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ORIGINAL CONTRIBUTION

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Susceptibility of table grape varieties grown in south-eastern Italy to *Drosophila suzukii*

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Abstract

Since 2008, *Drosophila suzukii*, the spotted wing drosophila, has colonized most of the American and European continents, being able to adapt to very different climates. In Italy, this pest has been captured from northern Alpine to southern Mediterranean regions where it can infest a broad range of fruits, including wine grapes. Studies have shown that oviposition levels and developmental rates of *D. suzukii* on wine grapes are lower than on other berries, although recent observations indicate that grapes may become a suitable host plant in particular conditions. Here, we report, for the first time, the results of a series of no-choice oviposition experiments using berries of five table grape varieties sampled in the provinces of Taranto and Bari (Apulia region, south-eastern Italy) during 2013 from both organic and conventional farming systems. The sugar content (SC) and skin hardness of each sample were analysed to assess the influence of these physiological parameters on the susceptibility of table grapes to *D. suzukii* infestation. A negative correlation was found between the number of eggs laid and berry skin penetration force, whereas there was a positive one between the number of eggs and Brix values, as well as sampling date. In organic grapes, SC and skin hardness of two varieties ("Crimson" and "Scarlotta") were measured, respectively, higher and lower than in conventional grapes, thus making them more susceptible to pest infestation. The study hence shows that in laboratory conditions *D. suzukii* is able to heavily infest and develop on table grapes and that susceptibility to its infestation significantly depends on both variety and farming system.

KEYWORDS
alien invasive pests, conventional and organic farming, fruit flies, pest control

1 | INTRODUCTION

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), also known as spotted wing Drosophila, is an invasive pest native to south-eastern Asia (Ometto et al., 2013), and after the first reports from United States and Europe (Cini, Ioriatti, & Anfora, 2012; Hauser, 2011), it has been recorded worldwide (Asplen et al., 2015). Thanks to its sclerotized and serrated ovipositor (Lee et al., 2015; Poyet et al., 2015), *D. suzukii* can lay eggs on a wide range of cultivated soft-Skinned fruits, thus causing loss of marketable value in a few days. Moreover, the oviposition wounds can give rise to secondary pathogen infestations that contribute to further fruit deterioration (Cini et al., 2012). While the ability of *D. suzukii* to infest berry fruits has been largely reported (Cini et al., 2012; Goodhue, Bolda, Farnsworth, Williams, & Zalom, 2011; Walsh et al., 2011), grape susceptibility and related economic damage are still a matter of debate (Rombaut et al., 2017).

To date, damage to *Vitis vinifera* L. (both table and wine grapes) has been recorded only in Japan (Kanzawa, 1939). In 2011, *D. suzukii* adults were trapped for the first time in vineyards in the Sauternes region (Bordeaux, France) and Michigan (USA), but no damage was reported (Rouzes, Delbac, Ravidat, & Thièry, 2012; Van Timmeren & Isaac, 2014). In Canada, the presence of *D. suzukii* in vineyards was observed in Quebec in both sentinel traps and grape bunches (Saguez, Lasnier, & Vincent, 2013). *D. suzukii* adults have also been found in table grape vineyards in Pennsylvania and Maryland (Joshi, Butler, Demchak, & Biddinger, 2017). Other field observations reported low levels of wine grape susceptibility to *D. suzukii* oviposition, but with significant differences between varieties (Andreazza et al., 2016; Linder, Martin, Laboisse, Chatelain, & Kehrli, 2014).

The potential impact of *D. suzukii* on wine grapes in a temperate climate was discussed for the first time by Ioriatti et al. (2015), who observed the ability of *D. suzukii* to lay eggs in undamaged soft-skinned berries (e.g., cv Schiava). On the other hand, a field study, carried out in Southern Wisconsin (USA) to assess grape susceptibility to the pest of cold hardy varieties, indicated that such varieties were generally resistant if the berry skin was intact but highly susceptible if it was damaged (Pelton, Gratton, & Guédot, 2017). The main factor that affects the grape susceptibility is skin hardness. In line with previous investigations on other fruits (Burrack, Fernandez, Spivey, & Krausa, 2013; Lee et al., 2016), Ioriatti et al. (2015) demonstrated that oviposition increased consistently as the skin hardness of the grape decreased. Other physiological changes that occur during ripening, such as an increase in sugar content (SC) and a decrease in acidity levels, can also play a role in host susceptibility.

