2848630.630 a	28513814.952 c
3	78550800.193 c	904196.449 a	760032.606 bc	11859913.541 b	900277.180 ab	1039600.334 a	103462613.976 c	130399.926 a	4113618.065 b	11334340.580 a	10423836.334 bc	2563838.368 a	647296720.228 b	1303200.517 b	5201360.186 a	3254301.898 a	2327466.076 b	22956911.779 d
5	134075940.509 a	851268.700 a	744986.395 bc	12109899.223 b	1345413.630 a	1219216.000 a	131058793.552 b	7538.247 b	846657.424 c	10840049.482 a	12847416.056 a	2340218.944 a	885498429.872 a	1827734.327 a	5683563.044 a	2582982.715 b	2714305.853 a	27271961.867 c
7	99978639.823 bc	1320374.744 a	586809.540 c	6646925.667 c	1272392.722 ab	1210221.562 a	109542412.188 c	294071.218 a	1876674.284 bc	8959870.585 b	11746267.051 ab	1905019.609 ab	609371061.063 bc	1340630.818 b	2272623.635 b	2458784.038 b	2104937.435 b	89337813.504 a
9	67577823.666 c	890796.113 a	1503057.099 a	18037199.803 a	926482.705 ab	1053207.935 a	175450258.176 a	0.000 b	10148264.299 a	6852404.718 b	9620673.321 c	1248755.579 b	550379817.201 c	1492565.970 b	6608629.848 a	2497445.130 b	1096234.140 c	52268433.758 b
Pr > F(Model)	<0,0001	0.010	<0,0001	0.001	0.001	0.040	<0,0001	0.677	0.007	<0,0001	<0,0001	0.016	<0,0001	<0,0001	<0,0001	0.001	<0,0001	<0,0001
Pr > F(OIV)	<0,0001	0.003	<0,0001	<0,0001	0.193	0.371	<0,0001	0.010	<0,0001	<0,0001	<0,0001	0.002	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

OIV class of resistance	Acetophenone	Benzaldehyde	2,5-Dimethyl-benzaldehyde	4-Ethyl-benzaldehyde	Benzeneacetaldehyde	Heptanal	Hexanal	Nonanal	Octanal	Hexyl acetate	Phenylmethyl acetate	Ethyl benzoate	(*E*)-3-Hexenyl butanoate	(*E*)-2-Hexenyl benzoate	Phenylmethyl formate	Ethyl octanoate	(*Z*)-2-Hexenyl acetate	4-Hexenyl acetate	Methyl salicylate
1	14295089.787 a	27395615.856 a	16259879.858 b	818696.749 bc	1233106.781 ab	6068239.576 b	16792706.174 b	80053292.975 b	14134999.478 bc	2551682.117 a	2792344.296 b	129189.964 b	2423322.927 a	738221.788 bc	481869.871 b	7582133.135 a	600023.077 a	33560573.882 b	515354.098 c
3	10821118.326 b	23397988.429 b	14243932.411 cd	1307921.526 ab	1119859.640 ab	5157596.209 b	15332949.498 b	59878924.834 c	12657906.763 c	1773408.756 b	2034743.573 c	581477.924 a	1233393.794 b	880600.105 a	384106.412 c	3954002.695 b	424691.935 b	22760792.939 c	370714.098 d
5	16266173.567 a	29701566.011 a	15945817.636 bc	2120071.191 a	1598311.578 a	21347176.490 a	19805265.557 a	93445303.321 a	14976810.069 ab	2901407.137 a	2664121.668 b	174950.914 b	1846038.430 a	836754.445 ab	350010.912 c	5106107.473 b	651679.072 a	45734851.948 a	263481.678 d
7	8262089.228 b	21016755.095 b	11687426.456 d	1297223.353 ab	666456.041 b	4327823.076 b	17090291.732 ab	94871407.122 a	16389322.995 a	2326279.113 ab	1873858.574 c	135901.300 b	675933.708 bc	570785.651 c	734926.823 a	5416602.109 b	571430.546 ab	38964960.356 ab	1525877.078 b
9	12991845.349 ab	31383735.136 a	25932133.735 a	283705.698 c	396118.467 b	2043913.662 b	17076903.931 ab	74761082.802 b	14373528.542 abc	2262802.916 ab	5474467.199 a	445120.908 ab	128037.357 c	786414.309 abc	597363.655 ab	4789600.824 b	131659.355 c	23800651.542 c	2861707.342 a
Pr > F(Model)	<0,0001	<0,0001	<0,0001	0.779	0.177	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	0.002	<0,0001	0.013	0.000	<0,0001	<0,0001	<0,0001	<0,0001
Pr > F(OIV)	0.005	<0,0001	<0,0001	0.001	0.045	<0,0001	0.000	<0,0001	0.000	<0,0001	<0,0001	<0,0001	<0,0001	0.008	<0,0001	<0,0001	0.001	<0,0001	<0,0001
Significant	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

OIV class of resistance	ß-Cyclocitral	(*Z*)-Linalool oxide	(*E*)-Linalool oxide	Citronellol	Eucalyptol	Geraniol	Geranyl vinyl ether	Menthol	Limonene	Linalool	Neral	*p*-Cymene	*α*-Terpineol	ß-Myrcene	ß-Ocimene	Geranylacetone	Nerol
1	7747993,730 a	1318993,955 b	404382,819 a	677041,724 b	7336805,470 b	19514910,235 a	424928,210 b	945051,344 a	58715092,617 a	3691203,839 a	1058900,665 a	2261660,209 b	475788,847 a	1566955,507 a	6263227,016 a	1798799,657 c	269191,150 b
3	8265381,171 a	902624,890 c	177552,468 c	612834,246 b	5971916,720 b	15422765,533 ab	362569,663 b	656815,675 ab	17599095,109 b	2268713,029 b	597381,786 bc	2321906,708 b	233663,452 b	2338510,717 a	6117107,471 a	1509132,085 d	209672,862 b
5	8361651,668 a	1208444,137 b	297393,174 b	440192,454 bc	2298019,640 b	11976721,181 bc	564473,682 a	724974,746 ab	19119126,280 b	1966201,396 b	425704,395 c	2123232,998 b	245556,418 b	747390,766 a	4265389,777 b	2022295,467 c	124360,536 c
7	7024442,964 ab	1096756,071 bc	426381,164 a	1564809,223 a	7644740,632 b	14047332,866 abc	326137,585 b	401886,407 b	27438729,927 b	2677798,355 ab	1045637,513 ab	1824470,060 b	595117,587 a	1161911,238 a	5803759,699 ab	3138412,491 b	390260,408 a
9	5765581,782 b	2087811,443 a	273988,657 bc	90212,485 c	20576137,755 a	3522222,296 c	478583,708 ab	526507,885 b	11019652,507 b	1372490,023 b	72032,695 c	3053543,180 a	621680,587 a	0,000 a	5155692,937 ab	3735686,109 a	100925,503 c
Pr > F(Model)	0,031	<0,0001	<0,0001	<0,0001	<0,0001	0,101	<0,0001	0,080	<0,0001	0,010	<0,0001	0,006	<0,0001	0,310	0,014	<0,0001	<0,0001
Pr > F(OIV)	0,001	<0,0001	<0,0001	<0,0001	0,002	0,008	0,002	0,007	<0,0001	0,000	<0,0001	0,005	<0,0001	0,171	0,028	<0,0001	<0,0001
Significant	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes

