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1 ***Scaphoideus titanus* Ball feeding behaviour on three grapevine cultivars** 2 **with different susceptibilities to Flavescence dorée**

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14

15 **Abstract**

16 *Scaphoideus titanus* (Ball) is a grapevine-feeder leafhopper, and the most important vector
17 of Flavescence dorée of grapevine (FD), a disease associated with phytoplasmas
18 belonging to ribosomal subgroups 16Sr-V–C and –D. FD is a major constraint to viticulture
19 in several European countries and, so far, its control has relied on roguing of infected
20 plants and insecticide applications against the vector. Detailed knowledge on different
21 levels of the multifaceted phytoplasma-plant-vector relationship is required to envisage
22 and explore more sustainable ways to control the disease spread. In the present work, *S.*
23 *titanus* feeding behaviour was described on three grapevine cultivars: Barbera (susceptible
24 to FD), Brachetto, and Moscato (tolerant to FD) using the Electrical Penetration Graph
25 (EPG) technique. Interestingly, no differences were highlighted in the non-phloem probing
26 phases, thus suggesting that the tested cultivars have no major differences in the
27 biochemical composition or structure of the leaf cuticle, epidermis or mesophyll, that can
28 affect the first feeding phases. On the contrary, the results showed significant differences
29 in leafhopper feeding behaviour in terms of the duration of the phloem feeding phase,
30 longer on Barbera and shorter on Brachetto and Moscato, and of the frequency of
31 interruption-salivation events inside the phloem, higher on Brachetto and Moscato. These

32 findings indicate a preference for the Barbera variety, that appears a more suitable hosts
33 for the leafhopper. *Scaphoideus titanus* feeding behaviour on Barbera correlates with an
34 enhanced FDp transmission efficiency, thus explaining, at least in part, the higher
35 susceptibility of this variety to FD. The mechanisms for the non-preference for Brachetto
36 and Moscato are discussed, and a possible antixenosis is hypothesized. We propose that
37 breeding for resistance against FD should take into account both plant traits associated
38 with the response to the phytoplasmas and to the vector.

39 **Keywords**

40 EPG; Electrical Penetration Graph; leafhopper vector; *Vitis vinifera*; cultivar

41 **1. Introduction**

42 The leafhopper *Scaphoideus titanus* Ball is the main vector of phytoplasmas associated
43 with the Flavescence dorée of grapevine (FD), a disease spread in most European
44 viticultural countries (EFSA, 2020) that causes severe reduction of yield and quality of
45 grapes, requires roguing of infected plants and leads to uneven-aged vineyards (Morone
46 et al., 2007). FD is associated with phytoplasmas belonging to the 16SrV group,
47 subgroups –C and –D (Davis and Dally, 2001; Lee et al., 2004; Martini et al., 2002), and it
48 causes severe losses to European viticulture (EFSA, 2016). Although different insect
49 species are competent for the transmission of FD phytoplasmas (FDp), *S. titanus* is by far
50 the most important vector, being strictly associated with *Vitis* plants and thus sustaining
51 both primary (from wild grapevines outside the vineyards to cultivated vines) and
52 secondary (from vine to vine within the vineyard) disease spread (Maggi et al., 2017;
53 Ripamonti et al., 2020). Control of FD relies on prophylactic measures, such as the use of
54 healthy propagation material, as well as on compulsory measures in infected vineyards,
55 namely roguing of infected plants, and insecticide treatments against the vector (Bosco
56 and Mori, 2013). However, the large-scale application of insecticides is a concern to
57 human health and environment, priming cascade ecosystem effects (Desneux et al., 2007)
58 with a strong negative impact on pollinators (Tosi et al., 2018). For this reason, recent
59 studies focused on identifying sources of resistance to FDp phytoplasmas within the
60 grapevine germoplasm (Eveillard et al., 2016; Ripamonti et al., 2021), that would represent
61 the best strategy to minimise damage and limit FD spread and insecticide applications.
62 Grapevine tolerance to FDp may be due to a direct response of the plant against the
63 pathogen or mediated by some resistance against the vector, or by a combination of the

64 two. Resistance against insects occurs when plant structural or chemical traits deter
65 herbivore feeding and thus minimize the amount of herbivore damage experienced by the
66 plant, while tolerance occurs when plant traits reduce the negative effects of herbivore
67 damage on crop yield (Mitchell et al., 2016). As an example, it was demonstrated that
68 resistant tea cultivars sustained lower phloem ingestions for *Empoasca vitis* (Miao et al.,
69 2014). Moscato and Brachetto are grapevine varieties tolerant to FD, as demonstrated by
70 Ripamonti et al. (2021) using transmission experiments with *S. titanus* under controlled
71 conditions (Ripamonti et al., 2021). The reduced *S. titanus* survival on Moscato observed
72 by the above mentioned authors, suggest that vector-host interaction could be the pivotal
73 factor underlying Moscato tolerance to FD. *S. titanus* is monophagous on *Vitis* species,
74 mainly *Vitis vinifera* and naturalized rootstocks of *V. riparia* in Europe, while in North
75 America, *V. labrusca* and *V. riparia* are reported as the preferred host plants (Chuche and
76 Thiéry, 2014). Although the species is regarded as monophagous, it shows a good level of
77 plasticity and can feed on plant species of different families, e.g. Vitaceae, Fabaceae,
78 Ranunculaceae (Caudwell et al., 1970; Trivellone et al., 2013). Plant resistance against
79 sap-sucking insects can be conveniently investigated by Electrical Penetration Graph
80 (EPG), that describes the nutrition pattern of a sucking insect on a given plant genotype,
81 by identifying possible altered nutrition on non-suitable genotypes (Backus et al., 2020;
82 Lucini et al., 2021).

83 Here we expand the first findings on *S. titanus* behaviour on grapevines, by analyzing the
84 vector probing behavior on three varieties with a different degree of susceptibility to FD:
85 one susceptible, Barbera, and two tolerant, Moscato and Brachetto, through the Electrical
86 Penetration Graph (EPG) (Backus and Bennett, 2009; McLean and Kinsey, 1964; Tjallingii,
87 1978).

88 EPG is a powerful tool to describe pierce-sucking insects' probing behaviour, previously
89 applied to describe *S. titanus* feeding behaviour on Cabernet-Sauvignon cuttings (Chuche
90 et al., 2017a, 2017b). EPG studies on different plant cultivars/genotypes provide precious
91 information for the epidemiology of vector-borne plant pathogens, also permitting the
92 identification of traits making a *Vitis* genotype unsuitable for the vector. A number of EPG
93 studies aimed at identifying plant resistance to insect vectors have been performed on
94 planthoppers (Kimmins, 1989), whiteflies (Jiang et al., 2001; Rodríguez-López et al., 2011)
95 and aphids (Caillaud et al., 1995a, 1995b; Garzo et al., 2018; Sauge et al., 1998). Among
96 these latter, EPG was applied to identify resistance factors involved in virus transmission
97 inhibition (Chen et al., 1997) as well as the presence of antixenosis (Kordan et al., 2019).

98 Besides those on *S. titanus* (Chuche et al., 2017a, 2017b), few EPG studies have been
99 conducted on Deltocephalinae leafhoppers (Carpane et al., 2011; Kawabe and McLean,
100 1980; Lett et al., 2001; Stafford and Walker, 2009; Trębicki et al., 2012), and very few of
101 these are relevant to phytoplasmas/mollicutes transmission (Carpane et al., 2011; Chuche
102 et al., 2017a).

103 The aim of this study was to compare *S. titanus* probing behaviour on three different
104 grapevine cultivars characterised by different susceptibilities to FD, in order to better
105 characterize the mechanisms underlying varietal tolerance/susceptibility to this
106 phytoplasma disease.

107 **2. Material & Methods**

108 **2.1. *S. titanus* collection and rearing**

109 To establish a *S. titanus* laboratory colony, in January/February 2019 two-year-old
110 grapevine canes with eggs were collected in vineyards of the Piemonte Region. The
111 selected sites were known to host a high population of the leafhopper in the previous
112 summer, as estimated by yellow sticky traps captures of adults. The collected canes were
113 stored in a cold room at $6\pm 1^{\circ}\text{C}$, covered with plastic film to avoid egg desiccation, until
114 use. When needed, grapevine canes were transferred into an insect-proof greenhouse at
115 $24 \pm 2^{\circ}\text{C}$ and maintained damp by daily water spraying. After four weeks, canes were
116 isolated in a cage together with a three-week-old broadbean plant as a food source for the
117 nymphs. After egg hatching, the broadbean plant was replaced every three weeks.
118 Nymphs were reared under controlled conditions inside a greenhouse chamber, $T = 24 \pm$
119 2°C , with no humidity and photoperiod control, from the beginning of April to the end of
120 September 2019. As FDp is not transovarically transmitted, and all the plants used for the
121 rearing and the experiments were phytoplasma-free, all *S. titanus* used in the experiments
122 were phytoplasma-free. For the EPG experiments, adults emerged from 7-21 days were
123 used (modified from Chuche et al., 2017a), since in this time frame they were sexually
124 mature, highly active and not subjected to high mortality (Bocca et al., 2020; Mazzoni et
125 al., 2009).

126 **2.2. Plant rearing**

127 The test plants were obtained from phytoplasma-free *V. vinifera* cuttings of three different
128 cultivars, Barbera N. - Clone I-AT 84, Brachetto N. - Clone I-CVT 20 and Moscato Bianco
129 B. - Clone I-CVT 190 as described in Ripamonti et al. (2021). Grapevine cuttings were

130 grown in a greenhouse at $24 \pm 2^\circ\text{C}$, with no humidity and photoperiod control, inside 0.9 L
131 pots (2:2:1 topsoil, clay, perlite), and watered once a week. Cuttings were used when
132 three- to five-months old, and periodically pruned in order to keep them within 80 cm
133 height . Broadbean plants used for *S. titanus* rearing were seedlings maintained in a
134 growth chamber ($24 \pm 2^\circ\text{C}$, with no humidity and photoperiod control) in 2.4 L topsoil, five
135 per pot, and watered twice a week.

