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Research article

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Laboratory Bioassays with Three Different Substrates to Test the Efficacy of Insecticides against Various Stages of *Drosophila suzukii* (Diptera: Drosophilidae)

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Abstract

Rapid worldwide spread and polyphagous nature of the spotted wing Drosophila *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) calls for efficient and selective control strategies to prevent severe economic losses in various fruit crops. The use of insecticides is one option for management of this invasive pest insect. Efficacy of insecticides is usually assessed first in laboratory bioassays, which are compounded by the cryptic nature of *D. suzukii* larvae and the fact that fruits used in bioassays often start to rot and dissolve before larvae have reached the adult stage. Here, we report on laboratory bioassays using three different types of substrates allowing a thorough screening of insecticides for their potential effects against *D. suzukii* eggs, larvae and adults. Suitability of our bioassays was validated in an assessment of the efficacy of four bioinsecticides and one synthetic insecticide against various developmental stages of *D. suzukii*. Water-apple juice agar used as a bioassay substrate allowed egg counting and observation of larval development due to its transparency, while apple-nutrition medium allowed complete metamorphosis. Use of grape berries in bioassays made it possible to assess effects of an insecticide present on a fruit's surface on oviposition and larval hatch from eggs. Insecticides tested in these three different bioassays with acetamiprid, spinosad or natural pyrethrins as active ingredients achieved a significant *D. suzukii* control if they were applied before egg deposition. Number of adult flies was significantly reduced if the bioassay medium was treated with an azadirachtin A containing insecticide both before or after egg deposition.

Key words: spotted wing *Drosophila*, bioassay, mortality, bioinsecticide

The spotted wing *Drosophila*, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is a highly polyphagous invasive pest native to East Asia. Among the numerous existing species of *Drosophila*, commonly known as vinegar flies, *D. suzukii* is one of the two species (the other being *D. subpulchrella*) known to puncture the skin of intact, ripening fruit to lay its eggs, as opposed to the vast majority of *Drosophila* species, which feed on rotting or overripe fruit (Atallah et al. 2014). The increasing global fresh fruit trade, together with the cryptic nature of larvae hidden inside fruit, facilitates the increasing distribution of this pest (Cini et al. 2012). First recorded as invasive in Hawaii in 1980 (Kaneshiro 1983) and then simultaneously in California (Hauser 2011) and in Europe in 2008 (Calabria et al. 2012), the pest is currently spreading rapidly across regions in Europe, North and South America. The impact of *D. suzukii* on fruit production is enormous due to the high number of pest generations per year (10–15), the short generation time, and the high reproductive rate (Mitsui et al. 2006, Walsh et al. 2011). The fly preferentially infests thin-skinned fruits. However, its host range is wide, including blackberries, blueberries, cherries, peaches, raspberries, strawberries, grapes (wine and table) as well as various non-crop plants (Kenis et al. 2016). Moreover, egg deposition on fruits that are damaged, dropped or split such as apples, apricots, loquat, greenhouse mandarins, persimmons and tomatoes have been reported (reviewed in Cini et al. 2012). The pest has a cryptic nature as the eggs develop into larvae within the fruit, and larval feeding and development cause a rapid deterioration, resulting in reduced crop yields and rendering the fruit unmarketable (Rota-Stabelli et al. 2013). Additionally, oviposition introduces infections from fungi and bacteria, as well as attacks from other insects (Walsh et al. 2011). Yield losses may reach up to 80–100%, depending on the crop and the area, leading to severe economic losses of fruit production. Estimated losses of more than 400 million dollars per year occur in the United States alone (Goodhue et al. 2011, Lee et al. 2011). In addition to the crop loss in the field, economic losses include cost-intensive selection of fruits in the storage facilities after harvest, as well as a shorter shelf life of fruit containing *D. suzukii* eggs (Lee et al. 2011).