Table S3 continued

OIV class of resistance	Caryophyllene	Caryophyllene oxide	(*Z*)-ß-Farnesene	Copaene	Humulene	Ylangene	α-Farnesene	α-Muurolene	ß-Guaiene	γ-Muurolene	(*E*)-ß-Ionone	5-Ethyl-2(5H)-furanone	Dihydroactinidiolide
1	12807763.160 a	23081.769 bc	493629.215 b	237984.258 a	3909389.160 a	1447137.654 a	26898819.908 b	718080.787 a	1683611.737 a	209307.434 ab	2227593.982 a	3822995.625 a	1309671.184 b
3	4513067.498 bc	142258.252 a	275977.343 c	270763.751 a	2009411.006 b	920530.358 a	8352355.745 c	622600.379 a	1152741.617 b	181696.789 ab	1722901.807 bc	3015335.554 b	1267794.800 b
5	8545446.882 ab	87472.846 ab	424119.998 b	215151.248 a	4086279.264 a	2405139.927 a	5233623.355 c	456334.219 a	1603207.380 a	424240.432 a	1931646.167 ab	3790082.657 a	1578403.054 a
7	8148892.753 ab	34866.465 bc	904892.243 a	71072.762 a	2364505.846 b	75465.269 a	106020707.901 a	157979.922 a	1008997.377 b	72676.978 ab	1893303.328 abc	3830505.148 a	1539457.258 ab
9	1032238.362 c	0.000 c	816920.382 a	0.000 a	2027701.448 b	46277.572 a	89359290.622 a	79239.527 a	641190.472 b	29245.364 b	1502065.358 c	2141200.565 c	1474284.947 ab
Pr > F(Model)	0.001	0.005	<0,0001	0.011	<0,0001	<0,0001	<0,0001	0.045	<0,0001	0.020	<0,0001	<0,0001	0.137
Pr > F(OIV)	<0,0001	<0,0001	<0,0001	0.546	<0,0001	0.128	<0,0001	0.317	0.000	0.125	<0,0001	<0,0001	0.033
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Significant	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes

Table S4 - Correlations (Pearson) between the sampling time from T_0 to T_3 and the absolute peak area of volatile organic compounds for each genotype and treatment (I - inoculated leaves; N - non-inoculated leaves). Bold values denote statistical significance at the $p < 0.05$ level.

Genotype	OIV class	Treatment	Acids								
			2-Hexenoic acid	(*E*)-3-Hexenoic acid	Benzoic acid	Heptanoic acid	2-Ethyl-hexanoic acid	Decanoic acid	Nonanoic acid	Octanoic acid	Pentanoic acid
Belina starohrvatska	1	I	**0.971**	0.677	0.436	-0.051	-0.059	0.447	0.264	0.051	0.029
Debit	1	I	0.922	0.817	0.539	-0.314	-0.343	-0.113	-0.389	-0.457	-0.293
Grk	1	I	**0.982**	0.896	0.210	-0.484	-0.068	-0.082	-0.395	-0.347	-0.854
Moslavac	1	I	0.945	**0.974**	**0.989**	-0.343	-0.219	0.913	0.706	0.216	-0.075
Plavac mali	1	I	0.898	-0.823	-0.873	-0.832	-0.912	-0.940	-0.859	-0.677	-0.756
Belina starohrvatska	1	N	**0.974**	0.211	0.744	0.673	-0.288	0.375	0.481	0.684	0.000
Debit	1	N	0.856	0.890	0.717	0.289	-0.008	0.250	0.195	-0.032	-0.293
Grk	1	N	0.470	0.581	0.425	-0.604	0.148	-0.053	-0.448	-0.802	0.042
Moslavac	1	N	0.923	**0.957**	0.891	-0.866	-0.896	0.036	-0.517	-0.746	-0.279
Plavac mali	1	N	-0.383	-0.280	-0.665	-0.831	-0.275	0.203	-0.255	-0.575	-0.773
Babić	3	I	0.551	**0.998**	-0.772	-0.086	0.191	0.509	-0.258	0.050	-0.293
Chardonnay	3	I	0.508	0.881	0.038	0.903	0.781	**0.986**	0.946	0.937	0.197
Kraljevina	3	I	**0.970**	0.248	0.654	0.374	0.406	0.856	0.698	0.530	-0.293
Plavina	3	I	0.205	0.882	0.570	0.854	-0.300	-0.683	0.735	0.751	0.937
Pošip	3	I	0.832	0.901	0.889	0.946	0.845	0.000	0.939	0.917	0.586
Škrlet	3	I	-0.531	**0.950**	0.643	0.860	0.892	0.000	0.928	0.879	-0.515
Tribidrag	3	I	0.809	0.942	0.730	0.759	0.729	0.799	0.720	0.818	0.496
Babić	3	N	-0.422	0.813	-0.860	-0.211	-0.236	0.156	0.808	-0.192	-0.251
Chardonnay	3	N	-0.007	-0.027	-0.393	0.684	0.343	-0.193	0.497	0.636	-0.293
Kraljevina	3	N	**0.994**	-0.657	0.700	-0.293	-0.601	-0.653	-0.591	-0.099	-0.293
Plavina	3	N	0.887	0.815	0.774	0.577	-0.225	-0.683	0.498	0.536	**0.956**
Pošip	3	N	0.617	0.227	0.777	0.570	0.308	0.000	0.494	0.602	0.748
Škrlet	3	N	-0.346	**0.952**	0.930	0.911	**0.959**	0.000	0.941	0.920	**0.980**
Tribidrag	3	N	-0.235	0.940	0.943	-0.800	-0.751	**-0.994**	-0.834	-0.941	-0.816
Malvazija istarska	5	I	0.829	0.738	0.136	-0.358	-0.388	0.572	0.147	-0.183	0.302
Ranfol	5	I	0.770	0.731	0.020	-0.398	0.511	-0.634	-0.774	-0.538	0.733
Teran	5	I	0.840	0.905	-0.631	-0.514	-0.817	0.204	-0.288	-0.720	-0.721
Malvazija istarska	5	N	-0.473	0.894	0.932	-0.771	-0.038	**0.968**	0.497	-0.912	0.898
Ranfol	5	N	0.409	**0.964**	0.360	0.406	0.153	0.621	0.681	0.414	0.280
Teran	5	N	-0.497	0.916	-0.928	-0.447	-0.646	-0.246	-0.485	-0.590	-0.731
Solaris	7	I	0.789	0.555	-0.121	-0.268	0.079	-0.168	-0.035	0.380	**-0.996**
Solaris	7	N	-0.042	**0.963**	0.697	0.191	-0.161	0.759	0.871	0.729	-0.023
Vitis riparia	9	I	0.823	0.887	0.804	-0.543	-0.439	0.775	0.301	0.050	-0.657
Vitis riparia	9	N	-0.607	**0.976**	0.719	0.891	0.933	0.120	0.045	0.816	-0.186

