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Research Article

Efficacy of an Autochthonous Strain of Entomopathogenic Fungi for the Control of *Drosophila Suzukii* Infestation in an Apulian Cherry Orchard

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Abstract

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is an invasive pest which has recently spread worldwide. The damage of this pest to fruit production is highest in the immediate pre-harvest period, thus making insecticide applications unsuitable. Sustainable management strategies, such as the use of specialized biological control agents able to suppress population outbreaks in the invaded areas, are presently scarce. In this study, the use of an Apulian strain (Southern Italy) of the entomopathogenic fungus *Metarhizium anisopliae* is proposed against *D. suzukii*, as a possible sustainable control strategy for autochthonous organic cherry orchards. Levels of natural *D. suzukii* infestation were monitored in the month of June both in 2019 and 2020 on untreated and *M. anisopliae* treated cherry trees. Such a study was carried out in open field to confirm previous positive results obtained under laboratory conditions. If the numbers of infested fruits and eggs laid by the pest are considered as the infestation parameters, treatments with the local strain of *M. anisopliae* generally reduced *D. suzukii* infestation and spread. Conversely, pyrethrin, a biological insecticide used as a comparison, did not reduce *D. suzukii* oviposition Finally, the level of natural *D. suzukii* infestation was significantly affected by the different climatic conditions registered in the two years considered.

Keywords: bio-pesticides, *Drosophila suzukii*, EPF, infested fruits, *Metarhizium anisopliae*

Introduction

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae), also known as Spotted Wing Drosophila (SWD), was reported in Europe for the first time in 2008. It originates from Asia and is presently diffused all over the world because of fresh fruits global commercialization, lack of quarantine applications and difficult detection in early infestation periods [1-2]. This species has a particular ovipositor by which it can lay eggs deep in healthy mature fruits near to be harvested; after hatching, larvae feed on the pulp of fruits, causing within few days their degeneration, favoring secondary infections by pathogens, and finally making the produce unmarketable [3-4]. This pest is reported on raspberries, blackberries, strawberries, and also on stone fruits including cherries [1,5-6]. This pest causes severe economic losses, directly via yield loss, shorter shelf life of infested fruits, extra labor and material costs for monitoring, field sanitation and post-harvest handling (especially in organic production). Indirectly, it causes the closure of international markets to fruits produced in infested areas [5-6]. Therefore, environmentally friendly and cost-effective strategies are urgently needed to manage the invasion of this species, as insecticide use is limited because of label restrictions on number of applications per season [5]. Today, a great number of tools are being studied to mitigate *D. suzukii* damage, including biological control agents (predators, parasitoids, entomopathogenic fungi (EPF), nematodes, and viruses), natural insecticides, cultural practices, and post-harvest treatments. In particular, EPF are used as valuable alternative to insecticides [7]. For instance, *Metarhizium anisopliae* (Ascomycota division, Hypocreales order) naturally grows in soils worldwide, and is the major fungal species that has been commercialized to control a number of pests [8-10].

In this context, this study evaluated the effectiveness of the locally isolated EPF *M. anisopliae* against *D. suzukii* infestation in an organic cherry orchard of Apulia, as it is generally recognized that local strains have higher capacity to adapt and survive in their own native areas [11]. This 2-year experiment (2019 and 2020) was carried out to confirm the efficacy of this local strain against *D. suzukii* oviposition in open field, as it had already been proved under laboratory conditions [12].

M. anisopliae was reported to significantly reduce *D. suzukii* oviposition and adult emergence, respectively, in treated compared with untreated fruits [13]. However, its effectiveness under field conditions was found to be negatively affected by extreme climatic parameters, particularly relative humidity, that can limit conidia survival and cause the failure of interaction with the pest body [14-15]. In this study, potassium silicate was used to enhance the efficacy of the selected strain. Indeed, it is demonstrated that potassium silicate reduces *D. suzukii* oviposition because it increases the resistance of fruits to penetration [12,16]. Therefore, it was considered relevant to evaluate the efficacy of such autochthonous EPF on *D. suzukii* egg laying activity in different climatic conditions.

Materials and Methods

Trials were carried out in an organically cropped cherry (*Prunus avium* L. cv. Ferrovia) orchard located in Southern Italy (Gioia del Colle, Bari Province) during two growing seasons (2019 and 2020). The trees used for this experiment were about 35-years old, cropped as vase-trained with a planting distance of 3 x 6 m. In both years, climatic parameters, as air temperature (T, °C) and relative humidity (RH, %), were recorded in each day of June directly in the field by data loggers placed in the canopies of trees; moreover, daily values were the mean of hourly based detections. Three treatments of biological control agents per month were carried out on cherry trees.

