Susceptibility to Flavescence dorée of different Vitis vinifera genotypes from North-Western Italy

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Running head: Grapevine susceptibility to Flavescence dorée

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Abstract

Aim of the 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 five and eight weeks post-inoculation (wpi) and the phytoplasma load was measured in leaves and roots at eight 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 behavior. To confirm these results, four *Vitis* cultivars, representing extremes of the FD susceptibility, from poorly to highly susceptible, were grafted onto Kober 5BB rootstocks and inoculated with lab-infected *S. titanus*, under semi-field conditions. The transmission experiments onto grafted cuttings confirmed that susceptibility to the disease depends on the scion genotype. These data indicated that none of the tested *V. vinifera* genotypes was resistant to FD, although some poorly susceptible cultivars 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 precious tool to support vine growers in their decision management, by helping them in choosing the most appropriate varieties according to their specific FD epidemiological contexts.

Introduction

Flavescence dorée 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 genetic loci (Arnaud et al., 2007; Rossi et al., 2019). In particular, genetic differences at the *vmp*A 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 & 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 shriveling causes severe yield reduction. Infected plants may die or recover (Caudwell et al., 1987; Maggi et al., 2017), although recovered vines are still prone to re-infections (Rossi et al., in press). The control of Flavescence dorée 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 symptomatic vegetation 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, impact on 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 explored (Oliveira et al., 2019) to support viticulture by stimulating grapevine defences with abiotic (Gutiérrez-Gamboa et al., 2019; Miliordos et al., 2017; reviewed in Oliveira et al., 2019) and biotic (reviewed in Oliveira et al., 2019) elicitors, and interfering with insect vector ability (Galetto et al., submitted; Gonella et al., 2019) or mating behavior (reviewed in Oliveira et al., 2019). In North-western area of Italy (Piedmont Region), FD is widespread, together with abundant and highly infective vector populations colonizing vineyards and wild surrounding 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 (cvs) 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 cvs are grown (Eveillard et al., 2016; Morone et al., 2007). However, evaluating cv 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

asymptomatic re-growth 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 defense 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* 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 inoculated 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. Aim of the present work was to evaluate the susceptibility of several grapevine varieties mostly grown in Piedmont, one of the most important wine production area of Italy. Several of the most well-known local *Vitis* genotypes were analyzed, taking into consideration the traditional single varietal wine production strategy of this area. Moreover, to confirm the results obtained with this protocol, four *Vitis* cvs, 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 lab-infected *S. titanus*, under semi-field conditions. Two local cvs, Moscato and Brachetto, showed low susceptibility to FD in both the experimental settings, although, for the white cv Moscato, preliminary results suggested that this behavior may result from cv-specific effects on vector fitness.

Materials and methods

Plant material

Plants from in vitro culture. Woody cuttings were collected from 12 *Vitis 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 which 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, Supplementary Table S1). For the cv Nebbiolo, which has a quite 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 non-clonal Barbera plants (Barbera NC). Micro-propagated 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 sub-culturing apical cuttings (3-4 cm long) on the same medium. After a four-week rooting and acclimatization period in Jiffy-7® peat pellets, plantlets were transplanted in 14 cm pots and grown under greenhouse condition, 16:8 L:D, 24±2°C with no humidity control. Sulfur was sprayed to control powdery mildew once per month, or at the onset of the first symptoms.

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

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

Insect rearings

Scaphoideus titanus laboratory colonies were initiated from two-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), 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 maintained damp by daily water misting. After four weeks, three-week old broad bean plants were introduced into the cage. After egg hatching, broad bean plants were replaced every three/four weeks. Nymphs were reared in these cages in the greenhouse, at 24±2°C, with no humidity and no photoperiod control. Healthy *Euscelidius variegatus* laboratory colonies were routinely maintained under controlled conditions on oat plants (Galetto et al., 2009).

Flavescence dorée phytoplasma isolate and acquisition by *Scaphoideus titanus* under controlled conditions

Flavescence dorée phytoplasmas (FD-C, Firrao et al., 2013) isolated in Piedmont Region was routinely maintained on *V. faba* plants with sequential transmission by *E. variegatus*, as detailed in Galetto et al. (2009). For acquisition by *S. titanus,* fourth/fifth instar nymphs were isolated onto four FD-infected broad bean plants for an acquisition access period (AAP) of two weeks, and then isolated onto four healthy broad bean plants for two-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.

