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Comparison of attractants, insecticides, and mass trapping for managing *Drosophila suzukii* (Diptera: Drosophilidae) in blueberries

Janine M. Spies^{1,*}, and Oscar E. Liburd¹

Abstract

The spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is a serious economic threat to the small fruit industry. Although there has been progress on identifying new insecticides for use against *D. suzukii* in berry crops, growers often reach the seasonal maximum use allowed for key insecticides, and there are issues with long pre-harvest intervals. The use of border sprays and mass trapping targets *D. suzukii* immigration into the field, reducing damage to fruits, and the amount of pesticides used. The purpose of this study was to investigate novel alternatives to conventional insecticide techniques for management of *D. suzukii* in blueberries. In laboratory bioassays, captures of adult *D. suzukii* were similar for yeast + sugar bait, wine + apple cider vinegar bait, and the commercially available RIGA® bait. In the field, more adult *D. suzukii* were collected in yeast bait traps placed in the control and alternative row spray treatments over the sampling period, compared with mass trapping and border spray treatments. In addition, more *D. suzukii* were reared from blueberries collected in the control treatment compared with berries collected in the border spray treatment. Our study provided evidence that border sprays and mass trapping could be an effective and sustainable alternative to conventional spraying techniques for controlling *D. suzukii* in blueberries. Also, we recommend spacing traps approximately 2 m apart to effectively manage *D. suzukii* immigration into blueberry fields.

Key Words: spotted wing drosophila, berry crops, border sprays, baits, mass trapping

Resumen

La drosophila de ala manchada, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), es una amenaza económica seria para la industria de frutas pequeñas. Mientras que se ha avanzado en identificar nuevos insecticidas para el uso contra *D. suzukii* en cultivos de bayas, los cultivadores a menudo alcanzan el uso máximo estacional permitido para los insecticidas clave y existen problemas con los largos intervalos de precosecha. El uso de aerosoles en los bordes del campo y la captura masiva de *D. suzukii* estan enfocados en la inmigración de la plaga al campo, reduciendo el daño a las frutas y la cantidad de pesticidas utilizados. El propósito de este estudio fue investigar alternativas novedosas a las técnicas de insecticidas convencionales para el manejo de *D. suzukii* en arándanos. En los bioensayos de laboratorio, la captura de adultos de *D. suzukii* fue similar para levadura + cebo de azúcar, vino + vinagre de sidra de manzana, y el cebo RIGA® disponible comercialmente. En el campo, se recolectaron más adultos de *D. suzukii* en tratamientos de la captura masiva y el rociado de borde. Además, se criaron más *D. suzukii* de arándanos recolectados en el tratamiento de control en comparación con las bayas recolectadas en el tratamiento de rociado en el borde. Nuestro estudio proporcionó pruebas de que las fumigaciones en los bordes del campo y la captura masiva podrían ser una alternativa eficaz y sostenible a las técnicas de pulverización convencionales para controlar *D. suzukii* en arándanos. Además, recomendamos separar las trampas de captura a una distancia de aproximadamente 2 m para manejar en una manera efectiva la inmigración de *D. suzukii* en los campos de arándanos.

Palabras Clave: drosophila de ala manchada, cultivos de bayas, rociadores de borde, cebos, atrapamiento masivo.

The spotted wing drosophila (SWD), *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is an invasive pest of fruit crops, and a serious economic threat to the US blueberry industry and other small fruit crops. Unlike most drosophilid flies that need overripe fruit on which to feed and oviposit, *D. suzukii* can feed and infest ripening fruit by means of a serrated ovipositor (Mitsui et al. 2006; Calabria et al. 2010). Fly larvae develop in the fruit causing it to become soft and rotten. Fruit infestation by *D. suzukii* larvae results in reduced crop yields and significant financial losses to farmers (Walsh et al. 2011).