Treatments with biopesticides

The tested EPF *M. anisopliae* was an autochthonous strain isolated from Apulia region by University of Bari [11] potassium silicate (PSi) was purchased by Siliplant and used in combination with *M. anisopliae*. A commercial formulate (Naturalis) containing another EPF, *Beauveria bassiana*, was used alone or in combination with PSi for comparison; a commercial organic pyrethrin-based insecticide (PyGanic 1.4) was used, as well. Controls were made by water treatments. Specifically, concentrations of formulates were set as follows

- *M. anisopliae*, 0.5 g/l (M).
- *M. anisopliae,* 0.5 g/l + PSi 5 ml/l (MS).
- *B. bassiana*, 1.66 ml/l (N).
- *B. bassiana*,1.66 ml/l + PSi, 5 ml/l (NS).
- Pyrethrin insecticide, 2.5 ml/l (P).
- Water (C).

Treatments were applied following good standard practices (https://www.eppo.int/ACTIVITIES/pppac-tivities) and the selected trees were far enough to avoid contamination among treatments. Treatments were applied to the canopies of trees in early morning or after sunset in order to avoid possible negative effects to bees or other pollinators (https://mccaa.org.mt/media/5564/guidance-document-for-non-professional-users-of-pesticides-final.pdf). Applications were carried out using a knapsack sprayer and two to three liters of treatment suspensions were applied per each canopy. Moreover, different knapsack sprayers were used for each treatment in order to avoid contamination.

Detection of infestation level

Sampling of cherries was carried out 3 days after treatment applications. Infestation level was determined on samples constituted by 10 cherries replicated five times per tree and reported as percentage of infested fruits. Moreover, the average number of infested fruits per 10 cherries was used for the creation of cumulative curves and the calculation of the correlation with climatic parameters. Moreover, the number of eggs was counted in each infested fruit and used for determining the average number of eggs per fruit.

Statistics analysis

Data were recorded in an excel file and after checking the normal distribution and the equal variances, one-way ANOVA was conducted for comparing the percentages of infested fruits and number of eggs per infested fruit of the same year. Significance of differences among treatments was determined by Fisher's Least Significant Difference (LSD) at a 5% probability level. Two-way ANOVA was conducted for number of infested fruits and number of eggs during the three applications and on cumulative values, following Fisher's Least Significant Difference (LSD) at a 5% probability level. The values in figure and tables with significant differences were labeled with different letters.

Results

Average temperature and relative humidity are reported per day of June in both 2019 and 2020 (Figure 1). In 2019, the highest air temperature recorded was 30 °C on June 22 and the lowest was 18 °C on June 1. Moreover, the highest RH value was 76% during the first days of the month, while the lowest was recorded on June 15th (47%). Overall, in 2020, June showed an average temperature lower than that in 2019; the highest temperature was 28 °C in the second decade of the month, while the lowest was measured few days later, in the middle of the month (18 °C). As it concerns RH, the trend of 2020 was opposite to temperatures, in fact, at the beginning of the second decade the lowest RH value was 54% and the highest was 89% on June 15th.

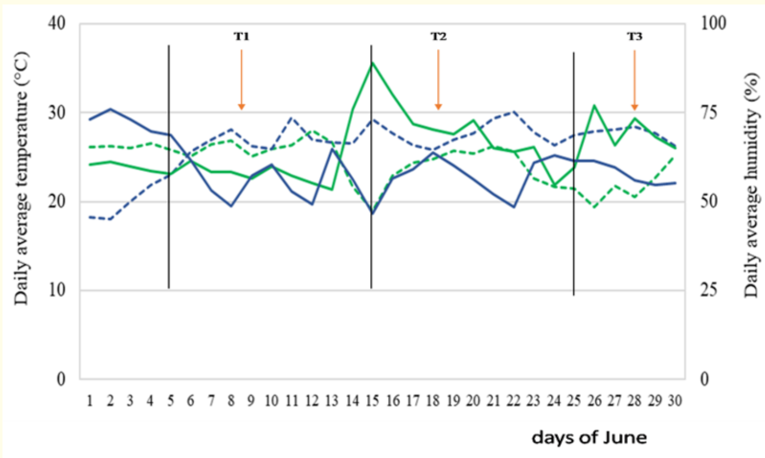

Figure 1: Variation of daily avarage temperature (°C) and humidity (%) during the month of June in 2019 and 2020. Solid lines indicate relative humidity values and dashed lines air temperatures. In blue data from 2019 and in green 2020. Black vertical lines indicate the application dates while sample collection was done 3 days after (arrows).

