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Xylem Vessel Size Is Related to Grapevine Susceptibility to *Phaeomoniella chlamydospora*

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Abstract: Grapevine trunk diseases are a threat to table- and grape-wine cultivation worldwide. *Phaeomoniella chlamydospora* (Pch) is a vascular fungus recognized as one of the most important pathogens associated with grapevine trunk diseases. The relationships between xylem vessel features and Pch susceptibility of 10 table- and 17 wine-grape genotypes, as well as 3 rootstocks, were investigated by image analysis of 50 µm cross-sections and artificial Pch inoculation on one-year-old vine cuttings. Vessels were grouped in the diameter classes 1–30, 31–60, 61–90, 91–120, and >120 µm. Among the table-grape varieties, 'Sable', 'Timco', and 'Red Globe' showed higher densities of large vessels (>120 µm) than 'Italia', 'Sugar Crisp', and 'Sugraone'. Among the wine-grape varieties, 'Minutolo', 'Montepulciano', 'Primitivo' CDTa19, and 'Verdeca' showed higher densities of large vessels than 'Aglianico', 'Nero di Troia', 'Sangiovese', and 'Susumaniello'. In the rootstocks, the vessel diameters were 50.8, 54.0, and 60.9 µm for '34 E.M.', '140 Ruggieri', and '1103 Paulsen', in that order. For table-grape varieties, Pch was re-isolated from 13.3% for 'Sugar Crisp' and 'Sugraone' cuttings up to 93.3% for 'Timco'. For wine-grape varieties, Pch re-isolation ranged from 51.1% ('Bombino nero', 'Negroamaro' D15, and 'Sangiovese') to 81.1% ('Montepulciano'), while for the rootstocks, the values were from 33 to 51%. A principal component analysis (PCA) revealed a positive correlation between the frequencies of large vessels and Pch re-isolation. In addition, in wine grapes and rootstocks, higher Pch re-isolation frequencies in the lateral parts of cuttings were correlated (r = 0.79) to a higher frequency of large vessels. The results highlight relationships between grapevine xylem vessel sizes and susceptibility to *P. chlamydospora* that are worthy of further research.

Keywords: grapevine trunk diseases; esca disease; vascular pathogens; in planta bioassay; image analysis

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1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the most important crops worldwide. Grapevine growing is widespread in the Mediterranean basin and Italy. In the Apulia region (southern Italy), there are favorable conditions for both table and wine grapes, and hence grapevine is grown on 118,759 ha [1]. Italy is the leading producer in Europe, with a stable production of around 1 million tons of table grapes per year, and the Apulia region is the greatest supplier, with a production of around 600,000 tons [1]. 'Italia' is still the most commonly grown cultivar, although work is ongoing to introduce new varieties, especially seedless ones [2]. On the other hand, autochthonous wine-grape varieties, such as 'Negroamaro', 'Primitivo', and 'Nero di Troia', are the predominant varieties that produce wines with a significant impact on international demand [3].

Although a lot of studies have focused on the resistance to abiotic and biotic stresses, grapevine is susceptible to numerous pathogens causing diseases both pre- and post-harvest, severely affecting production, grape quality, processing, and export [4]. Neverthe-

less, genetic improvement programs are often more focused on agronomical and qualitative features than on characteristics related to disease resistance.

Grapevine trunk diseases (GTDs) are important threats to grapevines. GTDs are caused by a complex of fungal pathogens, including Ascomycota belonging to the Botryosphaeriaceae, Diaporthaceae, Diatrypaceae, Phaeomoniellaceae, and Togniniaceae families, as well as the Basidiomycota of the Hymenochaetaceae family. They compromise the xylem system in vines, ultimately leading to plant death and inducing heavy economic losses to the grape industry [5,6]. One of the most common among the GTD-associated pathogens is the vascular fungus *Phaeomoniella chlamydospora* (Pch), endemic to all viticultural areas worldwide [7]. It is implicated in the discoloring of rootstock wood, rooted cuttings, or very young plants (young grapevine decline, slow dieback, and Petri grapevine decline), as well as wood alterations of young 8–10-year-old vines (young esca) [8,9].

Phaeomoniella chlamydospora resides mainly in vessels [10]. During systemic host colonization, Pch degrades structural cell wall polymers and produces phytotoxins [11]. Since the beginning of 2000, sodium arsenite has been banned due to its toxicity both to the environment and to humans. To date, there are no curative measures against GTDs [9,12,13].

Grape varieties and rootstocks differ in their susceptibility to Pch [12,14]. Recently, it was suggested that xylem vessel size affects Pch development, influencing the efficacy of the defense reaction of vines through vessel occlusion and pathogen compartmentalization. Hence, this feature can be helpful for the development of new tolerant or resistant grapevine genotypes to preserve plant health and prevent heavy economic losses [15–17]. In fact, it is known that vascular pathogens induce the plant to produce tyloses containing secondary metabolites (e.g., alkaloids, phenolic compounds, and terpenes) with antifungal activity [18,19]. Similarly, xylem vessel size in wine-grape varieties seems to be related to the response of grapevine genotypes to esca disease, while information on table grapes is scanty [18,20].

This paper reports the role of xylem vessel sizes in Pch susceptibility for ten table- and fifteen wine-grape varieties, as well as three rootstocks, by studying (1) the vessel features through image analysis of cross-sections; (2) the susceptibility to Pch by re-isolation on semi-selective medium after artificial inoculation; and (3) their relationships through a comprehensive principal component analysis (PCA).

2. Materials and Methods

2.1. Grape and Fungal Material

The table- and wine-grape varieties, including two clones of each 'Primitivo' and 'Negroamaro', and rootstocks used in this study and their features are summarized in Table 1. For each table- (experiment 1) and wine-grape variety and rootstock (experiment 2), 30 fragments (~50 cm in length; 7–11 mm diameter; ~4 internodes) were collected in the first half of January of 2020 (experiment 1) and 2021 (experiment 2) during the winter pruning from the basal portion of one-year-old canes from ten vines. Vines of table-grape varieties used for the sampling came from a total of four commercial 5–7-year-old vineyards, located in Turi (GPS coordinates: 40.947230, 16.991227; 40.927631, 17.012449) and Rutigliano (40.996696, 17.028576, 40.976901, 17.014358), Bari province, Italy, located less than 10 km from each other and subjected to the same agronomic and phytosanitary practices. Wine-grape varieties and rootstocks were all in an experimental vineyard at the Centro di Ricerca, Sperimentazione e Formazione in Agricoltura (Locorotondo, Bari; 40.755813, 17.341076). Temperature and precipitation data, as well as the agronomic and phytosanitary practices related to table-, wine-grape, and rootstock vineyards, are reported in Tables S1 and S2. Cane fragments of all varieties were stored in plastic bags at 4 °C until use.

