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Relationships between *Drosophila suzukii* and grapevine in North-western Italy: seasonal presence and cultivar susceptibility

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Abstract

Drosophila suzukii (Matsumura) is an Asian species, and is now widespread also in America and Europe. While it is acknowledged as a serious pest to many soft fruits, its impact on viticulture needs further investigation. This study focused on the relationships between *D. suzukii* and grapevine in Piedmont (North-western Italy), and was conducted for three seasons in 10 vineyards of six different grape varieties, surrounded by different crops and/or vegetation. Flies were collected using food traps, and grape berries were sampled to assess oviposition. The susceptibility of grape cultivars was analysed in the laboratory by exposing intact berries with a known skin hardness (i.e. skin break force mechanical property) and injured berries to female flies. A choice test was also performed to investigate the preference of *D. suzukii* for grape juice of different cultivars. Capture of adults in vineyards depended on sampling dates, surrounding vegetation, and years, but few eggs were observed in field-collected berries. Overall, in laboratory experiments, cultivars with soft-skinned berries were more exploited for egg-laying. However, among cultivars, the susceptibility changed depending on whether the berries were intact or injured. Concerning the latter, the most infested cultivars were also the most attractive to flies in the choice test. At present, *D. suzukii* may not be considered a threat to viticulture in Piedmont. However, considering the influence of grape skin hardness on oviposition and the attractiveness of some grape cultivars, further studies are needed to better understand the relationship between *D. suzukii* and grapevine.

Key words: spotted-wing drosophila, vineyard, egg-laying, berry skin hardness, trapping, grape ripening.

Introduction

Drosophila suzukii (Matsumura) (Diptera Drosophilidae), commonly known as the spotted-wing drosophila (SWD), is an invasive pest native to South-East Asia (Kanzawa, 1939), and is now present in several countries of America (Walsh *et al*., 2011; Burrack *et al*., 2013; Deprà *et al*., 2014; Lasa and Tadeo, 2015; Lue *et al*., 2017), Asia (Uchino, 2005; Asplen *et al*., 2015; Parchami-Araghi *et al*., 2015) and Europe (Calabria *et al*., 2010; Cini *et al*., 2012; Asplen *et al*., 2015). Contrary to many other species in the same family, *D. suzukii* females have a sclerotized and serrated ovipositor which makes them able to pierce and lay eggs in intact fruits (Lee *et al*., 2011a). This allows SWD to exploit a novel ecological niche and reduces its competition against other *Drosophila* species (Atallah *et al*., 2014). Moreover, *D. suzukii* has a wide range of hosts including cultivated, wild, and ornamental plants (Kinjo *et al*., 2013; Lee *et al*., 2015; Poyet *et al*., 2015; Tonina *et al*., 2016; Saeed *et al*., 2018). Severe economic losses on fruit crops, especially cherries, strawberries, blueberries, and raspberries, have been recorded in many countries where SWD has been accidentally introduced (Goodhue *et al*., 2011; Lee *et al*., 2011a; Kinjo *et al*., 2013; De Ros *et al*., 2015; Pajač Živković *et al*., 2018). Considerable damage has also been recorded in Northern Italy (Mazzetto *et al*., 2015a; Tait *et al*., 2018a). The first record of damage to the grape *Vitis vinifera* L. was reported by Kanzawa (1939) in Japan. More recently, traps set in vineyards confirmed the presence of *D. suzukii*: in France, adults were first captured in two vineyards in Bordeaux but no damage on grapes was recorded (Rouzes *et al*., 2012). On the other hand, surveys conducted in Quebec revealed the presence of *D. suzukii* in both traps and grape bunches (Saguez *et al*., 2013). A study conducted in Michigan on *V. vinifera* and *Vitis labrusca* (L.) confirmed the presence of SWD adults in vineyards, although without important yield losses (van Timmeren and Isaacs, 2014). Similarly, cold-hardy grape varieties (e.g., *V. labrusca*, *Vitis riparia* Michx., and hybrids of *V. vinifera*) seem to be resistant to *D. suzukii*, unless already damaged by other factors (Pelton *et al*., 2017). Nowadays the presence of *D. suzukii* in vineyards is confirmed in many other countries worldwide (Baroffio *et al*., 2014; Grant and Sial, 2016; Pajač Živković *et al*., 2018). In Italy, it was found in the North East region and in Apulia (Ioriatti *et al*., 2015; Baser *et al*., 2015; 2018; Antonacci *et al*., 2017). Although SWD damage on grapes is lower with respect to other fruits (Lee *et al*., 2011b; Kim *et al*., 2015; Pelton *et al*., 2017), a differential susceptibility among grape varieties was observed under laboratory conditions. Andreazza *et al.* (2016) identified high susceptibility in 3 out of 18 cultivar genotypes. The potential impact of *D. suzukii* on different cultivars has also been tested as a function of the physiological characteristics of grape berries such as degree Brix, pH, and skin penetration force. In particular, the highest number of eggs was found during the ripening period when both pH and berry firmness decrease, whereas sugar content increases (Ioriatti *et al*., 2015; Baser *et al*., 2018). However, among physiological features, penetration force seems to be the most important factor (Ioriatti *et al*., 2015; Baser *et al*., 2018; Rezazadeh *et al*., 2018; Entling *et al*., 2019). Furthermore, a possible influence of SWD on sour rot in grape clusters has also been considered. Rombault *et al.* (2017) suggested that grapes infested by *D. suzukii* were more attractive for *Drosophila melanogaster* Meigen, therefore increasing sour rot disease. A similar study found a clear implication of microorganisms associated with *D. suzukii* adults, which caused sour rot and spoilage (Ioriatti *et al*., 2018).

