ORIGINAL CONTRIBUTION

Scaphoideus titanus fitness on grapevine varieties with different susceptibility to Flavescence dorée phytoplasma

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Abstract

Scaphoideus titanus (Ball) is a Nearctic leafhopper (Cicadellidae: Deltocephalinae), monophagous on *Vitis* spp., naturalized in European vineyards since the 20th century. Following its introduction and establishment in Europe, *S. titanus* became the main vector of Flavescence dorée phytoplasma (FDp) to grapevine. FDp causes heavy economic losses to viticulture in Europe. The control of the disease mainly relies on insecticide applications against the vector and on the removal of infected plants. The exploitation of plant resistance or tolerance against pathogens and pests can represent a valuable tool for a more sustainable viticulture. The first step in identifying resistance traits in grapevine varieties towards *S. titanus* is the evaluation of insect fitness parameters on different grapevine genotypes. Therefore, in this work, nymph mortality and developmental time, adult survival and prolificacy of *S. titanus* have been studied on three grapevine varieties, characterized by different susceptibility to FD. *Scaphoideus titanus* showed highest fitness when reared on Barbera, whereas the worst performances were recorded on Moscato, with a significant reduction in nymph and adult survivals, a slowed nymph development, and a decreased number of available eggs. On Brachetto, *S. titanus* showed an intermediate level of fitness parameters. Consistently with previous studies on feeding behaviour, Barbera is the most suitable host for the FD-vector. The high suitability of Barbera for *S. titanus* may partly explain the high susceptibility to FD of this variety. On the contrary, the low suitability of Moscato, poorly susceptible to FD, may be due to antibiosis and antixenosis defence mechanisms that act against *S. titanus*.

KEYWORDS
developmental time, leafhopper, prolificacy, survival, vitellogenin, *Vitis vinifera*

1 | INTRODUCTION

Flavescence dorée of grapevine (FD) is a phytoplasma-associated disease transmitted by the Nearctic leafhopper *Scaphoideus titanus* Ball (Schvester et al., 1963). The vector and the disease are present in several European countries (Chuche & Thiéry, 2014; EFSA, 2020) and cause severe damages to viticulture. Control of FD largely relies on preventive measures, such as the use of healthy propagation material, and on compulsory control measures in infected vineyards, that is roguing of infected plants and insecticide treatments against the vector (Bosco

& Mori, 2013). There is an urgent need for developing new, innovative and environmentally friendly control strategies, as the current measures are expensive, have side effects on non-target insects and human health, and have no definitive effect, as FD phytoplasma (FDp) is still spreading (Jarausch et al., 2021).

The best sustainable strategy to minimize damages due to pathogens or parasites is the exploitation of plant resistance or tolerance. For arthropod-borne plant pathogens, plant resistance can exploit its activity against the pathogens or against insect vectors. Resistance against insects occurs when plant structural or chemical traits impair herbivore feeding and thus minimize the amount of herbivore damage experienced by the plant, while tolerance occurs when plant traits reduce the negative effects of herbivore damage on crop yield (Mitchell et al., 2016). Resistance against pathogens is the host ability to limit pathogen multiplication, while tolerance is the host ability to reduce the effect of infection on its fitness regardless of the level of pathogen multiplication (Pagán & García-Arenal, 2018). These definitions of the same terms against two different targets largely overlap. Indeed, resistance deters (insects) or limits (pathogens) the presence of the non-self being, while tolerance is the ability of the plant to live with it.

Resistance or tolerance towards pathogens is directed against viruses (Hashimoto et al., 2016), fungi (downy and powdery mildew: Yu et al., 2012; Riaz et al., 2020) and bacteria, such as grapevine-infecting (Riaz et al., 2018) and olive-infecting *Xylella fastidiosa* (D'Attoma et al., 2019) ones. Resistance or tolerance towards insects is directed against all kinds of phytophagous insects, including vectors of plant pathogens. Within the Hemiptera order, that includes many of the major plant pests (Koch et al., 2016), studies on insect-resistant/tolerant plant genotypes were conducted, among others, on aphids (Bowling et al., 1998; Kordan et al., 2019), planthoppers (*Nilaparvata lugens*, Srinivasan et al., 2015; Kang et al., 2019; Yue et al., 2019), splittlebugs (*Mahanarva fimbriolata*, Orozco-Restrepo et al., 2017) and leafhoppers (Brodbeck et al., 2004; Huang et al., 2020; Miao et al., 2014; Munyaneza & Upton, 2005). Life-cycle parameters, such as mortality/survival, developmental time and prolificacy, are the most common features used to evaluate phytophagous insect performances on plants under variable conditions (e.g. different plant varieties, temperatures and insecticides applications) (Akca et al., 2015; Akkopru et al., 2015; Xu et al., 2016; Zhang et al., 2018). Shorter survival and longer development are direct indicators of impaired fitness (Huang et al., 2020; Jandricic et al., 2010; Krechemer & Foerster, 2017; Munyaneza & Upton, 2005; Orozco-Restrepo et al., 2017). More recently, also feeding behaviour of sap-sucking insects has been widely and effectively applied to estimate plant acceptability, by comparing insect probing behaviour on susceptible and resistant genotypes (Baldin et al., 2018; Kordan et al., 2019; Miao et al., 2014; Ripamonti et al., 2022; Yorozuya, 2017).

In the present work, vitellogenin expression was also included to describe possible differences attributable to the grapevine varieties. In fact, the vitellogenin support during embryo development is well-known, as its abundance in oocytes of most insect species (Sappington, 1998) and its involvement in immunity regulation

(Amdam et al., 2004). Recently, more functions were highlighted for this protein, such as *in planta* immunity suppressor effector (Ji et al., 2021), target for viruses internalization (He et al., 2021), transmission (Huo et al., 2018) and transovarial transmission (Huo et al., 2014), transovarial carrier of immune priming signals (Salmela et al., 2015) and bacterial symbionts (Mao et al., 2020).