Table S4 continued

Genotype	OIV class	Treatment	Alcohols																		
			1-Heptanol	1-Hexanol	2-Ethyl-1-hexanol	1-Nonanol	1-Octanol	1-Octen-3-ol	(*E*)-2-Hexen-1-ol	2-Ethyl-2-hexen-1-ol	1-Butoxy-2-propanol	1-Methoxy-2-propanol	3-Hexen-1-ol	(*E*)-3-Nonen-1-ol	3,7-Dimethyl-3-octanol	2,4-Dimethyl-3-pentanol	6-Methyl-5-hepten-2-ol	*α,α*-Dimethylbenzyl alcohol	Benzyl alcohol	Eugenol	Phenylethyl alcohol
Belina starohrvatska	1	I	-0.925	**0.988**	-0.861	-0.696	-0.320	**-0.999**	**0.972**	0.944	-0.671	-0.706	0.742	0.364	0.094	0.288	-0.692	0.884	0.895	0.000	0.179
Debit	1	I	-0.806	**0.971**	-0.948	-0.847	-0.938	-0.890	0.702	0.635	-0.388	-0.727	0.757	0.545	-0.683	0.314	-0.683	-0.710	0.754	0.632	**0.956**
Grk	1	I	-0.818	**0.965**	-0.734	-0.584	-0.929	-0.923	**0.962**	0.899	-0.729	-0.779	**0.977**	0.944	-0.674	**0.980**	0.098	-0.556	0.376	0.000	**0.973**
Moslavac	1	I	0.652	**0.997**	-0.684	-0.941	0.852	-0.271	**0.955**	**1.000**	-0.113	-0.785	0.886	0.941	0.472	**0.985**	0.000	-0.358	0.757	0.000	**0.999**
Plavac mali	1	I	-0.856	**0.972**	-0.919	-0.726	-0.463	-0.898	**0.984**	0.908	-0.874	-0.715	0.843	0.296	-0.512	-0.729	0.000	-0.316	0.404	0.000	-0.725
Belina starohrvatska	1	N	**-0.951**	**0.978**	-0.935	-0.549	-0.168	0.463	0.822	0.852	-0.831	-0.710	0.836	0.260	-0.605	0.739	-0.305	-0.673	0.825	0.000	0.514
Debit	1	N	-0.854	0.772	-0.387	-0.521	-0.814	-0.633	0.877	0.335	-0.339	-0.730	0.721	0.557	-0.799	-0.481	-0.926	-0.652	0.718	0.615	**0.951**
Grk	1	N	-0.377	0.807	-0.321	-0.623	-0.654	-0.456	0.732	0.885	-0.592	-0.794	0.907	0.420	-0.496	0.368	0.000	-0.512	0.178	0.000	0.426
Moslavac	1	N	-0.402	**0.994**	-0.727	-0.661	0.837	0.909	0.862	0.905	0.024	-0.798	0.912	**0.981**	0.418	0.855	0.000	-0.259	0.587	0.000	**0.954**
Plavac mali	1	N	0.183	0.679	-0.883	-0.916	-0.239	-0.416	0.858	0.264	**-0.978**	-0.797	0.720	0.854	0.341	-0.509	0.878	-0.932	0.660	0.000	0.322
Babić	3	I	-0.909	**0.980**	-0.750	-0.562	-0.837	-0.726	0.904	**0.981**	-0.465	-0.730	0.928	0.712	0.292	-0.577	-0.631	-0.041	0.666	0.000	0.406
Chardonnay	3	I	-0.855	**0.985**	**-0.981**	**-0.989**	0.009	-0.225	0.859	0.910	**-0.987**	-0.774	0.947	**0.964**	-0.626	0.847	0.563	-0.569	0.567	0.845	**0.961**
Kraljevina	3	I	-0.462	0.934	-0.647	-0.647	-0.708	-0.933	**0.963**	0.302	-0.821	-0.767	0.532	0.732	-0.241	0.664	0.000	0.647	0.298	0.648	0.689
Plavina	3	I	-0.458	**0.967**	-0.848	**-0.960**	-0.412	-0.925	**0.954**	0.944	-0.749	-0.742	**0.951**	0.915	-0.084	-0.879	0.000	0.756	0.330	0.000	**-0.963**
Pošip	3	I	0.479	**0.963**	-0.796	-0.706	-0.313	0.758	**0.974**	0.732	-0.804	-0.686	**0.997**	0.645	0.839	-0.664	0.000	-0.206	0.873	0.000	**0.980**