Viticulture and wine industry are an important part of Italian agriculture, and Piedmont is one of the leading regions: vineyards cover approximately 46,000 ha, yielding 2.4-2.7 million hl of wine, counting more than 20,000 grapevine farms (ISTAT, 2018). Therefore, in terms of pest management, the discovery of exotic species feeding on grapes and/or vines is always a concern among vine growers, wine makers, and stakeholders. The present research was carried out to study the presence of SWD in different vine growing areas, on different grape varieties across different environments, and to assess the susceptibility to SWD oviposition of the same varieties under laboratory conditions.

Materials and methods

Study area

Field samplings were conducted from 2015 to 2017 in 10 vineyards settled in different grapevine growing areas of Piedmont, North-western Italy (table 1). Vineyards were sprayed twice a year against *Scaphoideus titanus* Ball (Alma *et al*., 2015), at the end of June and at the end of July with neonicotinoids or organophosphates (no insecticides were used at Site 2 only). Pruning followed the "Guyot" system except for Site 2, which was shaped as "pergola". Surrounding vegetation included dog rose (*Rosa canina* L.), elder (*Sambucus nigra* L.), bramble (*Rubus fruticosus* L.), pokeweed (*Phytolacca* spp.), orchards such as apricot and plum trees, hazelnuts, dogwood (*Cornus sanguinea* L.), and many broadleaf trees. In each vineyard, an experimental sampling plot approximately 3000 m^2 in size (width, 35-40 m with 24 grapevine rows; length, 100 m) having at least two edges close to surrounding vegetation and/or crops was set up. In vineyards smaller than 3000 m^2, the whole vineyard size was considered as the sampling plot.

Field sampling of adults and eggs

SWD adults were sampled with Droso-trap New® (Biobest Group NV, Westerlo, Belgium) fly traps, loaded with 250-300 mL of Droskidrink® food bait (75% apple cider vinegar and 25% red wine + 2 g/L raw brown sugar) (Prantil, Trento, Italy) (Grassi *et al*., 2015), adding a drop of detergent to promote drowning. One single trap was placed at the centre of the plot, at the same height of the clusters. Traps were placed at the beginning of July and emptied and reloaded every 2 weeks until harvest, which ranged from the end of August to the end of October, depending on grape variety and year of study. At each sampling, the trap contents were poured into glass jars and filtered. The insects were preserved in 70% ethanol inside Falcon tubes (diameter, 2.5 cm; length, 12 cm), and then observed under a stereomicroscope: SWD were counted and determined according to EPPO (2013).

Oviposition was investigated by randomly collecting 50 intact berries (1-2 per cluster) from each vineyard every two weeks, from veraison (end of July) to harvest. On the whole, a total of 450-700 berries per vineyard was sampled. The berries were placed in a single layer inside plastic boxes, and stored inside a cool chamber (4 °C). Within 24 hours, they were inspected under a stereomicroscope to detect SWD eggs.

Insect and grape sources for laboratory experiments

A population of *D. suzukii* was obtained by placing infested strawberries and blueberries inside insect-proof cages. The flies were reared on artificial diet (Mazzetto *et al*., 2015b) inside plastic Falcon tubes. The whole rearing process (and also both choice and no choice experiments) took place inside a climatic chamber (T = 25 ± 1 °C; RH = 50-60%; L:D = 16:8).

Six varieties of grape having different ripening times (precocious and late: before and after September 15, respectively), and colour (white or red) were harvested at ripening in many vineyards of Piedmont: Moscato and Erbaluce (precocious, white); Dolcetto and Schiava (precocious, red); and Barbera and Nebbiolo (late, red). All cultivars are widespread in North-western Italy, apart from cv. Schiava which was chosen as a precocious positive control for its sensitivity to *D. suzukii* (Ioriatti *et al*., 2015; 2018).

Table 1. Main features of the experimental sites. Barbera, Nebbiolo, Dolcetto: red grape varieties; Erbaluce, Favorita, Moscato: white grape varieties. SV: spontaneous vegetation 1: bramble; 2: dog rose; 3: elder; 4: pokeweed; 5: plum trees; 6: dogwood. P/A: presence/absence of SV as a random factor for GLMM.