With the aim of identifying sources of resistance or tolerance to FD phytoplasmas within the grapevine germplasm, two works have been conducted in France and Italy (Eveillard et al., 2016; Ripamonti et al., 2021). However, only very few information on the resistance/tolerance of the tested grapevine genotypes towards the insect vector *S. titanus* is available. Eveillard et al. (2016) observed lower survival rates of *S. titanus* on Merlot, a tolerant variety, when compared to Cabernet Sauvignon, a susceptible one. Similarly, Ripamonti et al. (2021) observed a lower survival rate of *S. titanus* on Moscato, a FD-tolerant variety. These preliminary hints suggest that the impact of grapevine genotype on vector fitness is worthy of investigation with the aim of understanding if the mechanism underlying the reduced susceptibility to FD acts against the phytoplasma or its vector. Therefore, to gain information on the resistance/tolerance mechanism and to test the hypothesis that different susceptibilities to FDp of some cultivars might be vector-mediated, a study on *S. titanus* fitness on the selected varieties was conducted here. Three *Vitis vinifera* varieties were chosen among the extremes of the FD tolerance range (Ripamonti et al., 2021), considering both their tolerance to FDp and the impact on *S. titanus* short-term survival. In particular, Barbera was chosen because it is highly susceptible to FD and the leafhopper showed high survival on this variety. Brachetto was picked as a tolerant variety for FD with none/little effects on *S. titanus* short-term longevity. Moscato was selected as tolerant to FD with possible negative effects on insect survival (Ripamonti et al., 2021). Some key fitness parameters, such as development time, survival and fecundity, can be regarded as markers of host plant acceptability by the insect. A description of longevity and fecundity of S. *titanus* on Kober 5BB, a hybrid of *Vitis riparia* that is considered its most preferred natural plant host (Bocca et al., 2020), can serve for comparative analyses of *S. titanus* fitness on different cultivars. Besides life cycle and demographic parameters, the feeding behaviour of *S. titanus* on different grapevine genotypes may have major consequences on its ability/efficiency in FDp transmission. This last feature has been addressed (Ripamonti et al., 2022), using the electropenetrography (EPG) technique, showing significant decrease in phloem ingestion for leafhoppers feeding on the two FDp tolerant varieties, Brachetto and Moscato, in comparison with a susceptible one (Barbera), thus suggesting the existence of vector-mediated resistance to FD in different grapevine cultivars.

Here, we describe four key fitness parameters of *S. titanus*: nymphal developmental time and survival, adult longevity, and female prolificacy, together with the expression of vitellogenin mRNA, in insects grown on three grapevine varieties and we discuss the possible implications of these data on grapevine tolerance to FD.

2 | MATERIALS AND METHODS

2.1 | *Scaphoideus titanus* collection and plant rearing

Scaphoideus titanus colony was reared in greenhouse condition, starting from eggs, as described in Ripamonti et al. (2021). Dormant wood with eggs were collected in winter in vineyards of the Piedmont Region where a high number of adult *S. titanus* were captured by yellow sticky traps during the previous summer.

To obtain *S. titanus* nymphs from eggs, broadbean plants were sown and maintained in an insect-proof greenhouse in 2.4 L top-soil, five per pot, watered twice a week. *Vitis vinifera* plants of three different cultivars, Barbera N.—Clone I-AT 84, Brachetto N.—Clone I-CVT 20 and Moscato Bianco B.—Clone I-CVT 190 (Ripamonti et al., 2021), grafted on Kober 5BB, were used for fitness experiments with *S. titanus*. Grapevines were grown in an insect-proof screenhouse, under natural photoperiod, potted in 9.5 L soil (3:1 clay-soil, perlite), watered once a week, regularly sprayed with copper- and sulphur-based fungicides to prevent downy and powdery mildew. One week before the scheduled beginning of every experimental replicate, one potted grafted cutting per variety was moved to the greenhouse for acclimation (T = 22 ± 3°C, photoperiod 16:8 L:D).

2.2 | Fitness tests

All tests were conducted under greenhouse conditions (T = 22 ± 3°C, photoperiod 16:8 L:D). Insect rearing conditions were the same for all the experiments and consisted in a Plexiglas-net cage (36 × 36 × 50 cm) per experiment per cultivar. In each cage, one well-developed shoot of a single cultivar was enclosed.

2.2.1 | Developmental time and nymph survival

The experiments were repeated five times: twice in 2019, twice in 2020 and once in 2021. In 2019, two groups (60 and 99) of *S. titanus* first instar nymphs were collected from the main rearing, one at the beginning of July and one at the beginning of September. In 2020, two groups of 210 first instar nymphs were collected, one at the end of May and one in mid-August. In 2021, a group of 450 first instar *S. titanus* nymphs were collected at the end of April. Nymphs were randomly subdivided and equally assigned to each cultivar treatment. Nymphs were left growing undisturbed and checked every day for the presence of newly emerged adults. As soon as adults emerged, they were collected, and sex and day of emergence were recorded. The total number of nymphs exposed to each cultivar treatment was the same (343 nymphs). The non-emerged nymphs were counted as dead during the development and included in the nymph survival analysis.

2.2.2 | Adult survival

Two batches (33 and 70) of fourth/fifth instar nymphs *S. titanus* were collected from the main rearing, one in summer 2018 and one in summer 2019, and maintained in a separate cage on broadbean until adult emergence. Newly emerged adults were collected twice, the day of the first emergences (day 0) and 2 days later (day 2), subdivided per sex, randomly assigned to one cultivar-treatment; the same ratio of males/females was caged on the three cultivars. Survival status was recorded every day, from the beginning of the test up to the death of the last insect. Dead insects were removed from the cage and discarded.