Inoculation of *Vitis* cvs with Flavescence dorée infected *Scaphoideus titanus*

Ex-vitro plants. At the end of LP, four infectious *S. titanus* were caged on each grapevine, for one-week inoculation access period (IAP). At the end 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 on 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 post inoculation (5 wpi), leaves of the inoculated grapevines were sampled (three leaves uniformly distributed in the plant) and tested for the presence of FDp. Eight weeks post inoculation (8 wpi), both leaves (five) and roots (up to one gram) were sampled and tested for the presence of FDp. When present, symptomatic leaves were preferred, while asymptomatic leaves and roots were randomly selected. For each cv, 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, over the inoculated ones for each experiment. At 8 wpi, the phytoplasma was also quantified in the leaf and root samples of all infected plants of the different cvs 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 of the different experiments. For each experiment, the inoculation efficiency of infective *S. titanus* was calculated as the percentage of infected plants of Barbera 84 over the total number of inoculated ones (same year).

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; this latter was included as control for inoculation efficiency. At the end of LP, four infectious *S. titanus* were caged onto a branch of the screenhouse grown grafted cuttings for a seven-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 on the total number of insects caged for IAP on each cultivar. Symptomatic leaves, when present, were sampled from each plant at the end of the vegetative season of the same year of 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 analyzed by Real-Time PCR to confirm their infectious status (see below).

Detection and quantification of **Flavescence dorée phytoplasma**

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 (Biorad), primers (300 nmol each), template (20 ng of total DNA). Cycling condition were first denaturation at 95°C for 30 s, then 45 cycles composed by a denaturation of 5 s at 95°C followed by 10 s of annealing/extention at 60°C. A melting curve analysis was run at the end of the PCR cycles, to confirm amplicon specificity. Grafted cuttings derived samples were analyzed using a commercial kit (Detection kit *Flavescence dorée et Bois Noir*, Multiplex Real-time PCR system, IPADLAB), through a Real-time PCR-based assay. A Taq Internal Positive Control IPC (TaqMan® Exogenous Internal Positive Control, Applied Biosystems®) was added to the reaction mix, in order to confirm absence of contaminations inhibiting the amplification process. Flavescence dorée phytoplasma relative quantification was performed according to Roggia et al. (Roggia et al., 2014), and the phytoplasma load was expressed as phytoplasma genome units per nanogram of plant DNA (FD GU/ng plant DNA).

Statistical analysis

All statistical analyses were conducted on the R software, version 3.6.2 (R Core Team., 2019), using multiple packages, as detailed in Supplementary File S1. Acquisition efficiencies, defined as the percentage of PCR-positive *S. titanus* at the end of the latency period, 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. p-values' multiple comparisons were adjusted with BH method (Supplementary Table S2 & 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 (Supplementary 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 was conducted on the same standardized variables, and represented cultivars were grouped according to clustering results. To test differences between the resulted groups after PCA, PERMANOVA test was utilized.

Results

Scaphoideus titanus infectivity and Flavescence dorée 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-value = 5.485e-07;

Supplementary Table S2). Under these conditions, inoculation efficiencies of *S. titanus* to Barbera 84 plants were 100%, 78.6%, 83.3% in 2016, 2017, 2018, with a mean value of 87.3% (Supplementary 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 into further statistical analyses (Fisher's Exact Test for Count Data: p-value = 4.173e-05; Supplementary Table S3).

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* cvs 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 first group of cultivars (Supplementary Table S4, Supplementary 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-value = 6.279e-05) than that on *ex vitro* plantlets under greenhouse conditions (Table 1).

Flavescence dorée susceptibility of *ex vitro Vitis* genotypes

***Phytoplasma infection*.** Moscato and Brachetto showed less 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 FD-infected at 8 wpi (Supplementary Table S5). In the 2019 experiment, Ruchè also showed higher infection rates compared to Barbera 84 (23.1% vs 13.3%, respectively). In the same experiment, about 15% and 7% of Merlot and Nebbiolo 423 inoculated plants were infected (Supplementary Table S1).

Supplementary Table S5 also details the numbers of FD phytoplasma-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 cvs. Indeed, for most of them (Arneis, Cortese, Brachetto, Erbaluce, Freisa, Nebbiolo 71 and Barbera NC), FDp was detected more frequently in roots than in leaves. In three cvs (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 (Supplementary Table S1).