Percentage of infested fruits indicates the fraction of cherries that were found to host *D. suzukii* eggs, on the total number of tested cherries. In both years, at the end of experimental time, samples treated with water (C) were found totally infested (Table 1).

By contrast, treatments caused a significant reduction of *D. suzukii* infestation after the third application with the only exception of M in 2019. Treatments, N and NS, based on *B. bassiana*, were associated to a much lower percentage of infested fruits in 2019

	Percentages of infested fruits (%)					
	2019			2020		
	T1	T2	T3	T1	T2	T3
M	30 ^{abc}	63 ^{a}	100 ^{ab}	43 ^{b}	87 ^{a}	93 ^{b}
MS	33 ^{ab}	80 ^{a}	83 ^{bc}	83 ^{a}	57 ^{b}	57 ^{c}
N	7 ^{c}	13 ^{b}	37 ^{e}	63 ^{ab}	93 ^{a}	83 ^{b}
NS	13 ^{bc}	13 ^{b}	57 ^{d}	80 ^{a}	90 ^{a}	87 ^{b}
P	10 ^{bc}	60 ^{a}	80 ^{c}	47 ^{b}	57 ^{b}	60 ^{c}
C	50 ^{a}	80 ^{a}	100 ^{a}	80 ^{a}	100 ^{a}	100 ^{a}

Table 1: Percentages of infested fruits per treatment with bio-pesticides during 2019 and 2020. Means were compared by using the least significant difference test (LSD test). Data with different letters indicate significant differences at $P \leq 0.05$.

but did not seem to be highly effective in 2020. On the other hand, P treatments seem to be effective in 2020 and much less effective in 2019. The tested strain of *M. anisopliae* (M) was not effective in 2019 and barely effective in 2020 while its use in combination with potassium silicate (MS) lead to good infestation reduction, comparable with P, in 2020 already after two applications.

Considering the cumulative curves of average number of infested fruits per 10 cherries, a higher infestation of the pest can be observed in 2020 with respect to 2019, throughout the experimental period (Figure 2).

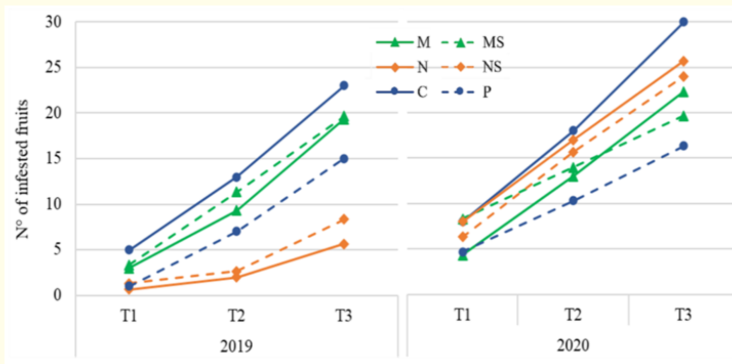

Figure 2: Cumulative curves of *D. suzukii* infestation level during 2019 and 2020, as detected by average number of infested cherry fruits in 10 cherries sub-samples.

In the year of the highest infestation (2020), P was the most effective control agent, whilst N and NS treatments were the best in 2019. With regards to *M. anisopliae* based treatments there is no clear difference among years that can be highlighted in terms of cumulative trends. Moreover, the cumulative curves during both years and their correlation with 10 days average temperature or relative humidity showed high r values for all treatments (data not shown). However, only M and N (r ≈ -1.00) were significantly ($P < 0.05$) correlated to temperature evolution in 2019.

Oviposition rate, measured as the number of eggs laid per each infested fruit, was found to be markedly higher for cherry fruits treated with water (C) 2019 than in 2020, while all EPF and pyrethrin treatments were able to decrease the oviposition rates (Figure 3).

