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NOTES ON *DROSOPHILA SUZUKII* MATSUMURA (DIPTERA DROSOPHILIDAE): FIELD SURVEY IN TUSCANY AND LABORATORY EVALUATION OF ORGANIC PRODUCTS

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Gargani E., Tarchi F., Frosinini R., Mazza G., Simoni S. – Notes on *Drosophila suzukii* Matsumura (Diptera Drosophilidae): field survey in Tuscany and laboratory evaluation of organic products.

Reported for the first time in Italy in 2009, the Spotted Wing Drosophila (SWD), *Drosophila suzukii* (Matsumura) (Diptera Drosophilidae), has rapidly spread throughout the country, causing serious damage on many fruit crops. The potential of infestation rate by *D. suzukii* is enormous: the females can complete many generations per year, and are able to attack the fresh, ripe fruit of many hosts by laying eggs under the soft skin; the larvae grow in the fruits and destroy the commercial value of them. The host range of SWD is reportedly wide, including both cultivated (soft fruits, sweet cherry, stone fruits etc…) and wild plants. In 2013, field surveys aimed at monitoring the presence of the insect on Tuscany territory, were regularly carried out: on an important district for the sweet cherry production with differently managed in control, on a biological blueberry orchard and even on other host plants neighboring the samples' areas. *D. suzukii* was present in the monitored areas and its numerically large populations and heavy infestations on sweet cherries have shown that the species has settled in these areas. In laboratory tests, labeled organic products and substances of natural origin - in accordance with the requirements of EU regulations which provides for the reduction of chemicals - were evaluated as concern toxicity and residual effect on the pest. As regards effects of direct toxicity, products based on *Beauveria bassiana* have shown some effectiveness in the control of SWD, other organic products have shown interesting results. The evidences acquired are discussed.

KEY WORDS: Spotted Wing Drosophila, toxicity, residual effects, cherry, blueberry.

INTRODUCTION

Drosophila suzukii (Matsumura, 1931) (Diptera Drosophiliidae), called Spotted Wing Drosophila (SWD), originated from South-Eastern Asia, is a dangerous invasive pest of soft fruits. The species was firstly found in North America (Santa Cruz, California, 2008) (HAUSER, 2011) and in Europe (Spain) (CALABRIA *et al.*, 2010) in 2008; in the same year, the insect was also found in Tuscany during a survey carried out in natural forest environments around San Giuliano Terme (province of Pisa) (EPPO, 2010b; RASPI *et al.*, 2011). In September 2009, SWD was detected in Italy on raspberry, high bush blueberry and strawberry in several cultivated fields in Trentino, north-eastern Italy (EPPO, 2010a; IORIATTI *et al.*, 2011). During the following three years, *D. suzukii* population has been reaching an extraordinary increase, spreading mainly on sweet cherries and soft fruits, also in many other Italian regions: Campania, Liguria, Lombardia, Marche, Piedmont, Sicily, Valle d'Aosta, Veneto, and Emilia Romagna (FRANCHI and BARANI, 2011; PANSA *et al.*, 2011; SÜSS and COSTANZI, 2011; GRIFFO *et al.*, 2012). Severe damages were locally recorded on sweet cherries, strawberries, raspberries, black and blueberries and, in some areas of the Northern Italy (Trentino), up to complete damage was registered on blackberry, raspberry and strawberry (GRASSI *et al.*, 2009); further damages caused by *D. suzukii* occurred on apricots, currants and figs such as new findings regarded grape varieties as well (GRASSI and PALLAORO, 2012; SINN, 2012).

Unlike other *Drosophila* spp. flies, which attack only decaying or rotten fruits, SWD is able to lay eggs in healthy, unwounded fruit by its serrated female ovipositor (SASAKI and SATO, 1995). Hence, ripening fruits are preferred to overriped ones (MITSUI *et al.*, 2006). Moreover, it exhibits a wide host crop range, infesting berries, stone fruits (peaches, cherries, apricots, etc.), grapes (wine and table), figs, kiwifruits, and damages fruits of apples, loquats, persimmons, and tomatoes (WALSH *et al.*, 2011). In addition, there are many wild and ornamental crops hosting SWD belonging to the family Aquifoliacee, Caprioliacee, Cornacee, Ebenacee, Elaeagnacee, Ericacee, Liliacee, Phytolaccacee, Rhamnacee, Rosacee, Solanacee, Taxacee and Vitacee (http://www.fruit.cornell.edu/spottedwing/cropshosts.htm).