Besides monitoring, growing crops under protected cultivation (Rogers et al. 2016) and various cultural management practices, growers rely heavily on the application of insecticides for controlling *D. suzukii* (Haye et al. 2016). Studies have highlighted in particular the use of active substances from the classes of pyrethroids, organophosphates, and spinosyns, while other substances, like azadirachtin, pyrethrins or neonicotinoids have shown poor efficacy (Bruck et al. 2011). Insecticides containing organophosphates and pyrethroids were the most effective in a study on a season-long control program against *D. suzukii* in blueberry crops (Diepenbrock et al. 2016). The situation for organic growers is especially challenging, as they need to rotate the use of spinosyns with a limited choice of other insecticides of natural origin, which are scarce though (Asplen et al. 2015). For organic plant protection, Grassi et al. (2011) have obtained a satisfactory degree of efficacy in field trials with natural pyrethrins and spinosad, but their residual impact was shown to be limited to a few days. Therefore, multiple applications of these products will be needed and certainly their rotation is indispensable (Beers et al. 2011). Van Timmeren and Isaacs (2013), on the contrary, found organic pyrethrum insecticides not effective against adults and pre-imaginal stages of *D. suzukii*. Moreover, it was recently shown, that the addition of sugar with or without yeast can greatly improve the effectiveness of various insecticides containing e.g. diamide and spinosyn for control of *D. suzukii* in various crops (Cowles et al. 2015, Knight et al. 2016).

When assessing the effects of an insecticide against *D. suzukii* both in the laboratory and under field conditions, the cryptic nature of this insect makes it difficult to exactly determine the effects on the number of eggs laid in a given substrate as well as putative effects on larvae hatching from eggs. Moreover, laboratory bioassays with mature fruits, which would permit larval development up to the adult stage, are often hampered by fruits rotting and liquefying before larvae have reached the adult stage. Here, we report on laboratory bioassays using three different types of substrates suitable for screening various insecticides for their potential effects against *D. suzukii* eggs, larvae, and adults. Moreover, suitability of our bioassays is validated in a study evaluating the efficacy of different bioinsecticides against all developmental stages of *D. suzukii*.

Material and Methods

D. suzukii Maintenance

D. suzukii individuals used in this study were obtained from a laboratory colony maintained at the University of Geisenheim since October 2014. Fly stock colonies were reared in breeding cages (46 × 30.5 × 33.5 cm) at 23–25 °C room temperature with a photoperiod of 16:8 (L:D) h. Apple-nutrition-medium (consisting of 2 litres unfiltered apple juice, 700 g apple puree, 40 g white sugar, 40 g wheat flour, 40 g agar, 100 g brewer's yeast, and 8 g Nipagin dissolved in 20 ml ethanol) dispensed in food-grade plastic containers (PAPSTAR GmbH, Kall, Germany) served as a food source for the emerging adults. A 30 ml plastic cup (Promet-Plast S.C., Poland) with a pierced lid and inserted cotton plug was filled with tap water and used as a water source. Nutrition medium as well as water source was exchanged three times a week.

Test Products

Four bioinsecticides and one synthetic insecticide (Table 1) were tested for their efficacy against different developmental stages of *D. suzukii* under the conditions of the laboratory bioassays using three different types of substrate. All assays included tap water as control.

Products were applied at rates shown in Table 1 in a volume of tap water equivalent to the use of 200 liters per hectare. Application was carried out using a pneumatic laboratory pesticide spraying apparatus ('SprayLab', Schachtner Gerätetechnik, Ludwigsburg, Germany) operating at 2.5 bars of pressure and speed of 1.25 km/h, equipped with a broadcast spray nozzle (Turbo TeeJet Wide Angle Flat Spray Tip - TT11001, TeeJetTechnologies, Glendale Heights, IL, USA). With these parameters a volume of 20 ml was sprayed onto a surface of 100 cm^{-1}.

Set-up of Bioassays

For all bioassays trial arenas consisting of plastic boxes (13.5 cm in diameter; 10.3 cm in height) with a hole on the bottom, sealed with a fine net, and two openings on the top were used. One opening, closed with a rubber plug, was used for releasing adults into the trial arena. The second one served for fixing a water source consisting of a 7 ml disposable plastic Pasteur pipette filled with tap water and having a cotton swab inserted at its end which was kept constantly wet. Bioassays were conducted using three different substrates (water agar with apple juice; grape berries; apple-nutrition-medium), allowing the assessment of the efficacy of the respective insecticide against eggs, larvae, and adults of *D. suzukii*. All types of substrates were treated with the insecticides either before egg deposition (set-up A, in which adult flies had contact with the insecticide), or after egg-deposition (set-up B, without adult flies). For both set-ups, 5 mated female flies with an age of 10–14 d, were manually transferred from the laboratory rearing container into each trial arena using a small glass vial. The substrate (treated or not treated with an insecticide) was exposed to *D. suzukii* females for 24 h, after which female mortality (for set-up A) was recorded and flies were removed by anesthetising them with a short dose of CO_2 gas released inside the trial arenas. For all assays, each treatment consisted of 10 replicates. In addition, bioassays 2 (on grape berries) and 3 (on apple-nutrition-medium) were conducted twice.

