# Comparative evaluation of insecticide efficacy tests against *Drosophila suzukii* on grape berries in laboratory, semi-field and field trials

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## Summary

As the period for field trials on grapevine is limited, we designed a laboratory test system to evaluate the effectiveness of selected insecticides against spotted-wing *Drosophila* (SWD), *Drosophila suzukii,* on different types of grape berries all year round. Tests were undertaken during winter and early spring with table grapes of different purchased varieties according to their seasonal availability and with wine grapes from experimental field plots in autumn. In preliminary experiments, we defined parameters for a standard laboratory test system for screening the effectiveness of several formulated insecticides in two different experimental set-ups: i) application before confining adults with berries and ii) application after confining adult *D. suzukii* with berries. These approaches allowed us to determine the contact activity of the products on adult *D. suzukii* or the impact on the larval development until the emergence of adult flies. The developed test system is suitable for screening substances with diverse types of activity on different grape types. In a second step, we combined laboratory bioassays with field applications in a semi-field persistence study and lastly we installed a randomized field plot in order to compare the effectiveness of selected insecticides in the laboratory and under field conditions. In all cases, the products Karate Zeon and SpinTor proved most efficacious in their contact mortality or as oviposition deterrents, while Mospilan SG and Coragen exhibited a good larvicidal activity. However, important disagreements occurred for the efficacy of currently authorized insecticides among laboratory, semi-field and practical field applications. The transferability of laboratory results into the field is discussed.

K e y   w o r d s : table grapes; wine grapes; laboratory bioassay; aged residue; field trial, control.

## Introduction

The invasive SWD has become a serious threat for European fruit growing and viticulture in a short time. While it is a well known pest in Asia (Kanzawa 1939) the first known damage to commercial small fruit in Italy was reported in Trentino during 2009 (Grassi *et al*. 2009, Calabria *et al*. 2010). Female *D. suzukii* oviposit into suitable ripening fruits using a serrated ovipositor (Lee *et al*. 2011). This is unique compared to other drosophilids, including the common fruit fly, *D. melanogaster*, which oviposit into overripe or previously damaged fruits (Tochen *et al*. 2016). *D. suzukii* is able to develop on a wide range of both cultivated and wild soft-skinned fruits with many host plants in the native and in the invaded areas, with berries being the preferred hosts (Cini *et al*. 2012). While SWD mainly attacks susceptible small and stone fruits, the *Vitis vinifera* case is still matter of verification (Cini *et al*. 2012). The poor capacity of grapes as a host for *D. suzukii* development is evidenced by several studies (Bellamy *et al*. 2013, Tochen *et al*. 2016). Laboratory experiments showed a significantly lower oviposition susceptibility and developmental rate of *D. suzukii* on grapes than on small fruits and cherry (Lee *et al*. 2011, Kim *et al*. 2015). However, recent observations in vineyards in Northern Italy indicate that *V. vinifera* can become a field host with soft-skinned varieties being more impacted. SWD can be found feeding on wine grapes that are negatively impacted by cracking, disease, and bird damage during the harvest period. When berries are compromised, as described above, SWD is expected to survive for longer periods, to lay more eggs, and to be a vector of *Acetobacter* spp. in wine grapes during the late season (Ioriatti *et al*. 2015).

As SWD is a novel pest in Europe, it is necessary to test registered insecticides for their efficacy to manage this pest in conventional and organic production. Current insecticide control measures are based on exceptional permissions for selected products. However, the insecticides that are currently available to growers for control of *D. suzukii* are not very effective since the use of highly efficient broad spectrum chemicals is being progressively restricted (Cini *et al*. 2012). Due to the lack of specific insecticides against immature stages of *D. suzukii* within fruits, research has so far focused on treatments based on insecticides targeting adults. Recent laboratory and field studies, both in the USA and in EU, revealed that among the registered insecticides organophosphates, timely applications of pyrethroids and spinosyns can provide good contact activity and residual impact for up to 12-14 d (Beers *et al*. 2011; Bruck *et al*. 2011). In contrast, the efficacy of the neonic-

otinoids as adulticides was not satisfactory (Bruck *et al.* 2011). Given that the impact of *D. suzukii* in vineyards so far is negligible control strategies with chemical products were mainly investigated in soft-skinned berry plots where the population densities in the field are sufficient to study insecticide efficacy. This is not the case in vineyards where problematic levels of infestation with *D. suzukii* only occur after previous damage by other pests or after harvest when overripe berries are left. Furthermore, only a small time frame is available in viticulture for field trials which renders studies of extensive control strategies nearly impossible. Hence, laboratory screening of potential products for managing this pest in viticulture is the only viable way so far. Therefore, future research needs to address not only identification of efficient active ingredients, but must also consider how test protocols can be optimized (Cini *et al.* 2012). The objective of our study was to establish a laboratory test system for evaluating insecticide effectiveness against adult and immature stages of *D. suzukii* in grape berries and to validate the obtained data in the field.