The biotic potential and infestation of SWD are enormous: females are extremely fecund and can lay on average about 400 eggs, over 7 up to 13 generations per year (KANZAWA, 1939). This fly develops through three larval instars, and the development from egg to adult lasts about 8 days at 25°C (KANZAWA, 1939; MITSUI *et al.*, 2006; WALSH *et al.*, 2011). Fruit damage is initially caused by SWD larvae feeding on flesh; secondary damages by other insects, fungi and bacteria may contribute to further fruit deterioration. The reproduction rate and the speed of infestation reflects on high endangerment potential on healthy soft fruits.

At the moment, there is no real efficient control strategy available. Due to the very high pest pressure, conventional insecticides, were quite unsuccessful in reducing the fruit damage under threshold; further, due to treatment very close to harvest, there is a serious matter for maximum residue limit (MRL).

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Drosophila suzukii posed an urgent challenge to research that led to the development of a transnational Euphresco project, 'Droskii', for insight on damage potential of SWD and development of risk management and control measures (SIMONI *et al.*, 2013). In the context of this European project, field surveys in order to monitor the presence of the insect on Tuscany territory and to assess in laboratory tests the efficacy of organic labeled products for controlling the pest, were carried out. The use of these substances was screened in the context and in accordance with the requirements of EU regulations which provides for the reduction of insecticides with high environmental impact. Given this data, by connecting to the aims of this work, crucial importance was devoted to monitoring, further the main plant hosts, for timely recording of the pest.

MATERIAL AND METHODS

FIELD SURVEYS

In 2013, a monitoring survey was carried out, in the area of Lari (Pisa province) and Castiglion della Pescaia (Grosseto province) within Tuscany. In the area of Lari, one of the most important and famous district for the sweet cherry production in Tuscany, the monitoring was in three farms where sweet cherry is cultivated: two plots were in organic orchard (Farm1-plot1: 43°33'52.80"N, 10°35'29.48"E; Farm1-plot2: 43°32'12.12"N, 10°36'4.44"E); the two other plots considered were in two farms conventionally run (Farm 2: 43°34'17.62"N, 10°34'20.15"E; Farm 3: 43°34'17.62"N, 10°34'20.15"E). The insecticides applied were mainly based on treatments with deltamethrin in the Farm 2 and with etophenprox in the Farm 3.

In these farms, the sweet cherry varieties most commonly grown were: Ferrovia, Bigarreaux, Moreaux, Marchiana and other native ones.

In the farm located in Castiglion della Pescaia (42°48'20.01"N, 10°57'24.21"E), the survey was carried out on a biological blueberry orchard where the predominant variety is Duke. This crop is present in the area for over 30 years and, with its six hectares area, represents a unique situation in the region.

From late May, before complete ripening of fruits, until the end of harvest and in some cases even a few weeks later, in the orchards and in warehouses, 47 traps (22 on sweet cherry, 4-7 traps/plot or farm, and 25 on blueberry) were positioned and changed once a week. Each trap consists of a plastic jar, baited with 150 ml of apple cider vinegar, 50 ml of red wine and a tea spoon of cane sugar, with 5-6 holes with a diameter of 4 mm on the top sides, to allow the entry of the attracted flies (IASMA, 2012). At the end of July in Farm 3, eight traps were moved from cherry to neighboring plum orchards; these observations lasted till mid September.

In addition, during summer 2013, *D. suzukii* 16 food traps were installed also in vineyards in Castiglion della Pescaia. The material collected from the traps was analyzed in the laboratories of CRA-ABP (Florence): all Diptera specimens were counted and the drosophilids were identified by specific dichotomous keys of VLACH (2010) and VAN TIMMEREN *et al.*, (2012).

In order to determine the presence of *D. suzukii*, more than 100 fruits per orchards, independently on the symptoms' evidence, were regularly collected and transferred in laboratories: for fruit variety, twenty five cherries and fifty blueberries were observed by stereo microscope and fifty of them were stored into boxes maintained in climatic chamber at 25°C and routinely checked for any emergence of adult drosophilids. These adults were collected and identified at specific level to confirm the SWD infestation in the fruits. A hundred fruits collected were analyzed after immersion in a saturated solution of Sodium Chloride inducing the escape of larvae and pupae from the fruits in order to count them.