No.	District	GPS (DD)	Elevation (m asl)	Area (m^2)	Grape variety	SV	P/A
1	Acqui Terme	44.702456°N 8.417744°E	385	7700	Barbera	-	A
2	Caluso	45.300666°N 7.909150°E	280	4200	Erbaluce	1, 3, 4	P
3	La Morra	44.658398°N 7.950170°E	258	12000	Nebbiolo	-	A
4	Monticello d'Alba	44.709014°N 7.921686°E	272	3900	Favorita	1, 2, 3, 5, 6	P
5	Novello	44.598088°N 7.936916°E	398	13000	Nebbiolo	-	A
6	Dogliani	44.548184°N 7.928400°E	335	2000	Dolcetto	1, 3	P
7	Fontanile	44.738202°N 8.398571°E	295	7200	Barbera	4, 6	P
8	Serralunga d'Alba	44.591737°N 8.002164°E	430	10000	Nebbiolo	-	A
9	Trezzo Tinella	44.669200°N 8.109400° E	423	3300	Moscato	1, 3, 6	P
10	Nizza Monferrato	44.783454°N 8.309432°E	197	2550	Barbera	-	A

Chemical and mechanical characteristics of the berries

To ensure the same level of ripeness within varieties, samples of berries (5 kg) were sorted into 10 density classes by densimetric flotation in salt solution, according to Rolle *et al*., (2011a; 2015). The floating berries were washed with water and visually inspected, discarding damaged ones. A sample of approximately 200 berries belonging to the most representative density class (i.e., maximum berry distribution percentage) for each variety was used to analyse chemical and mechanical properties.

Three replicates of 30-35 berries were used to measure technological ripeness parameters conventionally expressed by reducing sugars, pH, and titratable acidity parameters. In the juice obtained from these berries by manual grape crushing and centrifugation, pH was determined by potentiometry using an InoLab 730 pH meter (WTW, Weilheim, Germany), and titratable acidity (g L^{-1} as tartaric acid) was estimated according to OIV methods (2008). Reducing sugars (as sum of glucose and fructose, g L^{-1}) were determined using a high-performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA, USA), equipped with a refractive index detector and a diode array detector (DAD) set to 210 nm (Rolle *et al*., 2015). The skin hardness, evaluated by break skin force parameters (F_{sk}, measured in Newtons, N) of the remaining 60 berries was determined by puncture test according to Letaief *et al.* (2008). Berries were singularly numbered and perforated (one test per berry) in their equatorial position at 1 mm s^{-1} speed, using a P/2N SMS needle probe moved by a Universal Testing Machine TA.XTplus texture analyser (Stable Micro Systems, Godalming, Surrey, UK), equipped with a HDP/90 platform and a 5 kg load cell. Data were acquired at 500 points per second, and evaluated using Texture Exponent software (Stable Micro Systems).

No-choice trials

The influence of grape variety and skin condition of berries (intact or injured) on the oviposition of *D. suzukii* was evaluated by no-choice tests on 60 berries per type. Intact berries were the same used for measuring skin hardness values; the pedicel was not removed, in order to avoid damaging the skin and providing another route for oviposition. On the other hand, injured berries were obtained by slightly carving the skin with a razor blade; in this case, only eggs laid into the wound were counted.

Each berry was placed inside a Petri dish (height, 3.5 cm; diameter, 15 cm) on a moistened filter paper: the hole done by the penetrometer was concealed by placing it directly on the paper, to avoid flies laying eggs inside. SWD pairs (one male and one newly emerged female) were put inside plastic Falcon tubes and fed with honey drops for 48 hours. Afterwards, single mated females were moved to Petri dishes for another 48 hours to allow oviposition on berries, and then removed. The eggs in each berry were then counted under a stereomicroscope within 24 hours. Finally, the berries were put back into the Petri dishes and observed for 40 days to check for adult emergence.

Choice trials

The flavour attractivity of different cultivars to SWD adults was examined by means of a multi-choice test. Sugar content and acidity were homogeneous in grapes belonging to the same cultivar. Precocious and late ripening cultivars were tested separately.

Tests were carried out in a self-made choice arena (figure 1) consisting of a box made out of Plexiglas [Poly(methyl methacrylate), or PMMA] and fine, insect-proof mesh and provided with removable sinks on the bottom (five and three for precocious and late ripening cultivars, respectively, including 1 control sink for each ripening period). Each sink was made with a plastic jar (volume 100 mL) and a Falcon tube with a small hole on the tip to allow flies to fly through. A layer of soaked cotton was placed on the floor of the box to preserve the humidity inside. In each jar, we placed 13-17 g of pressed berries (including skin) of a specific cultivar, added with 40 ml of distilled water. As a negative control, we used distilled water alone.

Approximately 50 females aged 3-7 days were starved on 1.5% agar (15 mL) for 24 hours and released inside the arena for a further 24 hours. Afterwards, dead females found inside the box (no choice) and in jars (choice) were retrieved and counted, and the whole arena was cleaned with neutral soap (pH = 5.5, with no scents nor colorants added), distilled water and ethanol (70% volume), and water again. The experiment was replicated nine times for both precocious and late ripening cultivars. For each replicate, the jars bearing different varieties were switched between holes to minimise the effect of the position, whereas the same jar was always used for a given variety.