## Material and methods

1 . L a b o r a t o r y   b i o a s s a y s .  S o u r c e s   o f i n s e c t   a n d   f r u i t   m a t e r i a l : SWD used in the experiments originated from a laboratory colony maintained within bug-dorm insect cages (280 mm × 280 mm × 280 mm; Watkins and Doncaster, Leominster, UK) in an environmentally controlled room at 24 °C ± 2 °C, 75 % RH ± 5 °C and a photoperiod of 16:8 (L:D).The insects were reared on a particular *Drosophila* diet (25 g agar, 30 g wheat germ, 25 g maize meal, 25 g brewer's yeast, 25 g apple puree and 50 g sugar dissolved in 1200 mL water). Laboratory tests during winter and spring were conducted with supermarket-purchased table grapes of the varieties 'Crimson seedless' and 'Flame seedless'. Berries of the red wine grape varieties 'Pinot noir', 'Calandro', 'Regent', and 'Dornfelder' used for the tests in autumn were collected from an experimental field at Geilweilerhof (Siebeldingen, Germany).

T e s t   p r o d u c t s : The following commercial formulations of different insecticides, either authorized in horticulture or in viticulture, were tested for activity against SWD: Acetamiprid (Neonicotinoid, Mospilan SG 0.25 kg·ha⁻¹); Chlorantraniliprole (Coragen 0.07 L·ha⁻¹); Cyantraniliprole (Exirel 0.375 L·ha⁻¹); Spinosad (SpinTor 0.16 L·ha⁻¹); Spirotetramat (Movento 0.48 L·ha⁻¹); ʎ-Cy-

halothrin (Pyrethroid, Karate Zeon 0.05 L·ha⁻¹). All insecticides were applied at labeled field doses in 200 L·ha⁻¹; distilled water was used as control. A summary is given in Tab. 1.

Preliminary tests for defining parameters of a universal laboratory screening system: In a first part of our study we carried out some preliminary tests considering the attractiveness and suitability of different grape berries and the most suitable application method in order to determine the elements for a universally valid laboratory screening system for insecticide efficacy: Scratched berries, table grapes, spray application. Data of these pre-tests including related figures and tables are summarized in the electronic supplement.

P r o c e d u r e s   f o r   i n s e c t i c i d e   s c r e e n i n g w i t h   t h e   t e s t   s y s t e m : The effectiveness of insecticides was evaluated using two different experimental set-ups: i) Evaluation of the contact activity and the impact on the larval development when insecticides were "applied before confining adults with berries" and ii) determination of the effect on the larval development when insecticides were "applied after confining adults with berries". For each approach ten scratched berries were sprayed with the respective insecticides, dried for 2 h and placed in plastic boxes on sandbed into the insect cages. That way berries were stabilized and prevented fly mortality caused by fruit rolling. Ten male and 20 female adult *D. suzukii* (ca. 7 d old) were released into a cage with 20 berries total and confined adults with berries for 48 h at standard rearing conditions either after insecticide treatment (set-up i) or before insecticide application (set-up ii). To limit fly mortality a cup filled with a 10 % sugar water was placed in the cage. For set-up i), adult mortality was assessed after 24 h and 48 h, thereafter flies were removed from the cages. For set-up ii), egg deposition on untreated berries was determined 24 h after incubation with *D. suzukii* adults prior treatment. Then insecticides were applied on infested berries as described above. In any case boxes with treated berries were closed with a ventilated cover and incubated at standard rearing conditions (24 °C, 75 % rH, 16:8 L:D) for 14 d.

2 . A g e d   r e s i d u e   t r i a l : A semi-field persistence trial was performed in a vineyard with the red cultivar 'Dornfelder' at Albersweiler (South Rhineland-Palatinate) which was highly affected by *D. suzukii* in 2014. In a separate experimental block 7 rows were treated with the registered products (without repetition): Karate Zeon, Mospilan SG, and SpinTor. One untreated row served as control. All products were applied with a tunnel plot spray-