There is zero tolerance for larval *D. suzukii* infestation in berries that are destined to local or export markets (Burrack et al. 2015). Therefore,

fruit producers rely on weekly applications of organophosphate, pyrethroid, and spinosyn insecticides to manage this pest (Beers et al. 2011; Lee et al. 2011). Insecticide applications are initiated when *D. suzukii* adults are detected in the field and fruit begin to ripen. Applications can continue, often weekly, through the end of harvest (Van Timmeren & Isaacs 2013; Diepenbrock et al. 2016). Early detection of *D. suzukii* on farms is essential for implementing effective management measures in a timely manner to prevent larval infestation and damage of fruit.

Fruit growers have taken a proactive approach to control *D. suzukii* by protecting their crops through an extensive monitoring program. Adult *D. suzukii* populations are monitored by traps made from plastic

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containers with small holes that use fermentation products such as apple cider vinegar, yeast, or wine as baits (Kanzawa 1935; Beers et al. 2011; Landolt et al. 2011, 2012; Lee et al. 2011; Wang et al. 2016). In the past, apple cider vinegar has been used commonly because it is easily available, affordable, simple to apply in the field, and transparent enough for ease of fly identification in the bait liquid (Walsh et al. 2011; Lee et al. 2012). A mixture of sugar water with baker's yeast has been reported to be highly attractive to D. suzukii (Walsh et al. 2011; Iglesias et al. 2014). Indeed, yeast-baited monitoring traps are deployed regularly around blueberry plantings to detect adult D. suzukii to determine if and when crop protectants need to be applied in the field. Adding wine to apple cider vinegar increases the attractiveness of the bait to D. suzukii, resulting in captures comparable to the yeast + sugar baited traps (Landolt et al. 2011, 2012; Iglesias et al. 2014). Synthetic lures also are available for D. suzukii monitoring in fruit production, including Scentry® lures (Scentry Biologicals Inc., Billings, Montana, USA) and Pherocon® SWD Dual-Lure (Trécé Inc., Adair, Oklahoma, USA). Both products have been reported to be as attractive to adult D. suzukii as wine and apple cider vinegar (Cha et al. 2013; Briem et al. 2015).

Once adult *D. suzukii* are detected in the field, growers rely on frequent insecticide applications to suppress populations. Whereas there has been progress in identifying new insecticides for use against this pest in berry crops, growers in some situations reach the seasonal maximum use allowed for key insecticides. The problem is further complicated because growers cannot use many insecticide products due to long pre-harvest intervals and issues related to maximum residue levels. Current tactics involving a schedule-based insecticide program can have negative effects on non-target organisms (Roubos et al. 2014), including some of the important naturally occurring biological control agents helping to regulate *D. suzukii* populations. Furthermore, overdependence on chemical control to manage adult *D. suzukii* may result in secondary pest outbreaks and resistance development that ultimately increases production costs for growers (Van Timmeren & Isaacs 2013; Renkema et al. 2014; Roubos et al. 2014).

There is an urgent need to develop sustainable pest management strategies that reduce reliance on chemical inputs and are compatible with biological control efforts. Alternative strategies that eliminate area-wide broadcast of insecticides to entire fields in order to suppress *D. suzukii* damage in berry crops include alternate row sprays, border sprays, and mass trapping. These application methods may reduce pesticide residues on the crop, the amount applied to a field, and fruit knock-down due to pesticide application equipment (Chouinard et al. 1992; Prokopy et al. 2003; Klick et al. 2016a). The application of a border spray to the perimeter of a field targets pest immigration into the field from surrounding environments (Chouinard et al. 1992; Trimble & Solymar 1997; Blaauw et al. 2015). Border sprays could be a potentially effective strategy to manage adult *D. suzukii*, which has been shown to use wild hosts in wooded areas surrounding fruit crops (Lee et al. 2015; Briem et al. 2016; Klick et al. 2016a; Iglesias & Liburd 2017).