Out of experiment design, samples were collected on some possible host plants in the area of Stia (Arezzo province), Vada (Leghorn), around Florence and Montalcino (Siena province).

LABORATORY TRIALS

Products of natural origin - three multi-target ones already registered and two other products still under investigation for their perspective application - were selected for laboratory trials (Table 1). For trials on direct and residual effects, the tested products are reported in Table 1: Naturalis® and Botanigard®, registered bioinsecticides, both based on living spores of naturally occurring strains of the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuillemin, are authorized in organic farming and widely used against many insect pests in fruit and vegetable crops. The other products

Table 1 – List of active ingredients and label dose of commercial products tested in toxicity laboratory trials.

Active ingredients	Commercial product, dose	Crops
Beauveria bassiana strain GHA, g11.34 Equal to 2.26×10^{10} viable spores/g	Botanigard® 125ml/100lt	blueberry
Beauveria bassiana, strain ATTC74040, g 7.16 Equal to 2.3×10^{7} viable spores/ml	Naturalis® 75cc/100lt	blueberry
Polysaccharides	Agricolle 300ml/100lt	blueberry
Sophora flavescens (8%), Mn (1%), Zn (1%)	Deffort 200ml/100lt	blueberry
Brassicacee flour + mustard oil	Duofruit, (200g flour + 1lt oil product)/100lt	blueberry
Control	distilled water	blueberry

considered organically labeled without a specific and known insecticidal activity, were: "Agricolle", a product composed of some natural polysaccharides, derived from vegetal plant extracts, forming a sticky layer able to entrap small insects. "Duofruit", consisting of mustard oil fraction and one part of brassicacee' flour: the action of this product could contribute to the proper development in different phenological stages and it would strengthen the natural defenses of the plants. "Deffort" (*Sophora flavescens* Aiton, 8%), is a fertilizer liquid based on complexing micronutrients enriched with plant extracts with strong anti-stress action. All bioassays included a distilled water check.

The SWD adults used in the tests came from a population kept in the Foundation Edmund Mach Research and Innovation Centre facilities in Trento (FEM) (Italy), and were reared in CRA laboratories on *Drosophila* medium (consisting of soy flour, corn flour, yeast, sugar and agar – personal communication by Gianfranco Anfora) and maintained in a climatic chamber at 25°C and 75±5% RH.

Direct and residual toxicity test

As regard direct toxicity trials, about four hundreds healthy blueberries (derived from organic cultivation) were inserted in a cage (in a climatic chamber at 25°C) and exposed to 50-60 SWD adults for 72 hours. After adults' removal, 60 blueberries for each tested product were treated by immersion for 30 seconds in 100 ml of solution/emulsion prepared according to the label dose (Table 1).

Air dried fruits were kept in a cell at 25° C and 75±5% RH for 10 days and emerged adults were collected and counted under stereo microscope.

As concern residual contact toxicity test, 150 healthy blueberries were dipped in 100 ml of solution/emulsion prepared according to the label dose for each product and then air dried for two hours. For each product, 24 blueberries were treated: 16 blueberries were placed into Petri dish and the other eight were placed in individual slots on a "table" according to a randomized scheme. The whole material was kept in a cage with *D. suzukii* adults for 72 hours and stored in climatic chamber at 25°C and 75±5% RH. Inspections were made under stereo microscope in two steps: immediately after the removal of the adults in order to verify oviposition on fruits and five days later in order to count larvae and pupae.

DATA ANALYSIS

Concerning the evaluation of direct toxicity, the data obtained were subjected to one-way Anova and HSD Tuckey test to separate the means (SPSS, 2004). The efficacy of direct toxicity was evaluated by means of the Abbott's mortality index. The residual toxicity was determined for each pesticide (see OVERMEER, 1988) and expressed as:

$$E = 100\% - (100\% - M) \times R$$

where E is the coefficient of toxicity; M is the percentage of mortality calculated according to Abbott; R is the ratio between the average number of eggs and larvae counted for each product and the average number of eggs and larvae produced by females in the untreated thesis. Data on preference were processed by means of contingency tables and chi square test, all the procedures were performed by adopting the SPSS statistical program (SPSS, 1999).