However, as the *D. suzukii* larvae consistently showed low developmental and survival rates in grapevine berries, it is still questionable whether oviposition alone could cause economic damage even if in terms of subsequent outbreaks of sour rot (Ioriatti et al., 2015, 2017; Rombaut et al., 2017). It must also be pointed out that in the case of table grapes the economic threshold would be much lower than in wine grapes in that even small wounds in the skin can compromise the quality and marketability of table grape bunches.

In Europe, the production of table grapes mainly takes place in Mediterranean regions such as Italy, which is ranked third among exporters worldwide (Ferrara et al., 2014). Specifically, Apulia region produces more than 60% of Italian table grapes. In Apulia, *D. suzukii* was recorded for the first time in 2012, using vinegar traps placed in organic vineyards (Baser, Ouantar Broutou, Lamaj, Verrastro, & Porcelli, 2015). Subsequent monitoring campaigns revealed that *D. suzukii* has become well-established in the area and its population peaks occur in autumn (i.e., October and November) when table grapes have not yet been harvested (Baser et al., 2015; N. Baser, unpublished results).

The goal of this investigation was therefore to evaluate the susceptibility to *D. suzukii* of different table grape varieties grown in the Apulia region under laboratory-controlled conditions. We aimed at assessing whether the susceptibility was correlated with two organoleptic parameters, skin hardness and SC, over the ripening period. As a whole, we tested five table grape varieties in two different farming systems, for example, organic and conventional, to ascertain whether the farming system could determine distinct physiological properties on table grapes that in turn could affect their susceptibility to *D. suzukii*.

2 | MATERIALS AND METHODS

2.1 | Mass rearing of *D. suzukii*

The *D. suzukii* population used in the experiments was reared in the laboratory and originated from living wild adults collected in an experimental field at the Mediterranean Agronomic Institute of Bari (MAIB), in Valenzano (41°04′59.89″N, 16°88′43.69″E; Apulia, southern Italy) during the summer of 2012. Mass rearing of *D. suzukii* was carried out at the insect breeding facility of MAIB and periodically refreshed with individuals collected in the field, to avoid problems associated with multi-annual breeding. Insects were reared in Plexiglas® cages (30 × 30 × 30 cm) in laboratory conditions (T: 22–24°C, RH: 64% and 14-L:10-D photoperiod) and were constantly provided with a water wick and artificial diet (Dalton et al., 2011) that served both as a food source and an oviposition medium.

2.2 | Experimental design

Five different table grape varieties, three with red berries (Red Globe, Crimson and Scarlotta) and two with white berries (Victoria and Italia), were considered in this study. All varieties were grown under both organic and conventional farming systems in five different farms, located in the province of Bari and Taranto. The sampling period in each farm corresponded to harvest time (Table 1). Each sample consisted of a 2 kg lot of ripe fruit, from which two subsamples of 50 berries were randomly selected. Both subsamples were carefully examined under a stereoscope (Nikon SMZ 245T) to remove overripe and unhealthy berries. One of the subsamples was used for infestation trials, whereas the second was used to evaluate the physiological parameters of the berries. Both these activities were carried out within 24 hr of harvesting.

2.3 | Grape infestation trials and adult emergence

The berries from each sample were arranged at a distance of 5 × 5 cm in a HD-PVC plastic box (51 × 10 × 35 cm – l hw) containing a layer

TABLE 1 Sampling periods of the different table grape varieties

	Sampling period	
Variety	Organic	Conventional
Red Globe	21/09–10/10	03/10–11/12
Victoria	21/09–17/10	02/10–23/10
Italia	02/10–18/11	28/10–10/12
Crimson	05/10–24/10	10/10–18/12
Scarlotta	29/10–15/11	28/10–13/11

of 10-cm-thick clean river sand. Each berry was half-immersed in a sand bed with pedicels at the bottom. Wet paper and a medium diet were added to the boxes for insect feeding, and the containers were sealed with a 3-mm-thick glass sheet. Eight circular holes of 4 cm diameter were made along the lateral plastic walls of the box and closed with gauze to allow gas exchange and prevent the flies from escaping. Twenty couples of 5-day-old *D. suzukii* adults were released in each box and left in contact with the berries for 48 hr. After this time, the berries were scrutinized under the stereoscope and their infestation level was assessed as the percentage of berries containing at least one egg and the number of eggs per cluster. The five most heavily infested berries in each sample were put individually into a plastic cup covered with gauze and closed with a rubber band. The eggs were counted on each berry. Afterwards, the cups were observed every 3 days to monitor the number of laid eggs and emerging adults. Full-factorial two-way ANOVA was used to assess statistical differences between organic and conventional farming systems (first factor) and between grapevine varieties (second factor). Homoscedasticity (Levene's test) and normality (Shapiro–Wilk test) assumptions were tested before ANOVA. Tukey's HSD test was applied to separate the means. Five boxes for each variety and farming system were used in replications.

