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Susceptibility to flavescence dorée of different *Vitis vinifera* genotypes from north-western Italy

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Abstract

The aim of this work was to evaluate the susceptibility to flavescence dorée (FD) of 12 *Vitis vinifera* cultivars grown in Piedmont, and representative of the wine-making tradition of this area. The experiments were conducted under controlled conditions to ensure constant infection pressure. Test plants were ex vitro potted vines, singly inoculated with four *Scaphoideus titanus* infected by FD-C phytoplasma (FDp), under greenhouse conditions. Vines were tested for FDp at 5 and 8 weeks postinoculation (wpi) and the phytoplasma load was measured in leaves and roots at 8 wpi. Within the 14 *V. vinifera* accessions (belonging to 12 cultivars), three susceptibility clusters were identified. Cultivars within the low susceptibility group showed low phytoplasma loads and low percentages of infected plants, suggesting a tolerant behaviour. To confirm these results, four *Vitis* cultivars, representing extremes of FD susceptibility from low to high, were grafted onto Kober 5BB rootstocks and inoculated with laboratory-infected *S. titanus*, under semi-field conditions. The transmission experiments onto grafted cuttings confirmed that susceptibility to the disease depends on the scion genotype. The data indicated that none of the tested *V. vinifera* genotypes are resistant to FD, although some cultivars with low susceptibility are available, and can be explored for identifying genetic traits involved in disease tolerance/resistance. Moreover, ranking *Vitis* genotypes for their susceptibility to FD is in itself a valuable tool to support vine growers in their decision management, by helping them to choose the most appropriate varieties according to their specific FD epidemiological contexts.

KEYWORDS

flavescence dorée, grapevine cultivar, micropropagated *Vitis*, *Scaphoideus titanus*, susceptibility, tolerance

1 | INTRODUCTION

Flavescence dorée (FD) is a quarantine disease of grapevine that threatens viticulture in several wine-growing areas of Europe (EFSA Panel on Plant Health [PLH], 2014). The disease is caused by phytoplasmas (FDp) belonging to the 16SrV taxonomic group, showing genetic variation at several loci (Arnaud et al., 2007; Rossi et al., 2019). In particular, genetic differences at the *vmpA* locus, encoding a putative variable membrane protein, allow prediction of the ability of the leafhopper *Scaphoideus titanus* to transmit the disease (Malembic-Maher et al., 2020), as the protein is involved in specific molecular interactions with unidentified leafhopper

vector proteins (Arricau-Bouvery et al., 2018). The phytoplasma is transmitted in a persistent propagative manner mainly by the grapevine-feeder leafhopper *S. titanus* (Chuche and Thiéry, 2014), but some polyphagous leafhopper species may also transmit FDp from alternative hosts to grapevine (Malembic-Maher et al., 2020). Infected vines show a range of phytoplasma-specific symptoms, among which bunch shrivelling causes severe yield reduction. Infected plants may die or recover (Maggi et al., 2017), although recovered vines are still prone to reinfections (Rossi et al., 2020). The control of FD relies on different measures. Two to three compulsory insecticide treatments are applied in the infected areas to reduce vector population (Bosco and Mori, 2013). Roguing of infected plants and pruning of vegetation with symptoms during the vegetative season are implemented to minimize the inoculum source, and hot water-treated grafted cuttings are often employed for new plantations and to replace missing plants. Overall, these strategies are costly, affect the health of the environment and of wine growers, and raise concerns on insecticide residues in the final product. Hence, alternative FD management strategies are currently being explored (Oliveira et al., 2019) to support viticulture by stimulating grapevine defences with abiotic (Miliordos et al., 2017; Gutiérrez-Gamboa et al., 2019; reviewed in Oliveira et al., 2019) and biotic (reviewed in Oliveira et al., 2019) elicitors, and interfering with insect vector ability (Gonella et al., 2019) or mating behaviour (reviewed in Oliveira et al., 2019). In north-western Italy (Piedmont), FD is widespread, together with abundant and highly infective vector populations colonizing vineyards and surrounding wild areas (Ripamonti et al., 2020). As wild areas cannot be treated with insecticide for environmental concerns, a challenging landscape management is required. Therefore, FD disease is hard to control and its impact on vineyard productivity is of growing concern. The identification of cultivars with reduced susceptibility to the disease is a critical issue to support sustainable viticulture in Europe. Indeed, preliminary reports suggest that, under field conditions, FD incidence differs in vineyards where different cultivars are grown (Eveillard et al., 2016; Morone et al., 2007). However, evaluating cultivar susceptibility under field conditions is difficult due to uncontrolled environmental conditions affecting *S. titanus* presence and abundance (e.g., presence of abandoned vines as refuges for the insect), infection pressure (presence of symptomless regrowth branches of naturalized *Vitis* plants in abandoned vineyards and woods), accession routes to the vineyards (main wind direction, altitude of abandoned *Vitis* groves with respect to the vineyard, vineyard slope).

Two main mechanisms of plant defence against pathogens are known: resistance (the host's ability to limit pathogen multiplication), and tolerance (the host's ability to reduce the effect of infection on its fitness regardless of the level of pathogen multiplication; Pagán and García-Arenal, 2018). The two may also coexist, and result in low susceptible genotypes. To allow the contemporaneous evaluation of the FD susceptibility of different *Vitis* genotypes, a standardized protocol has been described to inoculate vine plants with infectious *S. titanus* under controlled conditions (Eveillard et al., 2016). This approach exploits ex vitro potted plantlets grown in uniform, semi-controlled conditions, therefore eliminating most of the confusing environmental effects described above, and inoculation with infective insects allowed to acquire FDp under controlled conditions. Indeed, this protocol has been applied to characterize several *Vitis* genotypes for their susceptibility to FD disease, including the most common rootstocks in France (Eveillard et al., 2016), but many other economically relevant cultivars grown in different viticultural areas still need to be characterized. The aim of the present work was to evaluate the susceptibility of several grapevine varieties commonly grown in Piedmont, one of the most important wine production area of Italy. Several of the most well-known local *Vitis* genotypes were analysed, taking into consideration the traditional single varietal wine production strategy of this area. Moreover, to confirm the results obtained with this protocol, four *Vitis* cultivars, representing extremes of the obtained FD susceptibility ranking, were grafted onto Kober 5BB rootstocks and their susceptibility to the disease was assessed upon inoculation with laboratory-infected *S. titanus*, under semi-field conditions. Two local cultivars, Moscato and Brachetto, showed low susceptibility to FD in both the experimental settings, although for the white cultivar Moscato, preliminary results suggested that this behaviour may result from cultivar-specific effects on vector fitness.