The Pch strain CIA43.2 was used for the in planta bioassay. The strain has a laboratory-induced resistance to the benzimidazole fungicide benomyl and is then distinguishable from common wild-type isolates and traceable in planta. The strain was stored in 10% glycerol at −80 °C and grown on malt extract agar supplemented with streptomycin and benomyl [MEASB: 20 g L^{-1} oxoid malt extract (Thermo Fisher Scientific, Milan, Italy);

20 g L^{-1} oxoid agar No. 3 (Thermo Fisher Scientific); 500 mg L^{-1} streptomycin sulfate (Sigma-Aldrich, St. Louis, MO, USA); and 10 mg L^{-1} benomyl (Du Pont de Nemours and Co., Wilmington, DE, USA)] at 25 ± 1 °C in the dark.

Table 1. Features of table- and wine-grape varieties and rootstocks used in this study.

Variety/Rootstock [1]	Berry Color [2]	Cuttings Features [3]		
		Cross-Section		Internode Length (cm)
		Shape	Diameter (mm)	
Table grapes (Experiment 1)				
Allison	Red	Slightly flattened	9.7 ± 0.3	8–12
Flame	Red	Rounded–elliptical	8.6 ± 0.1	9–12
Italia	White	Rounded–elliptical	8.1 ± 0.4	10–12
Red Globe	Red	Rounded	8.3 ± 0.1	8–12
Regal	White	Elliptical	9.2 ± 0.2	9–11
Sable	Black	Rounded–elliptical	10.0 ± 0.1	10–13
Sugar Crisp	White	Elliptical	8.4 ± 0.3	9–12
Sugraone	White	Rounded	8.5 ± 0.3	8–12
Timco	Red	Rounded	10.8 ± 0.3	9–12
Victoria	White	Rounded–elliptical	8.5 ± 0.2	8–10
Wine grapes (Experiment 2)				
Aglianico	Black	Elliptical	9.0 ± 0.2	8–12
Bombino Bianco	White	Rounded	9.0 ± 0.1	9–12
Bombino nero	Black	Rounded	8.7 ± 0.2	12–13
Cabernet Sauvignon	Black	Rounded–elliptical	8.5 ± 0.2	8–11
Malvasia nera di Brindisi	Black	Rounded–elliptical	9.3 ± 0.2	7–11
Merlot	Black	Slightly flattened	9.5 ± 0.3	7–10
Minutolo	White	Rounded	9.4 ± 0.2	8–11
Montepulciano	Black	Elliptical	9.3 ± 0.2	7–10
Moscato bianco	White	Elliptical	9.5 ± 0.2	9–12
Negroamaro D15	Black	Elliptical	9.1 ± 0.2	8–10
Negroamaro D18	Black	Rounded–elliptical	9.6 ± 0.3	8–10
Nero di Troia	Black	Elliptical	8.7 ± 0.2	8–12
Primitivo CDTA19	Black	Slightly flattened	10.0 ± 0.2	8–10
Primitivo UBA 55/A	Black	Slightly flattened	9.2 ± 0.2	8–10
Sangiovese	Black	Elliptical	9.2 ± 0.2	8–11
Susumaniello	Black	Elliptical	8.5 ± 0.2	7–10
Verdeca	White	Rounded–elliptical	9.7 ± 0.2	7–10
Rootstocks (Experiment 2)				
34 E.M.	-	Rounded	7.3 ± 0.3	12–15
1103 Paulsen	-	Rounded	8.2 ± 0.2	12–16
140 Ruggeri	-	Rounded	7.5 ± 0.2	12–15

[1]: D15 and D18 for the wine-grape variety Negroamaro and CDTA19 and UBA 55/A for Primitivo are the two different clones. [2]: Berry color and budbreak period information were obtained from http://catalogoviti.politicheagricole.it (accessed on 20 June 2021). [3]: Cuttings features data were recorded from the one-year-old cuttings used in this study. Figures represent mean diameters ± standard error.

2.2. Assessment of Vessel Features

A piece of ~3 cm in length was sampled at 5–6 cm from the basal part of each cutting. The rhytidome was removed, the cut surface was soaked with a drop of glycerol to avoid tissue flaking, and 50 µm cross-sections were obtained by using a rotary microtome (RM2245, Leica Microsystems, Milan, Italy). Sections were placed in glycerol on a microscope slide, and images were acquired at magnification 12× by using a steromicroscope (Leica MZ 125, Leica Microsystems, Wetzlar, Germany) equipped with a digital camera (Canon Power Shot S70, Tokyo, Japan). For both experiments, fifteen cross-sections were obtained and analyzed for each grape genotype.

Images were first processed with GIMP 2.10.20 (https://www.gimp.org/) to remove areas with no vessels, and then converted into a grayscale image. J-Microvision software ([21]; https://jmicrovision.github.io; last access: 27 April 2022) was calibrated with the image of a micrometric calibration slide acquired at the same magnification as the sections

and used for measuring the xylem area (mm^2), number of vessels, and their area, diameters, and perimeters (µm) in each GIMP image.

2.3. Response to P. chlamydospora Artificial Inoculation

One-node vine cuttings (~15 cm length) from the same cane used for the assessment of vessel sizes were artificially inoculated with the CIA43.2 strain. The basal 2 cm portion of cuttings, soon after cut, was immersed in 100 mL of conidial suspension (10^5 conidia mL^{-1}) and maintained for 24 h at 25 °C. Water was used for mock-inoculated canes as a control. Then, according to the protocol described by Ayres et al. [22], with a few adaptations, cuttings were placed vertically in holes in perforated polystyrene panels floating on 2 L of distilled water in plastic trays (54 cm × 38.5 cm), ensuring that a 2 cm basal portion of each cutting was constantly immersed in water (Figure 1a). Trays were maintained in a glasshouse at 25 ± 3 °C and a relative humidity of 70–80% under natural lighting (photoperiod of about 12 h). We first started with experiment 1 (table-grape varieties), assessing the response to Pch at 30 and 90 days after inoculation (DAIs), so a total of 30 cuttings were used per variety, 15 for each time point. Based on the results observed in experiment 1, in experiment 2 (wine-grape varieties and rootstocks), the response to Pch was assessed only at a single time point (50 DAIs), and 15 replicated cuttings per wine-grape variety and rootstock were used.

At 50 DAIs for wine grapes and rootstocks and 30 and 90 DAIs for table grapes (Figure 1b), single cuttings were longitudinally cut, and the severity of black wood discoloration was assessed using an empiric scale with four classes: 0 = no wood discoloration; 1 = 1–25% discolored wood surface; 2 = 26–50% discolored wood surface; 3 = >51% discolored wood surface (Figure 1c). Two wood fragments were aseptically collected from the surface of each cutting after removing the periderm at ~3 mm in depth and at 3, 6, and 9 cm from the inoculation point, and then placed on MEASB in 90 mm Petri dishes. For wine-grape varieties and rootstocks, the six wood pieces were collected separately from the dorsal and ventral (DV) parts and from the lateral (L) parts of the cuttings. Plates were incubated at 25 ± 1 °C in the dark and observed for the presence of fungal colonies starting after 7 days and up to 20 days. Fungal colonies were identified based on morpho-taxonomic features.