Škrlet	3	I	-0.373	**0.961**	-0.883	-0.761	0.609	-0.484	**0.968**	0.914	-0.456	-0.749	0.910	0.935	-0.545	0.433	0.000	-0.733	0.440	0.000	0.936
Tribidrag	3	I	-0.829	**0.951**	-0.845	-0.933	-0.746	-0.457	**0.968**	**0.954**	-0.276	-0.734	**0.983**	**0.973**	0.701	-0.781	-0.875	-0.444	0.236	0.000	0.633
Babić	3	N	-0.446	-0.141	-0.737	-0.349	-0.365	-0.907	0.158	0.477	-0.901	-0.822	0.884	-0.184	-0.810	-0.684	0.543	-0.676	0.339	-0.293	-0.580
Chardonnay	3	N	**-0.989**	0.818	-0.760	-0.574	-0.303	-0.940	0.902	0.808	-0.696	-0.759	0.562	0.039	-0.944	0.238	0.500	-0.555	0.281	0.000	0.004
Kraljevina	3	N	0.567	**0.971**	-0.779	-0.925	-0.716	0.003	**0.960**	0.844	-0.537	-0.702	0.244	0.751	0.832	0.876	0.000	0.904	0.558	0.711	0.616
Plavina	3	N	-0.749	0.936	-0.940	-0.628	-0.629	**-0.955**	0.902	0.877	**-0.987**	-0.752	0.816	0.715	-0.556	-0.548	0.000	0.078	0.457	0.000	0.294
Pošip	3	N	0.743	**0.955**	-0.854	-0.873	0.159	-0.364	0.837	**0.999**	-0.600	-0.716	**0.998**	0.778	**0.956**	0.352	0.000	-0.650	0.556	0.000	0.945
Škrlet	3	N	-0.222	0.744	-0.887	-0.886	0.585	-0.382	0.809	0.574	-0.705	-0.758	0.930	0.888	-0.281	0.547	0.000	-0.740	0.560	0.000	0.631
Tribidrag	3	N	-0.715	0.850	-0.895	-0.850	-0.894	-0.761	**0.967**	**0.977**	-0.250	-0.706	0.933	0.924	-0.351	-0.782	-0.878	**-0.988**	0.059	0.000	0.712
Malvazija istarska	5	I	-0.169	**0.976**	-0.881	-0.888	-0.241	-0.657	**0.980**	0.866	-0.758	-0.775	0.896	0.652	0.736	0.155	0.470	**0.950**	0.793	0.000	0.712
Ranfol	5	I	-0.869	0.829	-0.850	-0.827	0.129	-0.499	**0.950**	0.257	-0.769	-0.768	0.751	-0.093	-0.422	**0.966**	-0.683	-0.942	0.198	0.000	-0.668
Teran	5	I	0.468	0.902	-0.860	-0.872	-0.882	-0.949	**0.991**	0.786	-0.824	-0.854	0.940	0.764	-0.508	0.592	-0.683	-0.855	0.211	0.098	0.896
Malvazija istarska	5	N	-0.799	0.892	-0.881	-0.852	-0.230	-0.885	0.933	-0.634	-0.829	-0.691	0.745	0.898	-0.200	0.821	0.403	-0.900	0.837	0.000	0.936
Ranfol	5	N	-0.358	0.941	-0.899	-0.859	0.848	-0.448	**0.982**	0.829	-0.851	-0.852	**0.988**	0.550	-0.115	0.448	-0.850	-0.869	0.305	0.000	-0.475
Teran	5	N	-0.160	0.399	-0.771	-0.919	0.070	-0.926	0.750	0.892	-0.774	-0.785	0.941	-0.017	-0.636	0.088	-0.867	-0.782	0.204	0.000	0.747
Solaris	7	I	0.912	0.871	-0.806	-0.831	-0.842	0.108	0.877	**0.978**	-0.570	-0.705	0.626	0.854	0.928	-0.927	-0.552	0.020	0.314	0.875	0.752
Solaris	7	N	0.173	0.924	-0.846	-0.756	-0.346	-0.011	0.782	0.907	-0.907	-0.750	**0.961**	0.867	0.674	-0.756	-0.332	-0.678	0.661	0.936	0.922
Vitis riparia	9	I	0.142	**0.966**	-0.885	-0.781	0.557	-0.710	**0.953**	0.812	-0.322	-0.743	0.910	0.873	-0.934	-0.670	-0.172	-0.919	-0.187	0.000	**0.952**
Vitis riparia	9	N	-0.626	0.869	-0.910	-0.867	-0.051	0.549	0.725	**0.994**	-0.791	-0.742	**0.994**	0.734	-0.922	-0.897	-0.858	-0.888	-0.393	0.000	**0.996**