2 | MATERIALS AND METHODS

2.1 | Plant material

2.1.1 | Plants from in vitro culture

Woody cuttings were collected from 12 *V. vinifera* varieties, supporting the most economically important wine production in Piedmont. The cuttings were taken in winter from virus-free potted plants of specific clones that are the primary source of registered clones and are grown in the CE.PRE.MA.VI. screenhouse near Alba (CN; http://www.ipsp.cnr.it/projects/ce-pre-ma-vi/?lang=en; Table 1; Table S1). For the cultivar Nebbiolo, which has quite a large genetic variability, two biotypes were collected, Michet (Nebbiolo 71) and Picoutener (Nebbiolo 423). Two additional accessions were also included: Merlot (clone VCR489), from the IPSP grapevine collection field of Grinzane Cavour (CN), and a healthy Barbera plant from an old FD-infected vineyard made of nonclonal Barbera plants (Barbera NC). Micropropagated grapevines of the 14 genotypes were produced. Briefly, axillary buds (obtained by forcing the woody cuttings in water) were surface-sterilized and cultivated in vitro on a modified Murashige and Skoog (1962) medium (Gribaudo et al., 2007) without plant growth regulators; the resulting plantlets were multiplied by repeatedly subculturing apical cuttings (3–4 cm long) on the same medium. After a 4-week rooting and acclimatization period in Jiffy-7 peat pellets, plantlets were transplanted in 14 cm pots and grown under greenhouse condition, 16:8 hr light:dark, 24 ± 2 °C with no humidity control. Sulphur was sprayed

TABLE 1 Survival rate of infectious *Scaphoideus titanus* following a 7-day inoculation access period on either ex vitro plants or grafted cuttings of the different cultivars

Accession name	Cultivar	Clone code	Survival rate (%)	
			Ex vitro plants	Grafted cuttings
Cortese	Cortese	AL CO 2	47.8	–
Arneis	Arneis	AR CVT CN 32	64.8	–
Barbera 84	Barbera	BA AT 84	80.5	66.7
Barbera NC	Barbera	BA NC	74.2	–
Brachetto	Brachetto	BRA CVT 20	67.5	40.3
Dolcetto	Dolcetto	DO CVT 64	76.2	–
Erbaluce	Erbaluce	ER CVT TO 55	70.0	–
Freisa	Freisa	FR CVT 20	43.2	–
Merlot	Merlot	M VCR 489	78.6	25.0
Moscato	Moscato	MO CVT 190	17.5	22.4
Nebbiolo 71	Nebbiolo Michet	NE CVT 71	66.3	–
Nebbiolo 423	Nebbiolo Picoutener	NE CVT 423	70.8	–
Ruchè	Ruchè	RU CVT 10	67.9	–
Timorasso	Timorasso	TIM 18	73.8	–

to control powdery mildew once per month, or at the onset of the first symptoms.

2.1.2 | Grafted cuttings

Cuttings of Barbera 84, Brachetto, Merlot, and Moscato (White Muscat) grafted onto the Kober 5BB rootstock were potted in 80 L pots in a screenhouse made of insect-proof net in March 2018. Fungicide applications (copper- and sulphur-based treatments) followed the conventional calendar, while no insecticide treatments were applied.

2.1.3 | *Vicia faba* and *Avena sativa*

Plants of broad bean (*V. faba* 'Agua-dulce Supersimonia') and oats (*A. sativa*) were grown in pots in a greenhouse, at 24 ± 2 °C, no humidity and no photoperiod control, and used 2 weeks after sowing as host plants to maintain the FDp isolate (*V. faba*) or to rear healthy colonies of the leafhopper *Euscelidius variegatus* (*A. sativa*).

2.2 | Insect rearing

S. titanus laboratory colonies were initiated from 2-year-old canes collected in 2016, 2017, 2018, and 2019 (January to February) in Piedmontese vineyards known to host high populations during the previous seasons. The collected canes were stored in the cold (6 ± 1 °C), and covered with a plastic sheet to avoid egg desiccation until use. Grapevine canes were transferred to plexiglas cages in the greenhouse at 24 ± 2 °C and kept damp by daily water misting. After 4 weeks, 3-week-old *V. faba* plants were introduced into the cage. After egg hatching, plants were replaced every 3 or 4 weeks. Nymphs were reared in these cages in the greenhouse, at 24 ± 2 °C, with no humidity and no photoperiod control. Healthy *E. variegatus* laboratory colonies were routinely maintained under controlled conditions on *A. sativa* plants (Galetto et al., 2009).

2.3 | FDp isolate and acquisition by *S. titanus* under controlled conditions

Flavescence dorée phytoplasma (FD-C, Firrao et al., 2013) isolated in Piedmont was routinely maintained on *V. faba* plants with sequential transmission by *E. variegatus*, as detailed by Galetto et al. (2009). For acquisition by *S. titanus*, fourth or fifth instar nymphs were isolated onto four FD-infected *V. faba* plants for an acquisition access period (AAP) of 2 weeks, and then isolated onto four healthy *V. faba* plants for a 2-week latency period (LP). A representative number of *S. titanus* adults was collected at the end of the LP to assess the presence of FDp in the leafhoppers, and the acquisition efficiency (percentage of PCR-positive individuals out of the sampled ones) was measured for each experiment. Figure 1 details the experimental work-flow.

2.4 | Inoculation of *Vitis* cultivars with flavescence dorée infected *S. titanus*

2.4.1 | Ex vitro plants

At the end of the LP, four infectious *S. titanus* were caged on each grapevine for a 1-week inoculation access period (IAP). At the end