2.4 | Physiological characteristics of grapes and correlation with *D. suzukii* infestation

Sugar content and penetrating resistance (PR) were measured in the berries of the second sample and correlated with the level of *D. suzukii* infestation. Penetrating resistance was expressed as the penetration force (in cN) exerted by a 2-mm blunted needle probe at the point of penetration (Letaief, Rolle, Zeppa, & Gerbi, 2008). The SC (in °Brix) was measured by means of an electronic refractometer. Differences in SC and PR were tested by means of two-way ANOVA as previously described. Principal component analysis (PCA) was carried out to obtain the relative value for the contribution of each phenological parameter to *D. suzukii* egg-laying susceptibility (Ioriatti et al., 2015). Principal component analysis was based on two grape parameters (SC and PR) and sampling time during ripening (St). Samples were grouped by variety and classified at three infestation severity levels: low 0%–20%; medium 20%–50%; and high >50%. Infestation percentages referred to the fraction of berries with the presence of at least one laid egg. Pairwise correlation (Pearson product–moment correlation) was conducted to measure the correlation between each parameter and the number of eggs laid per berry. All statistical analysis was conducted using Statistica 64 software (version 13, Dell Inc., Tulsa, OK).

3 | RESULTS

3.1 | Grape infestation trials and adult emergence

Two-way ANOVA indicated a significant difference for both factors (farming system: $F_{1,40} = 8.4$, $p = .006$; variety: $F_{4,40} = 12.1$, $p < .001$) and for their interaction ($F_{4,40} = 7.2$, $p < .001$). The Italia and Victoria cultivars showed the lowest susceptibility in terms of numbers of eggs laid per berry, regardless of the farming system, while the highest number of eggs was observed in Scarlotta and Crimson. Organic systems were generally more susceptible than conventional farming systems ($F_{1,40} = 8.3592$, $p < .01$). However, only organic Scarlotta showed a significantly higher susceptibility than the conventional counterpart (Tukey's HSD test < 0.05), while the only other significant difference was found between conventional Crimson and Italia (Figure 1). The percentage of adult emergence deriving from eggs laid in the sampled berries ranged from 47% (organic Red globe) to 82% (organic Victoria and conventional Italia) (Table 2). However, we did not find any significant difference in adult emergence among the tested cultivars (farming system: $F_{1,40} = 0.3$, $p = .61$; variety: $F_{4,40} = 1.3$, $p = .30$; interaction: $F_{4,40} = 1.8$, $p = .14$).

3.2 | The physiological characteristics of grapes and correlation with *D. suzukii* infestation

Skin hardness varied from a minimum of 140 ± 30 cN (mean ± *SD*) in organic Scarlotta, to a maximum of 320 ± 40 cN in conventional Italia. While varieties grown with organic systems had lower skin hardness than those from conventional farming ($F_{1,40} = 14.8$, $p < .001$), only organic Italia and Scarlotta showed a significantly lower skin hardness (Tukey's HSD test < 0.05) than the conventional counterpart.

Sugar content of table grapes, expressed as Brix values, varied from 13.8 ± 0.6 (mean ± *SD*) (Red Globe) to 18.3 ± 0.9 (Scarlotta), and it was significantly higher in the grape berries grown under the organic system than those grown in the conventional system ($F_{1,40} = 7.8889$, $p < .01$). Italia and Scarlotta cultivars had higher SC in organic than in the conventional faming system ($F_{4,40} = 3.3$, $p < .02$) (Tukey's test: $p < .05$).

Principal component analysis (Figure 2) showed that the PR and SC of host grape berries were both important variance contributors to *D. suzukii* egg-laying capacity. Highly infested berries ($n = 20$) were placed on the negative side of the PC1 and samples from organic vineyards on the positive side of PC2. PC1 (53.5% of variance) was positively correlated with PR (0.76) and negatively with SC (−0.87), while PC2 (31.0%) was mostly correlated with sampling time (St) (0.83). The position of lower infested berries ($n = 17$) was relatively symmetrical in comparison with the positive side of PC1, with a certain trend towards the lower side of PC2, while the medium-level infested berries ($n = 13$) were located around the centre of the PCA diagram. These observations were confirmed by Pearson correlation analysis, with an infestation rate that was significantly correlated with PR (Linear r Pearson = −.83, $p < .0001$) and SC ($r = .64$, $p < .0001$) but not with the St ($r = −.24$, $p = .10$). Significant correlations were also found between SC and St ($r = −30$, $p = .037$) and between SC and PR ($r = −.48$, $p < .001$).