RESULTS

FIELD SURVEYS

The numbers of SWD adults captured in the traps during the monitoring period on sweet cherry orchards in Lari are reported in Fig. I. No great difference has been noted concerning the catching trends among the different managed orchards in particular, no relevant effect of treatments, made in the farms 2 and 3, was detected in comparison with the captured registered in the organic plots, in the Farm 1. The peak of captures was recorded in the Farm 2 after June, the 25^{th}, when the harvest was over and all the fruits, even those on the ground, had been taken away. On the whole, increasing linear trend in the number of SWD trapped was registered only in Farm 1 (Farm1-plot1: $F_{1,6}$ = 5.9, P<0.05, R^2=0.50; Farm1-plot2: $F_{1,6}$ = 41.8, P<0.01, R^2=0.86) where chemicals were not applied, while curvilinear, exponential or quadratic, trends were evidenced in Farm 2 and Farm 3.

All over the sampling period, the percentage of SWD trapped on the Diptera collected, ranged from 9 to 60% (Fig. II); on the whole, the SWD females trapped represented the 58% of the SWD captured adults (Fig. II).

The analysis in laboratory on the fruits' infestation, by counting SWD preimaginal stages, indicated: in Farm 1, by considering plot 1 and plot 2 cumulatively, an infestation percentage ranging between 28 and 44%; the average presence per single berry (SWD larvae/fruit) was between 0.5 and 2.4; in Farm 2, the infestation percentage registered ranged between 20 and 54% with an infesta-

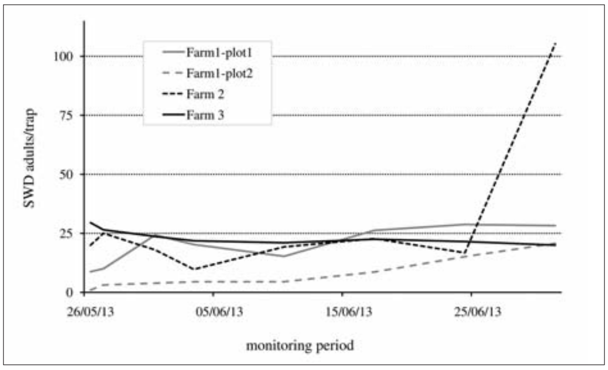

Fig. I – SWD adults captured in the traps during the monitoring period on sweet cherry orchards in Lari (Pisa) areas.

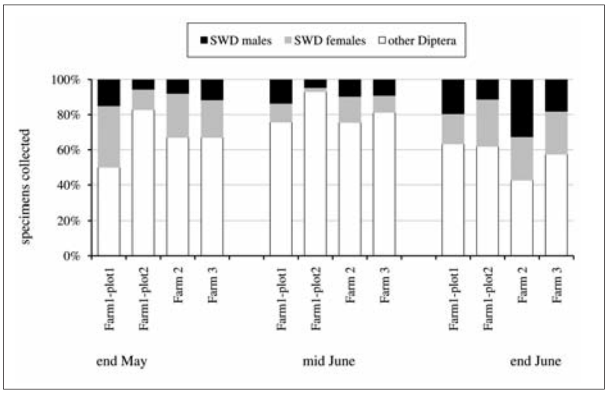

Fig. II – SWD, females and males, and Diptera collected in traps during the monitoring period, May-June 2013, on sweet cherry orchards in Lari (Pisa) areas.

tion/fruit between 0.2 and 0.9 SWD larvae/fruit; in Farm 3, the infestation percentage was between 24 and 41%, while the presence in the fruit was between 0.1 and 0.8 SWD larvae/fruit.

The monitoring performed on traps on plum denoted a shift in SWD spreading towards these plants (Fig. III): by quite linearly trend, *D. suzukii* increased its presence on plum from 0.5 SWD/trap/wk at the start of recording, last July decade, to more than 15 specimens at early September. Till mid August, SWD represented about 10% of all Diptera captured, since the last decade of August there was registered analogous captures for *D. suzukii* and all other Diptera (Fig. III).

Survey and monitoring results on the activities conducted in the Farm 4, near Castiglion della Pescaia (province of Grosseto) between June and October 2013, are synthesized in Fig. IV. In this area, the presence of *D. suzukii* was ascertained at the starting of the harvest of the blueberries. By considering all the 25 traps, in the whole monitoring period SWD represented an average about 7% of all Diptera captured (range 6.57-11); about ten SWD specimens were collected cumulatively at each sampling (Fig. IV); by considering all the samples the SWD females captured were about the 58% of all SWD flies. In the laboratory, no SWD larvae and pupae were found in the blueberry fruits.