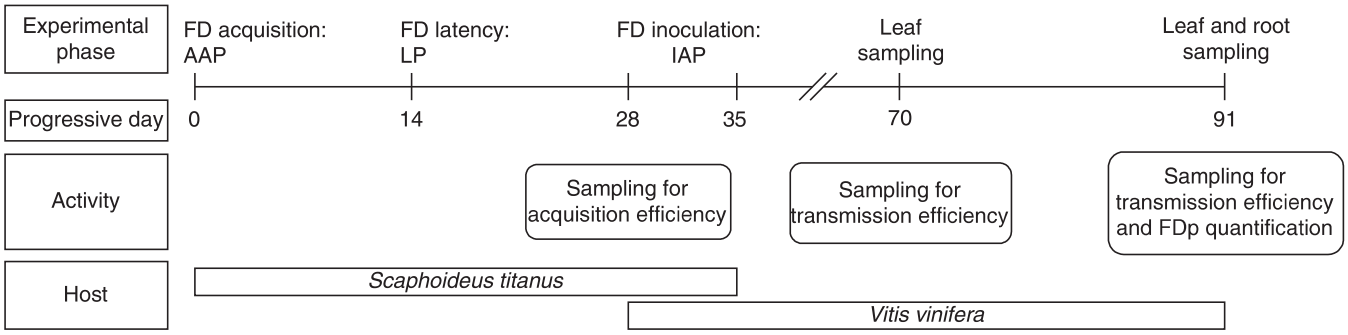

FIGURE 1 Experimental work-flow

of the IAP, dead and alive insects were collected, and stored under ethanol at −20 °C before total DNA extraction and phytoplasma detection. Survival rate of the infectious insects was calculated as the percentage of alive insects out of the total number of insects caged for IAP on each cultivar. Inoculated plants were maintained in a greenhouse at 24 ± 2 °C, without humidity and photoperiod control. Five weeks postinoculation (wpi), leaves of the inoculated grapevines were sampled (three leaves uniformly distributed in the plant) and tested for the presence of FDp. At 8 wpi, both leaves (five) and roots (up to 1 g) were sampled and tested for the presence of FDp. When present, leaves with symptoms were preferred, while symptomless leaves and roots were randomly selected. For each cultivar, the results were expressed as percentage of infected plants, assayed on leaves (5 and 8 wpi), roots (8 wpi), and percentage of infected whole plants (plants with infected leaves and/or roots) at 8 wpi, out of the total inoculated for each experiment. At 8 wpi, the phytoplasma was also quantified in the leaf and root samples of all infected plants of the different cultivars as described below. Four experiments were run, once a year, starting from 2016. Plants of Barbera 84 were included in each inoculation experiment as control. For each experiment, the inoculation efficiency of infective *S. titanus* was calculated as the percentage of infected plants of Barbera 84 out of the total number inoculated within the same year.

2.4.2 | Grafted cuttings

Four cultivars were selected for the validation of their susceptibility to FD, on the basis of their performances following the inoculation under greenhouse conditions: Brachetto, Merlot, Moscato, and Barbera 84; the latter was included as control for inoculation efficiency. At the end of the LP, four infectious *S. titanus* were caged onto a branch of the screenhouse-grown grafted cuttings for a 7-day IAP. A total of 35 plants (10 Barbera 84, 10 Brachetto, 5 Merlot, and 10 Moscato) were used for this experiment, and were inoculated with FD-C infectious *S. titanus*, as described above. Survival rate of the infectious insects was calculated as the percentage of alive insects out of the total number of insects caged for IAP on each cultivar. Leaves with symptoms, when present, were sampled from each plant at the end of the vegetative season of the same year as the inoculation (September), and at the beginning of the following growing season (June). In the absence of symptoms, five leaves uniformly distributed in the plant were randomly collected. Leaf samples from all plants were analysed by real-time PCR to confirm their infectious status (see below).

2.5 | Detection and quantification of FDp

Total DNA was extracted from midribs of five pooled leaf samples of the same plant, both from the plantlets issuing from greenhouse experiments and the grafted cuttings of the semi-field conditions, according to Pelletier et al. (2009). Real-time PCR for diagnosis of FDp on ex vitro plant samples was conducted with primer pairs mapFD-F/R (Pelletier et al., 2009), with a modified reaction mixture consisting of SYBR Green Master Mix (Bio-Rad), primers (300 nmol each), and template (20 ng total DNA). Cycling conditions were an initial denaturation at 95 °C for 30 s, then 45 cycles of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 10 s. A melting curve analysis was run at the end of the PCR cycles to confirm amplicon specificity. Samples derived from grafted cuttings were analysed using a commercial kit (detection kit *Flavescence dorée et Bois Noir*, Multiplex Real-time PCR system; IPADLAB), through a real-time PCR-based assay. An internal positive control (IPC; TaqMan Exogenous Internal Positive Control; Applied Biosystems) was added to the reaction mix in order to confirm the absence of contamination inhibiting the amplification process. FDp relative quantification was performed according to Roggia et al. (2014), and the phytoplasma load was expressed as phytoplasma genome units per nanogram of plant DNA (FD GU/ng plant DNA).

2.6 | Statistical analysis

All statistical analyses were conducted using R software v. 3.6.2 (R Core Team, 2019), using multiple packages, as detailed in File S1. Acquisition efficiencies, defined as the percentage of PCR-positive *S. titanus* at the end of the LP, and transmission efficiencies from *S. titanus* to Barbera, defined as the percentage of positive Barbera plants at the end of the 8 wpi period, were compared among years using Fisher's exact test. The *p* values'

multiple comparisons were adjusted with BH method (Tables S2 and S3). Survival rate of *S. titanus* on the 14 different *Vitis* genotypes at the end of the 7-day inoculation period were tested with a beta-regression model (Table S4; Figure S1). Comparisons among genotypes were computed with estimated marginal means (or least-squares means), followed by Tukey post hoc test with significance level set at 0.05. Hierarchical classification was conducted on four main variables: FD percentage of infection and mean FD load, both in leaves and roots, for every analysed *Vitis* accession. Variables were standardized with *Z*-score method. Euclidean distance and Ward's method were applied as similarity and association methods, respectively. Principal component analysis (PCA) was conducted on the same standardized variables, and represented cultivars were grouped according to clustering results. To test differences between the resulting groups after PCA, PERMANOVA test was used.

3 | RESULTS

3.1 | *S. titanus* infectivity and FDp transmission to the control clone Barbera 84

To test the susceptibility of the different *Vitis* genotypes, one experiment per year was performed from 2016 to 2019 (four experiments). The acquisition efficiencies of *S. titanus* ranged between 64% and 95%, with 2019 efficiency (64%) significantly different from those of the three other experiments (Fisher's exact test for count data: $p = 5.5 \times 10^{-7}$; Table S2). Under these conditions, inoculation efficiencies of *S. titanus* to Barbera 84 plants were 100%, 78.6%, and 83.3% in 2016, 2017, and 2018, respectively, with a mean value of 87.3% (Table S1). As the inoculation efficiency of the 2019 experiment was lower (13.3%) than those of the previous years and not comparable with them, data from this experiment were not included in further statistical analyses (Fisher's exact test for count data: $p = 4.2 \times 10^{-5}$; Table S3).

3.2 | Insect survival rate on different *Vitis* cultivars

Survival of the infectious *S. titanus* at the end of the 7-day IAP on the different *Vitis* cultivars is detailed in Table 1. Overall, more than 64% of the insects survived the 7-day IAP on most of the cultivars. In the case of Freisa and Cortese, *S. titanus* survival rates were 43% and 48%, respectively. Survival rate of the infectious insects on Moscato (17.5%) was significantly lower than that on the other cultivars with the exception of Freisa and Cortese (Table S4; Figure S1). Survival rates of the infectious vectors on the Barbera 84 and Brachetto grafted cuttings were higher than on Merlot and Moscato (Table 1). Survival rate on grafted Merlot was significantly lower ($\chi^2 = 16.017$, $df = 1$, $p = 6.3 \times 10^{-5}$) than that on ex vitro plantlets under greenhouse conditions (Table 1).