136 **2.3. EPG setup and data analysis**

137 Selected adults were collected and anaesthetised with carbon dioxide for 5 seconds in a
138 glass tube, then immobilised at the edge of a cut pipette tip connected to a vacuum pump
139 under a stereomicroscope. A small drop of water-based silver glue (EPG Systems,
140 Wageningen, The Netherlands) was placed on the pronotum of the insect, then a gold wire
141 of $18 \mu\text{m}$ (previously attached with solvent-based silver glue (Ted Pella Inc., USA) to a 3
142 cm copper wire in turn attached to a brass nail with melted stain) was positioned on the
143 dried drop, and covered with another small drop of silver glue. Before the EPG assay,
144 insects were starved for a 30-minute period, during which they were attached to the
145 electrode and hanged, inserting the nail in a polystyrene base.

146 The substrate voltage probe was inserted in well damped soil of a potted grapevine
147 cutting, and *S. titanus*, attached to the assembled electrode, was connected to a probe
148 and positioned onto the abaxial surface of a leaf. The feeding behaviour was then
149 monitored for 8 hours with a Giga-8dd DC-EPG amplifier (EPG Systems, Wageningen,
150 The Netherlands), inside a Faraday cage to isolate the system from external electrical
151 noise. Input resistance used was 1 giga Ohm, output set at 75x gain and plant voltage
152 adjusted so that the EPG signal fitted into +5V and -5V. All recordings were done between
153 June and August 2019, and started between 11:00 and 11:30 a.m. every day.

154 A total of 153 recordings were done, each day a total of 6 recording were run. Each single
155 recording was represented by a different plant-insect combination, one male or one female
156 on one grapevine plant. Potted plants of the three varieties were randomly arranged in the
157 Faraday cage for every recording and discarded after use. In case of falling from the leaf,
158 the insect was repositioned. At the end of the recording, dead insects were noted and
159 excluded from further analyses.

160 **2.4. EPG acquisition and marking of EPG files**

161 Recordings were acquired and marked using Stylet+ software (v01.30, Electrical
162 Penetration Graph Data Acquisition and Analysis, EPG Systems, Wageningen, The
163 Netherlands). Waveform marking was conducted accordingly to Chuche et al. (2017a) and
164 Stafford & Walker (2009), focusing on the following waveforms: np (non-probing activity),
165 pathway-phase (phase “C”), active ingestion (phase “G”) of mesophyll (<60 seconds) or
166 xylem sap (>100 seconds) (see Stafford & Walker, 2009), passive ingestion of phloem sap
167 (phase E), interruptions during ingestion (phase N of Chuche et al., 2017a). For more
168 details, see Supplementary File S1.

169 Once marked, all the recordings were singly selected for the successive analysis. In
170 particular, recordings with electrical noises, bad electric connections, or when insects fell
171 from the plant for more than 20% of the recording time, were discarded from further
172 analysis.

173 **2.5. Statistical analysis**

174 All the statistical analyses were conducted on R software v4.0.3 (R Core Team., 2020).
175 Selected recordings were analysed through a package of the software R ad-hoc produced
176 for the analysis on EPG recordings, called Rwaves (Chiapello,
177 <https://github.com/mchiapello/Rwaves>). Rwaves conducts summary statistics on the input
178 recordings on a set of variables of EPG analysis (Table 1), producing a table including the
179 values of all the variables for all the input recordings. The resulting table was composed as
180 follows: every row corresponded to a single recording (represented by the unique
181 combination of one leafhopper and one grapevine plant), while every column represented
182 a single EPG variable. Once obtained, the table was subjected to modifications to enhance
183 readability (packages dplyr, tidyr, stringr: (Wickham, 2019, 2020; Wickham et al., 2020),
184 and descriptive statistics were run (Tables 3, 4, 5, 6 and Supplementary File S2).
185 Univariate analyses were conducted starting from Generalised Linear Model (GLM) of
186 different families specific for the nature of the variable: quasi-Poisson or negative-binomial
187 for counts, Gamma or inverse-Gaussian for positive continuous variables, beta-regression
188 for proportions (packages stats, betareg, MASS: Cribari-Neto and Zeileis, 2010; Venables
189 and Ripley, 2002). Goodness-of-fit for every model was evaluated plotting half-normal
190 plots with simulated envelope against deviance residuals, with 95% confidence level (hnp
191 package: Moral et al., 2017). Homoscedasticity for every model was evaluated through
192 Levene’s test (car package: Fox and Weisberg, 2019). In case of rejection of the null
193 hypothesis, heteroscedasticity-consistent standard errors (sandwich package: Zeileis et

194 al., 2020) were calculated and considered for pairwise-comparisons. Comparisons among
 195 groups were conducted with least-square means method and Tukey method for p-value
 196 adjustment, at 0.05 significance level and 95% confidence intervals (packages emmeans
 197 and multcomp: Hothorn, Bretz, & Westfall, 2008; Lenth, 2020). Cultivar, Sex, and their
 198 reciprocal interaction were selected as explanatory variables. If no significant effects were
 199 found for Sex and Cultivar × Sex, the GLM was run with Cultivar as the only explanatory
 200 variable. GLMs summaries were reported in Supplementary File S3 using package jtools
 201 (Long, 2020). Packages ggplot2 (Wickham, 2016) and ggpubr (Kassambara, 2020) were
 202 used to produce Figure 1, and Supplementary File S4 and S5.

203 A multivariate Canonical Correspondence Analysis (CCA, Legendre & Legendre, 2012)
 204 was conducted through the vegan (Oksanen et al., 2019) and ggordiplots packages
 205 (Quensen, 2018), considering all the variables except multi-collinear ones, that were
 206 excluded from the analysis, based on a correlation coefficient higher than 0.95 (usdm
 207 package: Naimi et al., 2014), in order to strengthen the predictor value of the model.
 208 Starting from 25 variables, 5 variables were found to have collinearity problem, and were
 209 thus excluded from further analyses. The remaining variables were standardised (Hellinger
 210 method, Legendre & Gallagher, 2001) and subjected to CCA, with Cultivar, Sex and their
 211 interaction as explanatory variables. The CCA result was confirmed through a
 212 permutational Multivariate Analysis of Variance (perMANOVA; Anderson, 2001).

213 The complete R code will be made publicly available on GitHub ([https://github.com/matteo-](https://github.com/matteo-rpm)
 214 rpm).

215 **Table 1. EPG variable selected for the study.**

Variable	Abbreviation from Sarria et al., 2009 (implemented in Rwaves)	Abbreviation from Backus et al., 2007	Type (NS: non-sequential; S: sequential)
"Number of non-probing periods"	n_np	NWEi np	NS
"Total duration of non-probing periods [s]"	s_np	WDi np	NS
"Time from 1st np to 1st probe [s]"	s_npto1stprobe	-	S
"Duration of the 2nd non-probing period [s]"	s_2np	-	S
"Number of probes"	n_Pr	NPi	NS
"Total probing time [s]"	s_Pr	PDi	NS
"Total duration of pathway phase [s]"	s_C	WDi C	NS
"Number of active ingestion phases"	n_G	NWEi G	NS
"Total duration of active ingestion [s]"	s_G	WDi G	NS
"Number of phloem ingestions"	n_E2	NWEi E2	NS

"Number of sustained (> 600 s) phloem ingestion"	n_sE2	NWEi sE2	NS
"Total duration of phloem ingestions [s]"	s_E2	WDi E2	NS
"Mean duration of a single event of phloem ingestion [s]"	mean_E2	WDEi E2	NS
"Duration of the longest phloem ingestion [s]"	s_longestE2	-	NS
"Total duration of non-phloematic phases [s]"	s_notE	WDi C-G	NS
"Time from 1st probe to 1st phloem ingestion [s]"	t_1E2.exp	-	S
"Time from 1st probe to 1st sustained (> 600 s) phloem ingestion [s]"	t_1sE2.exp	-	S
"Time of 1st sustained phloem phase [s]"	t_1st_sE2	-	S
"Percentage of probing time spent in phloem ingestion [%]"	percprobtime_E2	-	NS
"Percentage of probing time spent in pathway-phase [%]"	percprobtime_C	-	NS
"Percentage of probing time spent in active ingestion [%]"	percprobtime_G	-	NS
"Potential E2 index [%]"	E2index	-	S
"Mean frequency of Np interruptions during phloem phase [mHz]"	mean_fr_Ninterrupt	-	NS
"Percentage of time spent in Np interruption during phloem phase [%]"	percNinterrupt_E2	-	NS
"Number of Np interruptions during phloem ingestion"	n_Ninterrupt_E2	NWEi Np	NS

216

217 3. Results

218 Number of recordings obtained from male and female *S. titanus* adults on the three
 219 grapevine varieties are summarised in Table 2. In particular, 51-cultivar specific recordings
 220 were acquired, of which a fraction was selected for further analysis (31 for Barbera, 32 for
 221 Brachetto, 37 for Moscato), as described in Material and Methods section.