4 | DISCUSSION

This work confirms that table grapes can be heavily infested by *D. suzukii* in laboratory conditions and that damage may differ according

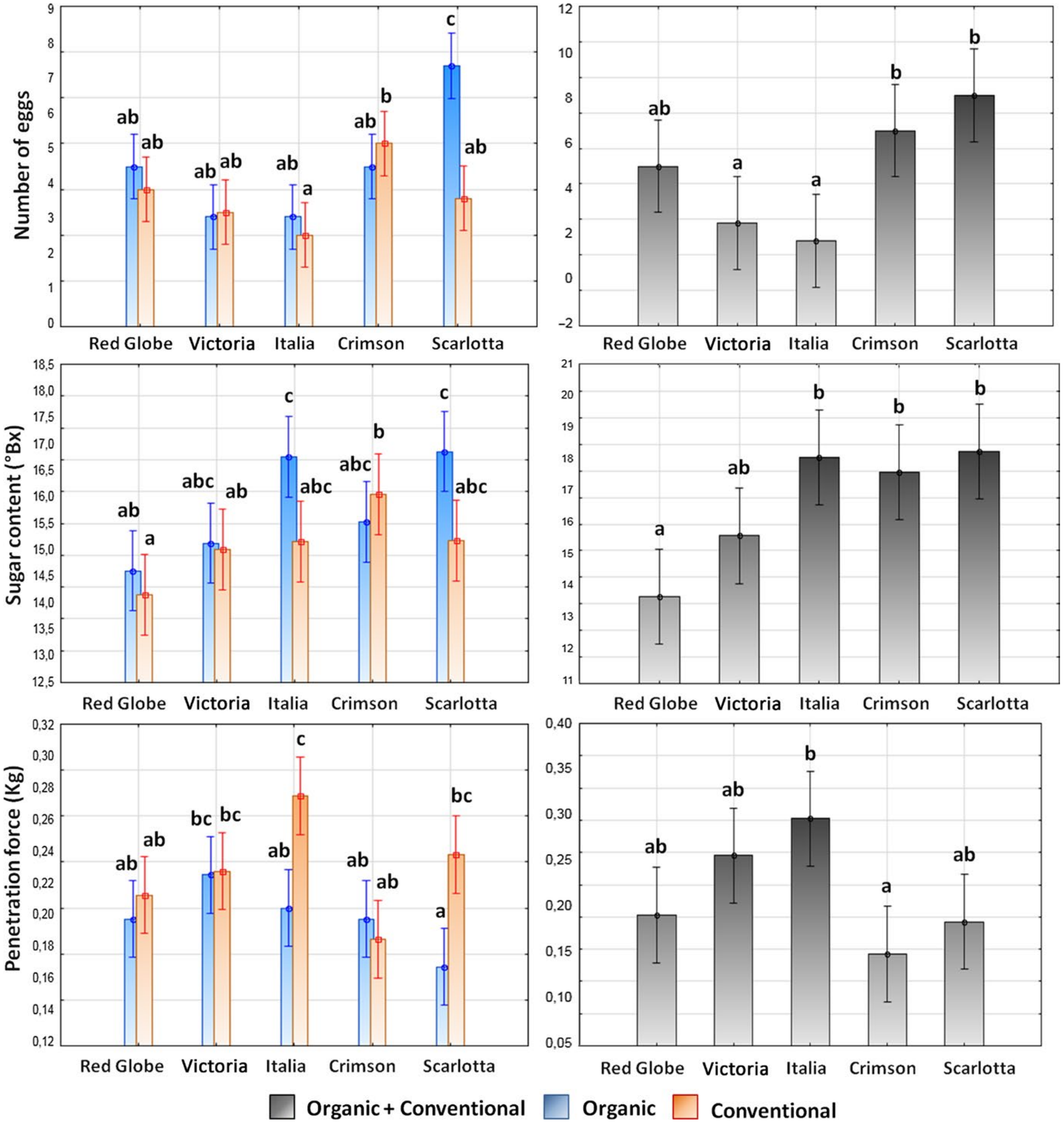

FIGURE 1 Number of eggs laid, sugar content and penetrating resistance of the different table grape varieties. Data are presented as mean ± *SD*. Different letters indicate significant differences between treatments (either within and between the farming systems) after two-way ANOVA followed by Tukey's HSD post hoc test ($p < .05$)