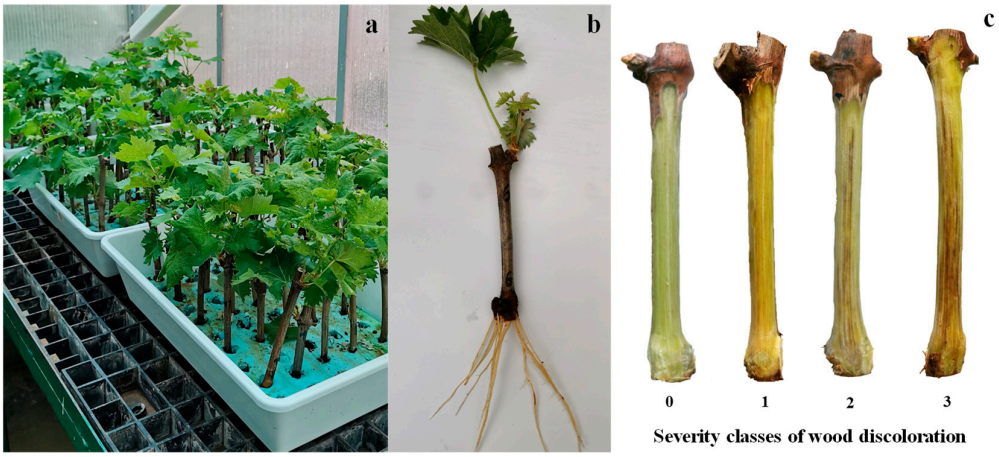

Figure 1. Floating system on perforated polystyrene panels (**a**), detail of a cutting after 50 days of incubation (**b**), and severity classes used to assess wood discoloration (**c**). The values of the classes for the severity of wood discoloration were the following: 0 = no wood discoloration; 1 = 1–25%; 2 = 25–50%; and 3 = >51% discolored wood surface.

2.4. Data Analysis

Experiments on table-grape (experiment 1) and wine-grape varieties and rootstocks (experiment 2) were considered separately because they were carried out at different times.

Mean values of vessel diameters and perimeters were analyzed, considering all vessels in each of the analyzed cross-sections of cuttings. Data on vessel features (diameter,

perimeter, and density) and percentage of Pch isolation were subjected to one-way ANOVA and Tukey's test (p = 0.05) by using Minitab software verision 19.2020.1 (State College, PA, USA).

Vessel density was calculated by dividing the number of vessels (v) by the xylem area (mm^2) of each section. Data on vessel sizes were also classified into five vessel diameter classes (1–30, 31–60, 61–90, 91–120, and >120 µm). Relationships among diameter class, vessel density, and variety were explored using two-way ANOVA, and data were used in correlation-based clustering in Minitab software.

Data were submitted to PCA based on Pearson's correlation coefficient using XLSTAT 2022, a plug-in for Microsoft Excel (www.xlstat.com).

For experiment 2, the relationship between the frequency of Pch isolation in the L and DV parts of the cuttings and the distribution of large vessels (LVs) and small vessels (SVs) within the xylem area was evaluated by correlation analysis. In detail, the isolations were performed separately from the L and DV parts of each cutting identified according to Pouzoulet et al. [23] (Figure S1a). LVs and SVs were discriminated using a threshold value of 60 µm, representing the average vessel diameter of all tested grapevine genotypes (Figure S1b). The portions of each cross-section containing predominantly LVs and SVs were manually defined, and their areas were measured using the J-Microvision software. In detail, for each cross-section, portions with fewer than 15 vessels with a diameter $\geq$ 60 µm (containing predominantly SVs) were first identified, and then the remaining ones were identified as portions containing LVs (Figure S1c). The ratios between the sizes of portions containing predominately LVs or SVs and between the frequencies of Pch isolation from the L or DV portions of the cuttings were calculated and submitted to correlation analysis.

3. Results

3.1. Xylem Vessel Features

Data referring to the anatomy of xylem vessels are reported in Table 2 and Figure S2. The mean values of vessel diameters were calculated from the area data and were therefore highly correlated (r > 0.90).

Regarding the table-grape varieties, 'Red Globe' had the largest vessels (average diameter 70.5 µm), which were not statistically different ($p > 0.05$) from those of 'Sable', 'Italia', and 'Timco' (63.4–65.5 µm). The smallest vessels were recorded in 'Allison' (58.8 µm), which, along with 'Flame', 'Regal', 'Sugar Crisp', 'Sugraone', and 'Victoria' (60.2–61.4 µm), were statistically different ($p \leq 0.05$) from 'Red Globe'. 'Sable' had the highest vessel density (29.7 v/mm^2), which was statistically different only from that of 'Flame' (24.8 v/mm^2). All the other varieties ranged from 28.4 ('Red Globe') to 25.0 v/mm^2 ('Regal') and were not statistically different ($p \leq 0.05$) from 'Sable' and 'Flame'.

As for the wine-grape varieties, 'Verdeca' and 'Merlot' had the largest vessels (diameters of 63.7 and 63.8 µm), followed by 'Minutolo', 'Bombino Nero', 'Montepulciano', and 'Negroamaro' D18 clone (61.1–62.4 µm). The mean diameter values of 'Malvasia nera di Brindisi', 'Cabernet Sauvignon', 'Moscato bianco', 'Bombino bianco', 'Negroamaro' D15, and both clones of 'Primitivo' ranged from 55.2 to 60 µm. The remaining varieties had mean diameters lower than 55 µm, reaching a value of 45.6 µm for 'Nero di Troia'. 'Bombino nero', 'Merlot', 'Minutolo', 'Montepulciano', 'Negroamaro' D18 clone, and 'Verdeca' statistically differed ($p \leq 0.05$) from 'Aglianico', 'Malvasia nera di Brindisi', 'Nero di Troia', 'Sangiovese', and 'Susumaniello'. High vessel densities (25.6 to 28.9 v/mm^2) were recorded for 'Sangiovese', 'Susumaniello', 'Minutolo', 'Verdeca', and 'Primitivo' CdTa19 clone, followed by 'Primitivo' UBA55/A clone, 'Montepulciano', 'Moscato bianco', and 'Malvasia nera di Brindisi' (21–25 v/mm^2), while values for the remaining varieties were in the range of 20–21 v/mm^2. Both 'Sangiovese' and 'Susumaniello' statistically differed from 'Aglianico', 'Bombino bianco', 'Bombino nero', 'Cabernet Sauvignon', 'Malvasia nera di Brindisi', 'Merlot', 'Negroamaro' D15, D18 clone, and 'Nero di Troia'.

As for the rootstocks, the vessels of 1103 Paulsen (diameter 59.9 µm) were statistically larger than those of 34 E.M. (49.7 µm), while 140 Ruggeri showed intermediate size

(54.3 µm). Vessel densities always ranged from 23.5 to 25.5 v/mm^2, with no statistically significant differences.

Table 2. Vessel characteristics for table- and wine-grape varieties and rootstocks.