Three more replicates were conducted, two in 2020 and one in 2021, with 67, 66 and 219 newly emerged adults, respectively. The newly emerged adults derived from nymphs developed on the three previously assigned cultivars.

The overall amount of newly emerged adults per cultivar was 215 for Barbera, 150 for Brachetto and 90 for Moscato, due to the differences in survival of the nymphs on the different cultivars during the development.

2.2.3 | Prolificacy

The main focus of the experiment was the estimate of *S. titanus* female prolificacy, as measured by counting the number of mature eggs and quantifying vitellogenin gene expression. Additional data were also acquired related to nymphal mortality, nymphal developmental time and adult survival.

In 2020, two groups (made of 210 insects each) of first instar *S. titanus* nymphs were collected from the main rearing, one at the end of May and one at mid-August. In 2021, a group of 450 first instar *S. titanus* nymphs were collected at the end of April. The nymphs were randomly subdivided and equally assigned to one cultivar treatment. They were left developing undisturbed until they reached the adult stage. Adults emerged from the same cultivar were grouped per day of emergence on the same cultivar, on a different branch, using a net cage (30 × 10Ø cm). Sex ratio was maintained at 1:1 or with an excess of males in every net cage. In case of absence of males due to protandry (Chuche & Thiéry, 2012) for the 'cultivar-day of emergence' combination, adult males were taken from the main rearing. Insects' survival status was recorded twice per week, during both nymphal and adult stages. At 14, 25 or 35 day post-emergence, females were sampled from every cultivar, their abdomen dissected, and eggs counted. Adults were left undisturbed until the scheduled day of dissection. As already mentioned, different sets of data were obtained in this experiment on the three cultivars, besides the one on female prolificacy: nymphal mortality, developmental time of nymphs and adult survival. Females were taken from the rearing of the adult survival for abdomen dissection and egg counting; they were defined as 'censored' and so considered in the analyses. Males remained

TABLE 1 List of primers used in the work

Application	Primer name	5'-3' sequences	Organism and target gene	Product size	qPCR efficiency	R^2	Melt peak	References
qPCR target gene	St_Vitel_F	AAGAGGAACATGCGCTCCTA	S. titanus vitellogenin	98 nt	96.2%	0.998	78.0°C	Ripamonti et al. (2022)
	St_Vitel_R	TTGCTGGGCAGGAAACTATC						
qPCR reference genes	St_EF1-α_F215	CCATTGACATTGCCCTGTGG	S. titanus elongation factor-1α	110 nt	98.0%	0.999	77.5°C	
	St_EF1-α_R325	CCTGAGAAGTTCCAGTAATCATG						
	St_GST1_F257	CTAAGGATGCCCAGAAACGA	S. titanus glutathione S-transferase	113 nt	94.5%	0.998	79.0°C	
	GST1_R369	TGGCGCTCCTCCAAACATCA						

alone in the net cage were eventually moved to a different branch, if needed, thus considered censored too. Dissections were conducted under a stereomicroscope (Leica S9E, Deutschland), females were CO_2 anaesthetized and then the abdomen dissected with two entomological needles in a 50 μl drop of PBS 1×. Only mature eggs were counted. Eggs were considered mature when elongated and with a curved tapering apex, as explained in Bocca et al. (2020). After egg counting, the single dissected female was collected, transferred in a 1.5 ml Eppendorf tube with the same buffer and stored at −80°C until RNA extraction.

2.3 | RNA extraction and gene expression

Total RNAs were extracted from single *S. titanus* females following dissection and egg count, with Direct-zol RNA Mini Prep kit (Zymo Research), following manufacturer's protocol and including the optional DNAse treatment step. Concentration, purity and quality of extracted RNA samples were analysed in a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific).

Quantitative RT-PCR (qRT-PCR) was used to quantify the possible effect of the cultivar on the expression of female vitellogenin mRNA (Table 1), in order to correlate the vitellogenin expression level with egg count. The vitellogenin sequences were retrieved from *S. titanus* transcriptome (Abbà et al., 2022). For each sample, cDNA was synthesized from total RNA (250 ng) with random hexamers using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The resulting cDNA was used as a template (1 μl) for qPCR in a 10 μl volume mix, containing 1× iTaq Universal Sybr Green Supermix (Bio-Rad) and 300 nM of each primer. All the primer pairs used for qRT-PCR are listed in Table 1.

Samples were run in triplicate in a CFX Connect Real-Time PCR Detection System (Bio-Rad). Cycling conditions were as follows: 95°C for 3 min and 40 cycles at 95°C for 30 s and 60°C for 60 s of annealing/extension step. The specificity of the PCR products was verified by a post-amplification melting curve analysis for all samples. No-template controls (water devoid of cDNA) were included in the plates. Primers targeting glutathione S-transferase and elongation factor-1α were used as housekeeping genes to normalize the cDNA among samples (Table 1). Normalized expression levels (ΔΔCq) of the target gene for each sample was calculated by CFXMaestro™ Software (Bio-Rad). The stability of the expression of reference genes was validated in a multiplate gene study using the M-value (Vandesompele et al., 2002) prov by the above-mentioned software (Supporting Information S1).

2.4 | Statistical analysis

All the statistical analyses were conducted on R software v 4.1.2 (R Core Team, 2021). Raw data were subjected to modifications to enhance readability (packages dplyr, tidyr, stringr: Wickham, 2019, 2020; Wickham et al., 2020). Nymphal overall mortality (Table 2)

was explored through a generalized linear mixed model with a Bernoulli distribution of the binomial family and Cauchit link function (Supporting Information S2; package lme4, Bates et al., 2015). Model performances were analysed with the 'performance' (Lüdecke et al., 2021) and 'DHARMa' packages (Hartig, 2022). Collected data were compared between treatments-cultivars, adding the different experiment replicates as random effect in the GLMM (Supporting Information S2). Summary statistics were reported (Table 2), adding the result of the comparisons based on the estimated marginal means (EMMs; package emmeans, Lenth, 2022) with Tukey's p-value adjustment followed by all-pairwise comparisons (package multcomp, Hothorn et al., 2008) on GLMM estimates.