Table 1

Specifications on test insecticides as registered in Germany

| Trade name | Active ingredient | Chemical classes | Translocation within plant/ mode of action | Dose rate·ha⁻¹ |
|---|---|---|---|---|
| Coragen | chlorantraniliprole | diamids | translaminar, neurotoxic | 0.07 L |
| Exirel | cyantraniliprole | diamids | translaminar, neurotoxic | 0.375 L |
| Karate Zeon | lambda-cyhalothrin | pyrethroids | non-systemic, neurotoxic | 0.05 L |
| Mospilan SG | acetamiprid | neonicotinoids | systemic, neurotoxic | 0.25 kg |
| Movento | spirotetramat | ketoenols | systemic, acaricide | 0.48 L |
| SpinTor | spinosad | spinosyns | systemic, neurotoxic | 0.16 L |

er ABS 6/25-TK (Schachtner Fahrzeug- und Gerätebau Ludwigsburg, Germany) at the same date (BBCH scale 81-85) without respecting their individual waiting time. Five grapes per treatment (pseudo repetitions) were collected 3 h, 7 d and 14 d after field application and prepared for the laboratory bioassay. Ten berries per grape and treatment were randomly sampled, scratched and placed into plastic boxes on a sandbed in insect cages. Ten scratched berries from untreated grapes acted as control for each treatment. Ten male and 20 female adult *D. suzukii* (ca. 7 d old) were released into a cage with 20 berries total and incubated for 48 h at standard rearing conditions. Adult mortality was assessed after 24 h and 48 h. After removal of all flies from cages, boxes were closed with a ventilated cover and incubated in a rearing chamber for 14 d at 24 °C and 75 % rH. After incubation the boxes were evaluated for emergence of adult flies and the berries were dissected to count pre-imaginal stages. All experiments were repeated 4 times.

3. Field trial: A comprehensive study was designed to evaluate the effectiveness of selected test insecticides under field conditions. The trial was performed in a separate block within the same 'Dornfelder' vineyard as for the aged residue trial but comprised in total 28 rows. The currently authorized products Karate Zeon, Mospilan SG, and SpinTor and the test products Coragen, Exirel and Movento were applied in a trial with random block design (n = 4 repetitions) at recommended field doses with an automatic plot sprayer (see aged residue trial) and according to the individual latency period of the different chemistries. SpinTor was applied twice with latency periods of 14 d and 7 d, respectively. Natural infestation of grapes was assessed weekly by evaluating pre-imaginal stages (eggs, larvae and pupae) in 50 randomly collected berries prior treatment and after insecticide application in 50 randomly sampled berries per treated and untreated rows. Given the low population density of *D. suzukii* inside and around the vineyard (monitored by vinegar traps) until harvest sampling of treated grapes for final evaluation was only carried out four weeks post-harvest. Five grapes per treatment (n = 4) were cut off and taken to the laboratory for evaluation. Ten berries were randomly collected around the grape and a total of 50 single berries per treatment was examined for infestation with *D. suzukii* larval stages. Berries from untreated grapes acted as control.

Data analysis: The effectiveness of insecticide treatments was calculated using Abbott's formula = 1-(x/y) where x is the number of living stages after treatments and y the number of living stages in the untreated control (Abbott 1925).

Statistical analysis was performed using the program R v2.9.0 and RStudio 3.0.3 (R Development Core Team, Vienna, Austria). At first data were subjected to a Shapiro-Wilk test to estimate normality of the distribution followed by a Bartlett test to determine the homogeneity of variance. As data did not fit the assumptions of homogeneity a Kruskal-Wallis rank sum test was used to determine differences among means. If the Kruskal-Wallis test revealed differences among means, a Duncan post-hoc test (*p* < 0.05) was adopted to rank these differences.

**Results**

Part 1 - Laboratory screening system for insecticide efficacy: Efficacy tests were performed according to the two experimental set-ups described above i) application before egg deposition and ii) application after egg deposition. During experimental set-up i) we determined the contact mortality and the impact on the emergence of adults from pupae and adults while for set-up ii) we calculated the survival rate based on the initial egg deposition before treatment. Fig. 1a-c demonstrates the effects of selected compounds on adult mortality, egg deposition and survival of larvae when applied before egg deposition (set-up i).
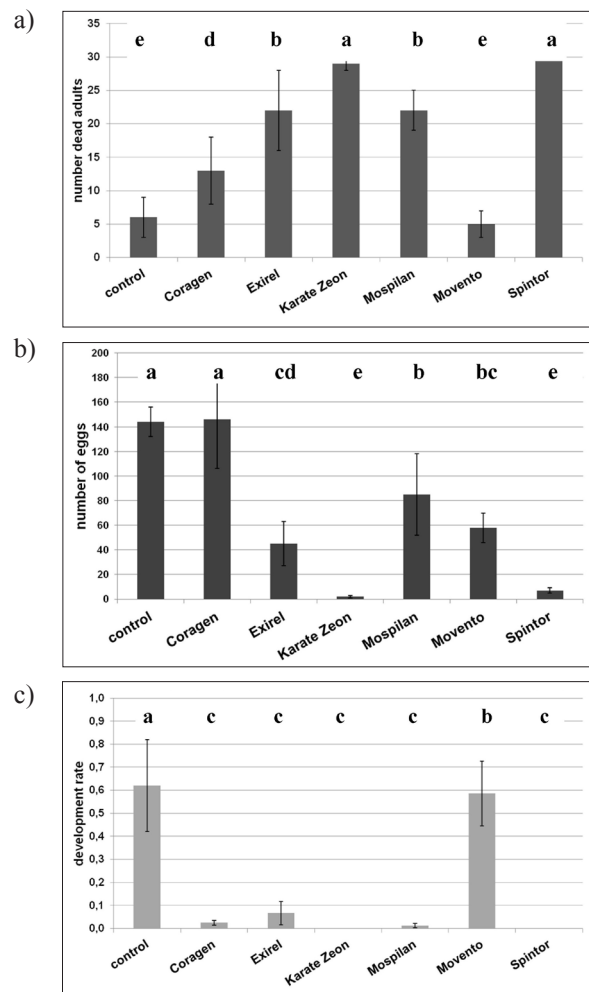