3.3 | FD susceptibility of ex vitro *Vitis* genotypes

3.3.1 | Phytoplasma infection

Moscato and Brachetto showed fewer than 30% infected plants (20% and 25%, respectively) at 8 wpi. At the same time, Freisa (36.4%) and Merlot (42.9%) showed an intermediate proportion of infected plants, while Cortese, Dolcetto, Erbaluce, and Timorasso showed about 70% of PCR-positive plants. More than 80% of the inoculated Barbera, both clone 84 and NC, both Nebbiolo 71 and 423, Ruchè, and Arneis plants were infected with FDp at 8 wpi (Table S5; Figure 2). In the 2019 experiment, Ruchè also showed higher infection rates compared to Barbera 84 (23.1% versus 13.3%, respectively). In the same experiment, about 15% and 7% of Merlot and Nebbiolo 423 inoculated plants were infected (Table S1).

Table S5 and Figure 2 also detail the numbers of FDp-positive leaf and root samples at 8 wpi. In particular, in the case of Merlot, FDp was never detected in leaf samples, while it was detected in the root samples of the three infected plants. On the other hand, the phytoplasma was found in the leaves and roots of the infected plants of all the remaining cultivars. Indeed, for most of them (Arneis, Barbera NC, Brachetto, Cortese, Erbaluce, Freisa, and Nebbiolo 71), FDp was detected more frequently in roots than in leaves. In three cultivars (Barbera 84, Moscato, and Ruchè), FDp was present with the same frequency in leaves and roots, while for Dolcetto, Nebbiolo 423, and Timorasso, FDp was more frequently detected in leaves than in roots. In the 2019 experiment, FDp was also more frequently detected in leaves than in roots of Barbera 84 and Nebbiolo 423 plants (Table S1).

Leaf samples were also collected for FDp detection at 5 wpi (Tables S1 and S5). More than 40% of the inoculated plants of Barbera 84, Nebbiolo 423, and Timorasso were already positive for the presence of FDp in their canopy at 5 wpi. At the same time, 5% of the Brachetto and Moscato inoculated plants were already infected, and two of the seven inoculated Merlot plants were positive for the presence of FDp in their canopy. A similar percentage of inoculated plants was already infected for Barbera NC (37.5%), Erbaluce (30%), and Nebbiolo 71 (33.3%). In the case of Merlot, two plants showed infected canopies at 5 wpi (Figure 2).

3.3.2 | Phytoplasma loads

FDp loads were measured in both leaves and roots of the infected plants at 8 wpi. Phytoplasma load was below the quantification threshold for about 40% of the infected plants, irrespective of the cultivar. For the remaining samples, FDp load ranged from 5×10^{-3} to 120 GU/ng plant DNA in leaves, and from 0.5 to 418 in roots (Figure 3). Phytoplasma load was below the quantification threshold for all Erbaluce, Merlot, and Moscato leaf samples of infected plants; for the remaining cultivars, it ranged from 24 to 39 GU/ng plant DNA in Arneis, Barbera NC, Dolcetto,

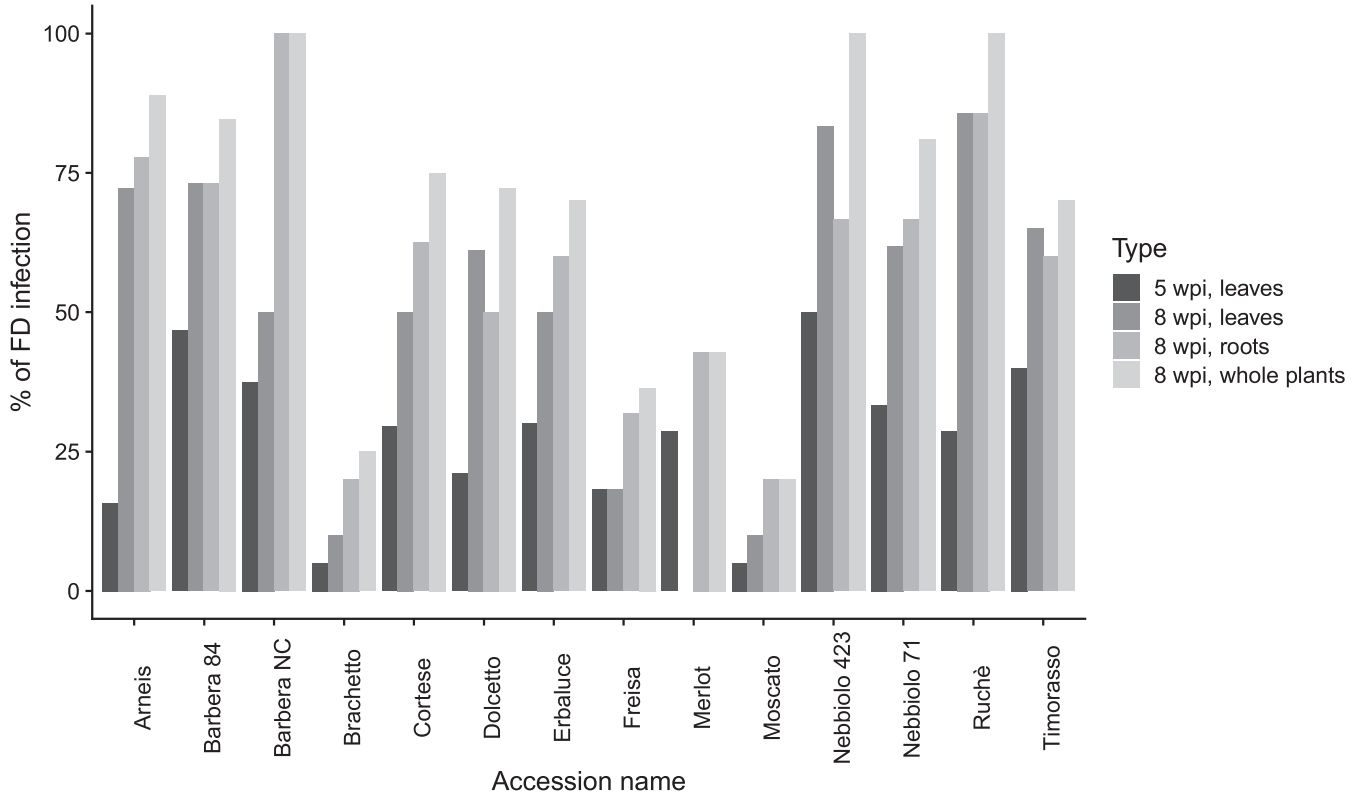

FIGURE 2 Percentages of flavescence dorée (FD)-positive leaf samples at 5 weeks postinfection (wpi), leaf and root samples at 8 wpi, and whole infected plants (showing FDphytoplasma-positive root and/or canopy) at 8 wpi. Percentages were calculated on plants from 2016 to 2018 experimental repeats pooled together