Leaf samples were also collected for FDp detection at 5 wpi (Supplementary Table S5& S1). More than 40% of the inoculated plants of the Barbera 84, Nebbiolo 423 and Timorasso cvs 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 Erbaluce (30%), Barbera NC (37,5%) and Nebbiolo 71 (33,3%). In the case of Merlot, two plants showed infected canopies at 5 wpi (Figure 2).

Phytoplasma loads. Flavescence dorée phytoplasma 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 5×10e-03 and 1.2×10e02 GU/ng plant DNA in leaves, and from 5×10e-01 and 4.18×10e02 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 cvs, it ranged from 2.4×10 and 3.9×10 GU/ng plant DNA (Arneis, Barbera

NC, Dolcetto, and Nebbiolo 423), and it was above 6.5×10 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 (5.6×10e-03 GU/ng plant DNA) (Figure 3, Supplementary 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 5.2×10e-01 in one of the four infected Moscato plants. For the other cultivars, FDp load was above 2.9×10e02 GU/ng plant DNA in Cortese and Barbera 84, and it ranged between 4.89×10 and 1.5×10e02 GU/ng plant DNA for Arneis, Barbera NC, Brachetto, Dolcetto, Erbaluce, Freisa, Nebbiolo 71, Ruchè, and Timorasso (Figure 3, Supplementary 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.

Hierarchical Classification and Principal Component Analysis

Hierarchical classification of four variables (percentage of infected leaves and roots, phytoplasma loads in leaves and roots) grouped Brachetto, Freisa, Merlot and Moscato separated 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 (Figure 4b). The PCA biplot suggested strong differences between groups, validated through a PERMANOVA test (*F*-ratio 12.813, p-value 1e-04, 9999 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 behavior. 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).

Susceptibility to Flavescence dorée of grafted cuttings under semi-field conditions

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

Discussion

Here we evaluated, under controlled conditions, susceptibility to Flavescence dorée phytoplasma (FDp) of 14 grapevine accessions. Grafted cuttings of selected cvs at the extremes of the disease susceptibility range were also inoculated with infective *S. titanus* under semi-field condition, to further confirm the results. None of the selected *Vitis* accessions was resistant to FD phytoplasma, when tested as plantlets from *in vitro* culture. In fact, under this experimental condition, all genotypes became infected upon vector inoculation, although in few instances only one to few plants were FD-positive, and a cluster of less susceptible accessions was found, including both non-coloured (Moscato) and coloured cvs (Brachetto, Merlot, and Freisa). These cvs confirmed their low susceptibility to FD upon grafting on Kober 5BB rootstock. Two of the least susceptible cvs, 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 cv, 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 cv during IAP was similar to that on Barbera 84 plants while phytoplasma load was low. Indeed, specific investigations evaluating *S. titanus* fitness and feeding behavior on the above-

mentioned poorly susceptible cvs are ongoing, to confirm the role of the plant/vector relationship in defining the degree of FD susceptibility of different *Vitis* genotypes. The feeding behavior 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 behavior 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 the roots of three inoculated plants, therefore challenging the hypothesis that FDp diffusion from the inoculation point may be hampered in this cv (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 upon 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 poorly susceptible cvs 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 on 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 re-infection 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.

Medium and high susceptible cvs 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 were already symptomatic and infected at 8 wpi (sometimes already at 5 wpi), while grafted plants in the field generally become infected one year after the inoculation. A similar situation was described by Eveillard et al. (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 poorly susceptible genotypes 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 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 high 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. (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 cvs. However, different survival rates of *S. titanus* on this cv were recorded, as in our experiments survival was much higher and comparable to those on 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). Since 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 three years. The introduction of a control cv, Barbera 84 (as suggested by Eveillard et al., 2016), allowed to separately analyze this experiment. The reasons for this low efficiency are unclear and possibly due to a low phytoplasma load in the source broad bean plants.

This work, together with the one of Eveillard et al. (Eveillard et al., 2016), shows that none of the explored genotypes is immune to FDp, but some poorly susceptible ones 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 precious tool to support vine growers in their decision management, by helping them in choosing the most appropriate varieties according to their specific FD epidemiological contexts.

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Data availability statement

Data available on request from the authors [dataset].

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z[dataset], n.d. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supporting information legends

Supplementary File S1. R packages used for data analysis.

Supplementary Table S1. Full database of the Flavescence dorée inoculation experiments onto *ex-vitro* plantlets of the different cultivars.