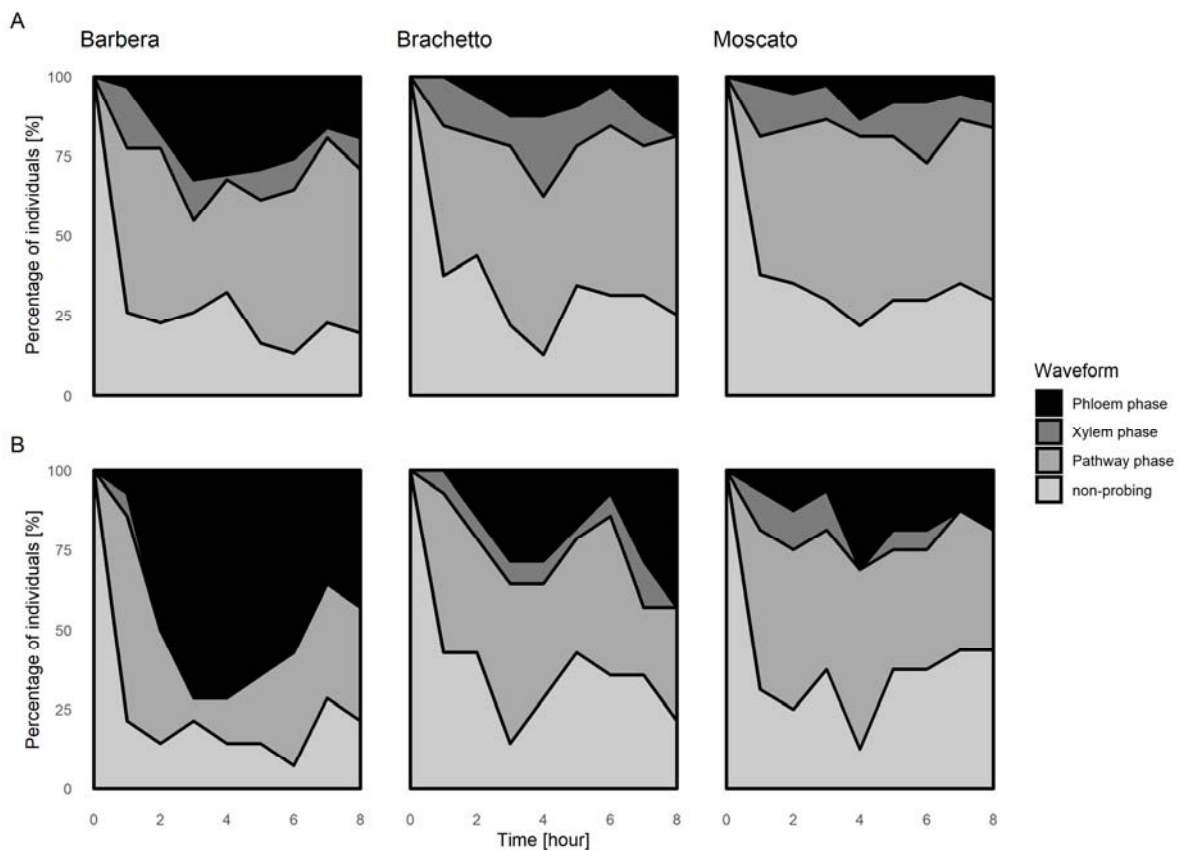
222 **Table 2. Number of total and selected recordings of *S. titanus* feeding behaviour on three**
 223 **grapevine cultivars.**

Cultivar	Total recordings (females, males)	Selected recordings (females, males)
Barbera	51 (25, 26)	31 (18, 13)
Brachetto	51 (25, 26)	32 (13, 19)
Moscato	51 (25, 26)	37 (16, 21)

224

225 No significant differences in acquiring successful EPG signals among cultivars, possibly
226 caused by human errors, were found (Pearson's Chi-squared test, X-squared = 0.36811,
227 df = 2, p-value = 0.8319). From now on, when referring to recordings, only the selected
228 ones will be considered, unless otherwise stated.

229 Irrespective of the cultivar, most of the insects started probing within the first minute from
230 their access to the leaf (median \pm SE = 41 \pm 21 s). Waveforms were graphically
231 summarised in a temporal progress representation (Figure 1). An overall larger area of
232 phloem phase was found for leafhoppers feeding on the Barbera variety.



233

234 **Figure 1. Temporal progress of *S. titanus* stylets activities on three grapevine cultivars**
235 **during the 8-h EPG recording.** Probing behaviours were represented as percentages of
236 leafhoppers in a given phase (non-probing, pathway phase, active ingestion of xylem sap, passive
237 ingestion of phloem sap) at 1 h intervals, starting from hour 0 (start of the recording) to hour 8 (end
238 of the recording). a) Graphs produced considering all recordings; b) graphs produced considering
239 only recordings where a phloem phase was present. The total number of recordings used to
240 produce Figure 1 are reported in the third column of Supplementary File S2 (all recordings) and
241 Table 3 (phloem recordings).

242 Values of non-phloem variables of all selected recordings are reported in Supplementary
243 File S2. No differences were identified in the variables among the three grapevine
244 varieties. The proportion of recordings with phloem phases were not significantly different
245 among cultivars (Supplementary File S6, Pearson's Chi-squared test, $X^2 =$
246 0.026378, $df = 2$, p -value = 0.9869), as almost half of the recordings (45% for Barbera,
247 44% for Brachetto, 43% for Moscato) showed phloem phases, irrespective of the cultivar
248 (Supplementary File S6). No differences were highlighted among cultivars for all the non-
249 phloem variables (Supplementary File S5), when considering the recordings without a
250 phloem-phase. Further, the non-phloem variables were analysed for recordings with
251 phloem phases (Table 3). Number of events and their duration for the non-phloem phases
252 did not differ among groups (Table 3). Interestingly, the total time spent by the insect with
253 stylets inserted in the plant tissues ("Total probing time") were similar among the three
254 *Vitis* genotypes. Some differences were found for the related variables "Number of non-
255 probing periods", and "Number of probes", as higher values were recorded for both
256 variables on Brachetto, compared to Barbera. On Barbera, females showed fewer
257 "Number of active ingestion (from mesophyll or xylem) phases" than males. No
258 differences were observed between sexes on the other varieties. No significant differences
259 among cultivars were found for the "Number of phloem ingestions", or for the "sustained"
260 (longer than 10 minutes) ones (Table 4). Although the "Mean duration of a single event of
261 phloem ingestion" did not differ significantly among cultivars, a longer duration of phloem
262 ingestion events on Barbera was evident. Indeed, significant differences were found for
263 "Total duration of phloem ingestions", "Duration of the longest phloem ingestion", and
264 "Time from first probe to first sustained phloem ingestion" between Barbera and the other
265 two grapevine varieties (Table 4). "Time from first probe to first phloem ingestion" was
266 shorter on Barbera compared to Moscato, with an intermediate duration recorded on
267 Brachetto (Table 5). This also suggests a preference of *S. titanus* for Barbera. For the
268 "Total duration of non-phloematic phases", for which an effect for the leafhopper sex was
269 found, a difference was recorded between *S. titanus* feeding on Barbera and on Moscato,
270 at least for females. *Scaphoideus titanus* also spent a higher percentage of time in the
271 phloem ingestion phase on Barbera, compared to Brachetto and Moscato varieties and,
272 consequently, less time in pathway- and active ingestion phases (Table 5). Since the
273 presence of "Np" (typical interruption between two different passive ingestion phases) in
274 phloem phases has been repeatedly recorded (Chuche et al., 2017a; Supplementary File
275 S1 of the present work), three variables were introduced for their description in the present

276 work and are reported in Table 6: “Mean frequency of Np interruptions during phloem
277 phase”, “Percentage of time spent in Np interruption during phloem phase”, and “Number
278 of Np interruptions during phloem ingestion”. The second and third variables showed
279 significant differences between leafhoppers feeding on Barbera and those feeding on the
280 other varieties, underlying different phloem feeding behaviour on the former variety.

281 **Table 3. Median ± SE of non-phloem variables related to recordings presenting phloem phases.** Every row reports a single combination of
 282 grapevine Cultivar and leafhopper Sex. Every column reports a specific variable. Comparisons between rows were done with a specific GLM family
 283 for every variable: quasi-Poisson or negative-binomial for counts, Gamma or inverse-Gaussian for continuous time variables, beta-regression for
 284 proportions. Cultivar, Sex and their interaction (Cultivar × Sex) effects for every variable were evaluated. In case of no effect for Sex and Cultivar ×
 285 Sex, the GLM was run with only Cultivar as explanatory variable (indicated in the tables with the * sign after the specific variable name). In case of
 286 effect for Sex or Cultivar × Sex, GLM was run with all the three explanatory variables (indicated in the tables with the ** sign after the specific
 287 variable name). Post-hoc comparisons were conducted with least-square means method and Tukey method for p-value adjustment, at significance
 288 level as 0.05 and 95% confidence intervals, and represented by letters for every specific group. GLMs specific details (family, coefficients,
 289 standard errors, AIC, BIC, R2) are reported in Supplementary File S3a-b.

Cultivar	Sex	n	Number of non-probing periods *	Total duration of non-probing periods [min] *	Time from 1st np to 1st probe [s] *	Duration of the 2nd non-probing period [s] *	Number of probes *	Total probing time [min] *	Total duration of pathway phase [min] **	Number of active ingestion phases **	Total duration of active ingestion [min] *
Barbera	female	8	9.5 ± 1.7 a	80.8 ± 22.8 a	33.5 ± 41.5 a	108 ± 143.4 a	9.5 ± 1.8 a	397.2 ± 22.8 a	96.1 ± 18.5 b	8 ± 6.9 a	3.2 ± 3.8 a
Barbera	male	6	14 ± 1.9 a	32.3 ± 47.7 a	19 ± 11.8 a	22.1 ± 43.8 a	12.5 ± 1.9 a	446.6 ± 47.5 a	124.9 ± 36.6 ab	42 ± 11.1 b	21.2 ± 8 a
Brachetto	female	6	21 ± 6.1 b	164.8 ± 32.1 a	48.5 ± 216.8 a	45.9 ± 80.9 a	21 ± 6.1 b	313.9 ± 31.8 a	175.1 ± 30.4 ab	52 ± 23.3 b	34.3 ± 13.7 a
Brachetto	male	8	16 ± 2 b	174.8 ± 20.9 a	63.3 ± 80.7 a	42.5 ± 48.5 a	15.5 ± 2 b	303.9 ± 20.5 a	160.3 ± 25 ab	51 ± 15.3 b	36.9 ± 11.6 a
Moscato	female	7	16 ± 3.3 ab	105 ± 24.9 a	87.6 ± 169.5 a	40.9 ± 96 a	16 ± 3.2 ab	374.5 ± 25 a	231.5 ± 36.5 a	47 ± 9.9 b	26.3 ± 7.5 a
Moscato	male	9	18.5 ± 3.5 ab	168.8 ± 28.3 a	54.6 ± 24.2 a	22.9 ± 79.5 a	17 ± 3.6 ab	310.5 ± 28.3 a	154.9 ± 24.7 ab	33 ± 14.3 b	22.5 ± 19.5 a

290

291 **Table 4. Median ± SE of phloem variables related to recordings presenting phloem phases.** The Table was drawn as detailed for Table 3.