and Nebbiolo 423, and it was above 65 GU/ng plant DNA in the leaves of Barbera 84, Cortese, Freisa, Nebbiolo 71, and Timorasso. In the case of Brachetto, FDp load was measurable in one of the two infected canopies (5.6×10^{-3} GU/ng plant DNA; Figure 3; Table S1). As for the roots of the infected plants, FDp load was below the quantification threshold in all the Merlot and Nebbiolo 423 infected plants, and it was 0.52 in one of the four infected Moscato plants. For the other cultivars, FDp load was above 290 GU/ng plant DNA in Cortese and Barbera 84, and it ranged between 49 and 150 GU/ng plant DNA for Arneis, Barbera NC, Brachetto, Dolcetto, Erbaluce, Freisa, Nebbiolo 71, Ruchè, and Timorasso (Figure 3; Table S1).

Overall, *V. vinifera* genotypes showing low numbers of infected canopies also hosted low phytoplasma loads in their leaves, while the situation was less clear at the root level.

3.4 | Hierarchical classification and PCA

Hierarchical classification of four variables (percentage of infected leaves and roots, phytoplasma load in leaves and roots) grouped Brachetto, Freisa, Merlot, and Moscato separate from Arneis, Barbera NC, Dolcetto, Erbaluce, both Nebbiolo clones, and Timorasso. Barbera 84, Cortese, and Ruchè formed a third cluster (Figure 4a). Using the clustering obtained by hierarchical classification, standardized variables were then explored with a PCA, where the first and second components explained 61.1% and 26.6% of the variability, respectively (Figure 4b). The PCA biplot suggested strong differences between groups, validated through a PERMANOVA test (F ratio = 12.813, $p = 1 \times 10^{-4}$, 9,999 permutations). In particular, the group including Brachetto, Freisa, Merlot, and Moscato showed an indirect relation with the original variable vectors, meaning a general low susceptibility behaviour. The group of Barbera 84, Cortese, and Ruchè shared a general direct relation with the original variables, confirming their susceptibility to the disease. The other cultivars were in an intermediate position, with some extremes in FD percentage of infection (Barbera NC and Nebbiolo 423).

3.5 | Susceptibility to FD of grafted cuttings under semi-field conditions

Following inoculation with FD-infective *S. titanus* on Kober 5BB-grafted vines, 3 out of 10 and 1 out of 5 of the inoculated Barbera 84 and Merlot plants were infected 1 year after inoculation, respectively. No infected plants were recorded among the 10 inoculated Brachetto and Moscato plants.

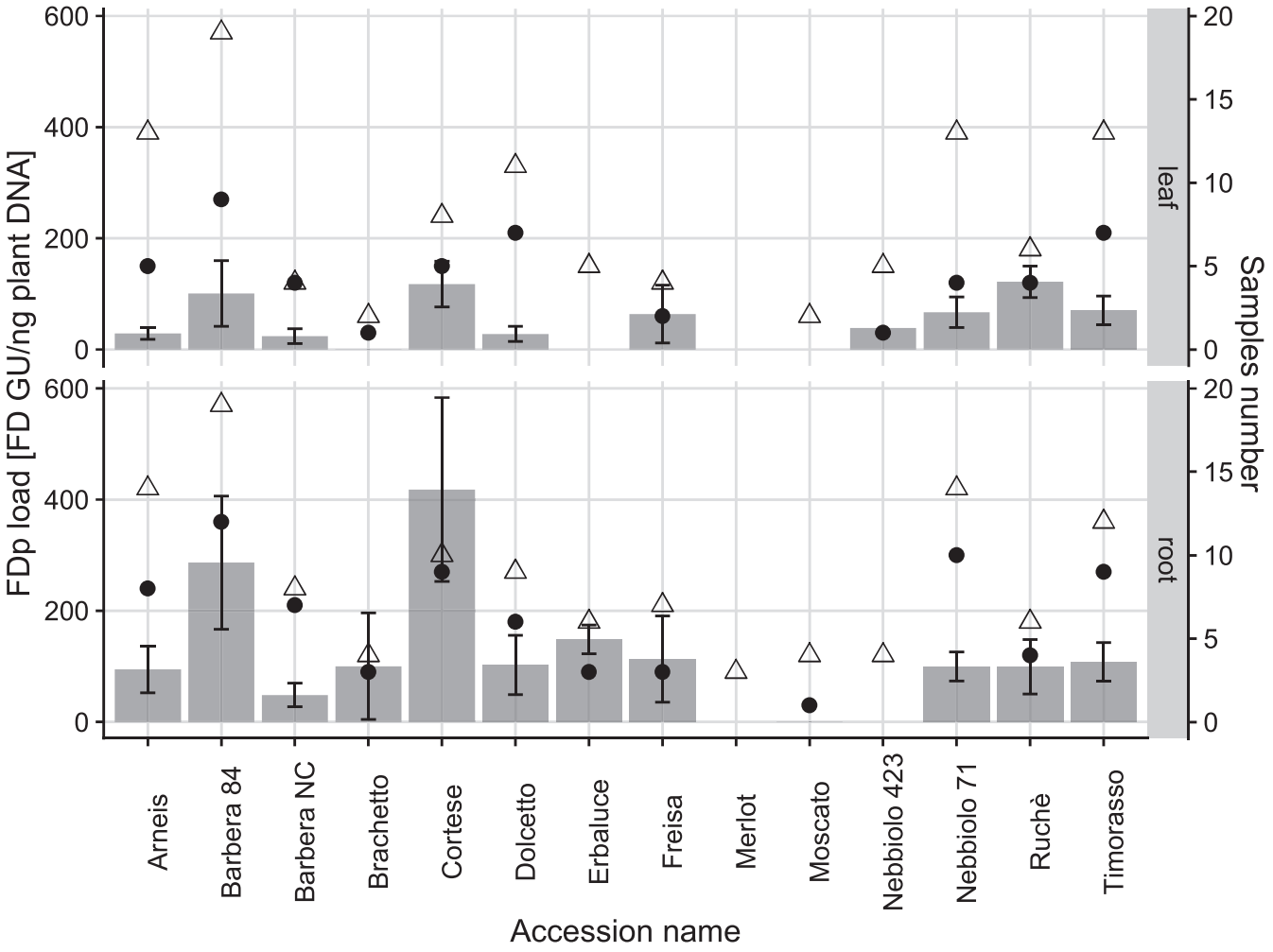

FIGURE 3 Mean flavescence dorée phytoplasma (FDp) load ± *SE* (left axis) in leaf (upper panel) and root (lower panel) samples (grey boxes). Numbers of FDp-positive samples (white triangles) and FDp-quantifiable samples (black circles) are indicated (right axis)