Bioassay 1: Water–Apple Juice Substrate

For the purpose of recording the number of eggs laid by female *D. suzukii* flies and estimating the percentage of larval hatching, water–apple juice agar was prepared and offered as a substrate for egg-laying. A ratio between the ingredients was chosen so as to provide very good transparency and visibility of the substrate which made it possible to count the number of eggs and larvae inside the substrate.

Table 1. Insecticides evaluated for their efficacy against different developmental stages of *D. suzukii*

Trade name	Active ingredient	Dose rate	Manufacturer
SpinTor	Spinosad (480 g/liter)	160 ml/ha	Dow AgroSciences LLS, Indianapolis, USA
NeemAzal-T/S	1% Azadirachtin A (10 g/liter)	3 l/ha	Trifolio-M GmbH, Lahnau, Germany
Spruzit Schädlingsfrei	Pyrethrin (4.59 g/liter), rapeseed oil (825.3 g/liter)	1%	W. Neudorff GmbH KG, Emmerthal, Germany
Piretro Verde	Pyrethrin (18.6 g/liter)	2.4 l/ha	Copyr S.p.A., Milan, Italy
Mospilan SG	Acetamiprid (200 g/kg)	375 g/ha	Cheminova GmbH & Co. KG, Stade, Germany

The agar contained distilled water and store-bought unfiltered apple juice in a ratio of 4:1 and 3% Bacto Agar (Difco Microbiology, USA) and was autoclaved at 115 °C for 5 min. 10 ml of the agar was dispensed under a laminar flow cabinet in disposable Petri dishes with ventilation cams inside the lids (55 mm in diameter, 14.2 mm in height; VWR International GmbH, Germany) using a Labmax bottletop dispenser (Labnet International, Inc., USA). Petri dishes were offered to adults for egg-laying either before or after being treated with an insecticide as described earlier. After 24 h, female mortality and number of eggs laid were recorded using a stereomicroscope. Petri dishes were further stored at 23–25 °C and a photoperiod of 16:8 (L:D) h for 3 d, after which the number of hatched larvae inside the agar was recorded using a stereomicroscope. Bioassay 1 was conducted in two separate series within four weeks.

Bioassay 2: Grape Berries as Substrate

A fruit bioassay was conducted to estimate larval development and survival in grape berries following an application of respective insecticides. Two different varieties of store-brought table grapes imported from Egypt were used in the bioassays (conventional *Vitis vinifera* var. "Early Sweet" in the first replicate and organic *V. vinifera* var. "Sugarone" in the second replicate of this experiment). Grapes were washed with warm water and dried before being used. Small clusters, consisting of five berries each, were cut from the stems, labelled and were offered to adults for egg-laying either before or after being treated with an insecticide. For insecticide application in the pesticide spraying apparatus, clusters were hung on a metal rod and two nozzles from opposite sides were mounted, allowing an uniform coverage of the berries. For egg-laying, clusters were placed for 24 h in the trial arenas (one cluster per arena), five females were released and were allowed to deposit their eggs for 24 h, followed by recording female mortality (in set-up A). After egg deposition clusters were placed in a 250 ml rectangular food-grade plastic container (PAPSTAR GmbH, Kall, Germany) lined with filter paper to absorb leaking berry juice and covered with a fine net and were stored under controlled conditions (23–25 °C and a photoperiod of 16:8 [L:D] h) for 5 d. The number of larvae was recorded by washing larvae out of the destemmed and crushed berries using 92 g of NaCl in 1 liter of tap water.

Bioassay 3: Apple-Nutrition-Medium as Substrate

In this bioassay, apple-nutrition-medium was chosen as an egg-laying substrate, which permitted the development of eggs up to the adult stage. The same apple-nutrition-medium as described earlier for rearing of *D. suzukii* flies was used. 30 ml of medium was poured in 60 ml round food-grade plastic containers (bikapack kg, Feldkirch, Austria) which were offered to adults for egg-laying either before or after being treated with an insecticide. After 24 h of egg-laying, female mortality was recorded, the containers were removed from the trial arenas and stored under the conditions described earlier. The number of emerging adult flies was recorded daily.