Supplementary Table S2. Counts and comparisons of *S. titanus* acquisition efficiencies among years.

Supplementary Table S3. Counts and comparisons of inoculation efficiencies onto Barbera 84 (control cultivar) among years.

Supplementary Table S4 and Figure S1. Pairwise comparisons of estimated marginal means of the beta-regression model on *Scaphoideus titanus* survival rates among the different cultivars, and its graphical comparisons.

Supplementary Table S5. Number of Flavescence dorée infected plants of each cultivar at five and eight week post inoculation (wpi) with infective *Scaphoideus titanus*. At five wpi leaves were sampled for the PCR assay; at eight wpi both roots and leaves were sampled and plants were considered infected when either their leaves or roots were positive at the PCR assay. n = total number of analyzed samples.

Figure legends

Figure 1. Experimental work-flow.

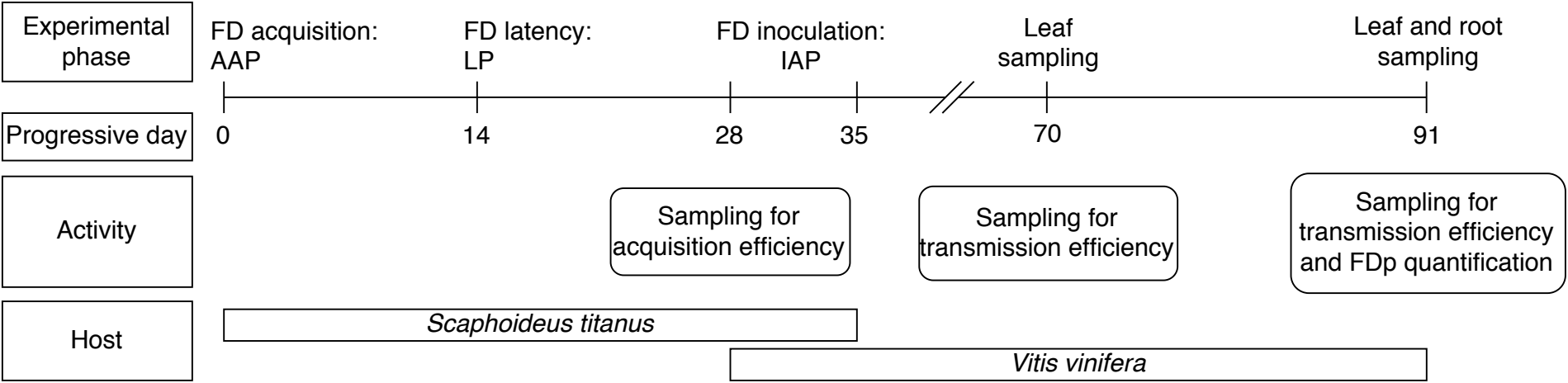
Figure 2. Percentages of FD-positive leaf samples at five weeks post infection (wpi), leaf and root samples at eight wpi, and whole infected plants (showing FDp positive root and/or canopy) at eight wpi. Percentages were calculated on plants from 2016 to 2018 experimental repeats pooled together.