Since the early August, SWD flies were recorded in the traps located in the neighboring vineyards (cv Cabernet Franc and Syrah) (Fig. IV). In the bunches collected for the laboratory analysis, no SWD individual was found.

Further the results registered in the experimental areas, the monitoring performed out of the experimental plan, firstly, highlighted the widespread presence of *D. suzukii* in Tuscany. The traps positioned in these areas allowed the finding of SWD in the province of Arezzo (Stia, on cherry trees and khaki), Leghorn (Vada, in urban gardens), Siena (Montalcino, in vineyards) and Florence (Cascine del Riccio, urban garden).

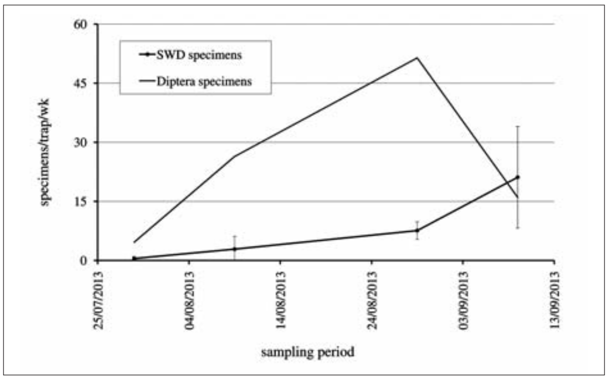

Fig. III – SWD and Diptera specimens collected in traps on plum plants in Lari (Pisa) areas during the monitoring period.

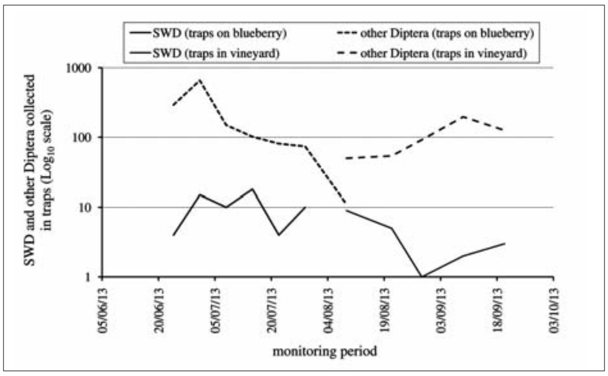

Fig. IV – SWD and Diptera specimens collected in traps on blueberry and in vineyard in the area of Castiglion della Pescaia (Grosseto) in June-October 2013.

Direct and residual toxicity test

Concerning the toxicity effects of the tested products, the results are summarized in Table 2.

With the exception of "Duofruit" and "Agricolle", all the other organically labelled products significantly reduced the vitality of SWD. In particular, Botanigard determined the highest Abbott mortality (84.6%), not significantly different from "Deffort" and Naturalis that reduced the population over 50%.

As concerns the residual contact toxicity, the number of hatched eggs was reduced by "Duofruit", Naturalis and Botanigard (Table 2). As consequence, the coefficient of

Table 2 – Direct and residual contact toxicity of the tested products on SWD. Direct toxicity was evaluated on the number of adults emerged after immersion of the infested blueberries in the different products and by calculating Abbott mortality index. Residual contact toxicity was evaluated on the means of preimaginal stages registered after exposition of treated healthy fruits to SWD females and by calculating the coefficient of toxicity (E)

	Direct Toxicity		Residual contact toxicity	
Products	Mean±SD	Abbott index	Mean±SD	E
Control	13.00±0.89 a	-	16.13±4.67 a	-
Deffort	5.67±2.73 bc	56.41	8.14±4.78 ab	81.27
Duofruit	11.33±2.74 a	12.82	7.50±3.07 b	81.71
Agricolle	10.00±3.90 ab	23.08	9.50±3.78 ab	68.38
Naturalis	6.00±2.68 bc	53.85	6.63±4.00 b	80.47
Botanigard	2.00±.89 c	84.62	7.88±5.74 b	76.37

Means followed by the same letter are not significantly different (HSD Tukey test, P=0.01).

toxicity (E) was higher than 80% for "Duofruit", "Deffort" and Naturalis (Table 2).