Variety/Rootstock	Vessel Characteristics [1]			Section Area (mm^2) [1]
	Diameter (µm)	Perimeter (µm)	Density (No./mm^2)	
Table grapes				
Allison	58.8 ± 3.9 b	214.8 ± 16.0 bc	26.3 ± 2.3 ab	20.1 ± 2.9
Flame	60.8 ± 3.4 b	222.8 ± 12.9 abc	24.8 ± 2.2 b	29.9 ± 2.7
Italia	64.2 ± 1.7 ab	220.3 ± 7.2 bc	26.6 ± 2.5 ab	29.5 ± 2.6
Red Globe	70.5 ± 2.9 a	251.0 ± 11.1 a	28.4 ± 1.5 ab	27.6 ± 1.1
Regal	60.1 ± 2.2 b	229.4 ± 7.2 abc	25.0 ± 1.8 ab	25.3 ± 2.1
Sable	65.5 ± 3.9 ab	244.7 ± 14.4 ab	29.7 ± 2.3 a	24.3 ± 1.8
Sugar Crisp	61.9 ± 3.6 b	219.1 ± 12.3 bc	26.0 ± 1.9 ab	18.5 ± 1.3
Sugarone	61.4 ± 2.9 b	224.4 ± 11.5 abc	28.0 ± 1.5 ab	30.7 ± 1.5
Timco	63.4 ± 4.5 ab	241.1 ± 16.9 abc	27.1 ± 2.0 ab	27.5 ± 2.1
Victoria	60.2 ± 2.3 b	212.0 ± 8.5 c	28.4 ± 1.5 ab	28.1 ± 1.2
Wine grapes				
Aglianico	54.8 ± 2.5 de	200.5 ± 10.0 cde	20.0 ± 2.0 e	42.2 ± 1.6
Bombino bianco	55.7 ± 1.9 cde	210.9 ± 7.7 bcd	20.6 ± 2.1 e	33.3 ± 1.2
Bombino nero	61.9 ± 3.3 ab	223.2 ± 11.3 abc	20.8 ± 1.6 e	29.1 ± 1.4
Cabernet Sauvignon	55.8 ± 2.0 cde	224.2 ± 13.8 abc	19.8 ± 3.0 e	46.0 ± 3.4
Malvasia nera di Brindisi	55.2 ± 1.9 de	201.8 ± 7.5 cde	21.2 ± 2.0 de	39.5 ± 2.1
Merlot	63.8 ± 1.4 a	240.9 ± 6.9 a	20.4 ± 1.4 e	50.6 ± 2.8
Minutolo	62.4 ± 2.2 ab	218.4 ± 6.7 abcd	26.6 ± 1.4 abc	31.7 ± 1.3
Montepulciano	61.2 ± 2.0 abc	207.0 ± 24.4 bcde	24.1 ± 1.9 bcde	30.7 ± 1.8
Moscato Bianco	57.9 ± 2.1 bcd	209.1 ± 9.0 bcd	24.1 ± 1.6 bcde	30.8 ± 1.5
Negroamaro D15	57.8 ± 3.8 bcd	220.7 ± 17.9 abcd	20.8 ± 1.6 e	33.3 ± 2.7
Negroamaro D18	61.1 ± 3.4 abc	225.8 ± 12.9 abc	20.5 ± 2.0 e	53.3 ± 3.8
Nero di Troia	45.6 ± 1.4 f	167.9 ± 5.0 f	20.3 ± 2.1 e	30.6 ± 2.0
Primitivo CdTa19	60.0 ± 4.0 abcd	221.2 ± 13.7 abcd	25.1 ± 3.9 abcd	32.3 ± 1.6
Primitivo UBA 55/A	59.0 ± 3.5 abcd	214.7 ± 12.2 bcd	22.0 ± 1.5 cde	31.5 ± 1.6
Sangiovese	54.7 ± 1.3 de	197.2 ± 4.8 de	28.9 ± 2.6 a	33.5 ± 1.5
Susumaniello	50.5 ± 1.8 ef	181.6 ± 6.5 ef	27.2 ± 2.1 ab	21.6 ± 1.0
Verdeca	63.7 ± 2.1 a	229.8 ± 6.9 ab	25.6 ± 2.1 abcd	38.1 ± 2.2
Rootstocks				
34 E.M.	49.7 ± 4.1 b	184.6 ± 15.3 b	25.5 ± 2.5 a	19.9 ± 2.4
1103 Paulsen	59.9 ± 2.6 a	228.9 ± 9.3 a	23.5 ± 1.7 a	29.6 ± 1.9
140 Ruggeri	54.3 ± 2.6 ab	209.1 ± 9.8 a	24.1 ± 2.3 a	24.7 ± 1.7

[1]: Figures are the mean values of all vessels in 15 examined cross-sections per genotype ± the standard error. For each parameter, different letters indicate statistical significance ($p \leq 0.05$) as determined by Tukey's test.

The vessel densities were grouped into five diameter classes (Figure 2). A two-way ANOVA was applied to explore if diameter classes, varieties, and their interactions affected vessel density. Statistical significance of the effects arose for table grapes (variety: F = 4.62 and $p \leq 0.001$; diameter class: F = 648.56 and $p \leq 0.001$; variety × diameter class: F = 3.87 and $p \leq 0.001$) and wine grapes (variety: F = 14.13 and $p \leq 0.001$; diameter class: F = 1873.20 and $p \leq 0.001$; variety × diameter class: F = 9.54 and $p \leq 0.001$). For rootstocks, a significant effect was observed for diameter classes (F = 646.83; $p \leq 0.001$) and the interaction of variety × diameter class (F = 5.20; $p \leq 0.001$) but not for variety (F = 0.28; $p = 0.75$). The statistically significant interactions were explored through correlation-based clustering in Minitab.

For the table-grape varieties, 'Red Globe' was characterized by the highest vessel density in the diameter classes >60 µm coupled to the lowest density in the classes <60 µm. 'Sugraone', 'Sugar Crisp', and 'Italia' showed similar features (similarity = 99.5%) and showed the lowest vessel density (0.8–1.1 v/mm^2) in the class >120 µm. The other varieties were included in another cluster (similarity = 99.7%) showing high vessel density in the classes <60 µm and >120 µm; 'Timco' and 'Sable' showed the highest density (2.4 and 3.2 v/mm^2) of the largest vessels (Figure 2).

The wine-grape varieties were essentially grouped into two main clusters: one cluster included 'Nero di Troia', 'Cabernet Sauvignon', 'Susumaniello', and 'Bombino bianco' (similarity = 98.8%), characterized by higher vessel density in the diameter class <60 µm; and the other cluster included the remaining varieties (similarity = 99.0%) and generally showed a high vessel density in the class >60 µm, with 'Verdeca', 'Minutolo', and 'Montepulciano' showing the highest densities in the classes >90 µm (similarity = 99.5%) and 'Sangiovese' showing high vessel density in the class 61–90 µm (Figure 2).

The three examined rootstocks ('34 E.M.', '1103 Paulsen', and '140 Ruggeri') showed similar behavior (similarity 99.8%), although '1103 Paulsen' showed higher vessel density in the classes >60 µm as compared to '140 Ruggeri' and '34 E.M.' (Figure 2).