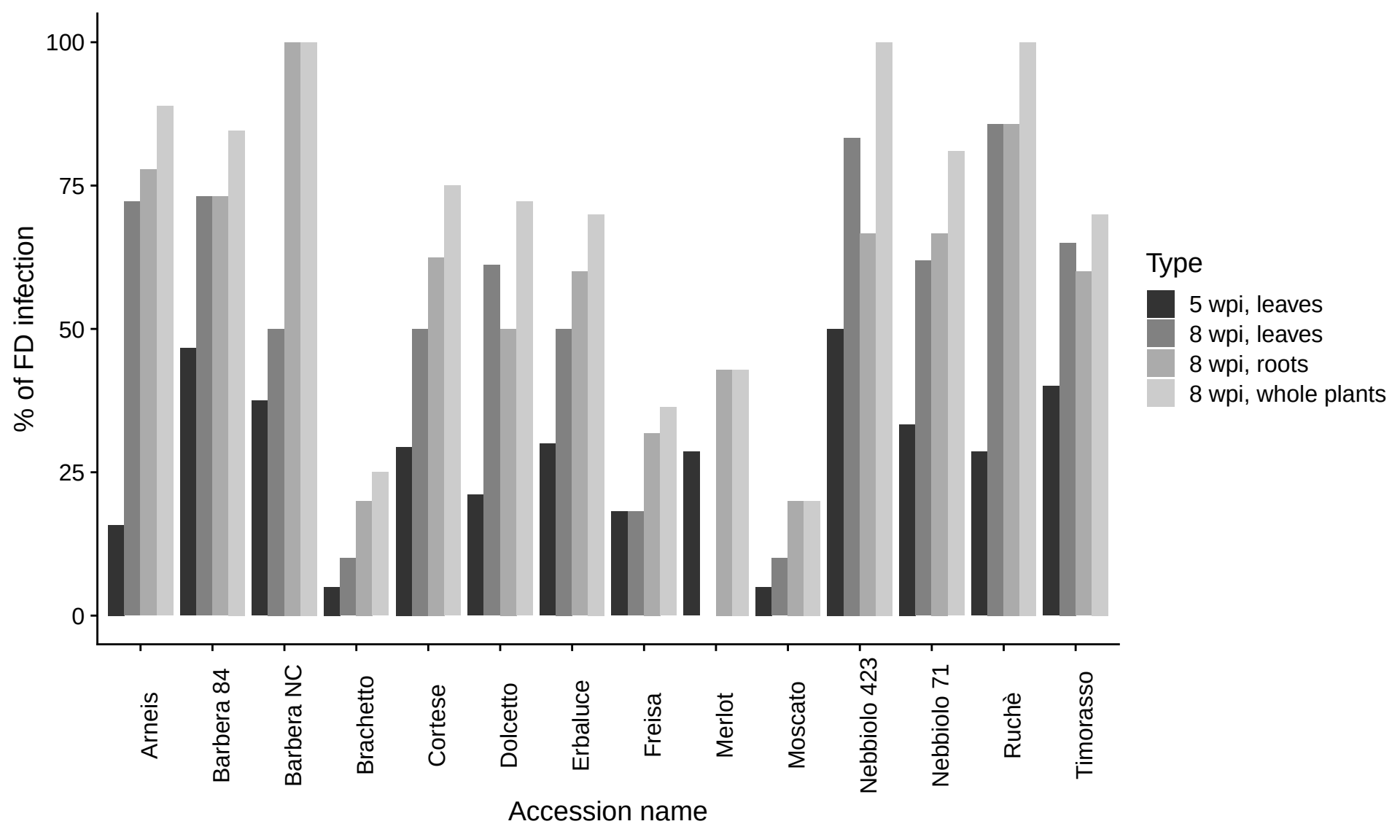
Figure 3. Mean Flavescence dorée phytoplasma load ± standard error (left axis) in leaf (upper panel) and root (lower panel) samples (grey boxes). Numbers of FD-positive samples (white triangles) and FD-quantifiable samples (black circles) are indicated (right axis).