Developmental time and adult survival/longevity were explored through Kaplan–Meier estimates (Kaplan & Meier, 1958), the former with an inverse transformation function (f[y] = 1- y), in order to emphasize the reaching of adult stage (development: Figure 1; survival: Figure 2). Generalized Additive Cox Models (Hastie & Tibshirani, 2017; Wood, 2011) were applied to the same datasets, with Peto's correction for ties and experimental replicates stratified (Supporting Information S3–S4). Covariate (cultivar and sex) effects were graphically represented by Aalen's Additive Regression Model (Supporting Information S3–S4; package survival, function aareg: Therneau, 2021). GAM results were subjected to EMMs comparisons (Lenth, 2022), with Tukey's p-value adjustment and all-pairwise comparisons (Supporting Information S3–S4; package multcomp, Hothorn et al., 2008). Summary statistics tables were reported (Tables 3 and 4), paired with the result of all-pairwise comparisons.

General Additive Model models were chosen due to non-proportional hazards of the Cox Hazard Ratio Models. All the different Kaplan–Meier estimates applied in the work measured the probability of survival or developmental time from the beginning of the experiment to the verification of the event of interest (death for survival estimates, adult emergence for developmental time). Individual adult females were censored (sampled when still alive) only in the prolificacy test, but the chosen model taken this possibility into account. Censored individuals were represented with an 'X' (Figure 2), and the total number was reported in brackets in the risk table (Figure 2).

The overall timespan of *S. titanus* presence as 'non-egg' stage was graphically reported in Figure 3, joining the Kaplan–Meier curves resulted from nymphal development and adult survival (Figures 1 and 2). Area drawn by joint development and survival was calculated as approximated integral of every function (describing the interaction Cultivar×Sex) using trapezoidal rule integration (Table 5; package pracma, 'trapz' function, Borchers, 2022).

A Linear Mixed Model (LMM) was applied on number of mature eggs (package nlme, Pinheiro et al., 2022), adding cultivar and day of dissection as fixed effects, and experimental replicate as random effect (Supporting Information S5). LMM performances were analysed with the 'performance' package, without raising any concern on the
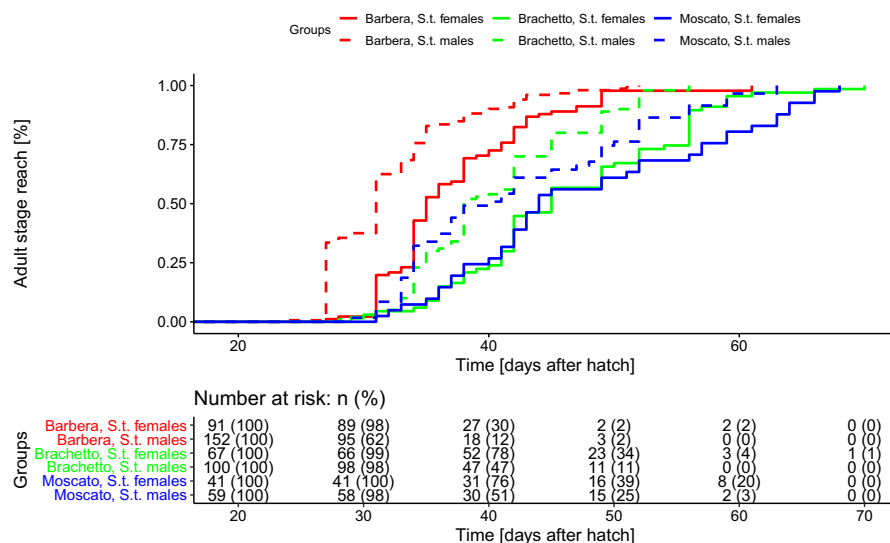
TABLE 2 Total number of dead nymphs and emerged adults in the experiments

Cultivar	Status	Sex	n	Pairwise comparisons
Barbera	Dead	NA	100	a
	Emerged	Female	91	
		Male	152	
Brachetto	Dead	NA	176	b
	Emerged	Female	67	
		Male	100	
Moscato	Dead	NA	243	c
	Emerged	Female	41	
		Male	59	

Note: Comparisons between rows (dead nymphs per cultivar) were conducted after a GLMM with Bernoulli distribution and Cauchit link function. Post hoc comparisons were conducted with least-square means method and Tukey's method for p-value adjustment, at significance level as 0.05 and 95% confidence intervals, and represented by letters for every specific group. GLMM and post hoc details are reported in Supporting Information S2.