Concerning susceptibility of fruits to the SWD ovipositing, after fruit dipping in the different products, the simultaneous proposal of the blueberries differently treated did not reveal any difference (chi square value =10.35, df =5, P=0.06). When the different treatments were proposed on the basis of a binomial choice test, the higher oviposition was registered in the untreated in comparison to each of the other five labelled organic products (chi square test, P<0.02).

DISCUSSION AND CONCLUSIONS

The monitoring actions performed in 2013 confirmed the fast spreading and settlement of *D. suzukii* population in different Tuscan areas. In particular, SWD can be connoted as key pest in one of the most important area of sweet cherry production as it was reported in other countries (BEERS *et al.*, 2011). In fact, as it happened in these last three years in other Italian regions, the fly confirmed its high potential diffusion; this can be mainly related not only to the presence and density of host plants but also to their susceptibility to SWD (BURRACK *et al.*, 2013). Several factors made the situation extremely serious in the Lari area in 2013. The farmers were well close to harvest sweet cherries before the presence of the fly was recorded. During the monitoring, the food traps captured not only SWD but also many other Diptera, as evidenced in our results, thus the count and taxonomic identification of the specimens were long and laborious. In the absence of identification, the infestations in the orchards were not timely diagnosable. As no warning that crop protection measures would be needed, this led to an underestimation of the problem. By monitoring the areas, it has become evident that, in considered orchards, SWD has found ideal conditions for its development, even as regards the micro-climatic conditions, characterized by cool and moist environment. In particular, organic sweet cherry growers have to face a serious challenge controlling SWD as the monitoring actions reported in this experience showed: a greater increase in infestation was registered in farm organically managed as it was reported by other Authors (BEERS *et al.*, 2011). Nevertheless, as it was detected in orchards monitored in the same area, in farms where treatments were applied, the effects of these interventions did not contain infestations at very low level.

Once more, as the linear increase in presence of SWD on plum in Lari after the harvest of sweet cherries, it is to be remarked as the mapping and closeness of possible host plants for SWD can constitute a crucial tool for the insect keeping and settling on the area.

Regarding the situation in the blueberry crops and in the vineyards monitored in the area of Castiglion della Pescaia, near the coast, at the moment it seems that SWD population is not settled: this result may be due to the fact that the spread of the species in this area is just at the beginning and/or to not so favorable environmental conditions, mainly regard to dry and airy conditions (WALSH *et al.*, 2011).

On the whole, an increased efficiency of food traps was noted when there is no competition of the fruits, as already described by other Authors (IASMA, 2012; GRASSI *et al.*, 2013). However, as the wide variety and the seasonality of the host plants, SWD population density tends to rapidly increase; due to the crucial importance in the set of timely monitoring actions, it will be necessary to develop better and more efficient types of traps. Insecticides will remain the principal method of control for SWD by the near term (ANGELI *et al.*, 2012). The combination of a long harvest period of multiple berry crops and the short generation time of SWD will likely increase the chances for SWD to develop resistance to some insecticide products. One tool to reduce selective pressure for resistance development can be to rotate insecticides with different modes of action (BRUCK *et al.*, 2011; VAN TIMMEREN and ISAACS, 2013). In these strategies, the use of organically labeled products could represent a further mean to avoid or late the resistance insurgence.

Our laboratory experiments provide preliminary data for *D. suzukii* control on blueberries, but many key issues have yet to be resolved. The results obtained have shown a certain control action of the fly by the two products based on *B. bassiana*, as already reported by other authors (BRUST, 2011). As for other products, some of them, "Deffort" and "Duofrut", deserve attention but it will be necessary to better understand their effects. Laboratory data become generally more variable as they are transferred to field, and the findings under controlled conditions must be tested on a broader scale before strong conclusions can be drawn. As concerns the residual contact effect of the tested products, no relevant effect was detected on the oviposition of SWD.

The evidences acquired can represent a basis and chronological reference for following studies on *D. suzukii* in Tuscany; much about the seasonal pattern of occurrence in the region and the relative crop risk needs to be established through further experimentation at all levels. It highlights the urgent need to deepen the study of the behavior and the epidemiology of the pest in relation to each crop, with the aim of rationalizing the phytoiatric intervention and, at the same time, concerting innovative actions for defense (*e.g.*, biocontrol agents, mass trapping and semiochemical) to achieve an effective and sustainable management of the pest in the field.

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