Cultivar	Sex	n	Number of phloem ingestions *	Number of sustained (> 600 s) phloem ingestion *	Total duration of phloem ingestions [min] *	Mean duration of a single event of phloem ingestion [min] *	Duration of the longest phloem ingestion [min] *	Total duration of non-phloematic phases [min] **	Time from 1st probe to 1st phloem ingestion [min] *	Time from 1st probe to 1st sustained (> 600 s) phloem ingestion [min] *	Time of 1st sustained phloem phase [min] *
Barbera	female	8	13 ± 2.7 a	2 ± 0.4 a	301.9 ± 37.7	23.4 ± 11 a	240.2 ± 38.4	107.4 ± 20.1 b	76.7 ± 9.5 b	111.7 ± 27.3 b	114.1 ± 27.1 b

					a		a					
Barbera	male	6	14 ± 1.3 a	2.5 ± 0.7 a	190.3 ± 48.4 a	12.7 ± 3.1 a	158.3 ± 41.3 a	156.2 ± 37 ab	104.4 ± 32.9 b	131.1 ± 34.2 b	133.5 ± 34.1 b	
Brachetto	female	6	7 ± 3.8 a	1 ± 0.6 a	83 ± 33.2 b	5.7 ± 4 a	50.2 ± 25.6 b	208.1 ± 43.2 ab	135.4 ± 15.3 ab	156.6 ± 63.7 a	157.1 ± 63.3 a	
Brachetto	male	8	18 ± 5.9 a	2 ± 0.6 a	53.9 ± 26.1 b	3.5 ± 7.9 a	38 ± 13.9 b	237.6 ± 25.6 a	167.1 ± 43.4 ab	229.2 ± 49.2 a	240.5 ± 47 a	
Moscato	female	7	10.5 ± 4.5 a	1 ± 0.4 a	37.6 ± 42.7 b	3.1 ± 3.5 a	20.2 ± 30.9 c	283.4 ± 44.6 a	187.1 ± 50.8 a	267.8 ± 62.3 a	270.7 ± 65.1 a	
Moscato	male	9	25.5 ± 3.8 a	1 ± 0.3 a	47 ± 11.8 b	2.3 ± 0.6 a	13 ± 7.1 c	257 ± 33.8 a	107.8 ± 54.6 a	203.9 ± 55.8 a	208 ± 55.5 a	

292

293 **Table 5. Percentage variables related to recordings presenting phloem phases.** The Table was drawn as detailed for Table 3.

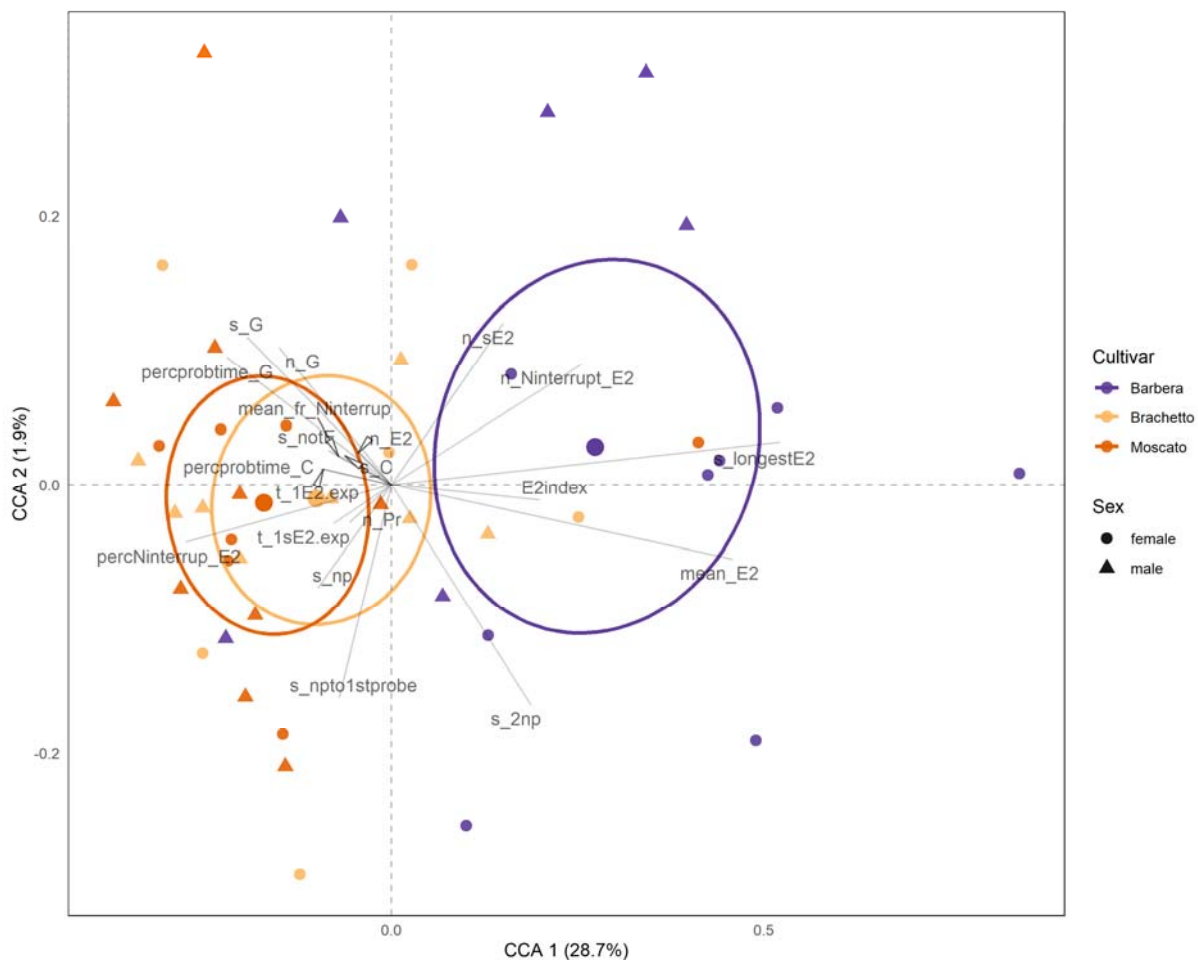
Cultivar	Sex	n	Percentage of probing time spent in phloem ingestion [%] *	Percentage of probing time spent in pathway-phase [%] **	Percentage of probing time spent in active ingestion [%] **	Potential E2 index [%] *
Barbera	female	8	75 ± 6.6 b	22.4 ± 6 a	0.7 ± 1.1 a	77.7 ± 8.1 a
Barbera	male	6	51.2 ± 9.4 b	38.5 ± 9.2 ab	8.3 ± 1.7 ab	66.1 ± 16 a
Brachetto	female	6	26.9 ± 9.3 a	60 ± 7.1 bc	13.4 ± 3.2 ab	29.1 ± 7.7 a
Brachetto	male	8	14.6 ± 7.4 a	54.3 ± 5.3 bc	9.5 ± 4.2 b	48.3 ± 10.2 a
Moscato	female	7	11.6 ± 10.9 a	75.9 ± 8 c	9.9 ± 2 ab	48.6 ± 14.2 a
Moscato	male	9	11.9 ± 4.4 a	55.4 ± 4.9 bc	7.5 ± 3.9 b	32.1 ± 9.5 a

294

295 **Table 6. "Np" phloem-interruptions variables related to recordings presenting phloem phases.** The Table was drawn as detailed for Table 3

Cultivar	Sex	n	Mean frequency of Np interruptions during phloem phase [mHz] **	Percentage of time spent in Np interruption during phloem phase [%] *	Number of Np interruptions during phloem ingestion *
Barbera	female	8	9.4 ± 0.8 b	1.4 ± 0.7 a	148.5 ± 27.2 b
Barbera	male	6	14.6 ± 1.9 ab	3 ± 1.6 a	167.5 ± 58 b
Brachetto	female	6	16.7 ± 1 ab	6.8 ± 5.8 ab	88 ± 27.8 a
Brachetto	male	8	19 ± 3.1 a	9.7 ± 7.3 ab	38.5 ± 26.2 a
Moscato	female	7	15.5 ± 3.4 a	16.2 ± 4.6 b	44 ± 20.6 a
Moscato	male	9	19.8 ± 2 a	11.8 ± 5.3 b	53 ± 16.1 a

296 A constrained Canonical Correspondence Analysis (CCA) was conducted to explore the
297 comprehensive effect of the explanatory variables Cultivar, Sex, and their interaction with
298 *S. titanus* feeding behaviour (Figure 2). The CCA is a graphical representation of the non-
299 multi-collinear variables more related to the different groups. In particular, considering the
300 absence of effect for Sex and Cultivar \times Sex (Table 7), ellipses were drawn containing
301 99% confidence intervals for the standard errors related to Cultivar variable. Again, this
302 representation highlighted the difference between *S. titanus* feeding behaviour on Barbera,
303 on one side, and on Brachetto and Moscato, on the other side. Moreover, CCA shows a
304 clear correlation between phloem variables and Barbera cultivar.