to the cultivar and its physiological characteristics. Cultivars with low skin firmness, such as Crimson and Scarlotta, were more heavily attacked than cultivars with high skin hardness, that is, Italia. The latter, despite a relatively high berry SC (>18 °Bx when organically farmed), showed low susceptibility to pest infestation. These findings confirm the results of previous studies carried out in Brazil, in which the Italia cultivar was reported to be resistant to *D. suzukii* oviposition (Andreazza et al., 2016). On the other hand, they also confirm that levels of PR are the principal indicators for evaluating grape susceptibility to *D. suzukii* attack (Ioriatti et al., 2015). This parameter involves direct measurement of the force that the pest has to apply to the fruit skin to lay eggs into the berry; thus, it looks reasonable to find it negatively correlated with pest oviposition rate (Burrack et al., 2013; Grant & Sial, 2016; Ioriatti et al., 2015;

TABLE 2 *Drosophila suzukii* adult emergence percentage in the different table grape varieties and at different farming systems

	Adult exclusion (%)	
Variety	Organic	Conventional
Red Globe	47 ± 16	53 ± 22
Victoria	82 ± 22	78 ± 19
Italia	68 ± 29	82 ± 22
Crimson	71 ± 12	72 ± 12
Scarlotta	62 ± 7	64 ± 17

All values are mean ± *SD*.

Kinjo, Kunimi, Ban, & Nakai, 2013; Lee et al., 2016). On the contrary, SC positively influences the deposition of eggs, probably because it makes the substrate more suitable for larval development (Broutou, 2014; Ioriatti et al., 2015; Lee et al., 2011). Nevertheless, in another recent study it is reported that fruit characteristics, such as °Brix, in some cold hardy grape varieties, were uncorrelated with *D. suzukii* performance (Pelton et al., 2017). It is likely that, independently from the SC, when the penetration force is over a certain threshold *D. suzukii* females prefer to move elsewhere to lay their eggs. *Drosophila* females are known to selectively choose egg-laying sites (Yang, Belawat, Hafen, Jan, & Jan, 2008), and this behaviour has been recently confirmed for *D. suzukii* females on grape as well (Ioriatti et al., 2017). At harvest time, females can choose among grapes characterized by highly variable PR, which depends on the variety and the phenological stage. The PR of wine grapes is reported to range between 30 and 225 cN (Ioriatti et al., 2015; Lee & Bourne, 1980; Letaief et al., 2008), while that of table grapes ranges between 50 and 180 cN (Ejsmentewicz et al., 2015; Lee & Bourne, 1980; Rolle, Giacosa, Gerbi, Bertolino, & Novello, 2013; Tarricone, Amendolagine, Di Gennaro, Masi, & Gentilesco, 2016). Ioriatti et al. (2015) observed a distinct threshold for skin hardness in wine grapes, corresponding to a PR lower than 40 cN, which would favour pest oviposition. In our trials, we observed that *D. suzukii* females were able to oviposit in berries of table grape varieties characterized by much higher PR (137–313 cN). However, lower skin penetration values were significantly associated with higher oviposition rate. Comparing this value with the skin penetration values reported in the literature during the ripening process, it is clear that most table grape varieties may become suitable hosts for *D. suzukii* before harvest (Abbal, Boulet, & Moutounet, 1992; Grotte et al., 2001; Lee & Bourne, 1980; Letaief, 2007). This would probably give the pest enough time to spread through the vineyard and lay eggs on a large number of berries, also favouring grey mould post-harvest infection (Hamby, Hernández, Boundy-Mills, & Zalom, 2012) provided that larvae develop inside the berry (Ioriatti et al., 2017).

Several studies in the literature have reported no differences between organic and conventional fruit production in terms of desirable components such as ethanol, sugar, total acid and extract (Alvarez, Carracedo, Iglesias, & Martinez, 1993; Danner, 1986; Lutz, 1990; Woese, Lange, Boess, & Bögl, 1997), whereas others reported a higher concentration of soluble solids in organic farming (Rembiałkowska, 2000, 2007; Zadoks, 1989). According to our analysis, organic farming induced significant increase in SC and significant decrease in skin hardness, thus exposing such organically grown grapes to heavier attack by the pest. In a temperate and Mediterranean climate, *D. suzukii* reaches its population peak between the end of summer and autumn (Baser et al., 2015; Rossi Stacconi et al., 2016; Wiman et al., 2014), when climatic conditions are the most favourable and there is a considerable availability of ripening hosts. During this period, *D. suzukii* finds large amounts of food and substrate for oviposition in vineyards, particularly when a large proportion of the berries are cracked because of high rainfall or lower temperatures (Saguez et al., 2013; Van Timmeren & Isaacs, 2013).