Data Analyses

Data for the numbers of eggs laid, numbers of larvae and numbers of emerged adults were subjected to square-root transformation. Data for the percentage larval hatching and the percentage female mortality were arcsine-square-root transformed in order to satisfy the assumptions of a parametric test. Normality of the distribution and homogeneity of variance were tested with Shapiro-Wilk and Bartlett test, respectively. Data on the percentage larval hatching and percentage female mortality did not fit the assumptions of normality and were analysed using Kruskal-Wallis test, followed by multiple comparisons test. For Bioassay 1, number of eggs laid was analysed using Welch's one-way ANOVA followed by Games-Howell post-hoc test, as the data violated the assumption of homoscedasticity despite the transformations. For Bioassay 2, data on the number of larvae washed out of grape berries was analysed using Kruskal-Wallis test, followed by multiple comparisons test as the data did not follow a normal distribution. For Bioassay 3, data on the number of emerged adults was subjected to one-way ANOVA and Tukey HSD test for means separation. All procedures were carried out using STATISTICA for Windows (StatSoft, Inc., Tulsa, 2005, version 7.1), excluding Games-Howell post-hoc test which was performed on an Excel spreadsheet available in Handbook of Biological Statistics (McDonald 2014). Treatment mortality data were corrected to account for control mortality using Abbott's formula (Abbott 1925).

Results

Effects on Female Mortality, Number of Eggs Laid and Percentage of Larvae Hatched: Water–Apple Juice Substrate

Mortality of mature females was only assessed for set-up A where adult *D. suzukii* flies were exposed to insecticide-treated water-apple juice substrate. Female mortality was significantly higher for water-apple juice substrate treated with the products SpinTor in the first series ($H = 31.646$; $P = 0.0000$; Table 2) and Piretro Verde in the second series of experiments ($H = 27.593$; $P = 0.0000$; Table 2) in comparison to the other treatments and the control. Abbott corrected mortality values were 100% for SpinTor and 56% for Piretro Verde, respectively.

When adult *D. suzukii* flies laid their eggs into insecticide-treated water-apple juice substrate (set-up A), treatments with SpinTor and Spruzit significantly reduced the number of eggs laid compared with the control ($F = 64.142$; df = 36; $P < 0.001$; Table 2) in the first series of this experiment. Same was evident for the second series, where water-apple juice substrate in Petri dishes treated with both Piretro Verde and Mospilan SG contained significantly fewer eggs than the control ($F = 21.172$; df = 27; $P < 0.001$; Table 2). The average percentage of hatched larvae after 3 days varied between 71 and 91.6%, with NeemAzal-T/S resulting in a significantly lower number of larvae hatched compared with SpinTor and Spruzit in the first series of experiments ($H = 25.164$, $P = 0.0000$; Table 2).

When untreated water-apple juice substrate dishes were offered to *D. suzukii* females for egg-laying (set-up B), the number of eggs laid within 24 h did not differ significantly between treatments (Table 2; $F = 0.198$; df = 36; $P = 0.897$ for series one; $F = 1.029$; df = 27; $P = 0.378$ for series two). Treatment of water-apple juice substrate with NeemAzal-T/S resulted in a significantly lower number of larvae hatched from eggs compared with the control and other treatments in the first series of this experiment ($H = 18.353$, $P = 0.0004$; Table 2).

Effects on Female Mortality and Number of Larvae Hatched: Grapes

Mortality of mature females was only assessed for set-up A where adult *D. suzukii* flies were exposed to insecticide-treated grape berries. Female mortality was significantly higher for grape berries treated with the products SpinTor and Mospilan SG in comparison to the other treatments and the control ($H = 74.068$; $P = 0.0000$; Table 3). Abbott corrected mortality values were 93% for SpinTor and 53.5% for Mospilan SG, respectively.

For set-up A (grapes were treated with the insecticides before egg-deposition), significantly fewer larvae were present inside grape berries 5 d after the start of the bioassay, if grapes were treated with SpinTor and Mospilan SG compared with the other treatments and the control ($H = 43.333$; $P = 0.0000$; Table 3). For set-up B (grapes were treated with the insecticides after egg deposition), a significant reduction in the number of larvae present inside berries was evident if grapes were treated with SpinTor, Spruzit, and Mospilan SG compared with NeemAzal/T/S and Piretro Verde ($H = 22.683$; $P = 0.0004$; Table 3). However, none of the insecticides tested showed a significant reduction in the number of larvae hatched from eggs compared with untreated control berries.

Effects on Female Mortality and on Number of Adults Developed: Apple-Nutrition-Medium

Mortality of mature females was only assessed for set-up A where adult *D. suzukii* flies were exposed to insecticide-treated apple-nutrition-medium. Female mortality was significantly higher for grape berries treated with the products SpinTor and Piretro Verde in comparison to the other tested insecticides and the control ($H = 98.983$; $P = 0.0000$; Table 4). Abbott corrected mortality values were 81% for SpinTor and 72% for Piretro Verde, respectively.