305

306 **Figure 2. Canonical Correspondence Analysis (CCA) on recordings with phloem phases.**

307 The new condensed CCA variables explained 28.7% (CCA 1, x axis) and 1.9% (CCA 2, y axis) of
308 the variability. Cultivar-specific recordings were grouped with ellipses, representing 99%
309 confidence intervals for the standard errors, and the centroid of each was represented. Every point
310 represents a single recording, colour refers to the grapevine cultivar and shape refers to the
311 leafhopper sex. Original variables were plotted and reported with their acronym (Table 1 for

312 acronym interpretation); all variables start from the intersection of the axes and are projected
313 according to their unique composition of CCA 1 and 2.

314 Results of the CCA were confirmed through a perMANOVA (Table 7), which highlighted
315 significant differences among Cultivars, while no significative differences were found for
316 Sex or the interaction of Cultivar and Sex.

317 **Table 7. perMANOVA results based on Bray-Curtis dissimilarities**, using all the non-multi-
318 collinear EPG variables (as described in Materials & Methods section). Df: degrees of freedom;
319 SumOfSqs: sequential sums of squares; F: F statistics values by permutations; Pr(>F): p-values,
320 based on 9999 permutations (the lowest possible p-value is 0.0001).

	Df	SumOfSqs	R2	F	Pr(>F)	signif
Cultivar	2	0.21459034	0.24924254	6.867267	0.0001	***
Sex	1	0.02922068	0.03393925	1.870225	0.1269	
Cultivar × Sex	2	0.02344144	0.02722678	0.750167	0.6142	
Residual	38	0.59371753	0.68959144	NA	NA	
Total	43	0.86096998	1	NA	NA	

321

322 4. Discussion

323 In this work, the probing behaviour of the FD leafhopper vector *S. titanus* on grapevine
324 varieties with different susceptibility to the disease was analysed, to highlight possible
325 differences that can account for different transmission efficiencies. As phytoplasmas are
326 phloem-limited in the plant, vector acquisition and transmission abilities are related to
327 phloem feeding phases, and thus a plant genotype that does not sustain efficient phloem
328 feeding may be less prone to infection.

329 To understand if probing behaviour of *S. titanus* may contribute to explain
330 tolerance/susceptibility mechanisms of grapevine genotypes, the FD highly susceptible
331 Barbera and the FD tolerant Brachetto and Moscato varieties (Ripamonti et al., 2021) were
332 compared. Indeed, *S. titanus* showed a feeding preference for the FD highly susceptible
333 Barbera variety. To describe *S. titanus*-grapevine interaction, total probing time was
334 subdivided into different probing phases, mainly related to the inter/intra-cellular
335 movements of the stylets (pathway-phase), the active ingestion of mesophyll or xylem sap,
336 the passive ingestion of phloem-sap.

337 A preference of the leafhopper for Barbera was suggested at first by the overall higher
338 proportion of *S. titanus* feeding on phloem of this variety (larger area under the phloem
339 phase), compared to Brachetto and Moscato in the temporal progress area graph.
340 However, it is possible that duration of phloem phases was underestimated in this study,
341 as well as in those of Chuche et al. (2017a, 2017b), and indeed longer recording times can
342 possibly highlight longer durations of phloem ingestion, as hypothesized for *Dalbulus*
343 *maidis* (Carpane et al., 2011). Under our experimental setting, eight-hour recordings were
344 long enough to allow 50% of the insects to reach the phloem phase, irrespective of the
345 cultivar. This recording time was chosen for the experiments as it represents a widely
346 used standard in EPG studies, and because in previous experiences, Chuche et al.
347 (2017b) showed that, in average, 27% of the *S. titanus* probing time was spent in phloem
348 feeding phases with four hour recordings. According to our results, most of the cultivar-
349 dependent differences in *S. titanus* lies in phloem feeding behaviour. Actually, leafhoppers
350 spent more than 50% of their probing time feeding on the Barbera phloem, while on the
351 other two cultivars spent less than 20%. This result is in line with an enhanced possibility
352 of acquisition and inoculation of phloem-limited agents, like FDP in the case of Barbera
353 (Galetto et al., 2014). Although the “Potential E2 index”, a parameter regarded as a
354 reliable indicator of phloem acceptability (Alvarez et al., 2006; Girma et al., 1992), was not
355 significantly different among the tested cultivars, higher values were recorded for Barbera,
356 further supporting a possible preference of the leafhopper for this cultivar. Moreover, since
357 only half of the vectors reached the phloem phase during the 8-hour recordings, we cannot
358 exclude that the amount of time was not sufficient to obtain a more descriptive feeding
359 behaviour from all leafhoppers. Dramatic differences were highlighted in the “Total
360 duration of phloem ingestions” on the different cultivars, while the “Number of phloem
361 ingestions” and “sustained phloem ingestions” were similar. The former was actually the
362 variable accounting for the highest differences in phloem phase among cultivars, and
363 suggests that *S. titanus* prefers Barbera phloem to Brachetto or Moscato ones. Since no
364 differences were recorded among cultivars in the percentage of leafhoppers reaching
365 phloem, but “Total duration of phloem ingestion” and “Duration of the longest phloem
366 ingestion” were higher on Barbera, it can be hypothesized that Brachetto and Moscato
367 phloem saps contain some repellent compounds disturbing phloem feeding. During the
368 phloem phase, the main waveforms were related to the passive ingestion of phloem and to
369 the interruption between two different passive ingestion phases (mainly “Np”). These
370 interruptions were already described for *S. titanus* by Chuche et al. (2017a) and for

371 *Circulifer tenellus* by Stafford and Walker (2009), and were suggested to represent
372 salivation events. Two are the main functions of saliva in piercing-sucking insects: i)
373 production of stylets sheath in the inter-cellular pathway phase (sheath saliva) or ii) dilution
374 of to-be-ingested sap and the suppression of defensive mechanism by the plant through
375 effectors (watery saliva) (Miles, 1972; Tjallingii, 2006; Will, Furch, & Zimmermann, 2013).
376 According to Chuche et al. (2017a) and Stafford & Walker (2009), the “Np” interruptions
377 found during *S. titanus* ingestion of phloem sap correspond to watery-salivation events.
378 This type of salivation is related to the inoculation of persistent-propagative agents from
379 the insect salivary glands into the plants tissues (Hogenhout et al., 2008). Therefore, the
380 greater number of interruption-salivation events on Barbera, that are a reflection of the
381 longer phloem phase, can explain, at least in part, the high susceptibility to FDp of this
382 cultivar. Phytoplasma spread can be regarded as a function of insect acquisition efficiency,
383 which is directly related to the duration of the phloem feeding phase, and of the inoculation
384 efficiency, which is putatively related to the absolute number of watery salivation events,
385 these latter also occurring during phloem feeding phase. According to this hypothesis, on
386 Barbera, the vector acquires and transmits efficiently, because it feeds longer in the
387 phloem and produces a higher number of salivation events compared to Brachetto and
388 Moscato. Indeed, Galetto et al. (2016) demonstrated that FDp acquisition by *S. titanus*
389 depends on the grapevine variety, with high efficiency from the most susceptible ones.
390 Also, on Brachetto and Moscato a high frequency of Np interruptions events were
391 recorded, but phloem phase was much shorter, leading to a lower absolute number of
392 salivation events. It is worth noting that, when the three grapevine varieties were exposed
393 to equally infected leafhoppers, Brachetto and Moscato showed a strong tolerance against
394 the infection (Ripamonti et al., 2021). This is a clear indication that either the inoculation,
395 more than acquisition, has a major impact on transmission efficiency, or plant genotype
396 account for different susceptibilities. The high frequency of Np interruptions on the tolerant
397 varieties can be explained by the presence of repellent compounds in the phloem saps.
398 Brachetto and Moscato are aromatic varieties (Pollon et al., 2019) and they are genetically
399 related (Raimondi et al., 2020). Their leaves contain high quantities of terpenoids (Mazza
400 et al., 2003), and this class of compounds can be transferred through the plant via the
401 phloem flux (Zhang et al., 2016) like other defence compounds (Will et al., 2013). Hence, it
402 can be speculated that *S. titanus* disturbed behaviour may be associated with the
403 presence of aromatic compounds, that act as repellents in Brachetto and Moscato
404 phloems. Repellent compounds can therefore act as antixenotic compounds. Antixenosis,

405 defined as the modification of herbivore behaviour by plant factors, which results in the
406 inability of a plant to serve as a host (Kogan and Ortman, 1978; Kordan et al., 2019), is a
407 well-known factor determining host plant resistance. Terpenoids and other volatile
408 compounds have well-known antixenotic activities in different plant-insect interactions
409 (Chand et al., 2017; Koul, 2008; Messchendorp et al., 1998). Antixenosis may represent
410 a valuable factor to be considered in the development of grapevine resistance against *S.*
411 *titanus*, *de facto* causing a reduction in the inoculation efficiency of FDp. Indeed,
412 leafhopper survival is reduced following a 7 day exposure to Moscato compared to
413 Barbera (Ripamonti et al., 2021). Further research is needed to clarify possible Moscato
414 antibiosis effect on *S. titanus*.