FIGURE 1 Developmental time curves (Kaplan–Meier estimates, inverted) for *Scaphoideus titanus* nymphs reared on three grapevine cultivars. *Scaphoideus titanus* sex is represented by line type (solid for females, dashed for males), while grapevine cultivar is represented by line colour (red for Barbera, green for Brachetto, blue for Moscato). Risk table is also reported, with number of residual nymphs in absolute number and percentage in brackets [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 2 Survival curves (Kaplan–Meier estimates) for adult *Scaphoideus titanus* reared on three grapevine cultivars. *Scaphoideus titanus* sex is represented by line type (solid for females, dashed for males), while grapevine cultivar is represented by line colour (red for Barbera, green for Brachetto, blue for Moscato). Risk table is also reported, with number of residual alive adults in absolute number and censored individuals in brackets [Colour figure can be viewed at wileyonlinelibrary.com]
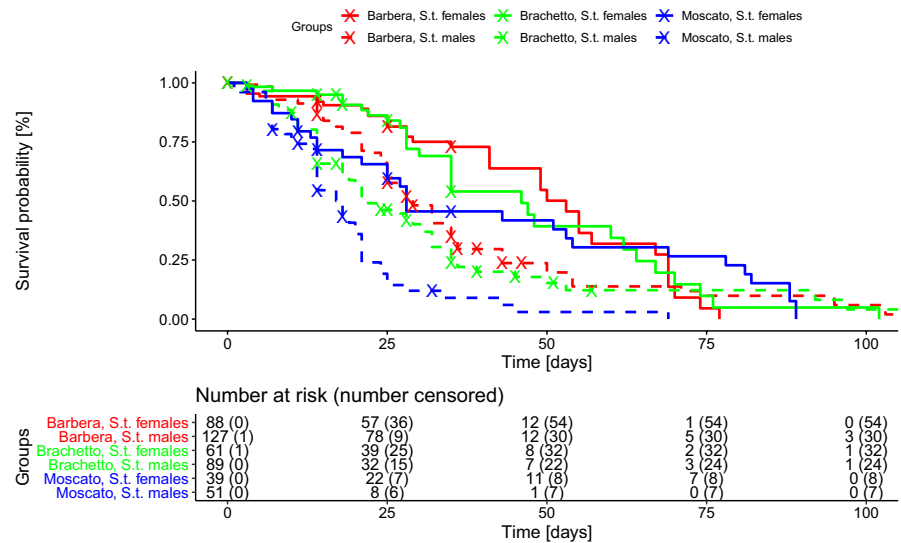
TABLE 3 Summary statistics for *S. titanus* developmental time on three grapevine cultivars, measured as days between egg hatching and adult emergence

Cultivar	Sex	*n*	Mean [days]	Median [days]	IQR [days]	Q1 [days]	Q3 [days]	Pairwise comparisons
Barbera	Female	91	37.4	35	7	34	41	d
Barbera	Male	152	32.0	31	7	27	34	e
Brachetto	Female	67	46.4	45	14	41	55	ab
Brachetto	Male	100	40.5	38	10	35	45	c
Moscato	Female	41	47.9	44	17	40	57	a
Moscato	Male	59	42.3	40	15.5	34	49.5	bc

Note: Comparisons between rows were conducted after a General Additive Model (GAM) with Cox proportional hazard family. Post hoc comparisons were conducted with least-square means method and Tukey method for *p*-value adjustment, at significance level as 0.05 and 95% confidence intervals, and represented by letters for every specific group. GAM and post hoc details are reported in Supporting Information S3.
Abbreviations: IQR, inter-quartile range; Q1, first quartile (25th percentile); Q3, third quartile (75th percentile).

TABLE 4 Summary statistics for *S. titanus* survival on three grapevine cultivars, measured as days between adult emergence and death

Cultivar	Sex	*n*	Mean [days]	Median [days]	IQR [days]	Q1 [days]	Q3 [days]	Pairwise comparisons
Barbera	Female	34	37.2	32	33.75	21.25	55	a
Barbera	Male	97	30.3	25	17	18	35	b
Brachetto	Female	29	40.0	35	35	25	60	a
Brachetto	Male	65	24.0	18	20	11	31	b
Moscato	Female	31	37.5	27	49.5	12	61.5	b
Moscato	Male	44	17.8	15.5	11.25	9.75	21	c

Note: Comparisons between rows were conducted after a General Additive Model (GAM) with Cox proportional hazard family. Post hoc comparisons were conducted with least-square means method and Tukey method for *p*-value adjustment, at significance level as 0.05 and 95% confidence intervals, and represented by letters for every specific group. GAM and post hoc details are reported in Supporting Information S4.
Abbreviations: IQR, inter-quartile range; Q1, first quartile (25th percentile); Q3, third quartile (75th percentile).

model assumptions. Comparisons between groups were conducted on LMM estimates with EMMs and Tukey's p-value adjustment on LMM, followed by all-pairwise comparisons.

A GLMM with Gamma distribution (package glmmTMB, Brooks et al., 2017) was applied on vitellogenin expression, adding cultivar and day of dissection as fixed effects, and experimental replicate as random effect (Supporting Information S6). GLMM performances were analysed with the performance package, without raising any concern on the model assumptions. Pairwise comparisons were not conducted for vitellogenin expression, due to non-significance found in the GLMM variable estimates.
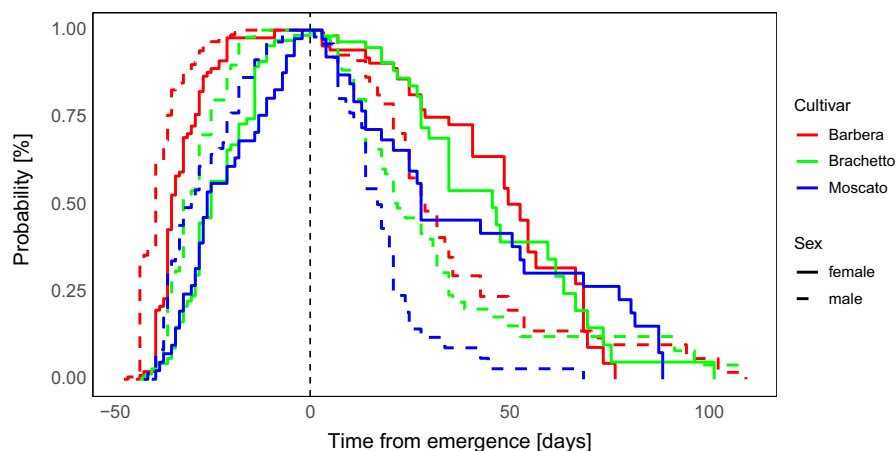
FIGURE 3 Joint developmental time and survival curves (Kaplan–Meier estimates) for *Scaphoideus titanus* reared on three grapevine cultivars. *Scaphoideus titanus* sex is represented by line type (solid for females, dashed for males), while grapevine cultivar is represented by line colour (red for Barbera, green for Brachetto, blue for Moscato). Figure 3 was produced by joining data from Figures 1 and 2, considering time from emergence as the zero point on the x-axis: on the left (negative numbers) nymph developmental time is reported, on the right (positive numbers) adult survival [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 5 Area drawn by joint development and survival Kaplan–Meier curves (Figure 3) for *Scaphoideus titanus* reared on three grapevine cultivars