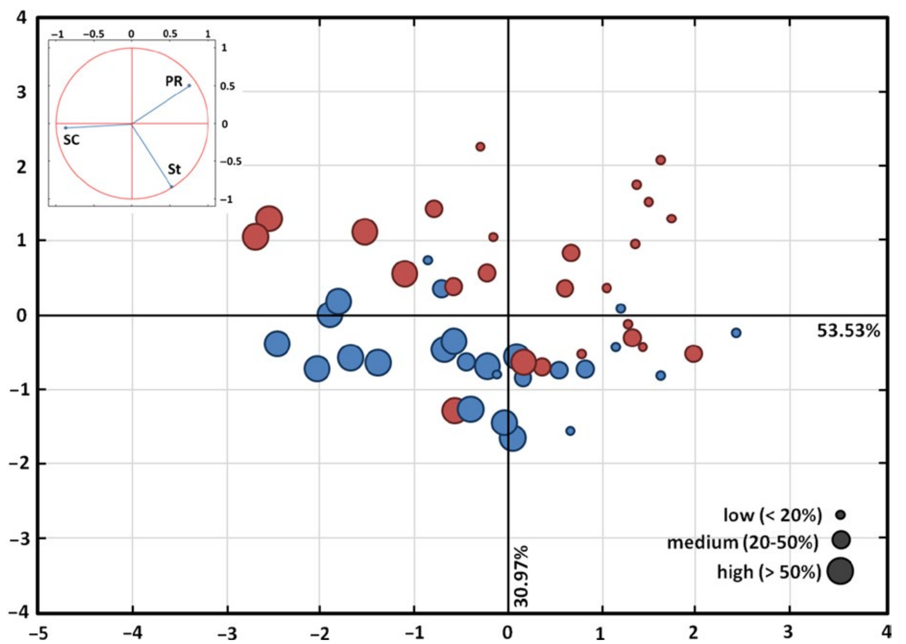

FIGURE 2 Principal correlation analysis based on three quality parameters: sugar content (SC), penetrating resistance (PR) and sampling time (ST). The degree of infestation has been categorized as low (<20%), medium (20%–50%) or high (>50%). Conventional (red) and organic (blue) farming systems are indicated for each variety. Cr: Crimson; It: Italia; Rg: Red globe; Sc: Scarlotta; Vi: Victoria

Conversely, *D. suzukii* oviposition occurs at a very low rate on undamaged wine grapes (Kim et al., 2015; Pelton et al. 2016). Atallah, Teixeira, Salazar, Zaragoza, and Kopp (2014) observed *D. suzukii* producing clusters of small and mostly sterile (i.e., no egg laying) oviposition punctures. This phenomenon is probably due to the difficulty in penetrating the grape skin with the ovipositor. Moreover, Ioriatti et al. (2015) found that the percentage of emerging adults from eggs laid on grapes was considerably low (0%–35%), varying according to different grape varieties. On the contrary, our experiment showed that the tested table grape varieties provided a suitable substrate for *D. suzukii* full development and that pre-imaginal mortality was comparable with the rates reported for other hosts (Tochen et al., 2014). These findings therefore suggest that the table grape is potentially a profitable host for *D. suzukii* and that if high population pressure is maintained over time, the crop is likely to be severely damaged by the pest.

In conclusion, our study indicates a risk for the table grapes grown in Apulia region to *D. suzukii* attacks. This raises the question of which pest control strategy could be suggested to farmers. As biological control against the pest is still at the stage of laboratory assays (Rossi Stacconi et al., 2015, 2017), farmers must rely on currently available preventive methods, such as insecticides and/or the use of exclusion netting (Chouinard, Firlej, & Cormier, 2016; Cormier, Veilleux, & Firlej, 2015; Del Fava, Ioriatti, & Melegaro, 2017) that should be applied at the end of summer (Baser et al., 2015).

AUTHOR CONTRIBUTION

All authors conceived the research. NB, FP and GA designed experiments. OB and NB conducted experiments. VM and MVRS elaborated data. NB, MVRS and VM wrote the manuscript. GA, CI, VV and FP revised the manuscript.

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