415 In our study, the leafhoppers started probing within the first minute, regardless of the
416 grapevine variety. No evident differences were highlighted in the non-phloem related
417 variables, as well as on total probing time. These results suggest that tested cultivars have
418 no major differences in the biochemical composition or structure of the leaf cuticle,
419 epidermis or mesophyll, that can impact the first feeding behaviour phases. Grapevine
420 trichomes are of the non-glandular type, subdivided in prostrate or erect (Gago et al.,
421 2016). Interestingly, Barbera has a highly dense trichomes surface in the abaxial leaf
422 blade (OIV, 2007), suggesting a possible repellence towards piercing-sucking insects
423 (Smith and Chuang, 2014). Nevertheless, Barbera was the most suitable variety for *S.*
424 *titanus* among those tested. For leafhoppers, data on trichome density acceptability are
425 available mainly for species of the Empoascini tribe, that are mostly insensitive to trichome
426 density on leaves. This is the case of *Empoasca vitis* on grapevine (Pavan and Picotti,
427 2009), *E. terminalis* on soybean (Nasruddin et al., 2014), and *E. fabae* on potato (Kaplan
428 et al., 2009). On the other hand, *E. fabae* and *Amrasca devastans* tend to avoid high
429 trichome density when feeding on edamame (*Glycine max* (L.)) and cotton, respectively
430 (Menger et al., 2018; Murugesan and Kavitha, 2010). As for *S. titanus*, it can be concluded
431 that a dense abaxially pubescence does not hamper nutrition on grapevine.

432 This work failed to identify clear differences in feeding behaviour of males and females.
433 Although small differences between sexes were recorded for some variables, no
434 differences were highlighted in the multivariate analysis conducted through CCA followed
435 by perMANOVA. On the contrary, Chuche et al. (2017b) reported that males feed more in
436 the phloem, compared to females. Following the analysis of our EPG recordings, we
437 conclude that no clear differences in feeding behaviour can be identified. Although

438 unlikely, we cannot exclude that the different grapevine varieties used in the studies may
439 explain for this difference.

440 Future research should focus on antixenotic compounds in *V. vinifera* genotypes, and their
441 role in vector-associated resistance to FD. On the other hand, plant secondary metabolites
442 involved in defense mechanisms against pathogens, such as polyphenols, particularly vein
443 flavonols and flavanonols (Kedrina-Okutan et al., 2019, 2018) may play a role in plant
444 resistance towards the phytoplasma. All these grapevine genetic traits should be regarded
445 as a natural resource to be exploited to obtain tolerant genotypes for a more sustainable
446 viticulture.

447

448 **5. Conclusions**

449 The results of the present work indicate that Barbera variety is a better food source than
450 Brachetto and Moscato for *S. titanus*. Indeed, the leafhopper showed longer phloem
451 ingestion, with an absolute higher number of watery-salivation events, on grapevines of
452 the Barbera cv. This latter feature is consistent with the high susceptibility of Barbera to
453 FDP, as watery salivation have been associated with the inoculation of persistent-
454 propagative agents from the insect salivary glands into the plants tissues. When feeding
455 on Brachetto and Moscato, *S. titanus* showed reduced phloem nutrition, possibly due to
456 antixenotic factors such as terpenoids, given the aromatic nature of the two varieties.

457

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465

466 **Author contributions**

467 Conceptualization: DB, CM, MR. Data curation: MR. Formal analysis: MR. Funding
468 acquisition: DB, CM. Investigation: MR, FM. Methodology: AF, DC, DB, MR. Software: MR.

469 Project administration: DB. Supervision: AF, DB, DC. Visualization: MR. Writing - original
470 draft: MR. Writing - review & editing: DB, CM, AF, DC.

471 References

- 472 Alvarez, A.E., Tjallingii, W.F., Garzo, E., Vleeshouwers, V., Dicke, M., Vosman, B., 2006. Location of
473 resistance factors in the leaves of potato and wild tuber-bearing Solanum species to the aphid
474 Myzus persicae. Entomol. Exp. Appl. 121, 145–157. [https://doi.org/10.1111/j.1570-](https://doi.org/10.1111/j.1570-8703.2006.00464.x)
475 8703.2006.00464.x
- 476 Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance: NON-
477 PARAMETRIC MANOVA FOR ECOLOGY. Austral Ecol. 26, 32–46. [https://doi.org/10.1111/j.1442-](https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x)
478 9993.2001.01070.pp.x
- 479 Backus, E.A., Bennett, W.H., 2009. The AC–DC Correlation Monitor: New EPG design with flexible input
480 resistors to detect both R and emf components for any piercing–sucking hemipteran. J. Insect
481 Physiol. 16. <https://doi.org/10.1016/j.jinsphys.2009.05.007>
- 482 Backus, E.A., Cline, A.R., Ellerseick, M.R., Serrano, M.S., 2007. *Lygus hesperus* (Hemiptera: Miridae) Feeding
483 on Cotton: New Methods and Parameters for Analysis of Nonsequential Electrical Penetration
484 Graph Data. Ann. Entomol. Soc. Am. 100, 296–310. [https://doi.org/10.1603/0013-](https://doi.org/10.1603/0013-8746(2007)100[296:LHHMFO]2.0.CO;2)
485 8746(2007)100[296:LHHMFO]2.0.CO;2
- 486 Backus, E.A., Guedes, R.N.C., Reif, K.E., 2020. AC–DC electropenetrography: fundamentals, controversies,
487 and perspectives for arthropod pest management. Pest Manag. Sci. <https://doi.org/10.1002/ps.6087>
- 488
- 489 Bocca, F.M., Picciau, L., Alma, A., 2020. New insights on Scaphoideus titanus biology and their implications
490 for integrated pest management. Entomol. Gen. <https://doi.org/10.1127/entomologia/2020/0977>
- 491 Bosco, D., Mori, N., 2013. Flavescence dorée vector control in Italy. Phytopathogenic Mollicutes 3, 40–43.
- 492 Caillaud, C.M., Di Pietro, J.P., Chaubet, B., Pierre, J.S., 1995a. Application of discriminant analysis to
493 electrical penetration graphs of the aphid Sitobion avenae feeding on resistant and susceptible
494 wheat. J. Appl. Entomol. 119, 103–106. <https://doi.org/10.1111/j.1439-0418.1995.tb01252.x>
- 495 Caillaud, C.M., Pierre, J.S., Chaubet, B., Di Pietro, J.P., 1995b. Analysis of wheat resistance to the cereal
496 aphid Sitobion avenae using electrical penetration graphs and flow charts combined with
497 correspondence analysis. Entomol. Exp. Appl. 75, 9–18. [https://doi.org/10.1111/j.1570-](https://doi.org/10.1111/j.1570-7458.1995.tb01904.x)
498 7458.1995.tb01904.x
- 499 Carpane, P., Wayadande, A., Backus, E., Dolezal, W., Fletcher, J., 2011. Characterization and correlation of
500 new electrical penetration graph waveforms for the corn leafhopper (Hemiptera: Cicadellidae).
501 Ann. Entomol. Soc. Am. 104, 515–525. <https://doi.org/10.1603/AN10052>
- 502 Caudwell, A., Kuszala, C., Bachelier, J.C., Larrue, J., 1970. Transmission de la Flavescence dorée de la vigne
503 aux plantes herbacées par l’allongement du temps d’utilisation de la cicadelle Scaphoideus littoralis
504 Ball et l’étude de sa survie sur un grand nombre d’espèces végétales. 2, 415–428.
- 505 Chand, R.R., Jokhan, A.D., Gopalan, R.D., 2017. A mini-review of essential oils in the South Pacific and their
506 insecticidal properties. Adv. Hortic. Sci. 295-310 Pages. <https://doi.org/10.13128/AHS-20833>
- 507 Chen, J.Q., Martin, B., Rahbé, Y., Fereres, A., 1997. Early intracellular punctures by two aphid species on
508 near-isogenic melon lines with and without the virus aphid transmission (Vat) resistance gene. J.
509 Plant Pathol. 103, 521–536.
- 510 Chucho, J., Backus, E.A., Thiéry, D., Sauvion, N., 2017a. First finding of a dual-meaning X wave for phloem
511 and xylem fluid ingestion: Characterization of Scaphoideus titanus (Hemiptera: Cicadellidae) EPG
512 waveforms. J. Insect Physiol. 102, 50–61. <https://doi.org/10.1016/j.jinsphys.2017.01.013>
- 513 Chucho, J., Sauvion, N., Thiéry, D., 2017b. Mixed xylem and phloem sap ingestion in sheath-feeders as
514 normal dietary behavior: Evidence from the leafhopper Scaphoideus titanus. J. Insect Physiol. 102,
515 62–72. <https://doi.org/10.1016/j.jinsphys.2017.01.014>
- 516 Chucho, J., Thiéry, D., 2014. Biology and ecology of the Flavescence dorée vector Scaphoideus titanus: A
517 review. Agron. Sustain. Dev. 34, 381–403. <https://doi.org/10.1007/s13593-014-0208-7>
- 518 Cribari-Neto, F., Zeileis, A., 2010. Beta Regression in R. J. Stat. Softw. 34, 1–24.
519 <https://doi.org/10.18637/jss.v034.i02>