Table S4 continued

Genotype	OIV class	Treatment	Carbonyls																
			(*E*,*E*)-2,4-Heptadienal	(*E*,*E*)-2,4-Hexadienal	(*E*,*Z*)-2,6-Nonadienal	2-Hexenal	(*E*)-2-Nonenal	(*E*)-2-Octenal	(*E*,*E*)-3,5-Octadien-2-one	4-Pentenal	6-Methyl-5-hepten-2-one	Acetophenone	Benzaldehyde	2,5-Dimethyl-benzaldehyde	4-Ethyl-benzaldehyde	Benzeneacetaldehyde	Heptanal	Hexanal	Nonanal	Octanal
Belina starohrvatska	1	I	-0.796	-0.598	**0.988**	0.493	-0.639	-0.863	-0.471	0.333	**0.981**	-0.871	-0.811	0.924	0.920	0.534	0.475	0.244	-0.819	-0.852
Debit	1	I	-0.398	0.112	0.818	0.817	-0.904	-0.682	-0.946	0.738	0.525	-0.813	-0.776	0.752	0.162	0.878	-0.644	0.927	-0.944	-0.422
Grk	1	I	-0.324	0.771	0.910	0.890	-0.852	0.229	0.389	-0.152	0.392	-0.577	-0.392	0.125	-0.805	0.211	-0.497	0.919	-0.862	-0.695
Moslavac	1	I	-0.132	0.338	0.743	0.798	0.405	-0.822	-0.815	-0.570	0.598	-0.438	-0.214	0.490	0.218	-0.172	-0.040	0.441	-0.843	-0.759
Plavac mali	1	I	0.368	0.526	**0.990**	0.147	-0.946	0.719	-0.828	0.111	**0.963**	-0.873	**-0.951**	**-0.979**	**-0.992**	-0.406	0.807	0.369	-0.793	-0.596
Belina starohrvatska	1	N	-0.203	0.429	0.939	0.802	-0.390	-0.646	0.594	0.782	**0.979**	-0.883	-0.794	0.515	0.231	0.507	0.197	0.657	**-0.976**	-0.580
Debit	1	N	0.250	-0.186	-0.169	0.863	-0.839	-0.498	**-0.960**	0.746	0.711	-0.880	-0.612	0.808	-0.771	0.694	-0.720	-0.047	-0.915	-0.351
Grk	1	N	-0.230	0.756	**0.998**	0.806	-0.817	**0.965**	0.339	-0.083	0.431	-0.845	-0.731	0.245	-0.768	-0.028	-0.421	0.846	**-0.981**	0.472
Moslavac	1	N	-0.141	0.853	0.114	0.915	0.636	-0.614	-0.218	-0.437	0.673	-0.543	0.272	0.757	0.659	-0.536	0.467	0.363	-0.653	-0.507
Plavac mali	1	N	0.514	0.901	0.941	**0.968**	-0.699	-0.347	0.743	0.224	**0.963**	-0.788	-0.852	0.361	-0.834	0.767	-0.620	-0.206	**-0.979**	-0.851
Babić	3	I	0.202	**0.959**	0.900	0.947	-0.919	-0.262	0.708	-0.650	**0.999**	-0.614	-0.310	0.814	**0.952**	0.840	-0.787	-0.037	**-0.985**	-0.774
Chardonnay	3	I	-0.905	-0.801	**0.978**	0.942	-0.806	-0.687	-0.717	-0.639	0.275	-0.647	-0.760	0.820	-0.932	0.740	**-0.979**	0.687	**-0.987**	-0.938
Kraljevina	3	I	-0.798	-0.784	0.774	0.789	**-0.977**	0.307	-0.711	-0.682	0.942	-0.635	-0.554	0.397	0.923	0.776	-0.654	**-0.974**	-0.763	-0.688
Plavina	3	I	-0.467	-0.930	0.821	0.812	-0.899	-0.109	-0.862	**-0.957**	**0.982**	-0.795	-0.806	0.810	-0.336	-0.887	0.192	0.634	-0.944	-0.690
Pošip	3	I	-0.916	0.305	-0.225	**0.997**	-0.454	-0.367	0.833	-0.698	0.911	-0.818	-0.252	-0.810	0.189	0.339	-0.674	-0.714	-0.839	-0.796
Škrlet	3	I	-0.727	0.799	0.209	0.715	-0.934	-0.776	0.842	-0.329	**0.972**	-0.891	**-0.986**	-0.928	0.329	-0.348	0.499	0.207	-0.859	-0.668
Tribidrag	3	I	0.187	0.761	0.673	0.937	-0.456	0.217	0.500	-0.043	0.882	-0.852	-0.862	-0.768	-0.731	-0.726	-0.784	-0.338	-0.864	-0.899
Babić	3	N	0.827	0.155	0.310	0.369	-0.641	0.521	0.496	-0.550	0.910	-0.531	-0.225	-0.330	-0.417	0.247	-0.856	-0.438	-0.772	-0.531
Chardonnay	3	N	**-0.964**	0.103	0.265	0.226	-0.772	-0.677	-0.819	-0.446	0.686	-0.552	-0.628	0.911	-0.922	**0.971**	0.008	**0.954**	-0.683	-0.791
Kraljevina	3	N	-0.787	-0.597	**0.976**	0.908	-0.850	-0.346	-0.901	-0.607	**0.968**	-0.858	-0.649	0.819	**0.976**	0.728	-0.841	0.488	-0.880	-0.285
Plavina	3	N	0.871	0.579	0.206	0.813	-0.791	**-0.978**	0.748	**0.985**	**0.985**	-0.878	-0.853	-0.342	-0.065	0.709	**-0.951**	0.292	-0.930	-0.916
Pošip	3	N	0.107	0.630	0.489	0.714	-0.412	-0.637	0.659	-0.693	**0.954**	-0.874	-0.818	0.079	-0.646	0.028	-0.480	-0.683	-0.873	-0.815
Škrlet	3	N	-0.818	0.696	0.075	0.725	-0.255	-0.475	0.289	-0.590	0.858	-0.846	-0.831	0.061	-0.696	0.520	-0.489	-0.047	-0.843	-0.107
Tribidrag	3	N	0.092	0.913	0.445	**0.964**	-0.135	-0.477	0.216	0.708	0.125	-0.843	-0.840	-0.239	-0.113	-0.801	-0.854	-0.237	**-0.984**	**-0.953**
Malvazija istarska	5	I	0.391	0.328	**0.970**	0.880	-0.666	**-0.971**	**-0.983**	-0.580	0.804	-0.705	-0.539	0.444	0.364	0.932	**-0.950**	-0.159	-0.755	-0.920
Ranfol	5	I	-0.636	-0.541	-0.048	0.008	**-0.971**	-0.935	-0.644	-0.771	**0.961**	-0.875	-0.861	-0.680	**-0.953**	0.718	0.819	**0.979**	-0.657	-0.690
Teran	5	I	0.536	0.786	0.502	0.883	-0.821	-0.128	-0.701	0.357	0.914	-0.882	-0.910	0.411	0.046	**-0.997**	-0.826	**-0.976**	-0.702	-0.856
Malvazija istarska	5	N	0.641	0.701	**0.978**	0.879	-0.874	-0.860	-0.670	0.105	0.874	-0.876	**-0.975**	-0.689	0.722	0.218	**0.994**	**0.982**	-0.853	-0.889
Ranfol	5	N	-0.555	0.254	-0.598	0.826	-0.683	-0.188	-0.873	-0.403	**0.992**	-0.869	-0.895	-0.918	-0.782	0.425	0.783	0.631	0.025	-0.624
Teran	5	N	0.699	0.825	0.624	0.882	-0.921	-0.074	-0.730	0.644	**1.000**	-0.661	-0.725	0.487	-0.303	-0.778	-0.694	-0.581	**-0.985**	-0.650
Solaris	7	I	0.813	-0.626	0.930	-0.656	-0.834	0.823	-0.040	-0.938	**0.982**	-0.888	-0.925	-0.885	0.368	0.905	-0.186	-0.310	-0.883	-0.699
Solaris	7	N	0.822	0.723	0.873	0.746	-0.780	-0.065	0.188	0.599	**0.968**	-0.848	-0.774	0.369	0.790	0.878	-0.725	-0.725	-0.801	-0.839
Vitis riparia	9	I	-0.226	0.523	-0.253	0.600	**-0.985**	0.814	-0.851	-0.863	**0.955**	-0.882	-0.899	0.527	-0.743	0.592	-0.480	0.685	**-0.987**	-0.899
Vitis riparia	9	N	0.477	0.494	0.248	0.514	-0.499	0.933	-0.860	0.175	**0.984**	-0.888	-0.682	-0.146	-0.892	0.374	-0.851	0.249	-0.737	-0.915