Mass trapping is another approach that targets pest immigration into a field from surrounding environments, and has the potential for management of this pest (Kanzawa 1935; Baroffio et al. 2017). This method consists of providing a dense barrier of traps baited with an attractant that is deployed along the edges of a field to intercept pest immigration. Mass trapping can be a successful approach if sufficient number of attractive traps are deployed area-wide, and it is cost effective. A recommendation of 60 to 100 traps per acre deployed around the perimeter of the crop and placed no more than 5 m apart has been suggested to reduce populations of *D. suzukii* in fruit crops (Lee et al. 2011; Quarles 2015). Although baits currently in use are moderately successful in luring adults to traps, improvements to the bait + trap system is needed to increase trap sensitivity and trap efficacy against these pests in fruit crops.

The purpose of this study was to investigate novel alternatives to conventional insecticide techniques for management of *D. suzukii* in blueberries. The specific objectives were to (1) evaluate the efficacy of the RIGA® AG commercial attractant for use in *D. suzukii* mass trapping, and (2) compare several field management strategies, including a border spray, a grower standard program of insecticides, and the RIGA® AG baiting system to control *D. suzukii* in blueberries.

Materials and Methods

INSECT COLONY

Adults of *D. suzukii* used in the laboratory bioassay were obtained from a colony maintained in the Department of Entomology and Nematology, University of Florida, Gainesville, Florida, USA. The laboratory colony was established from field populations of *D. suzukii* previously collected from blueberry (*Vaccinium* spp.) (Ericaceae) and blackberry (*Rubus* spp.) (Rosaceae). Emerged adults were collected and maintained for all future generations in 237 mL (8 oz) round bottom polypropylene Fisherbrand[™] Drosophila bottles (Thermo Fisher Scientific, Waltham, Massachusetts, USA) filled with approximately 10 g of Carolina[™] Formula 4-24[™] *Drosophila* medium (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 25 ml of deionized water, and 8 to 10 granules of Fleischmann's[®] Active Dry yeast (ACH Food Companies Inc., Memphis, Tennessee, USA). The colony was maintained under a 14:10 h (L:D) photoperiod at 23 °C and 65% relative humidity.

BAITS TO ATTRACT DROSOPHILA SUZUKII

The effectiveness of baits to attract D. suzukii for potential use in mass trapping was conducted. The laboratory bioassays were conducted in the Department of Entomology and Nematology, University of Florida, Gainesville, Florida, USA, using a 0.75 m³ wind chamber with a constant air velocity (< 1.6 kph) flowing from the top to the bottom of the chamber (Fig. 1). Four different baits were placed at opposing corners of the chamber in traps constructed using 1-L clear plastic deli cups with lids (Solo Cup Company, Lake Forest, Illinois, USA) and fifty 4-mm holes around the center of the cup (Iglesias et al. 2014). Bait treatments consisted of 200 mL: (1) commercial bait (RIGA® AG, Switzerland) of a blend of cider vinegar, red wine, and sugars; (2) yeast + sugar bait (4.3 g Baker's yeast, 11.6 g sugar, and 190 mL deionized water); (3) blueberry wine (Island Grove Wine Company, Hawthorne, Florida, USA) + apple cider vinegar bait (3:1); and (4) deionized water (control). Eight 30 mL (1 oz) Solo® polystyrene soufflé cups (Solo Cup Company, Lake Forest, Illinois, USA) with a cotton wick inserted through the lid contained deionized water and served as a water source. One hundred D. suzukii adults (50 female, 50 male, 4-10 d old) were released from a central point (equidistant to each trap) within the chamber. The number of adults found in each trap was recorded after 24 h, and flies were sexed using a 10× dissecting microscope. Baits were rotated for each trial to avoid positional bias, and the experiment was replicated 8 times.

FIELD MANAGEMENT STRATEGIES TO CONTROL *DROSOPHILA* SUZUKII IN BLUEBERRIES

Studies were conducted 16 Apr to 5 Jun 2015 on a conventional southern highbush blueberry farm in Hawthorne, Florida, USA (29.485944°N, 82.062083°W). *Vaccinium corymbosum* L. × *Vaccinium darrowi* Camp (both Ericaceae) highbush blueberry bushes were 4 to



Fig. 1. The laboratory assay conducted in a wind chamber testing the effectiveness of baits to attract Drosophila suzukii.