Fig. 1 a-c: Impact of selected insecticides on **a**) adult mortality, **b**) egg deposition and **c**) larval development when applied before egg deposition. Test were conducted in 2016 with ten scratched table grape berries for each product (n = 4) and incubation with 30 adult *D. suzukii* (10 males, 20 females) 2 h after treatment. **a**) Values represent mean numbers of dead adults 48 h post-treatment + SD (n = 4), Kruskal-Wallis χ2 = 32.37, df = 8, *p*-value = 7.99 e-05. **b**) Values represent the mean munber of eggs +SD (n = 4), Kruskal-Wallis χ2 = 32.77, df = 8, *p*-value = 6.76e-05. **c**) Development was calculated from the number of pupae and new adults emerged 14 d post-treatment, Kruskal-Wallis χ2 = 29.28, df = 8, *p*-value = 2.8e-04. Error bars indicate the variability among repetitions. A Duncan post-hoc test was adopted to compare means, confidence level: 0.95. Values accompanied by the same letters are not significantly different.

The contact activity of insecticides (Fig. 1a) measured as number of dead adults 48h after exposure showed important variation among the products. Thus, Karate Zeon and SpinTor cause a nearly 100 % mortality 48h post-inoculation while Exirel and Mospilan SG provided only intermediate control against adult SWD. The translaminar acting product Coragen was less effective regarding contact mortality while Movento did not exhibit any contact activity against adults.

The ovicidal and larvicidal effectiveness of selected insecticides is depicted in Fig. 1b (egg deposition 48 h post-treatment) and Fig. 1c (development rate after 14 d). Associated with the high contact mortality shown in Fig. 1a, egg deposition in SpinTor or Karate Zeon treatments was highly reduced and no further larval development occurred. Although Exirel and Mospilan SG exhibited similar contact mortality on adult *D. suzukii*, the toxicity patterns regarding ovicidal and larvicidal effectiveness were different. While Exirel showed intermediate potential to prevent egg deposition and further larval development, Mospilan SG was not effective related to egg deposition but strongly impaired further development indicating a larvicidal potential. A similar potential was observed for the product Coragen. While egg deposition was as high as in water controls, Coragen with the active ingredient chlorantraniliprole evolved a good residual activity toward further pre-imaginal stages. Unlike, Movento could slightly impact egg deposition but had no effect on further larval development.

In the second experimental set-up (ii) we investigated the impact of insecticides on the larval development 14 d after confining adults with larvae (dpc) with scratched berries. The initial number of eggs per rearing boxes of ten berries prior treatment was not significantly different for all repetitions (data not shown) and boxes were randomly assigned to the following particular insecticide treatments.

As shown in Fig. 2, the development rate after 14 d in the control of set-up ii is comparable with that of the approach i (Fig. 1c) indicating a basic reproducibility of the experiments. Also in this experimental layout Karate Zeon and SpinTor appeared highly effective in managing different stages of *D. suzukii*. The ovicidal or larvicidal impact of Coragen, Exirel and Mospilan SG when applied before egg deposition was also confirmed when treatment occurred after inoculation. Apart from Movento that had almost no effect on the development of *D. suzukii* the development rate after exposure with any other compound is similarly low and the statistical analysis discloses no significant differences between treatments.

Part 2 - Comparison of insecticide efficacy under laboratory conditions and in the field. Aged residue semi-field persistence trial: The contact mortality of currently authorized products aimed at manage *D. suzukii* was compared when applied in the laboratory test system or in a semi-field trial (Fig. 3).