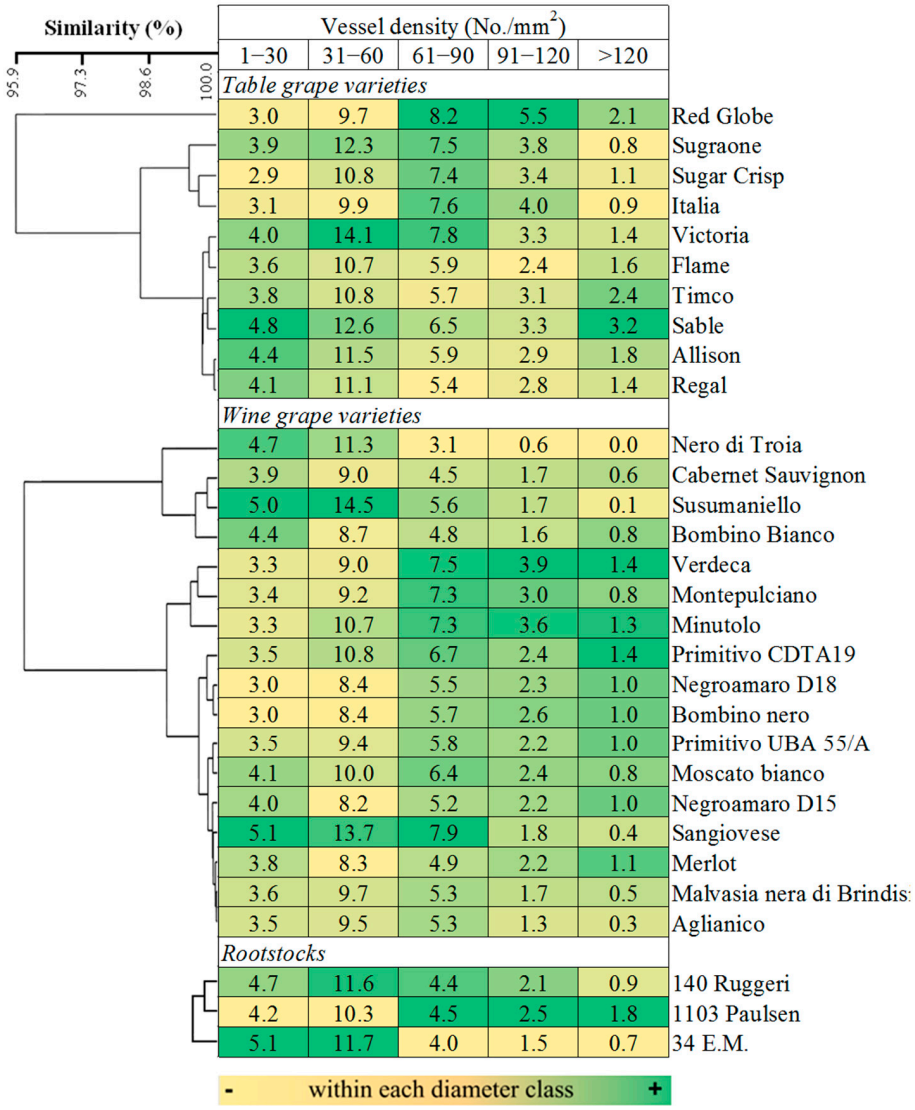

Figure 2. Vessel density of table- and wine-grape varieties and rootstocks in different diameter classes. Each data point corresponds to the mean value obtained by analyzing 15 different cross-sections. Similarity in data distribution among grapevine genotypes was explored through correlation-based clustering in Minitab.

3.2. Isolation of P. chlamydospora on Agar Medium

At 30 and 90 DAIs for table grapes or at 50 DAIs for wine grapes and rootstocks, Pch isolation on agar medium was performed to evaluate the frequency of colonized wood fragments (Figure 3). Colonies of other fungal genera (*Alternaria*, *Cladosporium*, *Penicillium*, etc.) were occasionally and uniformly recorded in all cuttings and sampling times.

For the table-grape varieties, at 30 DAIs, the highest frequency of Pch isolation was recorded for 'Regal' and 'Timco' (both 60%) and 'Allison' (53.3%), which were statistically different from 'Sugraone' (13.3%) and 'Sugar Crisp' (8.3%). At 90 DAIs, the frequency of Pch isolation increased for almost all varieties between 10% ('Regal') and 40% ('Red Globe'), but not for 'Sugraone'. The highest frequency values were recorded for 'Timco' (93.3%), which differed significantly from 'Flame' (43.3%) and 'Victoria' (53.3%), while the lowest values were shown by 'Sugar Crisp' and 'Sugraone' (both 13.3%).

For the wine-grape varieties and rootstocks, at 50 DAIs, the highest frequency of Pch isolations was recorded for 'Montepulciano' (81.1%), which was statistically different from 'Bombino nero', 'Negroamaro' D15 clone, and 'Sangiovese' (51.1%). The other varieties showed intermediate frequency values. The frequency of Pch isolation for the rootstocks was 50.0% for '1103 Paulsen', 46.7% for '34 E.M.', and 33.3% for '140 Ruggeri', and no significant differences were recorded.