Statistical analyses

Data of SWD captures were fitted to a generalised linear mixed model (GLMM), an extension of general linear models (GLMs) that is used to take into account both fixed and random effects (Bolker *et al*., 2009; Michel *et al*., 2017), and that is also suitable for handling incidence, binary, and count data. The general equation of a GLMM is as follows:

$$g(y) = X \cdot b + Z \cdot u + e$$

where *y* is the output (response, dependent) variable, $X \cdot b$ is the fixed part of the model (*X* the design matrix, and *b* the parameter matrix), $Z \cdot u$ the random part (*Z* and *u* the are design and parameter matrix, respectively), and *e* represents the residuals. The link function *g(y)* depends on the type of data distribution: as a rule of thumb, Poisson-distributed data (e.g., counts) require a *Log* link function (Michel *et al*., 2017). In the present research, the experimental sites were considered as subjects, and the number of captured SWD was the dependent variable. Week of sampling and presence/absence of spontaneous vegetation as a possible reservoir of SWD were the fixed effects, whereas year of sampling and grapevine variety were the random effects. Intercept was included in both fixed and random effects. We assumed a Poisson distribution (counts), with a *Log* link function. Post-hoc contrasts were performed using a sequential Bonferroni procedure.

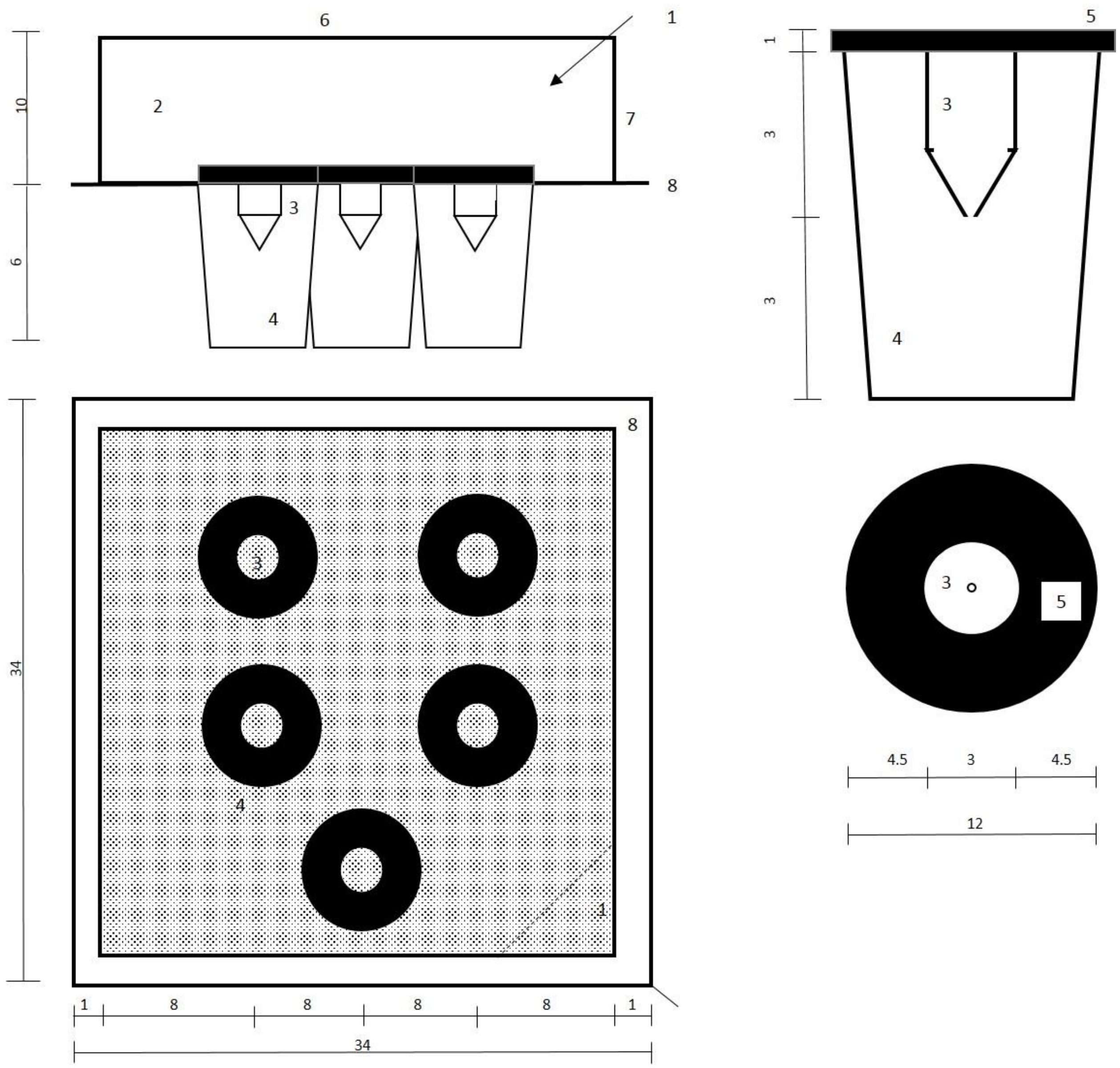

Figure 1. Choice test arena for *V. vinifera* cultivar preference of SWD. 1: entryway for flies; 2: choice arena; 3: Falcon tube glued to the lid (5) with a small hole at the tip; 4: plastic jar (100 mL) screwed to the lid; 5: lid of the jar, fitted and glued to a hole into the floor of the arena; 6: insect-proof mesh on the top of the arena; 7: Plexiglas walls of the arena; 8: Plexiglas bottom of the arena. Measurements are given in cm.