Table S4 continued

Genotype	OIV class	Treatment	Esters									
			Hexyl acetate	Phenylmethyl acetate	Ethyl benzoate	(*E*)-3-Hexenyl butanoate	(*E*)-2-Hexenyl benzoate	Phenylmethyl formate	Ethyl octanoate	(*Z*)-2-Hexenyl acetate	4-Hexenyl acetate	Methyl salicylate
Belina starohrvatska	1	I	0.392	0.928	-0.493	-0.788	-0.629	**0.963**	0.667	-0.874	-0.542	-0.477
Debit	1	I	0.891	0.424	-0.878	0.088	0.752	0.013	0.548	-0.430	-0.116	0.674
Grk	1	I	**0.998**	0.811	-0.695	-0.205	0.910	-0.089	-0.579	0.166	0.782	0.869
Moslavac	1	I	0.647	0.573	0.000	-0.498	-0.460	0.413	0.546	-0.389	0.163	0.159
Plavac mali	1	I	-0.673	0.113	0.000	-0.725	-0.654	-0.560	-0.930	-0.949	-0.810	0.000
Belina starohrvatska	1	N	0.930	0.934	-0.708	-0.798	-0.457	0.513	0.685	-0.599	-0.205	-0.603
Debit	1	N	0.887	0.597	-0.683	0.878	0.254	-0.229	0.509	-0.589	-0.730	-0.302
Grk	1	N	0.888	0.186	-0.683	-0.301	-0.171	-0.037	-0.002	-0.229	0.423	0.265
Moslavac	1	N	0.505	0.280	0.000	-0.735	-0.916	-0.267	0.683	0.505	-0.323	0.105
Plavac mali	1	N	-0.249	0.642	0.000	-0.733	-0.595	-0.678	0.176	-0.134	-0.701	0.000
Babić	3	I	0.878	0.622	-0.799	-0.159	0.855	0.806	0.723	0.115	0.867	0.878
Chardonnay	3	I	**0.990**	0.431	-0.683	0.000	**-0.969**	-0.642	0.511	0.113	0.265	0.265
Kraljevina	3	I	0.491	-0.017	-0.059	-0.897	0.016	0.828	-0.391	0.031	-0.442	-0.845
Plavina	3	I	0.753	0.330	0.896	-0.676	0.252	0.805	-0.819	0.493	0.614	**0.996**
Pošip	3	I	**0.971**	0.920	**0.978**	0.072	0.870	0.337	-0.014	0.234	**0.962**	0.878
Škrlet	3	I	-0.768	0.469	-0.044	**-0.952**	0.700	0.730	**-0.975**	-0.929	0.228	0.474
Tribidrag	3	I	0.829	0.855	0.878	0.880	-0.070	0.268	-0.933	-0.153	0.833	**0.971**
Babić	3	N	0.528	0.516	-0.876	-0.214	0.452	0.291	-0.272	-0.293	**0.982**	0.878
Chardonnay	3	N	-0.151	0.174	-0.683	-0.293	-0.487	-0.794	0.565	-0.869	-0.269	-0.444
Kraljevina	3	N	0.822	0.619	0.694	-0.594	-0.157	0.930	0.531	-0.118	-0.871	-0.902
Plavina	3	N	0.215	0.676	0.571	-0.722	0.696	-0.156	-0.918	0.436	-0.232	0.835
Pošip	3	N	0.670	0.758	0.001	-0.409	0.457	-0.806	0.904	-0.638	0.481	0.000
Škrlet	3	N	0.440	0.793	-0.196	-0.605	0.641	-0.161	-0.873	-0.603	0.936	0.580
Tribidrag	3	N	0.593	0.845	0.000	0.248	0.857	-0.832	-0.930	-0.690	**0.980**	**0.971**
Malvazija istarska	5	I	0.722	0.838	0.868	-0.596	0.262	0.837	0.742	-0.448	-0.722	0.000
Ranfol	5	I	-0.933	0.216	0.837	-0.849	0.020	-0.747	-0.407	-0.936	-0.085	0.840
Teran	5	I	-0.560	0.870	-0.697	**-0.995**	0.826	**-0.991**	-0.591	-0.941	0.100	-0.808
Malvazija istarska	5	N	0.871	0.459	-0.683	-0.683	-0.505	**-0.990**	0.866	0.144	0.483	0.000
Ranfol	5	N	0.202	0.304	0.000	-0.739	-0.409	-0.918	-0.346	-0.029	0.639	0.868
Teran	5	N	-0.760	0.869	-0.695	-0.465	-0.532	**-0.965**	-0.714	-0.809	-0.044	-0.785
Solaris	7	I	0.275	-0.152	0.313	-0.490	-0.731	**0.951**	-0.826	0.259	-0.025	0.549
Solaris	7	N	0.445	0.667	0.898	-0.878	**0.976**	0.904	0.493	0.518	0.502	0.879
Vitis riparia	9	I	0.467	0.152	0.948	0.098	0.558	0.838	-0.880	-0.630	0.239	0.858
Vitis riparia	9	N	0.084	0.007	-0.196	0.878	0.048	-0.211	-0.876	-0.683	0.401	0.806