In case *D. suzukii* eggs were deposited in insecticide-treated apple-nutrition-medium (set-up A), no adults developed in medium treated with SpinTor or Piretro Verde, respectively (Table 4).

Table 2. Average female mortality (%) (±SD), number of eggs deposited (± SD) and larval hatch (%) (± SD) in bioassay 1 after treatment of water–apple juice substrate with different insecticides or with water as control

		Set-up A (treatment before egg-deposition)			Set-up B (treatment after egg-deposition)	
	Treatment	Avg. female mortality (%) (± SD)	Avg. no. of eggs (± SD)	Avg. larval hatch (%) (± SD)	Avg. no. of eggs (± SD)	Avg. larval hatch (%) (± SD)
Series 1	Control	0.0 ± 0a	56.5 ± 11.5a	71.0 ± 11.9ab	107.4 ± 39.5a	80.9 ± 8.1a
	SpinTor	100.0 ± 0b	8.2 ± 4.9b	75.0 ± 30.0a	109.8 ± 27.5a	80.2 ± 8.4a
	NeemAzal-T/S	8.0 ± 10.3a	83.3 ± 28.6a	35.0 ± 10.4b	101.8 ± 41.8a	64.2 ± 10.9b
	Spruzit	2.0 ± 6.3a	20.8 ± 8.9c	91.6 ± 7.8a	110.0 ± 21.5a	86.4 ± 4.0a
Series 2	Control	0.0 ± 0a	40.7 ± 26.7a	82.5 ± 15.1a	38.9 ± 31.0a	75.4 ± 11.4a
	Piretro Verde	56.0 ± 24.6b	1.5 ± 1.4b	84.4 ± 34.3a	32.0 ± 33.1a	56.0 ± 25.5a
	Mospilan SG	0.0 ± 0a	4.4 ± 4.0c	76.0 ± 34.0a	54.7 ± 40.7a	74.3 ± 15.4a

Bioassay 1 was conducted in two separate series (1, 2), each with 10 replicates per treatment. Two different set-ups were assessed, where water-apple juice substrate was treated with insecticides before (A) or after (B) egg deposition. Different letters within the same column and series indicate significant differences between means ($P < 0.05$).

Table 3. Average female mortality (%) (± SD) and number of hatched larvae (± SD) in bioassay 2 after treatment of grape berries with different insecticides or with water as control

	Set-up A (treatment before egg-deposition)		Set-up B (treatment after egg-deposition)
Treatment	Avg. female mortality (%) (± SD)	Avg. no. of hatched larvae (± SD)	Avg. no. of hatched larvae (± SD)
Control	14.0 ± 24.4a	15.7 ± 13.3a	18.2 ± 11.6ab
SpinTor	94.0 ± 13.1b	3.2 ± 4.2b	12.0 ± 7.9b
NeemAzal-T/S	7.0 ± 21.8a	15.4 ± 10.7a	27.0 ± 16.2a
Spruzit	7.0 ± 16.25a	13.7 ± 11.4a	12.0 ± 12.3b
Piretro Verde	23.0 ± 33.23a	3.5 ± 3.8b	24.5 ± 14.8a
Mospilan SG	60.0 ± 31.11b	2.8 ± 3.6b	11.9 ± 9.0b

Two different set-ups were assessed, where water-agar was treated with insecticides before (A) or after (B) egg deposition. Data of two independent replicates of the experiment are presented (20 replicates per treatment in total). Different letters within the same column indicate significant differences between means ($P < 0.05$).

Table 4. Average female mortality (%) (± SD) and number of adults developed (±SD) in bioassay 3 after treatment of apple-nutrition-medium with different insecticides or with water as control

	Set-up A (treatment before egg-deposition)		Set-up B (treatment after egg-deposition)
Treatment	Avg. female mortality (%) (± SD)	Avg. no. of adults (± SD)	Avg. no. of adults (± SD)
Control	0.0 ± 0.0a	61.5 ± 13.7a	53.2 ± 15.8a
SpinTor	81.0 ± 18.9b	0.0 ± 0.0e	0.0 ± 0.0b
NeemAzal-T/S	1.0 ± 4.5a	36.5 ± 14.2b	35.0 ± 16.1c
Spruzit	7.0 ± 9.8a	20.9 ± 12.0c	52.4 ± 16.1a
Piretro Verde	72.0 ± 23.8b	0.0 ± 0.0e	10.5 ± 11.4d
Mospilan SG	6.0 ± 11.4a	7.2 ± 4.3d	13.6 ± 5.8d

Two different set-ups were assessed, where water-agar was treated with insecticides before (A) or after (B) egg deposition. Data of two independent replicates of the experiment are presented (20 replicates per treatment in total). Different letters within the same column indicate significant differences between means ($P < 0.05$).