Values of the berry skin force of the six cultivars tested in the oviposition experiment were analysed via the Kruskal-Wallis non-parametric test, with a Bonferroni adjusted post-hoc test for separation of means ($P < 0.05$).

A GLM with a negative binomial distribution and *Log* link function was performed to analyse egg-laying data (dependent variable) on both intact and injured berries. In the former, cultivar and skin hardness seemed to be related to each other: therefore, data were analysed separately to test the effect of both, which were considered as fixed factors. When analysing for skin hardness, the berries of each cultivar were divided into four classes according to skin break force parameter: F_{sk1} = 0.2-0.4 N; F_{sk2} = 0.4-0.6 N; F_{sk3} = 0.6-0.8 N; and F_{sk4} > 0.8 N. On the other hand, when analysing data of carved berries, the cultivar was considered as a fixed effect and the F_{sk} parameter was not considered. When fixed effects were significant, means were separated through the sequential Bonferroni post-hoc test. In the multi-choice test, the number of flies in the jars (including control and no choice) was compared with Friedman-analysis of variance (ANOVA) followed by Wilcoxon signed rank tests with Bonferroni correction ($P < 0.05$). All analyses were performed with SPSS Statistics 25.0 (IBM Corp, Armonk, NY, USA).

Results

Capture of SWD adults

On the whole, approximately 68,000 adults were captured over 3 years of sampling. The lowest value was recorded at Site 10 (approximatively 1,000), whereas the highest value was found at Site 2 (> 15,000). Captures decreased along with sampling years, scoring approximately 31,000, 26,000, and 10,000 from 2015 to 2017. The highest captures were recorded at Site 5 in 2015, and at Site 2 in 2016 and 2017, whereas the lowest captures were recorded at Site 10 in 2015 and 2016, and at Site 1 in 2017. Overall, the peaks of captures were recorded on 10/7 (Julian Day [JD] = 280), 10/21 (JD = 295), and 8/26 (JD = 238) from 2015 to 2017, respectively (figure 2).

In the GLMM model, all of the tested fixed effects were significant: date of sampling ($F = 21264.82$; df = 1, 204; $P < 0.01$), surrounding vegetation ($F = 28.38$; df = 1, 204; $P < 0.01$), and interaction ($F = 807.82$; df = 1, 204; $P < 0.01$). The coefficients of the fixed part of the model are listed in table 2. Concerning random effects, year of sampling was significant ($u = 0.34 \pm 0.16$, $S = 2.12$, $P = 0.03$), whereas grapevine variety was not ($u = 0.43 \pm 0.30$, $S = 1.41$, $P = 0.16$).

SWD eggs on field-collected berries

Only six eggs were found on field-collected berries, all in 2015 and all on red grape varieties from three different sites. In all cases, the eggs were found on a single berry out of 150 sampled (rate of infested berries: 0.7%). At Site 3 (cv. Nebbiolo), there were two eggs on a berry collected on September 16 (eggs/berry: 0.01). Again, at Site 5 (cv. Nebbiolo), three eggs on a single berry sampled were observed on September 16 (eggs/berry: 0.02). Finally, at Site 6 (cv. Dolcetto), one egg was found on September 30 (eggs/berry: 0.007).

Chemical and mechanical characteristics of the berries

Significant differences in skin break force (F_{sk}) values were found in different grape cultivars ($\chi^2 = 240.255$; N = 360; df = 5; $P < 0.001$). Cv. Barbera had the highest value, followed by Dolcetto and Erbaluce, Moscato and Nebbiolo, and finally Schiava (table 3). On the other hand, grape ripeness stage seems to have no influence: i.e., very small variations in F_{sk} values have been detected in Nebbiolo grapes from veraison to harvest (Rolle *et al.*, 2012b). The chemical composition and mechanical properties of each variety are reported in table 3. Overall, within the same cultivar, both the technological ripeness parameters and F_{sk} values were similar to those that are recorded at harvest in Piedmont (Torchio *et al.*, 2010; Rolle *et al.*, 2011b; 2012a).Concerning cultivar Schiava, results are consistent with those reported by Ioriatti *et al*. (2015).