Cultivar	Sex	Area
Barbera	Female	79.9
Barbera	Male	74.2
Brachetto	Female	69.5
Brachetto	Male	57.7
Moscato	Female	63.1
Moscato	Male	46.4

Note: The areas were calculated as approximated integrals of every function (describing the interaction Cultivar × Sex) using trapezoidal rule integration.

Figure 4 boxplots were produced based on raw data of mature eggs, resulted from counting, plus the expression level of vitellogenin mRNA, resulted after qRT-PCR, in the CFX Maestro software. Figure 4 includes, for the total number of mature eggs, groups resulting from all-pairwise comparisons (Supporting Information S5).

R packages used for analyses and production of figures were survival (Therneau, 2021), survminer (Kassambara et al., 2020), ggplot2 (Wickham, 2016) and patchwork (Pedersen, 2019).

The complete R code will be made publicly available on GitHub (https://github.com/matteo-rpm/papers), while the original datasets are available on OSF (Ripamonti, 2022).

3 | RESULTS

3.1 | Nymph survival

The total number of emerged and dead nymphs is reported in Table 2. In particular, nymph mortality was highest on Moscato, and, overall, *S. titanus* nymph performance decreased significantly on Brachetto compared with Barbera and was the lowest on Moscato.

3.2 | Developmental time

Our results highlighted substantial differences between *S. titanus* developmental times measured on the three *Vitis* varieties, and also between males and females (Table 3). In particular, as evidenced from the Kaplan–Meier curves (Figure 1) and the following pairwise comparisons (Table 3, Supporting Information S3), the well-known *S. titanus* protandry (Bocca et al., 2020; Chuche & Thiéry, 2012) was confirmed. Moreover, a considerable cultivar-related effect was present (Figure 1, Table 3). Leafhoppers developing on Barbera emerged significantly earlier than those on Brachetto and Moscato. A notable effect of the cultivar-related delay in the development was appreciable even between females emerged on Barbera and males on Brachetto and Moscato. The cultivar effect was higher than the impact of protandry, since Barbera females emerged significantly earlier than Brachetto and Moscato ones (Figure 1, Table 3).

3.3 | Adult survival

Summary data of adult survival are reported in Table 4. Adult longevity showed considerable differences in the survival probability for *S. titanus* reared on the three cultivars (Figure 2, Table 4, Supporting Information S4). In particular, males reared on Moscato survived for a shorter time compared with all other groups. Barbera- and Brachetto-reared males, together with Moscato-reared females, showed a similar survival, while Barbera- and Brachetto-reared females were the most long-lived (Table 4).
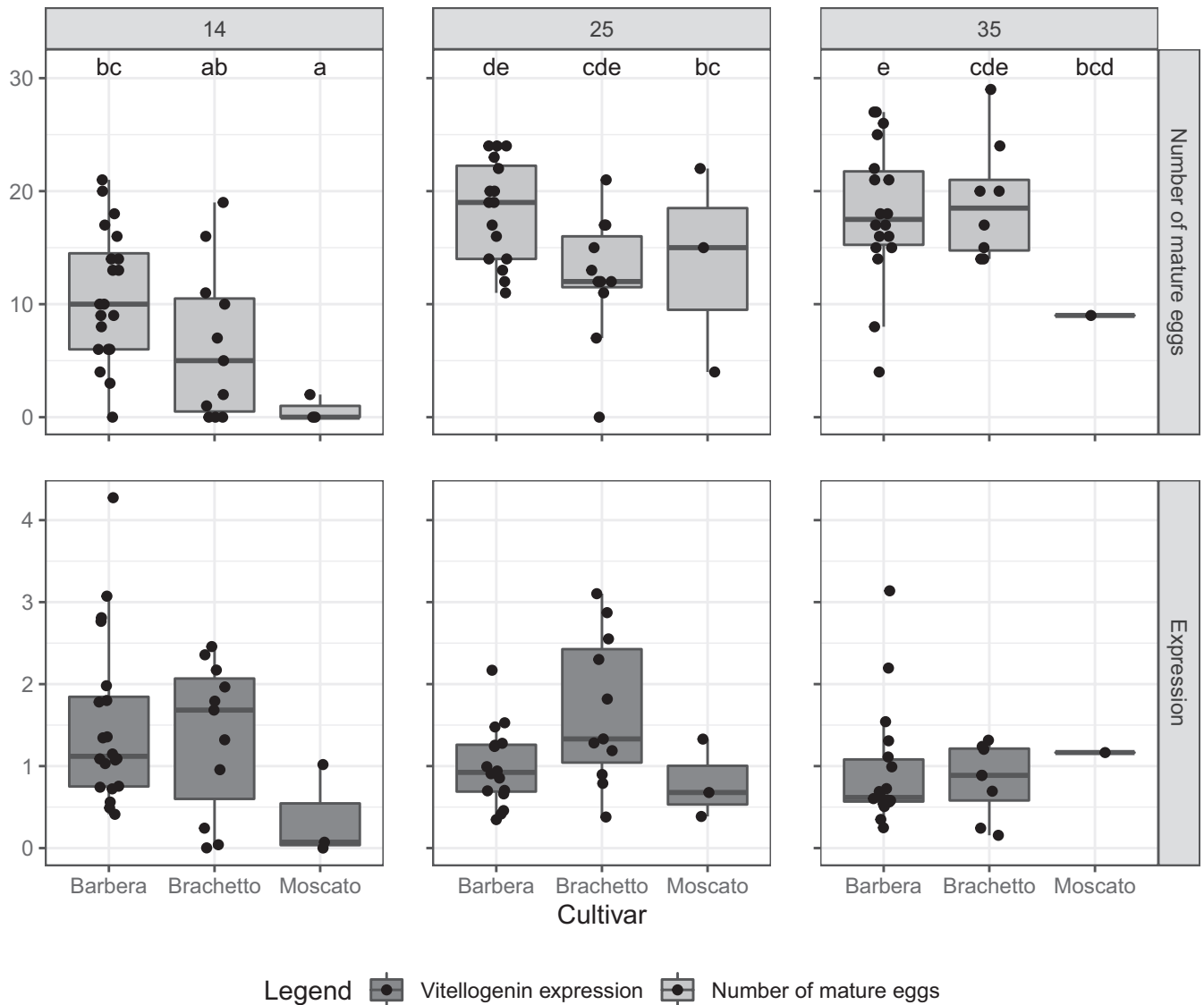
FIGURE 4 *Scaphoideus titanus* female prolificacy on three grapevine varieties. Number of mature eggs found in ovaries after dissection (upper row, light grey boxplots), and normalized expression of vitellogenin gene (lower row, dark grey boxplots), according to the day post-emergence at which females were sampled (columns). The x-axis reports the cultivar in which females were reared. The total number of dissected females (and prepared for RNA extraction) at 14, 25 and 35-day post-emergence is: 20, 16 and 18 for Barbera; 11, 11 and 9 for Brachetto; 3, 4 and 1 for Moscato. Post hoc comparisons, only for the number of mature eggs (upper row) were conducted with least-square means method and Tukey's method for *p*-value adjustment, at significance level as 0.05 and 95% confidence intervals, and represented by letters for every specific group (Supporting Information S5)