Moreover, all insecticides tested significantly reduced the number of adults emerging from the substrate compared with the untreated control, with significant differences being evident between the products ($F = 254.292$; df = 54; $p = 0.00$; Table 4). In case of set-up B (apple-nutrition-medium was treated with insecticides after egg deposition), all insecticides except Spruzit resulted in a significantly lower number of newly emerged adult flies relative to the water control, with no adults emerging in the SpinTor treatment ($F = 108.702$; df = 54; $P = 0.00$; Table 4). Treatments with the products Mospilan SG and Piretro Verde resulted in significant fewer number of adults developed than the NeemAzal-T/S treatment.

Discussion

The design of the bioassays reported here, all based on a similar setup but including three different types of substrates for oviposition and subsequent larval development, allowed assessment of the immediate toxic effects of insecticides against ovipositing female flies, the numbers of eggs laid by them, and the effects on subsequent larval development up to the adult fly. Depending on the expected mode of action of the respective insecticide or the research question of the given bioassay, we recommend using the quick-and-easy water-apple juice agar as a substrate in bioassays, which allows egg counting and observation of larval development due to its transparency, or the nutrient-rich apple-nutrition medium allowing complete metamorphosis. The third type of substrate used here—grape berries—makes it possible to assess effects of an insecticide present on a fruit's surface on oviposition and larval hatch from eggs. However, in this bioassay using grape berries, female mortality was observed in the untreated control, suggesting that flies were not able to utilize intact grape berries as a sufficient nutrient source. Ioriatti et al. (2015) observed that *D. suzukii* would prefer to feed on damaged grapes where they could find nutrients. Therefore, in case undamaged fruits are used for bioassays, it is essential to provide the flies nutrients such as a yeast suspension at the start of the bioassay in order to keep individuals vital and active.

We validated the suitability of these three types of bioassays using four different bioinsecticides and one synthetic insecticide applied both before or after oviposition by female *D. suzukii* flies and assessed the effects against various developmental stages of *D. suzukii*. Highest adult mortality based on residual contact was achieved in all bioassays with insecticides containing spinosad or a natural pyrethrin (Piretro Verde) as active ingredients. The same products also significantly reduced the average number of hatched larvae as well as the average number of emerging adult flies if they were applied before *D. suzukii* females have laid their eggs in the treated substrate. If applied after infestation (set-up B), these products were in most of the cases not significantly reducing egg or larval development, pointing to the necessity of applying products in particular based on pyrethrins onto a given fruit surface shortly before egg deposition.

Treatments with SpinTor (spinosad) caused the highest percentages of mortality of egg-laying females on all types of substrates, ranging from 78 to 100%, when females came into contact with a freshly treated surface. This quick knock-down effect to adult flies prevented oviposition. Similar results were achieved by Cuthbertson et al. (2014) who reported a complete protection of spinosad-treated blueberries from *D. suzukii* when the insecticide was applied before oviposition. Their trial demonstrated 100% mortality of adults and no development of larvae in fruits. In our bioassays, however, despite the high adult mortality, flies were able to lay a certain number of eggs in the given substrates. Yet, in spinosad-treated water–apple juice substrate or apple-nutrition medium, eggs or the newly hatched larvae did not develop further but quickly died. Nevertheless, in case of spinosad-treated grape berries, embryonic and larval development was possible to a certain extent, although significantly fewer larvae were found in the spinosad-treated berries compared with the water control. This corresponds to the findings of Beers et al. (2011) who reported an adult mortality of above 90% and a reduction in oviposition punctures, but still presence and development of larvae in spinosad-treated cherries. Apart from this quick lethal effect of spinosad against adult *D. suzukii* flies, one has to consider the length of residual control of this substance on a given fruit's surface, which may not be much longer than a few days. Residual control of this substance also e.g. after heavy rainfalls has to be further tested both in laboratory bioassays as described here and under field conditions (Beers et al. 2011, Cuthbertson et al. 2014).