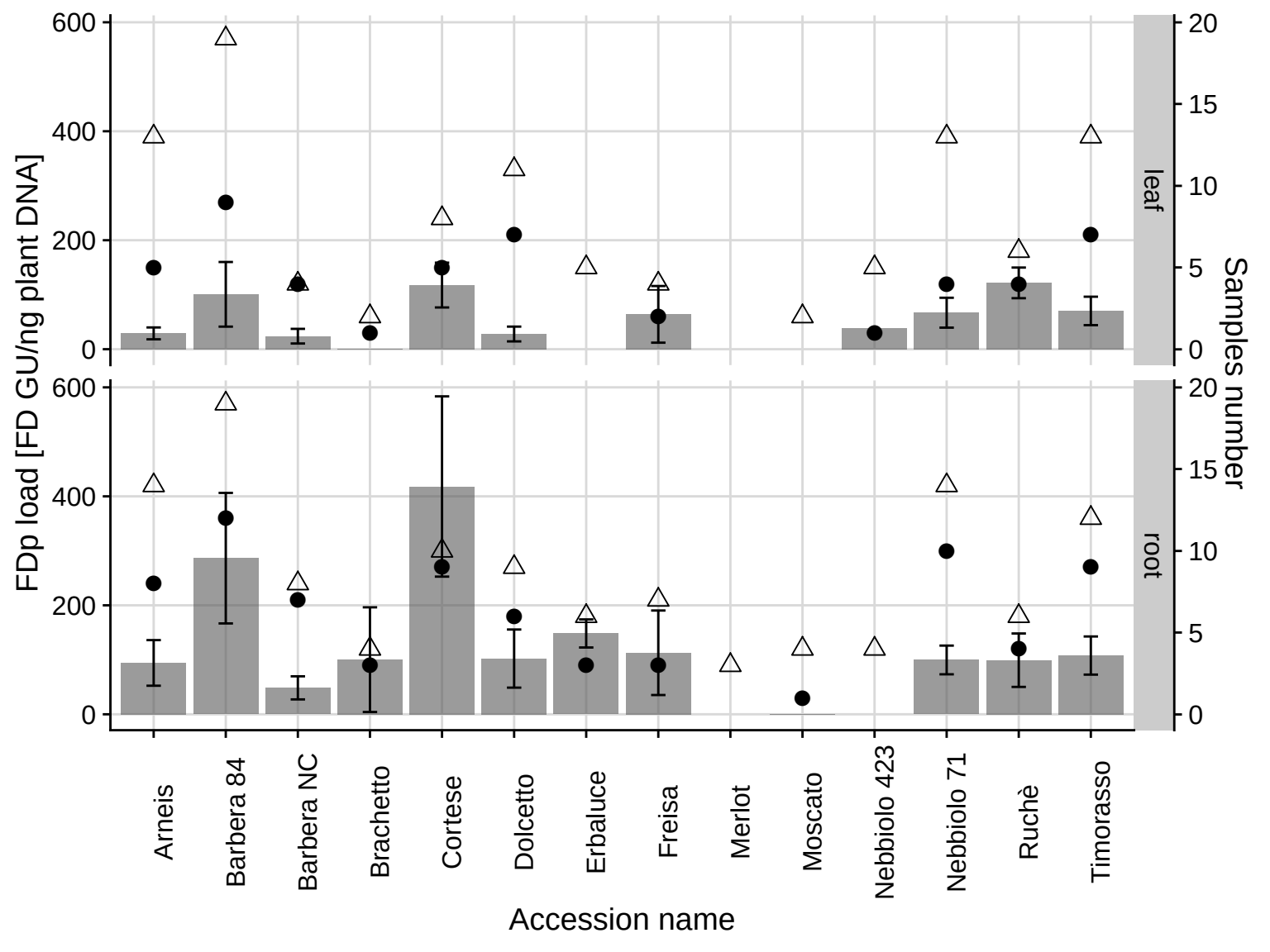
Figure 4. Hierarchical classification (a) and PCA biplot (b) of percentage of Flavescence dorée infected plants and phytoplasma loads in leaves and roots of infected grapevines of the different cultivars, at 8 weeks post inoculation. Cluster analyses identified three groups of cultivars characterized by high (purple, up), medium (orange, center), and low susceptibility (blue, bottom) (Similarity index: Euclidean distance; Association method: Ward). In panel 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 load for leaves (c) and roots (d). The new condensed PCA variables explained 61.1% of variability (Dim1, x-axis) and 26.6% (Dim2, y-axis).