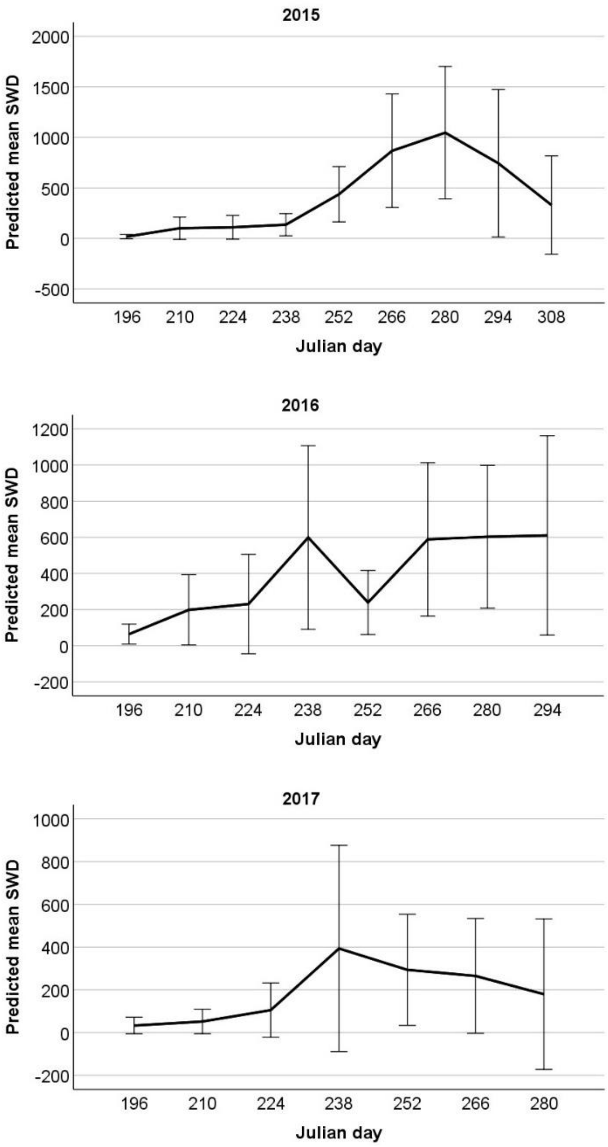

Figure 2. Seasonal trend in SWD captures as predicted by Generalized Linear Mixed Model (mean ± 95% confidence intervals).

No-choice trials

The number of SWD eggs laid on intact berries was significantly different depending on the cultivar (GLM; $\chi^2 = 134.777$; N = 360; df = 5; $P < 0.001$) (figure 3). The highest value was found in Nebbiolo (5.15 ± 0.63 eggs/berry), which was significantly different from all

Table 2. Significant fixed coefficients of GLMM applied to SWD captures.

Model term	Coefficient	Standard error	t	P	Lower 95% CI	Upper 95% CI
Intercept	1.40	0.35	4.00	< 0.01	0.71	2.09
Sampling date	0.12	0.00	79.71	< 0.01	0.12	0.12
Surrounding vegetation	−2.633	0.49	−5.33	< 0.01	−3.61	−1.66
Sampling date x surrounding vegetation	0.06	0.00	28.42	< 0.01	0.05	0.06

Table 3. Must parameters and berry skin break force values of selected grape berries. Berry skin break force (F_{sk}) values used for the no choice trials (n = 60) are expressed as mean ± standard error. Data of F_{sk} were analysed through Kruskal Wallis non-parametric test. When significant, mean values were indicated with different letters through Bonferroni adjusted post hoc test (P < 0.05). * as tartaric acid.

Cultivar	Selected density class [kg/m³]	Reducing sugars [g/L]	Titratable acidity [g/L]*	pH	Berry skin break force F_{sk} [N]	F_{sk} min [N]	F_{sk} max [N]
Moscato	1114	263	5.08	3.29	0.451±0.014 c	0.234	0.707
Erbaluce	1087	200	7.33	3.18	0.633±0.015 b	0.424	0.927
Schiava	1087	205	3.98	3.58	0.342±0.009 d	0.183	0.589
Dolcetto	1087	190	6.27	3.27	0.642±0.017 b	0.431	0.939
Barbera	1114	256	8.57	3.11	0.856±0.018 a	0.455	1.222
Nebbiolo	1106	242	5.64	3.21	0.487±0.014 c	0.267	0.878

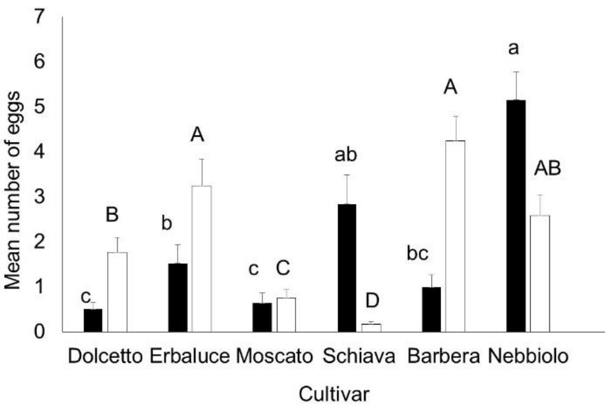

Figure 3. Eggs of SWD (mean ± s.e.) laid on berries of different grape cultivars (black: intact berries; white: injured berries). Precocious ripening cultivars: Dolcetto, Erbaluce, Moscato, Schiava; late ripening cultivars: Barbera, Nebbiolo.