4 | DISCUSSION

Here we evaluated, under controlled conditions, susceptibility to FDp of 14 grapevine accessions. Grafted cuttings of selected cultivars at the extremes of the disease susceptibility range were also inoculated with infective *S. titanus* under semi-field conditions, to further confirm the results. None of the selected *Vitis* accessions was resistant to FDp when tested as plantlets from in vitro culture. In fact, under this experimental condition, all genotypes became infected upon vector inoculation, although in a few instances only one to a few plants were FDp-positive, and a cluster of less susceptible accessions was found, including both noncoloured (Moscato) and coloured (Brachetto, Merlot, and Freisa) cultivars. These cultivars confirmed their low susceptibility to FD upon grafting on Kober 5BB rootstock. Two of the least susceptible cultivars, Moscato and Brachetto, share the presence of aromatic compounds in their leaves and berries, and are classified as aromatic varieties (Mazza et al., 2003; Pollon et al., 2019). Aromatic compounds may play a role in determining tolerance to FD, although the mechanism is not clear. In the case of Moscato, poor FD susceptibility may result from an indirect effect against the vector, as suggested by our preliminary results on the reduced vector survival during the IAP on this cultivar, with reduced chances to transmit the disease. In the case of Brachetto, the low susceptibility may act directly on the phytoplasma, as *S. titanus* survival on this cultivar during the IAP was similar to that on Barbera 84 plants while phytoplasma load was low. Indeed, specific investigations evaluating *S. titanus* fitness and feeding behaviour on the above-mentioned poorly susceptible cultivars are ongoing, to confirm the role of the plant–vector relationship in defining the degree of FD susceptibility of different *Vitis* genotypes. The feeding behaviour of *S. titanus* on Cabernet Sauvignon grapevine has been described by means of electropenetration graph analyses (Chuche et al., 2017), and this technique may indeed be helpful in unveiling possible differences in feeding behaviour of the vector on *Vitis* genotypes with different susceptibilities to FD. Among the cluster of poorly susceptible accessions, FDp was sporadically detected in the canopy of the Merlot plants only at 5 wpi, therefore confirming previous results based on both field and laboratory observations (Eveillard et al., 2016). Nevertheless, under our experimental conditions, phytoplasmas were detected (although below the quantification threshold) in

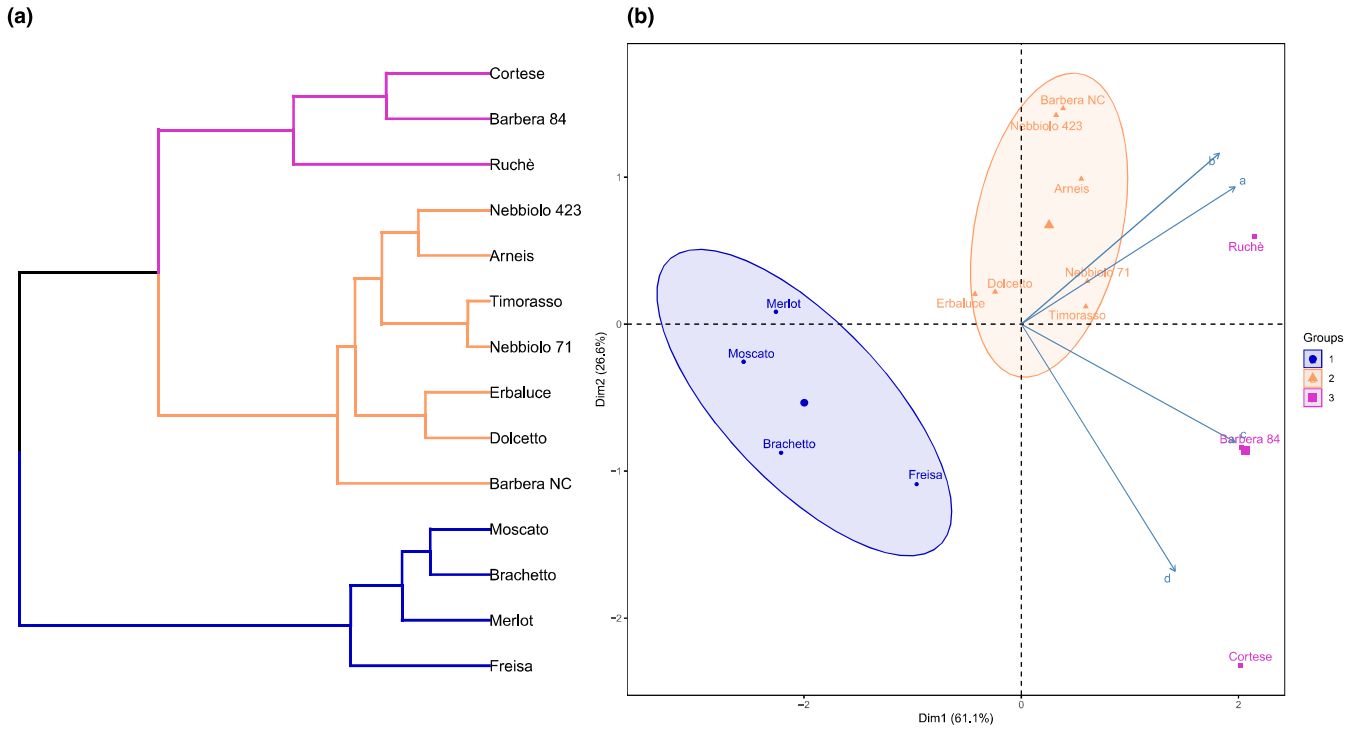

FIGURE 4 Hierarchical classification (a) and principal component analysis (PCA) biplot (b) of percentage of flavescence dorée (FD)-infected plants and phytoplasma loads in leaves and roots of infected grapevines of the different cultivars, at 8 weeks postinoculation. Cluster analyses identified three groups of cultivars characterized by high (purple, top), medium (orange, centre), and low (blue, bottom) susceptibility. Similarity index: Euclidean distance; association method: Ward. In (b), clusters were grouped with ellipses and the centroid of each was represented. PCA vectors represented the original variables: mean FD percentage of infection for leaves (a) and roots (b), and mean FD phytoplasma load for leaves (c) and roots (d). The new condensed PCA variables explained 61.1% (Dim1, *x* axis) and 26.6% (Dim2, *y* axis) of variability [Colour figure can be viewed at wileyonlinelibrary.com]