Table S4 continued

Genotype	OIV class	Treatment	Monoterpenes																
			ß-Cyclocitral	(*Z*)-Linalool oxide	(*E*)-Linalool oxide	Citronellol	Eucalyptol	Geraniol	Geranyl vinyl ether	Menthol	Limonene	Linalool	Neral	*p*-Cymene	α-Terpineol	ß-Myrcene	ß-Ocimene	Geranylacetone	Nerol
Belina starohrvatska	1	I	0.691	-0.570	**0.989**	**0.970**	-0.683	0.782	-0.932	0.939	0.943	-0.090	0.618	-0.353	0.851	-0.683	0.050	0.799	0.639
Debit	1	I	-0.780	-0.683	-0.514	0.755	0.000	**0.976**	-0.891	-0.542	0.890	-0.132	0.594	0.733	-0.383	0.332	0.825	0.473	0.764
Grk	1	I	0.833	-0.880	**-0.954**	0.912	0.000	**0.953**	-0.659	-0.426	0.012	0.862	0.598	-0.822	-0.277	0.000	0.357	0.179	0.596
Moslavac	1	I	-0.446	-0.084	0.587	0.878	-0.019	0.713	-0.452	-0.090	-0.616	0.109	0.878	0.137	0.796	0.878	0.932	0.870	0.493
Plavac mali	1	I	-0.048	**-0.973**	**0.964**	0.731	-0.782	0.649	-0.894	-0.311	0.546	0.213	0.798	-0.787	-0.572	**0.968**	-0.077	0.620	-0.650
Belina starohrvatska	1	N	0.221	-0.795	0.171	-0.286	0.622	-0.038	-0.938	-0.612	0.763	-0.212	0.429	-0.196	0.821	-0.683	-0.072	0.119	0.006
Debit	1	N	-0.166	-0.692	-0.216	0.083	0.000	0.517	**-0.957**	-0.127	0.842	0.035	0.382	0.737	-0.071	-0.693	0.659	0.534	-0.404
Grk	1	N	0.442	-0.663	0.106	0.251	0.000	0.525	-0.943	-0.193	-0.627	0.125	0.469	-0.276	0.012	0.000	0.352	0.058	-0.704
Moslavac	1	N	-0.255	0.382	0.442	**0.958**	0.257	-0.590	-0.591	-0.477	-0.514	0.253	0.878	0.365	0.774	0.000	0.838	0.926	0.752
Plavac mali	1	N	**0.958**	-0.380	0.261	0.842	-0.321	0.720	-0.742	-0.908	0.233	0.571	0.615	0.909	0.336	0.775	0.686	0.912	-0.575
Babić	3	I	0.864	0.892	0.814	0.770	0.000	0.549	-0.884	0.848	-0.683	0.387	0.384	-0.735	0.918	-0.683	0.181	0.384	0.431
Chardonnay	3	I	-0.765	0.845	0.000	0.845	0.527	**0.990**	-0.675	0.845	0.000	0.064	0.762	-0.167	0.000	0.000	0.845	-0.249	0.710
Kraljevina	3	I	-0.565	-0.457	-0.153	0.717	-0.664	0.710	-0.601	0.764	-0.045	0.404	0.842	-0.806	-0.137	0.098	0.708	0.085	0.683
Plavina	3	I	-0.877	-0.630	0.933	**0.962**	0.000	**0.990**	-0.800	0.217	0.943	0.811	**0.970**	-0.667	**0.987**	0.943	0.793	**0.993**	0.874
Pošip	3	I	0.452	0.733	**0.973**	0.057	-0.683	0.563	**-0.958**	**0.966**	**0.966**	0.565	0.487	0.026	0.926	0.060	0.546	0.932	0.806
Škrlet	3	I	-0.297	-0.638	-0.549	0.945	0.878	**0.966**	-0.928	-0.780	0.760	0.025	0.878	-0.704	0.602	0.878	-0.662	0.882	0.098
Tribidrag	3	I	-0.062	-0.338	0.898	0.878	0.000	0.833	-0.607	0.432	0.397	0.748	0.878	-0.735	0.776	0.000	-0.674	0.350	0.875
Babić	3	N	0.097	0.106	0.410	-0.299	0.000	-0.524	-0.787	-0.004	0.772	-0.660	-0.829	-0.570	0.568	-0.404	-0.834	-0.401	-0.545
Chardonnay	3	N	-0.866	0.373	0.364	0.000	0.878	0.530	-0.614	0.302	0.000	-0.022	-0.156	-0.792	0.098	-0.293	0.000	-0.388	-0.683
Kraljevina	3	N	-0.141	0.732	**0.953**	0.945	-0.697	0.905	-0.648	0.937	-0.595	0.815	0.918	0.937	**0.981**	0.098	0.933	0.491	0.906
Plavina	3	N	0.046	-0.192	0.652	0.931	0.000	0.765	-0.849	-0.359	0.340	0.369	0.801	-0.436	0.807	0.878	0.759	**0.955**	0.765
Pošip	3	N	0.567	0.165	0.846	0.867	-0.683	0.887	**-0.964**	-0.571	0.939	**0.972**	0.909	0.457	**0.996**	0.212	**0.954**	0.609	0.919
Škrlet	3	N	0.058	-0.755	-0.101	0.223	0.000	0.799	-0.820	-0.876	0.906	0.258	0.098	0.092	0.224	0.000	0.181	0.855	0.098
Tribidrag	3	N	0.658	-0.534	0.098	0.098	0.000	0.901	-0.937	-0.881	0.095	0.666	0.000	-0.583	-0.457	0.000	-0.637	-0.163	0.098
Malvazija istarska	5	I	-0.488	0.104	0.728	**0.966**	-0.683	0.602	-0.660	**0.974**	0.643	0.414	-0.003	0.727	0.760	0.652	0.674	0.672	0.476
Ranfol	5	I	-0.826	-0.709	0.572	0.907	-0.933	0.856	-0.901	-0.792	-0.712	-0.505	0.864	-0.861	**-0.993**	0.000	-0.816	**0.968**	0.563
Teran	5	I	0.227	**-0.993**	-0.118	0.479	-0.683	0.771	-0.844	-0.729	-0.343	0.121	0.000	0.259	-0.255	-0.413	-0.118	0.376	0.098
Malvazija istarska	5	N	0.538	0.552	0.911	0.000	-0.683	0.735	-0.917	-0.529	0.000	0.839	0.467	0.845	0.480	0.000	0.000	0.749	-0.683
Ranfol	5	N	-0.754	-0.315	**0.981**	0.000	-0.683	0.870	-0.870	-0.683	-0.570	0.130	0.909	-0.838	-0.683	0.000	-0.752	0.852	0.415
Teran	5	N	-0.562	-0.763	-0.608	0.098	-0.683	0.781	-0.641	-0.643	-0.695	-0.489	0.000	-0.340	-0.571	-0.827	0.230	0.465	0.000
Solaris	7	I	-0.030	**0.983**	0.920	0.947	-0.683	0.940	-0.880	-0.852	**-0.970**	0.883	0.904	**-0.957**	**0.969**	0.878	0.683	**0.960**	**0.954**
Solaris	7	N	0.470	**0.953**	0.748	0.778	-0.940	0.713	-0.819	-0.907	-0.387	0.824	0.898	-0.816	0.930	0.098	0.813	0.793	0.857
Vitis riparia	9	I	-0.562	0.701	0.520	0.000	-0.710	0.927	-0.942	-0.587	0.201	0.591	0.000	-0.014	0.583	0.000	0.641	0.936	-0.654
Vitis riparia	9	N	0.695	0.526	0.551	0.878	-0.444	**0.999**	-0.741	-0.583	0.098	0.857	0.878	-0.258	0.877	0.000	-0.014	**0.985**	0.584