While Karate Zeon and SpinTor resulted in essentially 100 % contact mortality in the laboratory their efficacy drastically dropped down when applied in the field. Only Karate Zeon treated berries showed some contact activity
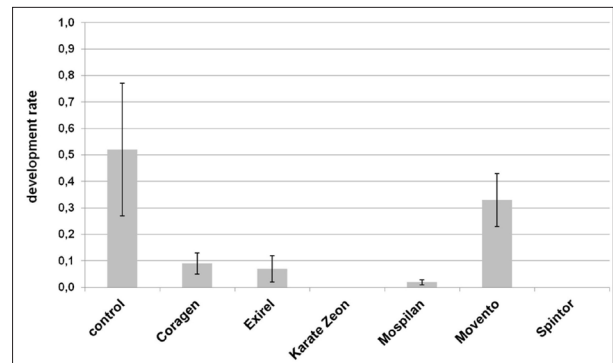


Fig. 2: Impact of several test insecticides on the development of *D. suzukii* from infested berries when applied after egg deposition. Tests were conducted with ten scratched table grape berries for each product (n = 4) and incubation with 10 male and 20 female *D. suzukii* 24 h before treatment. Egg deposition was monitored 24 h after incubation and prior insecticide application. After treatment boxes were closed with anti-insect mesh covers and maintained in climatic chambers for 14 d. Development rate was calculated from pupae and new adults 14 d post-treatment emerged from eggs deposited before treatment. Kruskal-Wallis $\chi2 = 30.32$, df = 8, *p*-value = 1.8e-04. Error bars indicate the variability among repetitions. A Duncan post-hoc test was adopted to compare means, confidence level: 0.95. Values accompanied by the same letters are not significantly different.
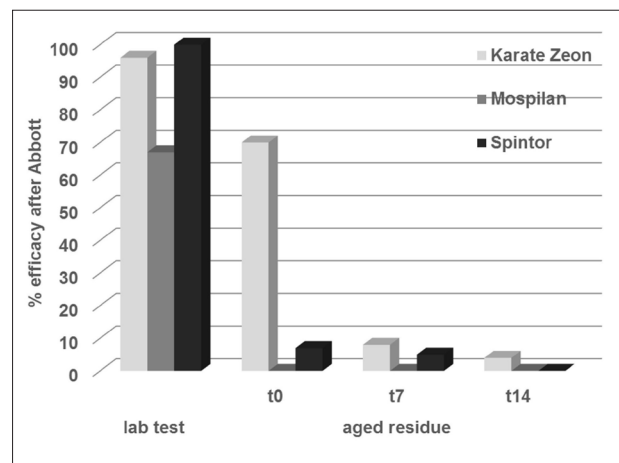


Fig. 3: Comparison of the efficacy (% Abbott) of selected test insecticides in laboratory tests and in an aged residue semi-field persistence trial. The effectiveness of products was assessed based on the number of dead adults 48 h after treatment (initial infestation with 30 adult *D. suzukii*) of berries in the laboratory test system and the number of dead adults after 48 h when insecticides where applied in an experimental field plot and berries were infested with 30 adult *D. suzukii* in the laboratory 3 h (t0), 7 d (t7) and 14 d (t14) after outdoor application.

when used immediately (t0) for exposure to adult *D. suzukii*. However, effectiveness decreased continuously with time after treatment. Interestingly, field application of SpinTor exhibited a negligible knockdown effect as compared with laboratory results but performed best at long-term with regard to Abbott corrected mortality. Mospilan SG never provided a satisfactory contact performance.

In assessing the potential of the selected products to act as oviposition deterrents we compared the oviposition 48h after application in laboratory bioassays and at differ-

ent times in the aged residue field trial and subsequently, following a further incubation of 14 d, the emergence of developmental stages of *D. suzukii* was recorded.

As summarized in Fig. 4, treatment with Karate Zeon and SpinTor provided 100 % control of *D. suzukii* in the laboratory test but did not hamper egg deposition and further development at each time in the semi-field application. The residual activity of Karate Zeon and SpinTor dropped drastically with time while Mospilan SG exhibited a delay of toxicity and performed best at long-term in controlling the development of *D. suzukii*.
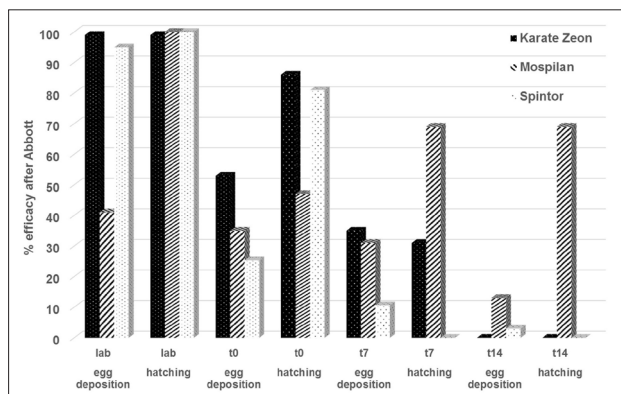