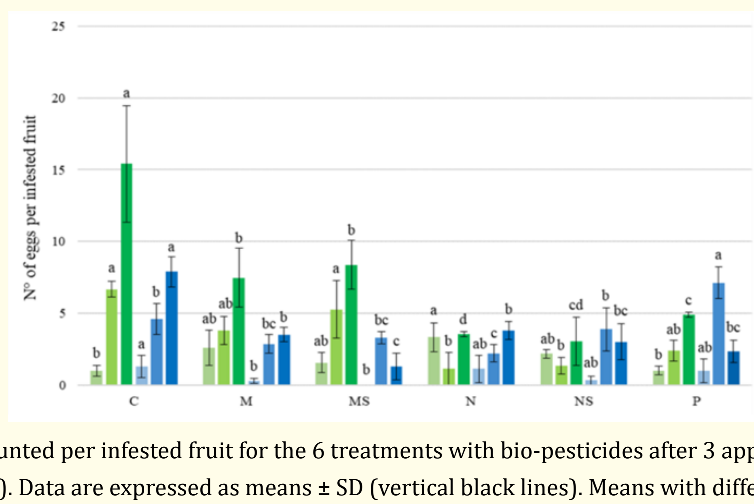

Figure 3: Number of eggs counted per infested fruit for the 6 treatments with bio-pesticides after 3 applications made in 2019 (green shade), and 2020 (blue shade). Data are expressed as means ± SD (vertical black lines). Means with different letters indicate significant differences at P ≤ 0.05, according to LSD test at a 5% probability level.

The lowest number of eggs, at the end of the application cycle, was found with N and NS treatments during 2019, while MS had the lowest oviposition rate in 2020. In table 2, all data are reported per each sampling time or cumulatively grouped for studying the effect of treatments and year on the development of *D. suzukii* population. N and NS treatments resulted in a consistent reduction of pest infestation (approx. 40%), similar to that induced by P, whereas M and MS treatments were less effective (approx. 20% reduction). Treatments N and NS were even more effective in reducing oviposition rates of pest (approx. 80% reduction), more than P (approx. 70%), while the reduction caused by M and MS treatments was slight but significant. Both considered factors (Treatment and Year) and their interaction significantly (P ≤ 0.05) affected the two selected parameters during all the sampling times and cumulatively. Nevertheless, the number of eggs per fruit at T1 seemed to be not significantly affected by the two considered factors, year and treatments, in 2020.

		No of infested fruits				No of eggs			
		T1	**T2**	**T3**	**Cumulative**	**T1**	**T2**	**T3**	**Cumulative**
Treatment	C	6.5 a	8.7 a	11 a	26.2 a	7.3	48.0 a	125.2 a	180.5 a
	M	3.7 cd	7.5 ab	9.7 b	20.8 b	4	25.2 b	53.5 b	82.7 b
	MS	5.8 ab	6.8 bc	7.0 cd	19.7 b	3.2	30.8 b	38.7 bc	72.7 bc
	N	3.5 cd	5.3 c	6.0 d	14.8 c	4.8	11.5 c	22.2 c	38.5 d
	NS	4.7 bc	5.2 c	7.2 c	17.0 c	2.8	18.8 bc	21.5 c	43.2 d
	P	2.8 d	5.5 bc	7.0 cd	15.7 c	2.8	27.3 b	26.5 c	56.7 cd
Year	2019	2.3 b	5.1 b	7.6	15.1 b	4.4	23.2	62.2 a	89.7 a
	2020	6.7 a	8.0 a	8.3	22.9 a	3.9	30.7	33.8 b	68.3 b
Significance	Treatment	0.000	0.003	0.000	0.000	0.318	0.000	0.000	0.000
	Year	0.000	0.000	0.068	0.000	0.729	0.052	0.000	0.004
	Treatment x Year	0.030	0.000	0.000	0.000	0.058	0.001	0.000	0.000

Table 2: Effects of treatments and year and their interaction on number of infested fruits and eggs. Means with different letters indicate significant differences at $P \leq 0.05$, according to LSD test at a 5% probability level.

Discussion

In Mediterranean area, the highest damaging impact of *D. suzukii* on cherry crop occurs during the period of maturation for sweet cherries in June, therefore we chose to carry out the field experiments in this month, in accordance with other previous studies [17-19]. Temperatures (T) and relative humidity (RH) values recorded in June of both 2019 and 2020 at the experimental field fell within the averages of the Mediterranean summers. The optimum temperature range for oviposition of *D. suzukii* is 18-30 °C, with highest egg-to-adult succession between 16 and 25 °C. Whereas the exact range of optimum RH could not be as well precisely identified, although high RH values were generally reported to be suitable [20]. The recorded temperatures during experimental time were always within the optimum temperature range for *D. suzukii* adult life and, certainly, RH values were favorable for their egg laying. Consequently, all the water treated cherries, used as controls, were found fully infested by *D. suzukii* at the end of experiment in late June. In addition, the higher temperature and lower RH registered in the final period of June 2019, with respect to 2020, negatively affected *D. suzukii* development, and consequently the spread of the pest on the trees, which is most favorite at the stable range of 20-26 °C [21-23].