Table S4 continued

Genotype	OIV class	Treatment	Sesquiterpenes									
			Caryophyllene	Caryophyllene oxide	(*Z*)-ß-Farnesene	Copaene	Humulene	Ylangene	α-Farnesene	α-Muurolene	ß-Guaiene	γ-Muurolene
Belina starohrvatska	1	I	0.741	0.000	0.833	**0.951**	0.784	0.945	**0.968**	0.930	0.948	0.852
Debit	1	I	0.485	-0.293	-0.822	0.000	0.235	0.862	0.287	-0.041	0.573	-0.248
Grk	1	I	0.896	0.000	-0.736	0.098	0.840	0.698	0.820	0.511	**0.969**	-0.879
Moslavac	1	I	0.430	0.000	0.581	0.098	0.482	0.878	0.536	**0.972**	0.801	-0.871
Plavac mali	1	I	0.804	-0.211	-0.699	0.878	0.179	0.905	**0.953**	**0.989**	0.817	-0.579
Belina starohrvatska	1	N	**0.954**	0.000	0.445	0.878	0.927	**0.958**	**0.977**	0.864	0.331	**-0.978**
Debit	1	N	0.107	-0.293	-0.432	0.000	0.284	-0.165	0.336	-0.184	-0.549	0.014
Grk	1	N	0.345	0.000	-0.091	0.000	0.443	0.823	0.192	0.073	0.451	-0.864
Moslavac	1	N	0.179	0.000	0.196	0.878	0.003	0.878	0.934	0.034	0.776	-0.830
Plavac mali	1	N	0.668	0.000	-0.225	0.000	-0.595	0.878	0.722	0.771	0.757	-0.860
Babić	3	I	0.645	0.000	0.748	-0.492	0.759	0.905	**0.969**	0.869	**0.975**	0.794
Chardonnay	3	I	0.881	-0.683	0.845	0.000	0.000	0.000	0.845	0.845	0.845	-0.683
Kraljevina	3	I	0.902	0.000	-0.286	**0.951**	**0.980**	0.929	0.939	**0.993**	0.906	0.638
Plavina	3	I	0.608	-0.485	0.310	**0.961**	0.786	0.921	0.917	**0.994**	0.910	-0.786
Pošip	3	I	0.365	0.678	-0.366	**0.965**	**0.996**	0.942	0.891	**0.971**	0.897	0.000
Škrlet	3	I	-0.020	-0.765	0.536	0.878	**0.982**	0.878	0.907	0.852	-0.245	-0.293
Tribidrag	3	I	0.915	0.501	0.249	0.878	0.763	0.912	**0.969**	0.883	0.928	0.350
Babić	3	N	0.290	0.359	-0.293	-0.683	0.833	0.087	0.916	0.089	-0.292	-0.293
Chardonnay	3	N	0.547	-0.683	0.098	0.000	0.000	0.000	0.907	0.000	0.000	-0.870
Kraljevina	3	N	**0.951**	-0.224	0.796	**0.962**	**0.990**	0.908	0.935	**0.975**	**0.985**	0.826
Plavina	3	N	0.417	**0.983**	0.320	0.415	0.495	0.870	**0.984**	0.730	0.386	-0.788
Pošip	3	N	0.447	0.369	-0.343	0.000	0.418	0.872	0.908	-0.015	0.946	-0.293
Škrlet	3	N	**0.997**	0.304	0.209	0.878	0.442	0.000	**0.977**	0.494	0.770	0.000
Tribidrag	3	N	0.532	-0.552	-0.683	0.098	-0.346	0.474	0.903	0.686	0.791	**-0.974**
Malvazija istarska	5	I	0.860	-0.512	0.307	**0.969**	0.626	**0.964**	0.751	**0.971**	**0.975**	0.922
Ranfol	5	I	0.455	0.000	-0.011	0.878	0.604	0.707	0.836	0.716	0.104	-0.683
Teran	5	I	0.698	-0.141	**-0.991**	0.000	0.220	0.938	**0.990**	-0.469	0.639	-0.846
Malvazija istarska	5	N	0.287	0.551	**0.974**	0.000	0.147	0.871	**-0.993**	0.000	-0.534	-0.878
Ranfol	5	N	0.155	0.000	-0.092	0.878	0.271	0.000	**0.983**	0.493	0.785	-0.637
Teran	5	N	0.753	0.098	-0.674	0.000	-0.054	**0.968**	**0.991**	-0.606	-0.706	-0.844
Solaris	7	I	0.818	-0.293	0.941	0.878	-0.022	0.878	**0.975**	0.690	-0.476	-0.726
Solaris	7	N	0.937	0.000	0.183	0.000	0.086	0.098	**0.971**	0.862	0.519	-0.860
Vitis riparia	9	I	0.900	0.000	-0.630	0.000	0.079	-0.293	0.920	-0.293	0.287	-0.683
Vitis riparia	9	N	0.824	0.000	-0.919	0.000	**0.994**	0.000	**0.972**	0.098	0.405	-0.760

Table S4 continued

Genotype	OIV class	Treatment	Other VOCs		
			(*E*)-*ß*-Ionone	5-Ethyl-2(5H)-furanone	Dihydroactinidiolide
Belina starohrvatska	1	I	0.908	0.689	0.876
Debit	1	I	0.343	0.684	0.693
Grk	1	I	**0.985**	**1.000**	0.910
Moslavac	1	I	0.131	0.776	-0.012
Plavac mali	1	I	0.418	0.789	-0.828
Belina starohrvatska	1	N	0.762	0.712	0.793
Debit	1	N	0.332	-0.429	0.734
Grk	1	N	0.333	0.696	0.553
Moslavac	1	N	0.598	0.924	0.703
Plavac mali	1	N	0.903	0.755	0.391
Babić	3	I	**0.972**	**0.969**	0.931
Chardonnay	3	I	-0.117	**0.990**	0.395
Kraljevina	3	I	0.766	-0.237	0.858
Plavina	3	I	-0.484	**0.988**	0.819
Pošip	3	I	0.717	0.470	0.553
Škrlet	3	I	-0.294	0.937	0.139
Tribidrag	3	I	**0.975**	**0.991**	**0.979**
Babić	3	N	-0.008	0.075	-0.291
Chardonnay	3	N	-0.090	0.497	-0.134
Kraljevina	3	N	0.670	-0.099	0.626
Plavina	3	N	0.677	**0.974**	0.930
Pošip	3	N	0.761	0.931	0.811
Škrlet	3	N	0.147	0.758	0.252
Tribidrag	3	N	0.923	0.584	0.884
Malvazija istarska	5	I	0.389	**0.975**	0.931
Ranfol	5	I	-0.312	0.452	0.253
Teran	5	I	0.656	0.409	0.750
Malvazija istarska	5	N	**0.968**	0.941	0.919
Ranfol	5	N	-0.481	0.628	0.582
Teran	5	N	0.540	0.592	0.809
Solaris	7	I	0.565	0.838	0.240
Solaris	7	N	0.767	0.874	0.794
Vitis riparia	9	I	0.729	0.500	-0.203
Vitis riparia	9	N	0.825	**0.973**	0.837