Fig. 4: Comparison of the residual activity of registered insecticides on the development of *D. suzukii* in laboratory bioassays and in an aged residue semi-field trial. The efficacy (% Abbott) was determined for the egg deposition after 48 h and the hatching of larvae, pupae and adults 14 d after treatment. Laboratory data are corresponding to field data at t0.

R a n d o m i z e d   f i e l d   t r i a l : A comparison of the efficacy of selected insecticides under practical field conditions was only possible 4 weeks after harvest due to the low infestation level in the vineyard before. Tab. 2 shows that only a small portion of berries was infested at final evaluation and there was no difference between untreated and treated plots.

The absolute number of eggs and pre-imaginal stages at final evaluation is depicted in Fig. 5. Corresponding with the initial percentage infestation by *D. suzukii* the number of eggs or further developmental stages is low and the statistical analysis revealed no significant differences between untreated control berries and any of the treatments.

Table 2

Natural infestation level of berries from a 'Dornfelder' experimental field trial collected from differently treated plots 4 weeks after harvest in 2016

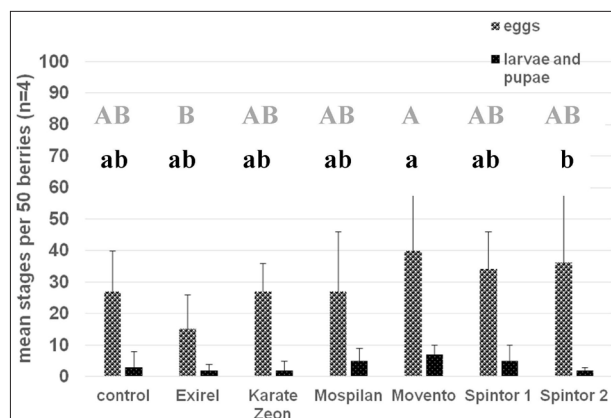| treatment | number of infested berries | % infested berries |
|---|---|---|
| control | 15 | 31 |
| Exirel | 18 | 18 |
| Karate Zeon | 13 | 26 |
| Mospilan SG | 12 | 24 |
| Movento | 17 | 35 |
| SpinTor 1 | 15 | 30 |
| SpinTor 2 | 16 | 33 |



Fig. 5: Residual activity of registered insecticides on the infestation with SWD under field scale conditions. A total of 50 berries per treatment was collected 4 weeks after harvest and evaluated for egg deposition or pre-imaginal stages of *D. suzukii*. Values represent the mean number of larvae and pupae + SD (n = 4). Treatment effects on egg deposition and developmental stages were analyzed using Welch's one-way ANOVA followed by a Duncan post-hoc test ($P = 0.05$) for means separation. Upper-case letters indicate statistical differences for eggs; lowercase letters are for larvae and pupae.

## Discussion

The presented study focused on the establishment of a laboratory screening system for insecticide efficacy against SWD and its validation in viticulture. Although it was confirmed that grape berries are a poor host for *D. suzukii* (LEE *et al.* 2011, BELLAMY *et al.* 2013, KIM *et al.* 2015) we exclusively used various grape berries as substrate for oviposition and larval development in our bioassays. Given the generally low infestation in vineyards, a meaningful testing of chemical products with different types of activity is difficult. Laboratory bioassays are becoming an important tool for identifying factors that determine susceptibility of *D. suzukii* to certain substances and assessing the specific efficacy of test products. We designed a modified laboratory test system following procedures of previous studies (BEERS *et al.* 2011, BRUCK *et al.* 2011, VAN TIMMEREN and ISAACS 2013, CUTHBERTSON *et al.* 2014) adjusted to our particular investigations on grape berries. As confirmed by other teams, females are not able to utilize intact berries as a sufficient nutrient source (PAVLOVA *et al.* 2017) and *D. suzukii* adults prefer to feed on damaged grapes (IORIATTI *et al.* 2015). Therefore, all our tests were performed with injured berries to facilitate egg deposition and to provide a nutrient source supplemented with an additional liquid source during the test duration. For both set-ups used in the laboratory screening tests - infestation after treatment or before treatment - the products SpinTor or Karate Zeon were the most effective products with mortality rates of adult *D. suzukii* between 96 and 100 % 48 h dpi. The good contact activity of spinosyns has already been repeatedly described (BEERS *et al.* 2011, BRUCK *et al.* 2011, CUTHBERTSON *et al.* 2014, ANDREAZZA *et al.* 2017, PAVLOVA *et al.* 2017). All authors confirm the quick knockdown effect of spinosyns toward adult *D. suzukii* in laboratory bioassays