- 520 Davis, R.E., Dally, E.L., 2001. Revised Subgroup Classification of Group 16SrV Phytoplasmas and Placement
521 of *Flavescence Dorée* -Associated Phytoplasmas in Two Distinct Subgroups. *Plant Dis.* 85, 790–797.
522 <https://doi.org/10.1094/PDIS.2001.85.7.790>
- 523 Desneux, N., Decourtye, A., Delpuech, J.-M., 2007. The Sublethal Effects of Pesticides on Beneficial
524 Arthropods. *Annu. Rev. Entomol.* 52, 81–106.
525 <https://doi.org/10.1146/annurev.ento.52.110405.091440>
- 526 EFSA Panel on Plant Health (PLH), Jeger, M., Bragard, C., Caffier, D., Candresse, T., Chatzivassiliou, E.,
527 Dehnen-Schmutz, K., Gilioli, G., Jaques Miret, J.A., MacLeod, A., Navajas Navarro, M., Niere, B.,
528 Parnell, S., Potting, R., Rafoss, T., Rossi, V., Urek, G., Van Bruggen, A., Van Der Werf, W., West, J.,
529 Winter, S., Bosco, D., Foissac, X., Strauss, G., Hollo, G., Mosbach-Schulz, O., Grégoire, J., 2016. Risk
530 to plant health of *Flavescence dorée* for the EU territory. *EFSA J.* 14.
531 <https://doi.org/10.2903/j.efsa.2016.4603>
- 532 European Food Safety Authority (EFSA), Tramontini, S., Delbianco, A., Vos, S., 2020. Pest survey card on
533 *flavescence dorée* phytoplasma and its vector *Scaphoideus titanus*. *EFSA Support. Publ.* 17.
534 <https://doi.org/10.2903/sp.efsa.2020.EN-1909>
- 535 Eveillard, S., Jollard, C., Labroussaa, F., Khalil, D., Perrin, M., Desqué, D., Salar, P., Razan, F., Hévin, C.,
536 Bordenave, L., Foissac, X., Masson, J.E., Malembic-Maher, S., 2016. Contrasting Susceptibilities to
537 *Flavescence Dorée* in *Vitis vinifera*, Rootstocks and Wild *Vitis* Species. *Front. Plant Sci.* 7.
538 <https://doi.org/10.3389/fpls.2016.01762>
- 539 Fox, J., Weisberg, S., 2019. *An {R} Companion to Applied Regression.*, Third edition. ed. Sage, Thousand
540 Oaks CA.
- 541 Gago, P., Conéjéro, G., Martínez, M.C., Boso, S., This, P., Verdeil, J.-L., 2016. Microanatomy of leaf
542 trichomes: opportunities for improved ampelographic discrimination of grapevine (*Vitis vinifera* L.)
543 cultivars. *Aust. J. Grape Wine Res.* 22, 494–503. <https://doi.org/10.1111/ajgw.12226>
- 544 Galetto, L., Miliordos, D., Pegoraro, M., Sacco, D., Veratti, F., Marzachi, C., Bosco, D., 2016. Acquisition of
545 *Flavescence Dorée* Phytoplasma by *Scaphoideus titanus* Ball from Different Grapevine Varieties. *Int.*
546 *J. Mol. Sci.* 17, 1563. <https://doi.org/10.3390/ijms17091563>
- 547 Galetto, L., Miliordos, D., Roggia, C., Rashidi, M., C, M., Bosco, D., 2014. Acquisition capability of the
548 grapevine *Flavescence dorée* by the leafhopper vector *Scaphoideus titanus* Ball correlates with
549 phytoplasma titre in the source plant. *J. Pest Sci.* 87, 671–679.
- 550 Garzo, E., Fernández-Pascual, M., Morcillo, C., Fereres, A., Gómez-Guillamón, M.L., Tjallingii, W.F., 2018.
551 Ultrastructure of compatible and incompatible interactions in phloem sieve elements during the
552 stylet penetration by cotton aphids in melon. *Insect Sci.* 25, 631–642.
- 553 Girma, M., Wilde, G.E., Reese, J., 1992. Russian Wheat Aphid (Homoptera: Aphididae) Feeding Behavior on
554 Host and Nonhost Plants. *J Econ Entomol* 85, 395–401.
- 555 Hogenhout, S.A., Ammar, E.-D., Whitfield, A.E., Redinbaugh, M.G., 2008. Insect Vector Interactions with
556 Persistently Transmitted Viruses. *Annu. Rev. Phytopathol.* 46, 327–359.
557 <https://doi.org/10.1146/annurev.phyto.022508.092135>
- 558 Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous Inference in General Parametric Models. *Biom. J.* 50,
559 346–363.
- 560 Jiang, Y.X., Nombela, G., Muñiz, M., 2001. Analysis by DC-EPG of the resistance to *Bemisia tabaci* on an Mi-
561 tomato line. *Entomol. Exp. Appl.* 99, 295–302. <https://doi.org/10.1046/j.1570-7458.2001.00828.x>
- 562 Kaplan, I., Dively, G.P., Denno, R.F., 2009. The costs of anti-herbivore defense traits in agricultural crop
563 plants: A case study involving leafhoppers and trichomes. *Ecol. Appl.* 19, 864–872.
564 <https://doi.org/10.1890/07-1566.1>
- 565 Kassambara, A., 2020. *ggpubr: “ggplot2” Based Publication Ready Plots.*
- 566 Kawabe, S., McLean, D.L., 1980. Electronic measurement of probing activities of the green leafhopper of
567 rice. *Entomol. Exp. Appl.* 27, 77–82. <https://doi.org/10.1111/j.1570-7458.1980.tb02948.x>
- 568 Kedrina-Okutan, O., Novello, V., Hoffmann, T., Hadersdorfer, J., Occhipinti, A., Schwab, W., Ferrandino, A.,
569 2018. Constitutive Polyphenols in Blades and Veins of Grapevine (*Vitis vinifera* L.) Healthy Leaves. *J.*
570 *Agric. Food Chem.* 66, 10977–10990. <https://doi.org/10.1021/acs.jafc.8b03418>

- 571 Kedrina-Okutan, O., Novello, V., Hoffmann, T., Hadersdorfer, J., Schneider, A., Schwab, W., Ferrandino, A.,
572 2019. Polyphenolic diversity in *Vitis* sp. leaves. *Sci. Hortic.* 256, 108569.
573 <https://doi.org/10.1016/j.scienta.2019.108569>
- 574 Kimmins, F.M., 1989. Electrical penetration graphs from *Nilaparvata lugens* on resistant and susceptible rice
575 varieties. *Entomol. Exp. Appl.* 50, 69–79. <https://doi.org/10.1111/j.1570-7458.1989.tb02317.x>
- 576 Kogan, M., Ortman, E.F., 1978. Antixenosis-A New Term Proposed to Define Painter's "Nonpreference"
577 Modality of Resistance. *Bull. Entomol. Soc. Am.* 24, 175–176.
578 <https://doi.org/10.1093/besa/24.2.175>
- 579 Kordan, B., Stec, K., Słomiński, P., Laszczak-Dawid, A., Wróblewska-Kurdyk, A., Gabryś, B., 2019. Antixenosis
580 Potential in Pulses Against the Pea Aphid (Hemiptera: Aphididae). *J. Econ. Entomol.* 112, 465–474.
581 <https://doi.org/10.1093/jee/toy349>
- 582 Koul, O., 2008. Phytochemicals and Insect Control: An Antifeedant Approach. *Crit. Rev. Plant Sci.* 27, 1–24.
583 <https://doi.org/10.1080/07352680802053908>
- 584 Lee, I.-M., Martini, M., Marcone, C., Zhu, S.F., 2004. Classification of phytoplasma strains in the elm yellows
585 group (16SrV) and proposal of 'Candidatus Phytoplasma ulmi' for the phytoplasma associated with
586 elm yellows. *Int. J. Syst. Evol. Microbiol.* 54, 337–347. <https://doi.org/10.1099/ijs.0.02697-0>
- 587 Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data.
588 *Oecologia* 129, 271–280.
- 589 Legendre, P., Legendre, L., 2012. *Numerical Ecology*, 3rd English ed. ed. Elsevier.
- 590 Lenth, R., 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means.
- 591 Lett, J.-M., Granier, M., Grondin, M., Turpin, P., Molinaro, F., Chiroleu, F., Peterschmitt, M., Reynaud, B.,
592 2001. Electrical penetration graphs from *Cicadulina mbila* on maize, the fine structure of its stylet
593 pathways and consequences for virus transmission efficiency. *Entomol. Exp. Appl.* 101, 93–109.
594 <https://doi.org/10.1046/j.1570-7458.2001.00895.x>
- 595 Long, J., 2020. jtools: Analysis and Presentation of Social Scientific Data.
- 596 Lucini, T., Panizzi, A.R., de F. Bueno, A., 2021. Evaluating resistance of the soybean block technology
597 cultivars to the Neotropical brown stink bug, *Euschistus heros* (F.). *J. Insect Physiol.* 131, 104228.
598 <https://doi.org/10.1016/j.jinsphys.2021.104228>
- 599 Maggi, F., Bosco, D., Galetto, L., Palmano, S., Marzachi, C., 2017. Space-time point pattern analysis of
600 flavescence dorée epidemic in a grapevine field: Disease progression and recovery. *Front. Plant Sci.*
601 7. <https://doi.org/10.3389/fpls.2016.01987>
- 602 Martini, M., Botti, S., Marcone, C., Marzachi, C., Casati, P., Bianco, P.A., Benedetti, R., Bertaccini, A., 2002.
603 Genetic variability among flavescence dorée phytoplasmas from different origins in Italy and
604 France. *Mol. Cell. Probes* 16, 197–208. <https://doi.org/10.1006/mcpr.2002.0410>
- 605 Mazza, G., Cascio, P., Barbieri, E., 2003. Free and Glicosidically Bound Volatile Compounds from *Vitis*
606 *vinifera* Cv. White Muscat Leaves and Grape Berries. *Riv. Vitic. E Enol.* 2–3, 57–74.
- 607 Mazzoni, V., Lucchi, A., Čokl, A., Prešern, J., Virant-Doberlet, M., 2009. Disruption of the reproductive
608 behaviour of *Scaphoideus titanus* by playback of vibrational signals: *Disruption of mating behaviour*
609 *in Scaphoideus titanus*. *Entomol. Exp. Appl.* 133, 174–185. <https://doi.org/10.1111/j.1570-7458.2009.00911.x>
- 610
- 611 McLean, D.L., Kinsey, M.G., 1964. A Technique for Electronically Recording Aphid Feeding and Salivation.
612 *Nature* 202, 1358–1359. <https://doi.org/10.1038/2021358a0>
- 613 Menger, J., Hanson, A.A., Koch, R.L., 2018. Evaluation of insect pests on edamame varieties in Minnesota. *J.*
614 *Econ. Entomol.* 111, 2272–2280. <https://doi.org/10.1093/jee/toy204>
- 615 Messchendorp, L., Schoonhoven, L.M., Loon, J.J.A. van, 1998. Terpenoid antifeedants against insects: a
616 behavioural and sensory study. Wageningen University, Wageningen, The Netherlands.
- 617 Miao, J., Han, B.-Y., Zhang, Q.-H., 2014. Probing behavior of *Empoasca vitis* (Homoptera: Cicadellidae) on
618 resistant and susceptible cultivars of tea plants. *J. Insect Sci.* 14.
619 <https://doi.org/10.1093/jisesa/ieu085>
- 620 Miles, P.W., 1972. The Saliva of Hemiptera, in: *Advances in Insect Physiology*. Elsevier, pp. 183–255.
621 [https://doi.org/10.1016/S0065-2806\(08\)60277-5](https://doi.org/10.1016/S0065-2806(08)60277-5)