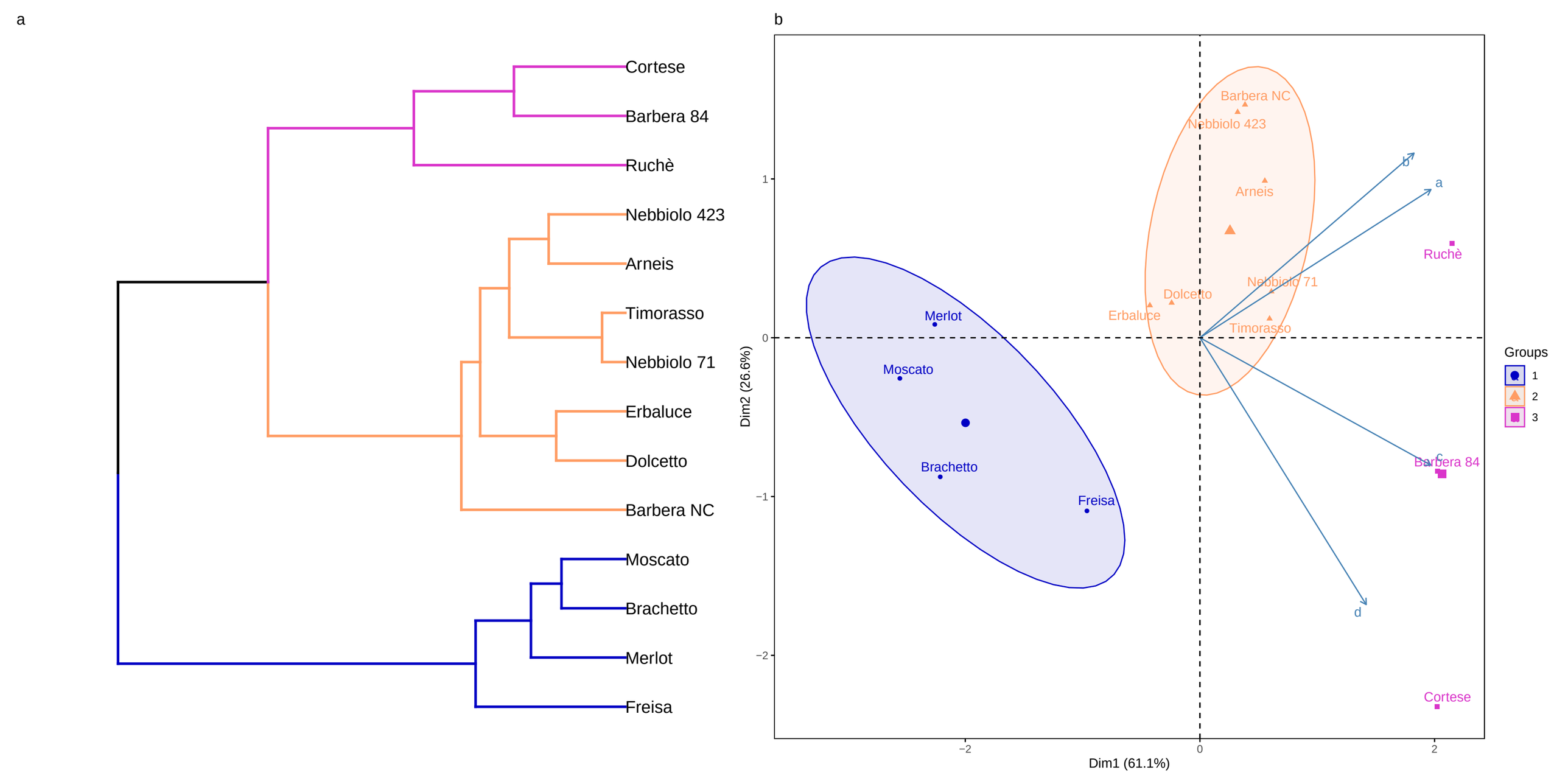
Table 1. Survival rate of infectious *Scaphoideus titanus* following one week inoculation access period on either *ex vitro* plants or grafted cuttings of the different cultivars.

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	-


Experimental phase
FD acquisition: AAP
FD latency: LP
FD inoculation: IAP
Leaf sampling
Leaf and root sampling
Progressive day
0
14
28
35
70
91
Activity
Sampling for acquisition efficiency
Sampling for transmission efficiency
Sampling for transmission efficiency and FDp quantification
Host
Scaphoideus titanus
Vitis vinifera


100
75
50
25
0
% of FD infection
Arneis
Barbera 84
Barbera NC
Brachetto
Cortese
Dolcetto
Erbaluce
Freisa
Merlot
Moscato
Nebbiolo 423
Nebbiolo 71
Ruchè
Timorasso
Accession name
Type
5 wpi, leaves
8 wpi, leaves
8 wpi, roots
8 wpi, whole plants


FDp load [FD GU/ng plant DNA]
Samples number
leaf
root
600
400
200
0
20
15
10
5
0
Arneis
Barbera 84
Barbera NC
Brachetto
Cortese
Dolcetto
Erbaluce
Freisa
Merlot
Moscato
Nebbiolo 423
Nebbiolo 71
Ruchè
Timorasso
Accession name


a
Cortese
Barbera 84
Ruchè
Nebbiolo 423
Arneis
Timorasso
Nebbiolo 71
Erbaluce
Dolcetto
Barbera NC
Moscato
Brachetto
Merlot
Freisa
b
Barbera NC
Nebbiolo 423
Arneis
Nebbiolo 71
Dolcetto
Erbaluce
Timorasso
Merlot
Moscato
Brachetto
Freisa
Ruchè
Barbera 84
Cortese
a
b
c
d
Dim2 (26.6%)
Dim1 (61.1%)
1
0
−1
−2
−2
0
2
Groups
1
2
3