but also in semi-field tests that employed fruits with field-aged residues in laboratory bioassays (van Timmeren and Isaacs 2013). Although lambda-cyhalothrin (Karate Zeon) is registered in many countries for use in horticulture and viticulture, little information is published on the efficacy of this pyrethroid in controlling *D. suzukii*. Karate Zeon appeared highly effective and performed equally well as SpinTor in our laboratory tests. Beers *et al.* (2011) found that lambda-cyhalothrin provided good control of adult *D. suzukii* when the flies were exposed to either treated strawberry leaves or fruits. In our laboratory screening tests, SpinTor and Karate Zeon did not only show a quick contact mortality but also acted as oviposition deterrent and exhibited a negative effect on larval development. This ovicidal and larvicidal effect corroborates data from other teams (Beers *et al.* 2011, Andreazza *et al.* 2017). The latter authors also reported a good larvicidal activity of cyantraniliprole (Exirel) during dipping bioassays using strawberry fruits previously infested with larvae of *D. suzukii*. This is in agreement with results from our laboratory tests where Exirel treatment considerably affected egg deposition and the development of immature stages relative to the control and other materials tested. As all diamids, Cyantraniliprole shows a translaminar distribution within leaf tissues and exhibits neurotoxic activity. Therefore, its unsatisfactory contact mortality is likely balanced by a long-lasting ovicidal or larvicidal effect.

A special issue for discussion remains the performance of the products Mospilan SG and Coragen. In our laboratory screening tests the active ingredients acetamiprid and chlorantraniliprole, respectively, showed an intermediate performance: None of these substances exhibited good contact efficacy and oviposition in Coragen-treated berries was as high as in the water control. Nevertheless, both materials were effective in controlling larval development with Coragen performing better when applied before infestation and Mospilan SG when applied after infestation. A similar result for Coragen-pretreated blueberries was reported by Cuthbertson *et al.* (2014) but they also noticed a high adult mortality after 48 h. The modes of action of acetamiprid and chlorantraniliprole, respectively, are different: While the neonicotinoid Mospilan SG is systemic, Coragen has a translaminar dispersal within the plant tissue. As both ingredients act neurotoxically it is likely that they kill developing larvae in the fruit. An important potential of Mospilan SG for controlling larval development and adult emergence was reported only recently by Pavlova *et al.* (2017). During similar laboratory trials they found that the neonicotinoid Mospilan SG caused significant mortality of adult females but also reduced numbers of developing larvae in grape berries and emerging adults from an apple-nutrition-medium. Wise *et al.* (2015) described that 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*. However, within a winegrape field trial Bruck *et al.* (2011) figured out that acetamiprid provided a level of control significantly greater than the untreated control only on day 3. This aspect addresses a problem when efficacy tests of insecticides are

only assessed in the laboratory system under controlled conditions, because a couple of outdoor factors might influence the performance of a product.

We analysed the activity of the registered insecticides SpinTor, Karate Zeon and Mospilan SG as well in a semi-field bioassay as in a randomized complete block design and compared field data with the results of our laboratory screening tests. While Karate Zeon and SpinTor exhibited nearly 100 % efficacy in complete laboratory application, their residual contact activity decreased substantially with time in laboratory bioassays on field-aged residues. Only Karate Zeon still exhibited some contact effect at the day of outdoor treatment. Grassi *et al.* (2012) reported that lambda-cyhalothrin provided an adequate level of control, but at high population densities repeated applications by alternating pyrethroids and spinosad in strawberry plantations only reduced the damage immediately after the treatment and had negligible impact at the end of the harvest time. A better accordance between laboratory and semi-field application of the respective products was achieved regarding the ovicidal and larvicidal effects. While Karate Zeon and SpinTor treatment mainly affected egg deposition and decreased continuously with time after application the residual activity of Mospilan SG rather targeted further pupation and eclosion and amazingly increased with time. This confirms the long-term potential of acetamiprid identified during the laboratory screening tests and indicates the systemic properties of neonicotinoids. Our results are corroborated by data from previous semi-field investigations. Van Timmeren and Isaacs (2013) reported that acetamiprid continued to cause high larval control in fruits of highbush blueberries even with corresponding low adult mortality and they determined an activity of acetamiprid of up to five days. Beers *et al.* (2011) concluded that the poor adult mortality of acetamiprid and imidacloprid in fruit assays with sweet cherries allowed higher levels of oviposition to occur, but with about the same numbers surviving to the adult stage as the other materials tested. Despite their unsatisfactory activity toward adults, Bruck *et al.* (2011) conceded systemic properties and potentially beneficial long-term sublethal effects of neonicotinoids. Wise *et al.* (2007) showed that neonicotinoids possess compounds that penetrate into the plant tissue and can thus provide opportunity for post-infestation control of the eggs and the larvae. Similarly Hoffmann *et al.* (2009) determined diminishing effects of curative sprays with some neonicotinoid compounds continuing to kill large larvae up to 14 d post-infestation. And Wise *et al.* (2015) concluded that the curative activity by neonicotinoids may be an important mechanism for achieving overall control of larval infestation of berries. However, the last proof for meaningful control properties of an insecticide must be provided within practical field applications.