the roots of three inoculated plants, therefore challenging the hypothesis that FDp diffusion from the inoculation point may be hampered in this cultivar (Eveillard et al., 2016). In other pathosystems involving herbaceous host plants, rapid phytoplasma movement from the inoculation point to the root has been described (Saracco et al., 2005), although the root system has been excluded from the analyses of FDp presence in different grapevine organs over time (Prezelj et al., 2013), probably due to difficulties in retrieving *Vitis* roots under field conditions. This work showed that FDp spreads rapidly to the roots, where it accumulates to higher loads than in the plant canopy. Our results confirm that cultivars with low susceptibility host low phytoplasma loads (Eveillard et al., 2016), at least at the leaf level. Nevertheless, this trend was less evident at the root level. Phytoplasma presence in the roots raises the question of its epidemiological role. Indeed, the mere presence of phytoplasmas in the root system does not imply that phytoplasmas actively multiply there, as they can be translocated from epigeal sites. Also, FDp routes for colonization of the aerial part of the plant from the root have never been explored, and, as the phloem runs basipetally, the roots may represent a dead-end accumulation site, rather than the source of plant reinfection over time. However, phytoplasma movement towards sinks of the canopy through acropetal flow cannot be ruled out. Also, phloematic flux towards the roots may differ in ex vitro plantlets compared to field grapevines. Further investigations are needed to clarify the interactions between roots and phytoplasmas in grapevine.

Cultivars with medium and high susceptibility hosted high FDp loads, but no evident relationship with the number of infected plants was found. Susceptibility of ex vitro Barbera 84 and both Nebbiolo clones was similar, although Nebbiolo is reported as less susceptible to FD under field conditions (Roggia et al., 2014). Ex vitro plantlets already showed symptoms and were infected at 8 wpi (sometimes already at 5 wpi), while grafted plants in the field generally become infected 1 year after the inoculation. A similar situation was described by Eveillard et al. (2016). These discrepancies can be due to the different physiology of herbaceous micropropagated plants and woody grafted ones, as the genotype is identical. Nevertheless, three genotypes with low susceptibility identified in this study maintained their phenotype upon inoculation as grafted rootstocks. The two tested Nebbiolo genotypes behaved similarly, both falling within the intermediate susceptible cluster, despite a genetic difference between them that has been described as based on the specific functional category "responses to pathogens" (Gambino et al., 2017). The Barbera NC fell within the intermediate cluster, separated from the Barbera 84 clone, included in the highly susceptible cluster. The low number of tested Barbera NC plants may prevent robust conclusions, and genetic differences between the two accessions have not been explored. Together with the work of Eveillard et al. (2016), different *Vitis* genotypes have

been tested for FDp susceptibility, and Merlot (of different clones) was the only common genotype included in the studies, and both ranked it among the least susceptible cultivars. However, different survival rates of *S. titanus* on this cultivar were recorded, as in our experiments survival was much higher and comparable to those on the most suitable varieties. From an epidemiological perspective, it is worth noting that FDp load in infected grapevines is not a good predictor of phytoplasma spread by the vector, as infected Moscato is a poor source of inoculum for *S. titanus*, while Brachetto is a better one, thus proving that variety is an important factor, independently from the phytoplasma load (Galetto et al., 2016). Because the diffusion of the disease depends, among other factors, on the probability of a competent vector feeding on an infected plant, vineyards of poorly susceptible varieties have a limited number of infected plants, thus slowing secondary spread of FD (from vine to vine within the vineyard).

In the 2019 experiment, phytoplasma acquisition and transmission efficiencies by *S. titanus* were lower compared to the previous 3 years. The introduction of a control cultivar, Barbera 84 (as suggested by Eveillard et al., 2016), allowed this experiment to be analysed separately. The reasons for this low efficiency are unclear and possibly due to a low phytoplasma load in the source *V. faba* plants.

This work, together with that of Eveillard et al. (2016), shows that none of the explored genotypes is immune to FDp, but some with low susceptibility are available for identifying genetic traits involved in FD tolerance/resistance. This step is crucial for successive traditional or cisgenic breeding applications, and for targeted genome editing through CRISPR/Cas9 technology (Ren et al., 2019). The possibility of ranking *Vitis* genotypes for their susceptibility to this very important disease is a valuable tool to support vine growers in their decision management, by helping them to choose the most appropriate varieties according to their specific FD epidemiological contexts.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

OPEN RESEARCH STATEMENT

The data that support the findings of this study are openly available in the Open Science Framework (OSF) at http://doi.org/10.17605/OSF.IO/UEQ87.

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REFERENCES

Arnaud, G., Malembic-Maher, S., Salar, P., Bonnet, P., Maixner, M., Marcone, C. et al. (2007) Multilocus sequence typing confirms the close genetic interrelatedness of three distinct flavescence dorée phytoplasma strain clusters and group 16SrV phytoplasmas infecting grapevine and alder in Europe. *Applied and Environmental Microbiology*, 73, 4001–4010.

Arricau-Bouvery, N., Duret, S., Dubrana, M.P., Batailler, B., Desque, D., Beven, L. et al. (2018) Variable membrane protein A of flavescence doree phytoplasma binds the midgut perimicrovillar membrane of *Euscelidius variegatus* and promotes adhesion to its epithelial cells. *Applied and Environmental Microbiology*, 84, e02487–e02517.

Bosco, D. & Mori, N. (2013) Flavescence dorée vector control in Italy. *Phytopathogenic Mollicutes*, 3, 40–43.

Chuche, J., Backus, E.A., Thiéry, D. & Sauvion, N. (2017) First finding of a dual-meaning X wave for phloem and xylem fluid ingestion: characterization of *Scaphoideus titanus* (Hemiptera: Cicadellidae) EPG waveforms. *Journal of Insect Physiology*, 102, 50–61.

Chuche, J. & Thiéry, D. (2014) Biology and ecology of the flavescence dorée vector *Scaphoideus titanus*: a review. *Agronomy and Sustainable Development*, 34, 381–403.

EFSA Panel on Plant Health (PLH). (2014) Scientific opinion on pest categorisation of grapevine flavescence dorée. *EFSA Journal*, 12, 3851.

Eveillard, S., Jollard, C., Labroussaa, F., Khalil, D., Perrin, M., Desqué, D. et al. (2016) Contrasting susceptibilities to flavescence dorée in *Vitis vinifera*, rootstocks and wild *Vitis* species. *Frontiers in Plant Science*, 7, 1762.