- 622 Mitchell, C., Brennan, R.M., Graham, J., Karley, A.J., 2016. Plant Defense against Herbivorous Pests:
623 Exploiting Resistance and Tolerance Traits for Sustainable Crop Protection. *Front. Plant Sci.* 7.
624 <https://doi.org/10.3389/fpls.2016.01132>
- 625 Moral, R.A., Hinde, J., Demétrio, C.G.B., 2017. Half-Normal Plots and Overdispersed Models in R: The hnp
626 Package. *J. Stat. Softw.* 81. <https://doi.org/10.18637/jss.v081.i10>
- 627 Morone, C., Boveri, M., Giosuè, S., Gotta, P., Rossi, V., Scapin, I., Marzachi, C., 2007. Epidemiology of
628 flavescence dorée in vineyards in northwestern Italy. *Phytopathology* 97, 1422–1427.
629 <https://doi.org/10.1094/PHYTO-97-11-1422>
- 630 Naimi, B., Hamm, N.A.S., Groen, T.A., Skidmore, A.K., Toxopeus, A.G., 2014. Where is positional uncertainty
631 a problem for species distribution modelling? *Ecography* 37, 191–203.
632 <https://doi.org/10.1111/j.1600-0587.2013.00205.x>
- 633 Nasruddin, A., Gassa, A., Melina, M., 2014. Field evaluation of several cultivated soybean varieties against
634 *Empoasca terminalis* (Hemiptera: Cicadellidae). *Fla. Entomol.* 97, 995–1001.
635 <https://doi.org/10.1653/024.097.0357>
- 636 OIV, The International Organisation of Vine and Wine, 2007. OIV descriptor list for grape varieties and *Vitis*
637 species (2nd edition). Descriptors 84-87.
- 638 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O’Hara, R.B.,
639 Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. *vegan*: Community
640 Ecology Package. R package version 2.5-6.
- 641 Pavan, F., Picotti, P., 2009. Influence of grapevine cultivars on the leafhopper *Empoasca vitis* and its egg
642 parasitoids. *BioControl* 54, 55–63. <https://doi.org/10.1007/s10526-008-9151-3>
- 643 Pollon, M., Torchio, F., Giacosa, S., Segade, S.R., Rolle, L., 2019. Use of density sorting for the selection of
644 aromatic grape berries with different volatile profile. *Food Chem.* 276, 562–571.
645 <https://doi.org/10.1016/j.foodchem.2018.10.040>
- 646 Quensen, J., 2018. *ggordiplots*: Make ggplot Versions of Vegan’s Ordplots.
- 647 R Core Team., 2020. R: A language and environment for statistical computing. R Foundation for Statistical
648 Computing, Vienna, Austria.
- 649 Raimondi, S., Tumino, G., Ruffa, P., Boccacci, P., Gambino, G., Schneider, A., 2020. DNA-based genealogy
650 reconstruction of Nebbiolo, Barbera and other ancient grapevine cultivars from northwestern Italy.
651 *Sci. Rep.* 10, 15782. <https://doi.org/10.1038/s41598-020-72799-6>
- 652 Ripamonti, M., Pegoraro, M., Morabito, C., Gribaudo, I., Schubert, A., Bosco, D., Marzachi, C., 2021.
653 Susceptibility to flavescence dorée of different *Vitis vinifera* genotypes from north-western Italy.
654 *Plant Pathol.* 70, 511–520. <https://doi.org/10.1111/ppa.13301>
- 655 Ripamonti, M., Pegoraro, M., Rossi, M., Bodino, N., Beal, D., Panero, L., Marzachi, C., Bosco, D., 2020.
656 Prevalence of Flavescence Dorée Phytoplasma-Infected *Scaphoideus titanus* in Different Vineyard
657 Agroecosystems of Northwestern Italy. *Insects* 11, 301. <https://doi.org/10.3390/insects11050301>
- 658 Rodríguez-López, M.J., Garzo, E., Bonani, J.P., Fereres, A., Fernández-Muñoz, R., Moriones, E., 2011.
659 Whitefly resistance traits derived from the wild tomato *Solanum pimpinellifolium* affect the
660 preference and feeding behavior of *Bemisia tabaci* and reduce the spread of Tomato yellow leaf
661 curl virus. *Phytopathology* 101, 1191–1201.
- 662 Sarria, E., Cid, M., Garzo, E., Fereres, A., 2009. Excel Workbook for automatic parameter calculation of EPG
663 data. *Comput. Electron. Agric.* 67, 35–42. <https://doi.org/10.1016/j.compag.2009.02.006>
- 664 Sauge, M.-H., Kervella, J., Rahbé, Y., 1998. Probing behaviour of the green peach aphid *Myzus persicae* on
665 resistant *Prunus* genotypes. *Entomol. Exp. Appl.* 89, 223–232. <https://doi.org/10.1046/j.1570-7458.1998.00403.x>
- 666
- 667 Smith, C.M., Chuang, W.-P., 2014. Plant resistance to aphid feeding: behavioral, physiological, genetic and
668 molecular cues regulate aphid host selection and feeding: Plant resistance to aphid feeding. *Pest*
669 *Manag. Sci.* 70, 528–540. <https://doi.org/10.1002/ps.3689>
- 670 Stafford, C.A., Walker, G.P., 2009. Characterization and correlation of DC electrical penetration graph
671 waveforms with feeding behavior of beet leafhopper, *Circulifer tenellus*. *Entomol. Exp. Appl.* 130,
672 113–129. <https://doi.org/10.1111/j.1570-7458.2008.00812.x>
- 673 Tjallingii, W.F., 2006. Salivary secretions by aphids interacting with proteins of phloem wound responses. *J.*
674 *Exp. Bot.* 57, 739–745. <https://doi.org/10.1093/jxb/erj088>

- 675 Tjallingii, W.F., 1978. ELECTRONIC RECORDING OF PENETRATION BEHAVIOUR BY APHIDS. *Entomol. Exp.*
676 *Appl.* 24, 721–730. <https://doi.org/10.1111/j.1570-7458.1978.tb02836.x>
- 677 Tosi, S., Costa, C., Vesco, U., Quaglia, G., Guido, G., 2018. A 3-year survey of Italian honey bee-collected
678 pollen reveals widespread contamination by agricultural pesticides. *Sci. Total Environ.* 11.
679 <https://doi.org/10.1016/j.scitotenv.2017.09.226>
- 680 Trębicki, P., Tjallingii, W.F., Harding, R.M., Rodoni, B.C., Powell, K.S., 2012. EPG monitoring of the probing
681 behaviour of the common brown leafhopper *Orosius orientalis* on artificial diet and selected host
682 plants. *Arthropod-Plant Interact.* 6, 405–415. <https://doi.org/10.1007/s11829-012-9192-5>
- 683 Trivellone, V., Jermini, M., Linder, C., Cara, C., Delabays, N., Baumgärtner, J., 2013. Rôle de la flore du
684 vignoble sur la distribution de *Scaphoideus titanus*. *Rev Suisse Vitic Arboric Hortic* 45, 222–228.
- 685 Venables, W.N., Ripley, B.D., 2002. *Modern Applied Statistics with S* 504. [https://doi.org/10.1007/978-0-](https://doi.org/10.1007/978-0-387-21706-2)
686 387-21706-2
- 687 Wickham, H., 2020. *tidyr: Tidy Messy Data*.
- 688 Wickham, H., 2019. *stringr: Simple, Consistent Wrappers for Common String Operations*.
- 689 Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
- 690 Wickham, H., François, R., Henry, L., Müller, K., 2020. *dplyr: A Grammar of Data Manipulation*.
- 691 Will, T., Furch, A.C., Zimmermann, M.R., 2013. How phloem-feeding insects face the challenge of phloem-
692 located defenses. *Front. Plant Sci.* 4. <https://doi.org/10.3389/fpls.2013.00336>
- 693 Zeileis, A., Köll, S., Graham, N., 2020. Various Versatile Variances: An Object-Oriented Implementation of
694 Clustered Covariances in *R*. *J. Stat. Softw.* 95. <https://doi.org/10.18637/jss.v095.i01>
- 695 Zhang, P., Fuentes, S., Wang, Y., Deng, R., Krstic, M., Herderich, M., Barlow, E.W.R., Howell, K., 2016.
696 Distribution of Rotundone and Possible Translocation of Related Compounds Amongst Grapevine
697 Tissues in *Vitis vinifera* L. cv. Shiraz. *Front. Plant Sci.* 7. <https://doi.org/10.3389/fpls.2016.00859>
698