In terms of number of infested fruits, pyrethrin was not the most effective pest control agent among the tested treatments as detected at the third sampling time in both years. In 2019, treatments with N and NS led to a lower *D. suzukii* infestation than pyrethrin, although such a performance was not repeated in 2020. On the opposite, MS treatment had the lowest percentage of infested fruits only in 2020. These results suggest that efficacy of these EPF formulates may depend on climatic conditions, and particularly on temperature changes, as previously reported [24]. *B. bassiana* was observed to have the highest performance on insects at 30 °C and high values of RH [25-27]. *M. anisopliae* infestation rate was reported to be highest at 95% RH and between 20-30 °C [28]. However, it was concluded that high temperature, but not RH, increased the effectiveness of *M. anisopliae* against insects [29]. The above-reported difference in the optimum ranges of T and RH between *B. bassiana* and *M. anisopliae* may explain the results of this work that show different impacts against the insect pest in the two tested years, as average temperatures in these years markedly differed particularly in the third decade of June. This statement is also corroborated by the significant ($P \leq 0.05$) correlation among cumulative infestation curves of M and N and temperature values.

Differently from N and NS, performance of P, M, and MS treatments did not meaningfully change in the two tested years in terms of cumulative curves of infestation. The addition of silicate to M and N did not substantially change the performance of the formulates. This result apparently differs from that reported in [12], in which the use of potassium silicate led to a reduction of *D. suzukii* oviposition by creating a protecting layer on berry fruits. However, the contrasting results observed in our study can easily be explained by the different experimental conditions used in [12], in which a laboratory assay under controlled climatic conditions was performed.

If the infestation is considered in terms of eggs laid per fruit, after three applications, N and NS treatments had similar suppressive effects as P treatment in 2020 and even better ones in 2019. Conversely, M and MS treatments worked better in 2020 than in 2019. *M. anisoplia* affects the fertility of *Plutella xylostella* by reducing number of laid eggs, as well [30]. Moreover, the combined use of potassium silicate to *M. anisoplia* significantly reduced the number of eggs during the month of June. This result complies with what was found by [12], where the use of potassium silicate created a protecting layer on berries fruits and led to a reduction of *D. suzukii* oviposition.

Two-way ANOVA analysis revealed the good performance of N and NS, compared with P treatments, after each of the 3 applications and as the whole procedure, in reducing both spread and oviposition of *D. suzukii*. However, treatment based on the autochthonous selected strain of *M. anisoplia* led to a weak reduction of infestation and oviposition, slightly bettered by the climatic condition and combination with potassium silicate.

Haviland and Beers [31] proposed a selection of insecticides to control *D. suzukii* population, to be provided with a minimum number of applications in a sequence tailored on pest pressure and the number of days before harvest. In our experiment, except for pyrethrin (organically allowed product with mandatory days of shortage), schedule of treatments did not have to take into account harvesting times and applications could be applied all over the cherry maturation period [31]. Although the costs for the obtained *D. suzukii* pest management were not considered in this study, it is presumable that a similar level of control obtained by the continued use of insecticides would be both economically and environmentally less sustainable [32]. On the other hand, loss of incomes for growers, due to uncontrolled *D. suzukii* attacks, can be a graver economic burden [33]. According to [34], organic farming better responds economically to *D. suzukii* infestation because of the adoption of more preventive strategies than in conventional agriculture. In this sense, the use of *B. bassiana* and *M. anisopliae* might be easily included into a plan of integrated pest management.

Conclusion

Because the damage of *Drosophila suzukii* is to ripe fruits, farmers urgently need environmentally friendly plant protection products that do not put human health at risk, and that can be used close to harvest. For these reasons, although biological control systems may be plant protection tools that can provide an immediate solution to this issue, the results of field trials are still insufficient. Moreover, there is a lack of solutions allowed in organic agriculture, tailored to the local contest and that can be applied closely before harvesting. In this study, we wanted to test an autochthonous strain of *M. anisopliae*, with proved virulence against *D. suzukii* in laboratory condition, in an in-field experiment with cherry fruits.

According to the results of this 2-year field experiment, performance of the autochthonous strain of *M. anisopliae* was variable because the climatic conditions in 2019 and 2020 were different, particularly during the last decade of June. In particular, we found that the local EPF prevented *D. suzukii* from laying eggs for a long time and reduces fruit infestation in 2020. *M. anisopliae* had better performances than controls but was a more effective control agent than the commercial product only when climatic conditions were favorable. On the other hand, further studies and longer lasting observations are needed for fully understanding the real efficacy of *M. anisopliae* and, generally, the suitability of field applications of local EPF strains against alien pests, such as *D. suzukii*.

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