The pyrethrin-based product Piretro Verde caused both rather high female mortality rates when applied on artificial medium and very strong suppression of egg or larval development in artificial medium and grape berries, when applied before egg deposition. Unsatisfactory efficiency was recorded for pyrethrum in the study of Van Timmeren and Isaacs (2013), who exposed *D. suzukii* adults to one-day-old residues of this insecticide sprayed on blueberries. The recorded mortality and the number of larvae developing in the fruit were not significantly different from numbers present in the control. Apparently, this active ingredient has short residual activity in the field, requiring frequent applications to achieve sufficient control (Bruck et al. 2011). This is especially important for organic growers, who are limited in their choice of active substances against *D. suzukii*. Therefore, in organic horticulture or viticulture pyrethrin applications may be alternated with spinosad to delay or prevent the development of resistance (Bruck et al. 2011). Taking into consideration the quick action of pyrethrin against flies and its short preharvest interval, a spray shortly before harvest might fit well in a management strategy. Efficacy of the second pyrethrin-containing insecticide (Spruzit) tested in this study was in most cases not significantly different to the control and thus did not come close to the efficacy achieved by Piretro Verde. This might be simply because Piretro Verde contains an approximately four times higher amount of the active ingredient pyrethrin in grams per litre in comparison to Spruzit.

The bioinsecticide NeemAzal-T/S containing the active ingredient azadirachtin A, did not cause significant adult mortality in any of the bioassays. This corresponds to other studies which have assessed direct mortality of *D. suzukii* flies after application of azadirachtin (Van Timmeren and Isaacs 2013, Cuthbertson et al. 2014). However, on apple-nutrition medium allowing complete metamorphosis, the number of individuals developing to the adult stage was significantly reduced if the medium was treated with NeemAzal-T/S both before or after egg deposition. This corresponds to the well-known biological effects of azadirachtin A as an insect growth regulator affecting metamorphosis (Schmutterer 1990).

The neonicotinoid insecticide Mospilan SG (acetamiprid) included in the present study caused significant mortality of adult females when exposed to treated grape berries and apple-nutrition medium. In addition, this product reduced numbers of developing larvae in grape berries and emerging adults from apple-nutrition medium. In previous studies, acetamiprid was found to be able to pass through the grape skin, thus providing sufficient levels of toxicity to eggs or larvae of *D. suzukii* (Wise et al. 2015). In testing the curative potential of acetamiprid, Bruck et al. (2011) obtained unsatisfactory results with neonicotinoids as adulticides against *D. suzukii*.

However, the authors pointed out the systemic properties and potentially beneficial long-term sublethal effects (i.e. antifeeding, repellency, ovipositional deterrence) of this substance class. According to Wise et al. (2015) curative activity by neonicotinoids may be an important mechanism for achieving overall control of larval infestation of berries. Thus, an application post-infestation would not result in insect-free fruit, but it could prevent survival of larvae (Wise et al. 2015).

The recent rapid worldwide spread as well as the polyphagous nature and high pest potential of *D. suzukii* call for the development of efficient control strategies against this pest insect. Besides cultural management practices, application of insecticides represent one option both for integrated as well as organic horticulture and viticulture. In this regard, bioassays as described here are the basis for comprehensive evaluations of the effects of insecticide applications against different developmental stages of *D. suzukii* and thus for providing sound recommendations to the growers.

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References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Asplen, M. K.G. Anfora, A. Biondi, D. S. Choi, D. Chu, K. M. Daane, P. Gibert, A. P. Gutierrez, K. A. Hoelmer, W. D. Hutchison, et al. 2015. Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities. J. Pest Sci. 88: 469–494.

Atallah, J., L. Teixeira, R. Salazar, G. Zaragoza, and A. Kopp. 2014. The making of a pest: The evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. P. Roy. Soc. B-Biol. Sci. 281:

Beers, E. H., R. A. Van Steenwyk, P. W. Shearer, W. W. Coates, and J. A. Grant. 2011. Developing *Drosophila suzukii* management programs for sweet cherry in the western United States. Pest Manag. Sci. 67: 1386–1395.

Bruck, D. J., M. Bolda, L. Tanigoshi, J. Klick, J. Kleiber, J. DeFrancesco, B. Gerdeman, and H. Spitler. 2011. Laboratory and field comparisons of insecticides to reduce infestation of *Drosophila suzukii* in berry crops. Pest Manag. Sci. 67: 1375–1385.

Calabria, G., J. Maca, G. Bachli, L. Serra, and M. Pascual. 2012. First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. J. Appl. Entomol. 136: 139–147.

Cini, A., C. Ioriatti, and G. Anfora. 2012. A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. B. Insectol. 65: 149–160.