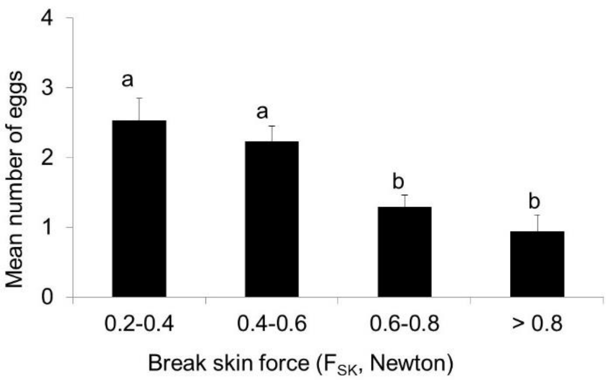

Figure 4. Eggs of SWD (mean ± s.e.) laid on berry of grapes having different break skin force (F_{sk}).

others except Schiava (2.83 ± 0.64). As well, Schiava was significantly different from Dolcetto and Moscato but not from Barbera and Erbaluce. Finally, Dolcetto and Moscato were significantly less affected than all others except Barbera (figure 3).

Significant differences in egg-laying on intact berries were also found among different skin hardness classes (GLM; χ^2 = 23.355; N = 360; df = 3; P < 0.001). More eggs were laid in berries belonging to lower F_{sk} classes (F_{sk1} and F_{sk2}) compared to higher ones (F_{sk3} and F_{sk4}) (figure 4). A small number of SWD adults emerged, and only from eggs laid on Barbera (emergence rate = 5.70 ± 4.23%) and Schiava (6.23 ± 2.87%).

Finally, significant differences among cultivars were found in the number of eggs laid on injured berries (GLM; χ^2 = 117.915; N = 360; df = 5; P < 0.001). The highest values were observed in Barbera (4.29 ± 0.55 eggs/berry), Erbaluce (3.23 ± 0.60), and Nebbiolo (2.58 ± 0.46), without significant differences among them. In addition, Nebbiolo did not differ from Dolcetto. The lowest oviposition rates were detected in Moscato (0.75 ± 0.18) and Schiava (0.17 ± 0.05), which were significantly different both from each other and from all others (figure 3). On injured grapes, adults emerged from eggs laid in Dolcetto (emergence rate = 4.17 ± 3.26%), Moscato (2.63 ± 2.63%), and Erbaluce (0.57 ± 0.40%). No adults emerged from Barbera, Nebbiolo, or Schiava.

Choice trials

Overall, significant differences were detected in the numbers of flies caught in the jars, both in precocious (Friedman-ANOVA, N = 9; df = 4; χ^2 = 25.650; P < 0.001) and late cultivars (Friedman-ANOVA, N = 9; df = 2; χ^2 = 14.889; P = 0.001). In precocious cultivars, significantly more flies were caught in Dolcetto (choice rate = 34.5 ± 6.4%) than in Schiava (3.7 ± 0.8%) and control (0.3 ± 0.3%), and in Erbaluce versus control (27.5 ± 6.6%). No differences were observed between Moscato (11.5 ± 2.6%) and all other cultivars including control. The rate of no choice in each replicate was always lower than 25% (figure 5). Relating to late cultivars, no differences were found between Barbera (37.2 ± 7.7%) and Nebbiolo (29.1 ± 5.0%), but both had a significantly higher choice rate than control (0.8 ± 0.4%). The rate of no choice in each replicate was always lower than 35% (figure 6).

Discussion

This research showed that adults of *D. suzukii* are widespread in piedmontese vineyards. Our data are consistent with many studies conducted in other countries (Rouzes *et al.*, 2012; Saguez *et al.*, 2013; Baroffio *et al.*,

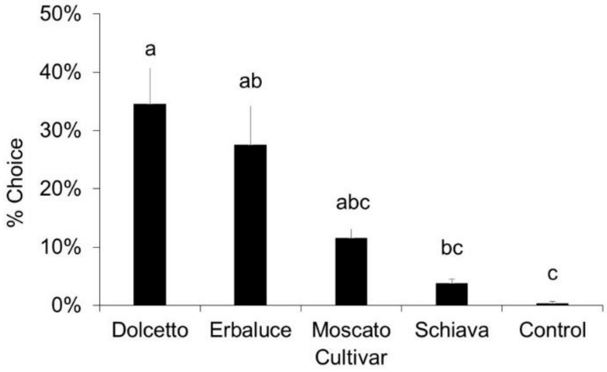

Figure 5. Choice of SWD adults (mean % ± s.e.) among pressed berries of precocious ripening grape cultivars.