Firrao, G., Martini, M., Ermacora, P., Loi, N., Torelli, E., Foissac, X. et al. (2013) Genome wide sequence analysis grants unbiased definition of species boundaries in "Candidatus *Phytoplasma*". *Systematic and Applied Microbiology*, 36, 539–548.

Galetto, L., Miliordos, D., Pegoraro, M., Sacco, D., Veratti, F., Marzachì, C. et al. (2016) Acquisition of flavescence dorée phytoplasma by *Scaphoideus titanus* Ball from different grapevine varieties. *International Journal of Molecular Sciences*, 17, 1563.

Galetto, L., Nardi, M., Saracco, P., Bressan, A., Marzachì, C. & Bosco, D. (2009) Variation in vector competency depends on chrysanthemum yellows phytoplasma distribution within *Euscelidius variegatus*. *Entomologia Experimentalis et Applicata*, 131, 200–207.

Gambino, G., Dal Molin, A., Boccacci, P., Minio, A., Chitarra, W., Avanzato, C.G. et al. (2017) Whole-genome sequencing and SNV genotyping of 'Nebbiolo' (*Vitis vinifera* L.) clones. *Scientific Reports*, 7, 17294.

Gonella, E., Tedeschi, R., Crotti, E. & Alma, A. (2019) Multiple guests in a single host: interactions across symbiotic and phytopathogenic bacteria in phloem-feeding vectors – a review. *Entomologia Experimentalis et Applicata*, 167, 171–185.

Gribaudo, I., Ruffa, P., Cuozzo, D., Gambino, G. & Marzachì, C. (2007) Attempts to eliminate phytoplasmas from grapevine clones by tissue culture techniques. *Bulletin of Insectology*, 60, 315–316.

Gutiérrez-Gamboa, G., Romanazzi, G., Garde-Cerdán, T. & Pérez-Álvarez, E.P. (2019) A review of the use of biostimulants in the vineyard for improved grape and wine quality: effects on prevention of grapevine diseases. *Journal of the Science of Food and Agriculture*, 99, 1001–1009.

Maggi, F., Bosco, D., Galetto, L., Palmano, S. & Marzachì, C. (2017) Space-time point pattern analysis of flavescence dorée epidemic in a grapevine field: disease progression and recovery. *Frontiers in Plant Science*, 7, 1987.

Malembic-Maher, S., Desqué, D., Khalil, D., Salar, P., Bergey, B., Danet, J.-L. et al. (2020) When a Palearctic bacterium meets a Nearctic insect vector: genetic and ecological insights into the emergence of the grapevine flavescence dorée epidemics in Europe. *PLoS Pathogens*, 16, e1007967.

Mazza, G., Cascio, P. & Barbieri, E. (2003) Composti volatili liberi e glicoconiugati presenti nelle foglie e negli acini di *Vitis vinifera* cv Moscato Bianco [Free and glycosidically bound volatile compounds from

Vitis vinifera cv. White Muscat leaves and grape berries]. *Rivista di Viticoltura e di Enologia*, 2–3, 57–74.
Miliordos, D., Galetto, L., Ferrari, E., Pegoraro, M., Marzachì, C. & Bosco, D. (2017) Acibenzolar-S-methyl may prevent vector-mediated flavescence dorée phytoplasma transmission, but is ineffective in inducing recovery of infected grapevines. *Pest Management Science*, 73, 534–540.
Morone, C., Boveri, M., Giosuè, S., Gotta, P., Rossi, V., Scapin, I. et al. (2007) Epidemiology of flavescence dorée in vineyards in northwestern Italy. *Phytopathology*, 97, 1422–1427.
Murashige, T. & Skoog, F. (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15, 473–497.
Oliveira, M.J.R.A., Roriz, M., Vasconcelos, M.W., Bertaccini, A. & Carvalho, S.M.P. (2019) Conventional and novel approaches for managing "flavescence dorée" in grapevine: knowledge gaps and future prospects. *Plant Pathology*, 68, 3–17.
Pagán, I. & García-Arenal, F. (2018) Tolerance to plant pathogens: theory and experimental evidence. *International Journal of Molecular Sciences*, 19, 810.
Pelletier, C., Salar, P., Gillet, J., Cloquemin, G., Very, P., Foissac, X. et al. (2009) Triplex real-time PCR assay for sensitive and simultaneous detection of grapevine phytoplasmas of the 16SrV and 16SrXII-A groups with an endogenous analytical control. *Vitis*, 48, 87–95.
Pollon, M., Torchio, F., Giacosa, S., Segade, S.R. & Rolle, L. (2019) Use of density sorting for the selection of aromatic grape berries with different volatile profile. *Food Chemistry*, 276, 562–571.
Prezelj, N., Nikolić, P., Gruden, K., Ravnikar, M. & Dermastia, M. (2013) Spatiotemporal distribution of flavescence dorée phytoplasma in grapevine. *Plant Pathology*, 62, 760–766.
R Core Team. (2019) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
Ren, F., Ren, C., Zhang, Z., Duan, W., Lecourieux, D., Li, S. et al. (2019) Efficiency optimization of CRISPR/Cas9-mediated targeted mutagenesis in grape. *Frontiers in Plant Science*, 10, 612.
Ripamonti, M., Pegoraro, M., Rossi, M., Bodino, N., Beal, D., Panero, L. et al. (2020) Prevalence of flavescence dorée phytoplasma-infected *Scaphoideus titanus* in different vineyard agroecosystems of north-western Italy. *Insects*, 11, 301.
Roggia, C., Caciagli, P., Galetto, L., Pacifico, D., Veratti, F., Bosco, D. et al. (2014) Flavescence dorée phytoplasma titre in field-infected Barbera and Nebbiolo grapevines. *Plant Pathology*, 63, 31–41.
Rossi, M., Mori, N., Lavezzaro, S., Beal, D., Veratti, F., Galetto, L. et al. (2020) Are grapevine plants recovered from flavescence dorée susceptible to new infections by this phytoplasma? *IOBC-WPRS Bulletin*, in press.
Rossi, M., Pegoraro, M., Ripamonti, M., Abba, S., Beal, D., Giraudo, A. et al. (2019) Genetic diversity of flavescence doree phytoplasmas at the vineyard scale. *Applied and Environmental Microbiology*, 85, e03123-18.
Saracco, P., Bosco, D., Veratti, F. & Marzachì, C. (2005) Quantification over time of chrysanthemum yellows phytoplasma (16Sr-I) in leaves and roots of the host plant *Chrysanthemum carinatum* (Schousboe) following inoculation with its insect vector. *Physiological and Molecular Plant Pathology*, 67, 212–219.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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