5 yr old and approximately 1.2 m tall. Standard blueberry production practices with regard to watering regime, fertilizers, fungicide applications, and pruning were followed (Childers & Lyrene 2006). The experiment followed a block design in which 3 treatments were compared with an untreated control. Treatments were applied to 4 different blocks within a 1.2 ha section of the blueberry farm and replicated 4 times. Each treatment was separated by a 6 m buffer zone (untreated blueberries). The first 2 treatments utilized a weekly rotation of 3 insecticides: Delegate® WG (25% spinetoram) (Dow AgroSciences, Indianapolis, Indiana, USA) at 420 g per ha; Exirel[®] (10.2% cyantraniliprole) (Dupont, Newark, Delaware, USA) at 1.5 L per ha; and Malathion® 8 Flowable (79.5% malathion) (Gowan Co., Yuma, Arizona, USA) at 2.92 L per ha, in that order. Insecticides were applied at the manufacturer's maximum labelled rate with an air-blast sprayer (model Storm 828, Leinbachs Inc., Rural Hall, North Carolina, USA) with a total of 2 rotations as either (1) a border spray applied to a 6 m wide perimeter section of the blueberry field, or (2) a conventional method of spraying every other row (alternate rows) of the blueberry field. The third treatment employed mass trapping using a commercially available readyto-use attractant trap from RIGA® AG (Zurich, Switzerland). The RIGA® AG trap consisted of a small plastic cup (5.5 cm in height, 7 cm in diam) filled with 80 mL of RIGA® AG bait (cider vinegar, red wine, sugar, and berry juice) (Fig. 2) (Kehrli et al. 2013). Entrance holes were pierced in the aluminum lid, and traps were rain-protected with a white plastic roof. Bait was replenished as needed, and traps replaced after 3 wk of sampling. Traps were placed 5 m apart along the perimeter of the field.

Adult *D. suzukii* monitoring traps were constructed as described above for the wind chamber bioassay (Iglesias et al. 2014). These were baited with a 200 mL mixture of Fleischmann's® Active Dry yeast (4.2 g), 11 g white granulated sugar (Publix, Lakeland, Florida, USA), approximately 200 mL deionized water, and 0.3 mL odorless dish detergent (Palmolive Pure and Clear, Colgate-Palmolive Company, New York, New York, USA). Four baited traps were placed at least 20 m apart in each treatment. One trap was placed in each replicate plot by securing them 1 m from the ground inside the blueberry bush. Traps were serviced weekly for 6 wk, and consisted of pouring all liquid bait into a labeled collection container in the field and refilling the trap with 200 mL of fresh bait. Forty blueberries were collected weekly from each treatment (10 per replicate) in the center of plots to monitor the rate



Fig. 2. Commercially available trap from RIGA® AG used in mass trapping.

of infestation within the field. Collected berries were placed in Choice[®] 60 mL (2 oz) polystyrene soufflé cups (Solo Cup Company, Lake Forest, Illinois, USA), covered with lids with breathing holes. Two wk later, emerged male and female adult *D. suzukii* were identified using a 10× dissecting microscope, and counted for each sample collected.

STATISTICAL ANALYSIS

Bioassay data were analyzed using a 1-way analysis of variance (ANOVA) procedure to test the difference in adult *D. suzukii* numbers across treatments. Treatment means were separated by least significant differences (LSD) test. Data from the field experiment were analyzed using the repeated measures ANOVA procedure to investigate insect population density over time. Sample date was the repeated measure and treatment means separated by LSD test (PROC GLM) (SAS 2009). Insect counts were log transformed when necessary to meet the assumptions of normality. For all tests, statistical significance was determined as $P \le 0.05$. Reported means were from non-transformed data.