3.4 | Time span from eclosion to death

Figure 3 reports an overview of the time presence for *S. titanus* at 'non-egg' stage, from newly emerged nymph to adult death, in the previously described rearing conditions. The area drawn by the different curves was calculated to quantify the cultivar acceptability. In fact, faster development and/or longer survival constitute a reliable indication of host acceptability by the insect (Huang et al., 2020; Munyaneza & Upton, 2005; Orozco-Restrepo et al., 2017; Zhang et al., 2018). The area for the Barbera-reared leafhoppers was quite larger than those for the other two cultivars, especially when Moscato was considered (Table 5).

3.5 | Prolificacy and vitellogenin gene expression

Alive females at defined post-emergence intervals were dissected and mature eggs counted. The high nymphal mortality of *S. titanus* reared on Brachetto and Moscato together with the high adult mortality on Moscato explained the reduced number of females that could be dissected for egg count, especially for Moscato variety (Figure 4).

Egg counts derived from the dissected females are reported in Figure 4, paired with the level of vitellogenin mRNA expressed in the same samples.

Focusing on the Barbera, a significant difference in the number of mature eggs per female was found. As expected, the number

of mature eggs increased significantly over time: at 14-day post-emergence (dpe), with an estimated marginal mean of ~10 eggs, then at 25 and 35 dpe with ~17 and ~19 eggs, respectively (Figure 4, Supporting Information S5). A similar positive trend was found also for the other two cultivars, with increasing numbers of counted eggs per female upon time (~7, ~14 and ~16, Brachetto females, and ~2, ~10 and ~11, Moscato females; Figure 4, Supporting Information S5). The total number of eggs at every sampling time in Barbera females was significantly higher than in Moscato females. Brachetto- and Moscato females showed similar amounts of mature eggs at all dpe. Interestingly, a delay in egg maturation was evident in Brachetto- and Moscato-reared females, compared with Barbera ones.

The vitellogenin expression was similar among females from the three groups (Supporting Information S6). The relation between the number of mature eggs and vitellogenin relative transcription of the same female was investigated (Supporting Information S7). Maximum vitellogenin expression was reached when the female beared ~10 eggs.

4 | DISCUSSION

Vitis vinifera cv Barbera is highly susceptible to FD, while Brachetto, Merlot and Moscato show some degree of resistance (Eveillard et al., 2016; Ripamonti et al., 2021), and preliminary observations on Moscato have suggested that this behaviour may result from cultivar-specific effects on vector fitness (Ripamonti et al., 2021). In this work, we showed that the ampelophagous *Scaphoideus titanus* leafhopper performs better on Barbera variety compared with Brachetto and Moscato, both at nymphal and adult stages. Actually, all the fitness parameters selected for the study, nymphal developmental time, nymphal mortality, adult longevity and female prolificacy, point out better performances on Barbera.

In particular, egg-to-adult developmental time on Moscato was delayed compared with Barbera, suggesting that this latter variety is more acceptable for the leafhoppers at the nymphal stage. Indeed, delayed development is, in general, an index of negatively impacted fitness (Huang et al., 2020; Munyaneza & Upton, 2005), and, for example, *Lobesia botrana* reared on non-preferred grapevine varieties shows delayed development (Moreau et al., 2006). Nevertheless, there are exceptions, where non-preferred varieties induce faster development, as in the case of the spittlebug *Mahanarva fimbriolata* on sugarcane (Orozco-Restrepo et al., 2017). The high nymph mortality recorded on Moscato confirmed the poor performance of the leafhopper on this genotype. Nymph performance on Brachetto cultivar was somehow intermediate between Barbera and Moscato for both parameters, clearly indicating that, at this stage, the leafhopper performed less efficiently on the two FD-tolerant cultivars. Incidentally, males developed faster than females, thus proving that protandry occurs in this species, as already noticed by Chuche and Thiéry (2012) and Bocca et al. (2020).