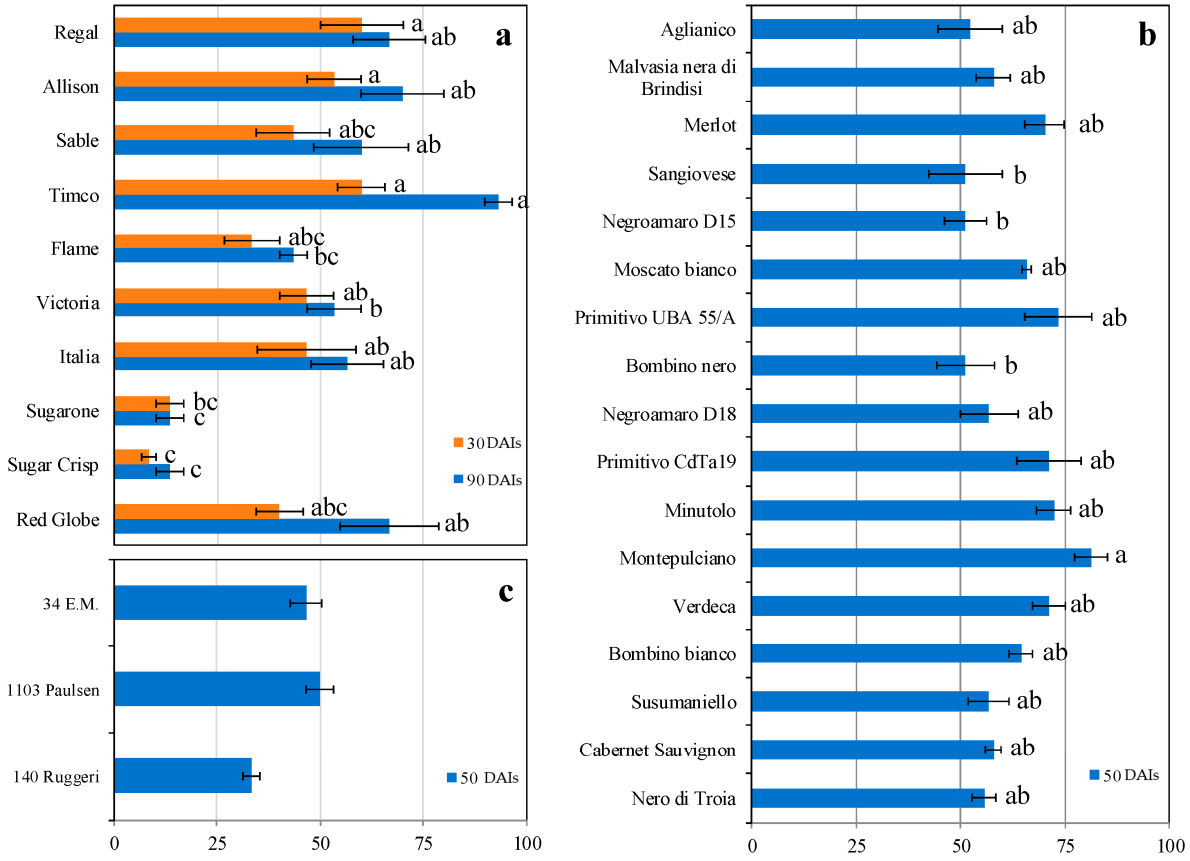

Figure 3. Frequency of isolation on agar media of *Phaeomoniella chlamydospora* from wood fragments of cuttings of table grapes (**a**), wine grapes (**b**), and rootstocks (**c**) artificially inoculated with the fungus. Data are the mean values obtained by the analysis of 15 cuttings per genotype ± the standard error. For each chart, different letters indicate the statistical difference ($p \leq 0.05$) among the genotypes, as determined by Tukey's test performed in Minitab. In (**a**), data were elaborated separately for 30 and 90 DAIs.

The frequency of Pch isolation was highly correlated ($r \geq 0.9$) with the severity of wood discoloration observed on the inoculated cuttings at 90 (table-grape varieties) and 50 (wine-grape varieties and rootstocks) DAIs (Figure 4).

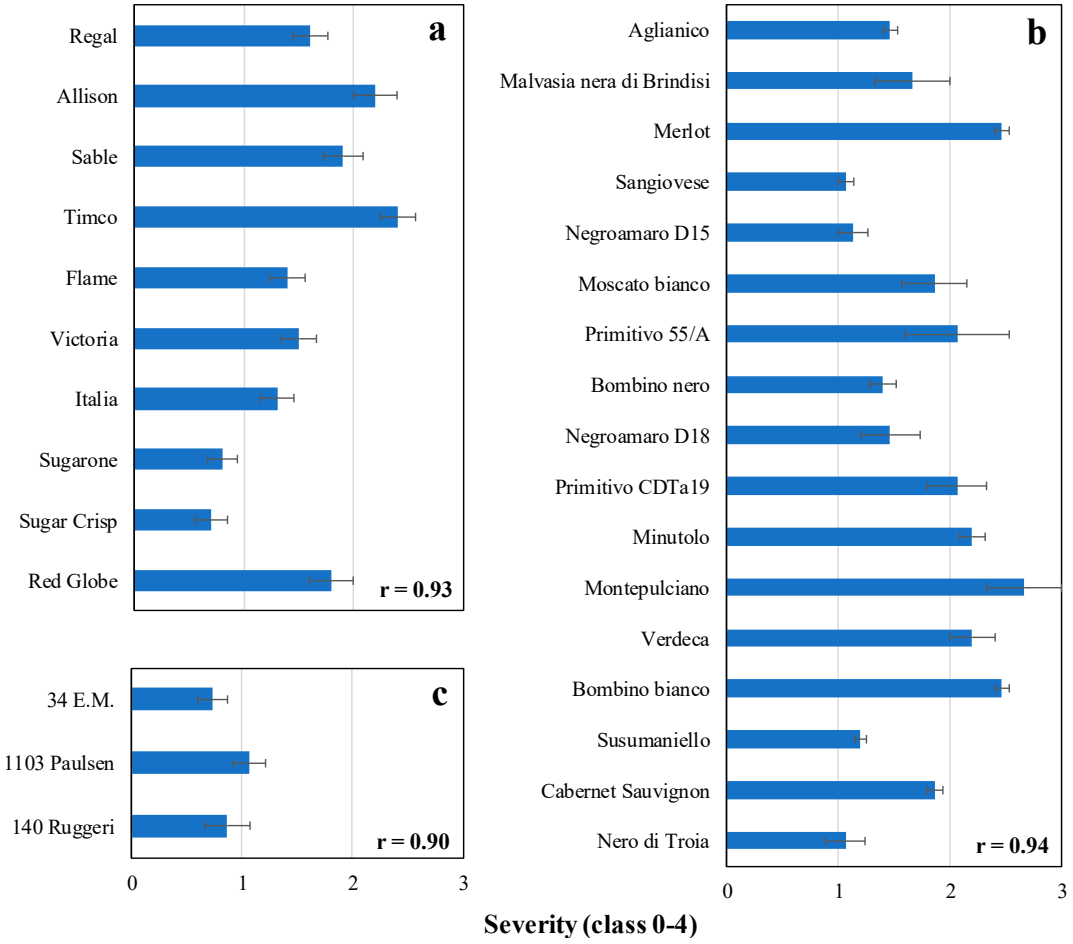

Figure 4. Severity of wood discoloration observed in cuttings of table grapes (**a**), wine grapes (**b**), and rootstocks (**c**) following artificial inoculation with *Phaeomoniella chlamydospora*. The "r value" within each graph corresponds to the correlation between the severity of wood discoloration and *P. chlamydospora* isolation frequency (Figure 3). Data are the mean values obtained by the analysis of 15 cuttings per genotype ± the standard error.

3.3. Vessel Sizes and Response to P. chlamydospora

The relationship between the frequencies of Pch isolation, vessel features (variables), and grape varieties (observations) was investigated by PCA separately for the table- (Figure 5a) and wine-grape varieties (Figure 5b).

For the table-grape varieties, the first two principal components accounted for 80.4% of the total variance (F1: 55.7% and F2: 24.7%). A high positive correlation was observed between the percentages of Pch isolation at 30 and 90 DAIs (E and F). Both variables were positively correlated with average vessel diameter (A), vessel density (B), number of vessels in the class with diameter > 120 µm (C), and average vessel perimeter (D). 'Sable', 'Red Globe', and 'Timco' were clearly grouped due to their large vessels; in addition, 'Timco' was also the variety showing the highest percentage of Pch isolation at both 30 and 90 DAIs. Another group was characterized by smaller vessels and included 'Italia', 'Victoria', 'Flame', 'Allison', and 'Regal'. Finally, 'Sugar Crisp' and 'Sugraone' were well separated from the previous groups due to their small vessels and low frequency of Pch isolation (Figure 5a).

As for the wine-grape varieties, the first two principal components accounted for 79.1% of the total variance data (F1: 60.5% and F2: 18.6%). The percentage of Pch isolation (E) was positively correlated with the average vessel diameter (A), vessel density (B), number of vessels in the class with diameter > 120 µm (C), and average vessel perimeter (D). 'Minutolo', 'Montepulciano', 'Primitivo' CdTa19, and 'Verdeca' formed a distinct group

characterized by a high frequency of Pch isolation and large vessels. Another group included both 'Negroamaro' clones, 'Bombino nero', and 'Merlot', which shared medium–large vessels not strictly related to the percentage of Pch isolation. In the group including 'Aglianico', 'Cabernet Sauvignon', 'Malvasia nera di Brindisi', and 'Moscato bianco', the low frequency of Pch isolation was combined with small–medium-sized vessels. 'Sangiovese' and 'Susumaniello' constituted another distinct group showing a low percentage of Pch isolation, small-sized vessels, and high vessel density (Figure 5b).