Cowles, R. S., C. Rodriguez-Saona, R. Holdcraft, G. M. Loeb, J. E. Elsensohn, and S. P. Hesler. 2015. Sucrose improves insecticide activity against *Drosophila suzukii* (Diptera: Drosophilidae). J. Econ. Entomol. 108: 640–653.

Cuthbertson, A., D. Collins, L. Blackburn, N. Audsley, and H. Bell. 2014. Preliminary screening of potential control products against *Drosophila suzukii*. Insects 5: 488.

Diepenbrock, L. M., D. O. Rosensteel, J. A. Hardin, A. A. Sial, and H. J. Burrack. 2016. Season-long programs for control of *Drosophila suzukii* in southeastern US blueberries. Crop Prot. 81: 76–84.

Goodhue, R. E., M. Bolda, D. Farnsworth, J. C. Williams, and F. G. Zalom. 2011. Spotted wing drosophila infestation of California strawberries and raspberries: economic analysis of potential revenue losses and control costs. Pest Manag. Sci. 67: 1396–1402.

Grassi, A., L. Giongo, and L. Palmieri. 2011. *Drosophila (Sophophora) suzukii* (Matsumura), new pest of soft fruits in Trentino (North-Italy) and in Europe. IOBC/WPRS Bull. 70: 121–128.

Hauser, M. 2011. A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest Manag. Sci. 67: 1352–1357.

Haye, T., P. Girod, A. G. S. Cuthbertson, X. G. Wang, K. M. Daane, K. A. Hoelmer, C. Baroffio, J. P. Zhang, and N. Desneux. 2016. Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world. J. Pest Sci. 89: 643–651.

Ioriatti, C., V. Walton, D. Dalton, G. Anfora, A. Grassi, S. Maistri, and V. Mazzoni. 2015. *Drosophila suzukii* (Diptera: Drosophilidae) and its potential impact to wine grapes during harvest in two cool climate wine grape production regions. J. Econ. Entomol. 108: 1148–1155.

Kaneshiro, K. 1983. *Drosophila (Sophophora) suzukii* (Matsumura). Proc. Hawaiian Entomol. Soc. 24: 179.

Kenis, M., L. Tonina, R. Eschen, B. van der Sluis, M. Sancassani, N. Mori, T. Haye, and H. Helsen. 2016. Non-crop plants used as hosts by *Drosophila suzukii* in Europe. J. Pest Sci. 89: 735–748.

Knight, A. L., E. Basoalto, W. Yee, R. Hilton, and C. P. Kurtzman. 2016. Adding yeasts with sugar to increase the number of effective insecticide classes to manage *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in cherry. Pest Manag. Sci. 72: 1482–1490.

Lee, J. C., D. J. Bruck, A. J. Dreves, C. Ioriatti, H. Vogt, and P. Baufeld. 2011. In Focus: Spotted wing drosophila, *Drosophila suzukii*, across perspectives. Pest Manag. Sci. 67: 1349–1351.

McDonald, J. H. 2014. Handbook of biological statistics. 3rd edition. Sparky House Publishing, Baltimore, MD.

Mitsui, H., K. H. Takahashi, and M. T. Kimura. 2006. Spatial distributions and clutch sizes of *Drosophila* species ovipositing on cherry fruits of different stages. Popul. Ecol. 48: 233–237.

Rogers, M. A., E. C. Burkness, and W. D. Hutchison. 2016. Evaluation of high tunnels for management of *Drosophila suzukii* in fall-bearing red raspberries: Potential for reducing insecticide use. J. Pest Sci. 89: 815–821.

Rota-Stabelli, O., M. Blaxter, and G. Anfora. 2013. *Drosophila suzukii*. Curr. Biol. 23: R8–R9.

Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol. 35: 271–297.

Van Timmeren, S., and R. Isaacs. 2013. Control of spotted wing drosophila, *Drosophila suzukii*, by specific insecticides and by conventional and organic crop protection programs. Crop Prot. 54: 126–133.

Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. Lee, D. J. Bruck, V. M. Walton, S. D. O'Neal, and F. G. Zalom. 2011. *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening soft fruit expanding its geographic range and damage potential. J. Integr. Pest Manage. 2: G1–G7.

Wise, J. C., R. Vanderpoppen, C. Vandervoort, C. O'Donnell, and R. Isaacs. 2015. Curative activity contributes to control of spotted-wing drosophila (Diptera: Drosophilidae) and blueberry maggot (Diptera: Tephritidae) in highbush blueberry. Can. Entomol. 147: 109–117.