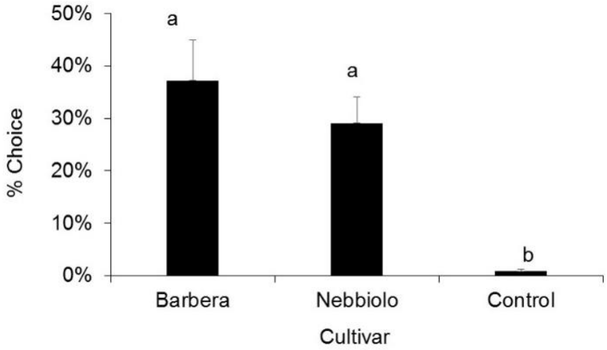

Figure 6. Choice of SWD adults (mean % ± s.e.) among pressed berries of late ripening grape cultivars.

2014; van Timmeren and Isaacs, 2014), and in other Italian regions (Ioriatti *et al*., 2015; Baser *et al*., 2018). Captures were significantly different depending on the sampling date, being quite low in the first part of summer (July), immediately after grapevine flowering, and increasing quickly just before harvest (end of August and beginning of September), depending on the season. This is also consistent with SWD flight dynamics on other crops (Mitsui *et al*., 2010; Hamby *et al*., 2014; Mazzetto *et al*., 2015a). Despite high levels of captured flies, no or very few eggs were detected on grape berries collected in vineyards. Moreover, eggs were found on berries only late in the season; i.e., on late-ripening varieties (e.g., Nebbiolo), possibly because of either the increase in the number of flies or the reduction of alternative hosts. As well, the presence of surrounding spontaneous vegetation suitable for SWD oviposition increased the capture of flies inside vineyards. Similar results were obtained with other crops (Poyet *et al*., 2015; Kenis *et al*., 2016). In particular, some plants, such as pokeweed, bramble, and elder, are highly affected by *D. suzukii* (Baroffio *et al*., 2014; Lee *et al*., 2015), and their presence in the surrounding area may cause an increase in the number of flies in vineyards. Captures of SWD were significantly different between years. This may be a consequence of different harvest times, depending on seasonal climatic factors (e.g., temperature). Generally, harvest occurs earlier following warm and dry weather (Jones and Davis, 2000; Meléndez *et al*., 2013), as observed in 2017. Harvest time also depends on the variety: usually, white grapes are harvested earlier than red ones. However, the presence of SWD does not depend on grape variety as we found no differences in fly captures among different varieties. A study conducted in Brazil (Andreazza *et al*., 2016) found an overall low susceptibility among different grapevine cultivars, apart from three genotypes. As well, we found a higher susceptibility of cvs. Nebbiolo and Schiava. The latter was already deemed vulnerable to SDW in northern Italy (Ioriatti *et al*., 2015). In fact, in our experiments these cultivars also showed a lower F_{sk} value compared to others, in accordance with the literature (Ioriatti *et al*., 2015; Baser *et al*., 2018; Entling *et al*., 2019). In particular, Barbera was more resistant and had the highest F_{sk} value. On the other hand, SWD laid few eggs on Moscato, despite a F_{sk} as low as in cv. Nebbiolo, possibly because of grape skin colour, flavours, and other chemical parameters. In fact, the overall situation changed slightly when SWD laid eggs on injured berries, and was also consistent with the results of the grape juice attraction choice test. Similarly, flavour blend of grape flesh, sugar parameters, and volatile acidity may have decreased the susceptibility and attractiveness of injured berries in Schiava, whereas Dolcetto and Barbera were more preferred. The influence of flavour blend on SWD has been studied in other fruits (Yu *et al*., 2013; Abraham *et al*., 2015; Revadi *et al*., 2015; Feng *et al*., 2018; Tait *et al*., 2018b) but still requires further investigation in grapes. On the other hand, the effects of sugar content and volatile acidity have been studied in many grape cultivars other than those tested in the present research (Entling *et al*., 2019).

A small number of adults emerged from eggs laid on the berries, both intact and injured, without any influence of cultivar and skin hardness. Grapes do not seem therefore a suitable host for SWD, in accordance both with the literature (Rouzes *et al*., 2012; van Timmeren and Isaacs 2014; Kim *et al*., 2015; Pelton *et al*., 2017) and with our field sampling results.

In conclusion, while the present research has demonstrated the importance of grape skin break force parameter (F_{sk}) for the oviposition of *D. suzukii*, another factor that should be investigated is the volatile blend emitted by grape skin and flesh, both in red and white cultivars. At the moment, SWD may not be considered a serious threat to viticulture in the Piedmont region of Italy. However, attention must be paid to late ripening cultivars, especially if grapes are damaged by other factors (e.g., hail and European grapevine moths), which may trigger sour rot disease (Ioriatti *et al*., 2018).

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FM and FL designed the experiments, sampled insects and grapes, conducted laboratory tests, analysed data, and wrote the manuscript. SG and LR performed laboratory analyses on grape features and contributed in writing the manuscript. AA conceived and designed the research. All Authors declare that they have no conflict of interest.

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