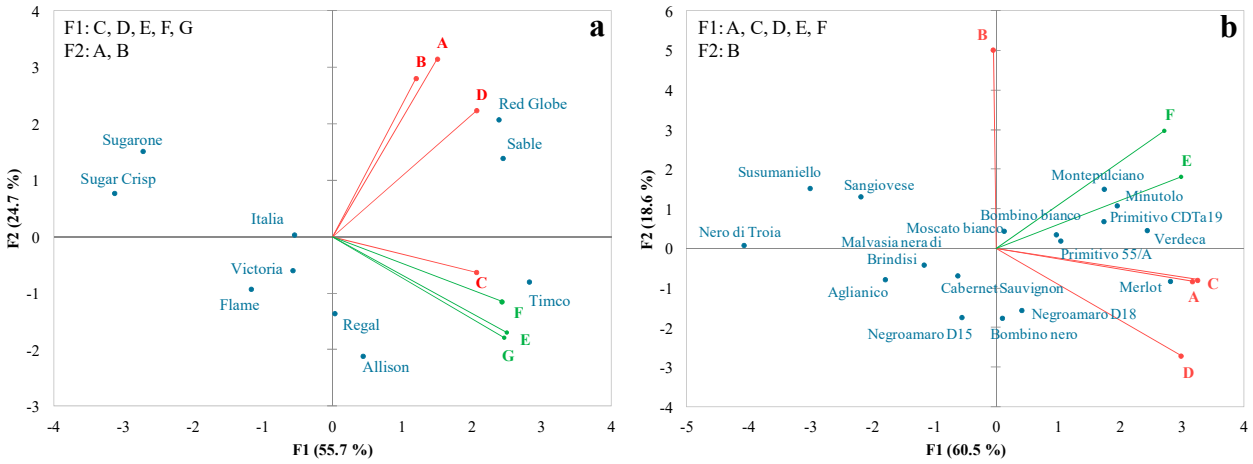

Figure 5. Results of PCA. (**a**) Table grapes; and (**b**) wine grapes. The analyzed variables were vessel average diameter (A); density (B); density of vessels with diameter ≥ 120 µm (C); average perimeter (D); severity of wood discoloration (E); frequency of *Phaeomioniella chlamydospora* isolation on agar media at 30 (table grapes), 50 (wine grapes) (F), and 90 DAIs (G). Variables related to *P. chlamydospora* are in green, and those related to grapevine vessels are in red. For each biplot, the variables correlated with the principal components F1 and F2 are shown at the top left.

We observed more abundant LVs in the DV than in the L part of the cuttings. Therefore, in experiment 2, we performed Pch isolations separately for the L and DV parts of each cutting. Meanwhile, in the cross-sections, we measured the xylem area containing predominantly LVs and SVs that were discriminated by using a threshold diameter size of 60 µm (Figure S1).

The frequency of Pch isolation was very often lower in the L than in the DV parts of the cuttings, with few exceptions (e.g., 'Bombino nero' and 'Minutolo'). The area including LVs was always larger than that including SVs, their ratios being from 95:5 ('Minutolo') to 76:24 ('140 Ruggeri'). The frequency of Pch isolation from the L parts of the cuttings was higher for the grapevine genotypes with high LV:SV area ratios and, conversely, lower for the genotypes with low ratios (Table 3). The positive correlation was corroborated by statistical significance, with r = 0.79.

Table 3. Ratios of average percentages of *Phaeomoniella chlamydospora* (Pch) isolation from lateral (L) and dorsal–ventral (DV) parts of the cuttings and the average proportions of xylem areas with prevalently large vessels (LVs) or small vessels (SVs) in the cross-sections.

Variety/Rootstock	Pch Isolation (%) [1]	Proportion of Xylem Area (%) [1]
Aglianico	43:57	87:13
Bombino bianco	42:58	88:12
Bombino nero	50:50	94:6
Malvasia nera di brindisi	40:60	83:17
Minutolo	51:49	95:5
Montepulciano	47:53	91:9
Moscato bianco	39:61	86:14
Nero di Troia	47:53	83:17

Table 3. *Cont.*

Variety/Rootstock	Pch Isolation (%) [1]	Proportion of Xylem Area (%) [1]
Negroamaro D15	36:64	80:20
Negroamaro D18	43:57	80:20
Primitivo CdTa19	41:59	82:18
Primitivo UBA55/A	38:62	79:21
Sangiovese	47:53	85:15
Verdeca	48:52	90:10
Susumaniello	44:56	81:19
Merlot	39:61	86:14
Cabernet Sauvignon	48:52	88:12
34 E.M.	36:64	69:31
1103 Paulsen	47:53	88:12
140 Ruggeri	37:63	76:24

[1]: The two ratios were positively correlated, with r = 0.79.

4. Discussion

GTDs represent one of the most important threats to viticulture and can be caused by a complex of fungal pathogens, compromising the xylem system in vines [5,6]. Pch is one of the most common GTD-associated pathogens in all viticultural areas worldwide [7].

Studies on xylem morphology are usually addressed in the field of plant hydraulics due to its link to water transport capacity, reflecting plant productivity [24]. However, it was previously reported that the spatial organization of xylem, which represents a niche for vascular pathogens, also plays an important role in resistance/tolerance against microorganisms [5,25,26]. Recent studies on grapevine showed that the size of xylem vessels affects the ability to compartmentalize Pch and, hence, its susceptibility to the pathogen [16,27]. In the present study, the features of xylem vessels from 28 grapevine genotypes were examined, including 10 table grapes, 15 wine grapes, and 3 rootstocks. The possible relationship with the response to Pch by artificial inoculation of grape cuttings was also analyzed. Information regarding table- and wine-grape varieties more tolerant to Pch was provided and can be useful for the development of new genotypes in grapevine-breeding programs. The comparative characterization of xylem vessel features has been mainly studied on wine-grape varieties so far [20,23], and to the best of our knowledge, this is the first time being studied on table-grape varieties.

First, it should be considered that, in addition to genetic traits, environmental conditions (e.g., water availability) can affect xylem vessel size [28,29]. To minimize this variation source, we collected all the canes for each experiment from a single (wine grapes) or a few (table grapes) vineyards.

Vessel density can affect susceptibility to vascular pathogens in grapevine [30], and the density of large xylem vessels was demonstrated to be important for the susceptibility to Pch [16]. Significant differences among the assayed grapevine genotypes were recorded through the analysis of the density of vessels with different sizes. In agreement with previous reports [16], most grapevine varieties predominantly showed xylem vessels with diameters lower than 120 µm. 'Red Globe', 'Sable', and 'Timco' among table grapes and 'Montepulciano', 'Verdeca', and 'Minutolo' among wine grapes showed the highest densities of vessels belonging to the class with diameters > 120 µm. According to the information on the parents (*Vitis* International Variety Catalogue VIVC; www.vivc.de; last access: 20 June 2024), it is not possible to prove the existence of consistent genetic relationships among the analyzed genotypes. However, 'Timco', a cross of 'Red Globe' × 'Princess', was similar to 'Red Globe' for both the presence of LVs and the susceptibility to Pch. Among the wine-grape varieties, 'Susumaniello' shared a high presence of SVs and a lower susceptibility to Pch with 'Sangiovese', the only known parent.