Consequently, as a last step we tested the performance of currently registered products and further test products within a comprehensive field experiment as complete randomized block design. Owing the low infestation pressure in vineyards in both experimental years, 2015 and 2016, a reliable evaluation of the efficacy of the tested insecticides

was not possible. Since the number of eggs and developmental stages in the untreated control was not significantly different or even lower as in treated plots, no residual activity could be assessed. This points out the limit to transferring laboratory data to the field. Especially in viticulture we are facing the situation that natural susceptibility of *D. suzukii* is highly variable while infestation pressure is quite similar and so far the impact in viticultural areas was generally low apart from the exceptional year 2014 in Germany. However, wine growers are also demanding preventive control measures enabling them to react in years of risk. Strategies to protect fruits from infestation by this insect are currently dominated by insecticide application, so producers need information on relative efficacy and residual activity of insecticides to be able to select effective treatments (van Timmeren and Isaacs 2013). Data from laboratory, field-laboratory and field experiments became generally more variable as they approached conditions of typical commercial settings, and the findings under controlled conditions must be tested on a broader scale before firmer conclusions can be drawn (Beers *et al.* 2011). Although Bruck *et al.* (2011) found a good conformity between results from laboratory bioassays that way that products that performed well in the laboratory also performed well in the field they concluded that screening of new insecticides in the laboratory is a worthwhile exercise. Comparing our laboratory data with results from semi-field trials we can corroborate the same trend of performance for selected products albeit the absolute range for efficacy is different. Pavlova *et al.* (2017) stated that when assessing the effects of an insecticide against *D. suzukii* both in the laboratory and under field conditions, the cryptic nature on 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 larval hatching from eggs. For identifying the specific type of activity of an ingredient laboratory bioassays seem to be an appropriate tool while semi-field and practical field trials better display outdoor conditions. Hence, laboratory bioassays with field-aged residues represent a good compromise for testing insecticide efficacy. However, it will also be essential to determine how results from semi-field assays with a high degree of coverage of treated fruit translate into real-world settings where coverage may not be as complete and surviving flies can choose where they fly (van Timmeren and Isaacs 2013). Indeed, despite professional application procedures satisfactory coverage of fruits when applied in the field is a crucial factor for comparability of laboratory and field data. It is easy to imagine that not all berries in a grape cluster will receive the same amount of a product when applied in the field compared to separated berries used in laboratory screening tests. This could be an explanation for the insufficient efficacy of our test products obtained for the semi-field approach. A good option is to simulating the treatment of compact clusters in the laboratory using a pesticide spraying apparatus as described by Pavlova *et al.* (2017). However, other abiotic or biotic outdoor factors might influence the performance of insecticides in the field such as rainfall, UV sensitivity, temperature or microbial degradation of active ingredients. A symbiotic degradation of pesticides is not novel and Smirle *et al.* (2017) suggested that microbial complexes on the fruit could act to metabolise toxins. Furthermore, considering the high fecundity, the short generation time and the permanent migration into the vineyard it is evident that an efficiency of a product less than estimated 95 % is not sufficient to manage this pest. Albeit our investigations enabled us to identify the stage-specific type of activity of a product it still remains a big challenge to evaluate the conditions under which its application is ecologically and economically sustainable. The fast generation turnover requires many chemical interventions at ripening stage, which can increase the risk of residues in fruits, promote insect resistance and negatively affect beneficial species (Cini *et al.* 2012). Therefore, application of insecticides represents only one option for controlling this insect. An intelligent and environmentally friendly management program suitable for application in integrated as well as organic viticulture should also include cultural management practices as well as other innovative control measures.

## Acknowledgements

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