Adult leafhoppers also differed in their survival rate on the three cultivars, and *S. titanus* reared on Moscato lived significantly less than on the other two varieties. In this case, decreased survival of males was measured on the three cultivars, but adult lifespan of males was half that of females, when reared on Moscato. Survival of *S. titanus* on Barbera is in line with that recently reported by Bocca et al. (2020), and minor differences may be ascribed to the different *Vitis* species (American hybrids versus European grapevine varieties) and rearing conditions of the two experimental settings (detached shoots in small cages versus grafted cuttings branches inside larger cages). The overall timespan for *S. titanus*, from 1st instar nymph to adult death, differed considerably between sexes and particularly among rearing cultivars. Indeed, lifespan was reduced of about 20% and up to 50% for females and males, respectively, when reared on Moscato compared with Barbera. This would lead to a reduced presence of the vector in a Moscato vineyard with consequent decreased possibility of acquiring and spreading FD. This observation, coupled with the shorter phloem feeding duration and higher frequency of interruption-salivation events compared with Barbera (Ripamonti et al., 2022), would make Moscato the right cultivar for vineyards in areas where the FD disease is endemic.

Although prolificacy tests were heavily affected by the mortality experienced by nymph and adult of *S. titanus* on Moscato, females reared on this cultivar showed a delay in egg maturation and vitellogenin expression. On the contrary, in *S. titanus* females reared on Barbera, the number of mature eggs increased with time. When female fitness was addressed by estimation of the vitellogenin expression (Ge et al., 2017; Liu et al., 2015), gene transcription showed a similar delay as for egg maturation, reaching its expression peak at the 1st or at the 3rd sampling date for *S. titanus* reared on Barbera or Moscato, respectively. A decrease in vitellogenin mRNA level should lead to a reduction in its bioavailability. The temporal shift of the vitellogenin expression peak in Moscato-reared females is a further clue of decreased performance of young females on this cultivar. Liu et al. (2015) described the expression profile of vitellogenin mRNA in *Chrysopa septempunctata*, showing a transcript peak at 10-day post-emergence (dpe), followed by a drop in transcript accumulation and a consequent significant reduction in laid eggs and egg hatching rate. Similarly, in our work we found a vitellogenin mRNA peak at 14 dpe in females reared on Barbera. Despite the different experimental design, the results are consistent with those of Bocca et al. (2020), as indeed they set the median time for the start of oviposition approximately a 14 dpe, when Barbera-reared females are already carrying mature eggs. Moreover, in this work, covering only a window of 21 days for oviposition, we estimated a median total load of about 30–40 eggs per female, a result consistent with the average of more than 60 eggs per female over a median oviposition period of about 45 days (Bocca et al., 2020). A delayed production of egg, coupled with reduced longevity, results in a lower population rate of increase (Birch, 1948). Our results suggest that *S. titanus* fitness is impaired on Moscato, and partially on Brachetto. Besides delaying time to reach sexual maturity in females, Moscato affects *S. titanus* survival and development time.

Further research on cultivar-dependent prolificacy should better clarify the role of grapevine cultivars on this *S. titanus* fitness

parameter. Indeed, we have shown that life history and prolificacy parameters are useful to estimate grapevine variety suitability for the FDp vector *S. titanus*. Different adaptation of the vector to a plant genotype may be a clue of preference/non-preference of the vector for different plant genotypes, that can in turn explain, at least in part, susceptibility/resistance to vector-borne diseases. Actually, susceptibility/resistance of a plant genotype to an arthropod-borne pathogen can be due to a response of the plant to the pathogen or to the vector, or to both. In this case, antibiosis, 'the adverse effects of a resistant plant on the survival, development, or fecundity of an arthropod' (Smith & Clement, 2012), may explain the lower suitability of the FD-tolerant varieties Moscato and Brachetto compared with the susceptible Barbera for *S. titanus*. Indeed, a different probing behaviour on the three grapevine varieties has been demonstrated (Ripamonti et al., 2022), clearly indicating that Barbera, very susceptible host of FDp, is also a much-liked host for the vector, while, among the FD-tolerant varieties, Moscato is definitely a non-preferred host. Resistance/tolerance to FDp may result not only from a direct plant response against the phytoplasma, but also against the vector, as low numbers of visiting insect vectors, with less efficient feeding in the phloem, may explain the low incidence of this phloem-limited pathogen in some grapevine varieties. Identification of the genetic traits underlining antibiosis, as shown in this work, and antixenosis ('modification of herbivore behavior by plant factors, which results in the inability of a plant to serve as a host' (Kogan & Ortman, 1978; Kordan et al., 2019), as shown in Ripamonti et al. (2022), should be included in programmes of breeding for resistance or New Genomic Techniques applications (European Commission, 2021) against this major grapevine pathogen.

AUTHOR CONTRIBUTIONS

Matteo Ripamonti, Luciana Galetto, Cristina Marzachì and Domenico Bosco conceived research. Matteo Ripamonti and Federico Maron conducted experiments. Cristina Marzachì and Domenico Bosco contributed material. Matteo Ripamonti analysed data and conducted statistical analyses. Domenico Bosco and Cristina Marzachì secured funding. Matteo Ripamonti wrote the manuscript. Luciana Galetto, Cristina Marzachì and Domenico Bosco revised the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on GitHub (https://github.com/matteo-rpm/papers) and OSF (https://doi.org/10.17605/OSF.IO/9CWUV, Ripamonti, 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.