Grapevine susceptibility to Pch was previously assessed in different studies by artificial inoculation and assessment of black wood discoloration [31–34]. The outcomes of the artificial inoculation were assessed by measuring the extension of black wood discoloration

as well as by isolating the Pch strain on the semi-selective medium MEASB from the wood at different distances from the basal cut, since Pch can move systemically through the transport of conidia and/or mycelial fragments into xylem vessels [35,36]. In this study, the artificial inoculation was performed using a Pch strain that had laboratory-induced resistance to benzimidazole fungicides coupled with unaltered fitness compared with its wild type [37]. In this way, all the possible outcomes of natural infections were excluded from the assessments of Pch isolation.

For the table-grape varieties, the surveys were carried out at two time points, 30 and 90 DAIs, yielding similar results; the frequencies of Pch isolation increased at 90 DAIs, essentially due to the isolation of the pathogen from wood fragments collected from more distal positions of cuttings. Among the table-grape varieties, the highest frequencies of Pch isolation were recorded for 'Allison', 'Red Globe', 'Regal', 'Sable', and 'Timco' (up to 90% at 90 DAIs). The frequencies were much lower (up to 13.3%) for 'Sugar Crisp' and 'Sugraone'. The isolation frequencies were remarkably less variable for wine grapes (51.1 to 81.1%), with the highest values (>70%) recorded for a few of them, including 'Minutolo', 'Montepulciano', 'Primitivo', and 'Verdeca'.

Previous studies conducted in vitro and in vivo on the field showed that grapevine genotypes with SVs were less susceptible to vascular fungal pathogens, including Pch, and reported a positive correlation between susceptibility and the density of large vessels [12,16]. In our study, PCA revealed that the frequency of Pch isolation was highly correlated to the density of LVs more than other vessel parameters. In the experiment concerning wine grapes and rootstocks, we could evaluate the frequency of Pch isolation separately from the DV and L portions of the cuttings. It was recently reported that LVs are predominantly distributed in the DV rather than the L parts of canes [23], and this finding was confirmed by our observations of cross-sections for all the examined grapevine genotypes. Additionally, we observed that LVs are aggregated and generally distributed in the external part of the cross-section. Moreover, we observed that the width of the xylem area, including LVs, in the cross-sections was different among the varieties, and we found a positive correlation between the size of the xylem area, including LVs, and the frequency of Pch isolation in the L parts of the cuttings. These findings corroborate previous reports on the relevant role of large vessels in grapevine wood colonization by Pch [12,16,23].

The biplot generated by PCA highlighted differences among the tested grapevine genotypes. The table-grape variety 'Timco' was characterized by an abundance of LVs and a high frequency of Pch isolation from artificially inoculated cuttings. On the contrary, 'Sugar Crisp' and 'Sugraone' were well separated from the other varieties for both their high density of SVs and low frequency of Pch isolation. The wine-grape varieties showed broader differences in terms of density of vessels with different sizes. The group including 'Minutolo', 'Montepulciano', 'Verdeca', and 'Primitivo CdTa19′ was characterized by an abundance of LVs and a high frequency of Pch isolation. Conversely, 'Nero di Troia', 'Sangiovese', and 'Susumaniello' were less susceptible to Pch and presented the highest density of SVs. The relationship between vessel features and Pch colonization in the wood was less obvious in other cases, for instance, 'Red Globe', 'Sable', and 'Italia' among the table grapes and 'Merlot', 'Bombino bianco', and 'Moscato bianco' among the wine grapes. But this was not an unexpected result since numerous plant genotypic traits other than vessel features, such as activation of defense systems, availability of starch reserves, differentiation of anatomical barriers, and cell wall reinforcement, even if they come from different genetic origins (Table S3), may modulate the response to the pathogen [38–40].

Pch is associated with Petri disease in young vines [8], and infected propagation materials from the nursery might be the primary source of the pathogen, resulting in diseased vines at a young age [41]. The three rootstocks analyzed in this study showed only a moderate susceptibility to Pch since the frequency of fungal colonization in the wood fragments was generally lower than that recorded for the *Vitis vinifera* varieties. This finding agrees with the report by Csótó et al. [42], who showed that hybrid *Vitis* cultivars with American or Asian ancestries show higher tolerance to GTDs than *V. vinifera*. Nevertheless,

differences among rootstocks were observed, and '1103 Paulsen' showed a higher density of LVs coupled to a higher frequency of Pch isolation than '34 E.M.' and '140 Ruggeri'. This result confirms our previous findings on the susceptibility of rootstocks to Pch in the nursery (unpublished data) and agrees with Eskalen et al. [14], who reported that '1103 Paulsen' is more susceptible than 3309, 420A, 110R, 5C, Schwarzmann, St. George, and Salt Creek. Although not conclusive, these findings would suggest that the anatomical traits of xylem vessels can play a role in determining the response to Pch also in rootstocks.

5. Conclusions

In the present study, the size and density of xylem vessels from 10 table-grape, 15 wine-grape, and 3 rootstock genotypes were examined. At the same time, their susceptibility to Pch was assessed by the artificial inoculation of one-year-old cuttings. Significant differences among the assayed genotypes were recorded through the analysis of the density of vessels with different sizes as well as by the assessment of black wood discoloration and re-isolation of the fungus on semi-selective agar medium. A comprehensive PCA of the data showed that the frequency of Pch isolation from wood was highly correlated with the density of large vessels. These results corroborate previous reports on the relevant role of the density and size of xylem vessels in wood colonization by Pch in wine grapes. To the best of our knowledge, this is the first time that this finding may also be applied to table-grape varieties. The results obtained improve our knowledge of plant–pathogen interactions and might be useful for the development of new tolerant or resistant grape genotypes for improving integrated crop protection management against GTDs.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae10070750/s1: Figure S1: Workflow used to study the relationships between the frequency of Phaeomioniella chlamydospora isolation and vessel sizes in cross sections of grapevine genotypes: (a) identification of dorsal-ventral (DV) and lateral (L) parts of cuttings for P. chlamydospora isolation on agar media; (b) discrimination of large (LVs, green) and small (SVs, red) vessels using a threshold value 60 µm (means value of diameters for all tested grapevine genotypes; (c) manual recognition of portions of the cross section including predominance of LVs (blue) and SVs (orange); Figure S2: Representative cross sections of table grape (a) and wine grape varieties (b) and rootstocks (c); Table S1: Rain and temperature data obtained from meteorological station close to the vineyards used for the collection of table-grape and wine-grape and rootstocks cuttings; Table S2: Agronomic and protection practices used for table- and wine-grapes and rootstcks vineyards used for cuttings collection; Table S3: Genetic origin of table- and wine-grape varieties and rootstocks used in this study.

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Data Availability Statement: Data are contained within the article or the Supplementary Materials. Datasets are available upon request from the authors.

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