Results

BAITS TO ATTRACT DROSOPHILA SUZUKII

The average response rate of *D. suzukii* adults to baits in the wind chamber bioassay regardless of treatment was 71 ± 1.3%. Generally, there were significantly more adult *D. suzukii* captured in treatment baits compared with those in traps with deionized water (Fig. 3; $F_{3,28}$ = 12.98; $P \le 0.0001$). Specifically, more *D. suzukii* were captured in the wine + apple cider vinegar and RIGA® baits compared with the yeast + sugar bait; however, there was no significant difference between baits (Fig. 3). Similar to the combined male and female *D. suzukii* counts, more female *D. suzukii* were captured in treatment baits compared with deionized water ($F_{3,28} = 14.38$; $P \le 0.0001$), but there was no significant difference between baits (Fig. 4). However, there were significantly more male *D. suzukii* captured in the RIGA® bait compared with yeast + sugar bait, but captures in the wine + apple cider vinegar bait were not significantly different from that in the RIGA® bait or yeast + sugar (Fig. 4; $F_{3,28} = 9.24$; P = 0.0002).

FIELD MANAGEMENT STRATEGIES TO CONTROL *DROSOPHILA SUZUKII* IN BLUEBERRIES

Mean abundance of adult *D. suzukii* captured in yeast + sugar bait traps was low across treatments over the 6 wk sampling period (Fig. 5).

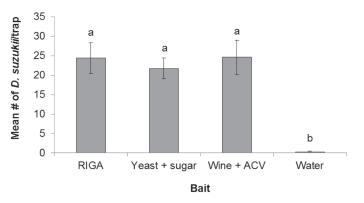


Fig. 3. Mean (\pm SE) number of adult *Drosophila suzukii* captured in baited traps suspended in a wind chamber. Treatments with the same letter are not significantly different (P > 0.05).

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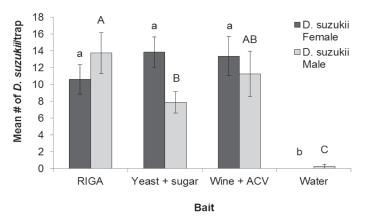


Fig. 4. Mean (\pm SE) number of female and male *Drosophila suzukii* captured in baited traps suspended in a wind chamber. Treatments with the same letter case (lower and upper letters are for female and male, respectively) are not significantly different (P > 0.05).

Adult *D. suzukii* counts were significantly different between treatments ($F_{3,72} = 3.24$; P = 0.0270), and over time ($F_{5,72} = 6.83$; $P \le 0.0001$), but there was no treatment × time interaction ($F_{15,72} = 1.19$; P = 0.3027). Treatment differences were observed in the third and fourth wk of sampling that corresponded with applications of Exirel and malathion, respectively. Fewer adult *D. suzukii* were collected in yeast + sugar bait traps placed in the mass trap and border spray treatments compared with alternative row spray treatments and control plots (Fig. 5). Within the mass trap treatment, the total number of 27 adult *D. suzukii* captured in the RIGA® bait traps placed around the perimeter was greater than the total number of 8 adult *D. suzukii* collected from the yeast + sugar bait traps within the field.

The majority of adult *D. suzukii* collected from yeast + sugar bait traps were females. Female *D. suzukii* counts were significantly different between treatments ($F_{3,72} = 3.49$; P = 0.0199) and over time ($F_{5,72} = 5.42$; P = 0.0003), with differences observed in the third and fourth wk of sampling. There was no treatment × time interaction ($F_{15,72} = 1.36$; P = 0.1920). Significantly fewer females were captured in yeast + sugar bait traps placed in the mass trap treatment compared with alternative row sprays and control plots (Fig. 6). There were also significantly fewer females in yeast + sugar bait traps placed in the border spray treatment

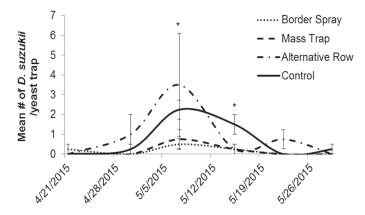


Fig. 5. Mean (± SE) number of adult *Drosophila suzukii* captured in yeast + sugar traps placed in a blueberry field in Hawthorne, Florida, USA, blocked into 4 separate treatments: border spray, mass trapping, alternative row spray, and an untreated control. Populations monitored weekly during a 6-wk period; asterisks indicate those treatments that were significantly different ($P \le 0.05$) during a sample period.

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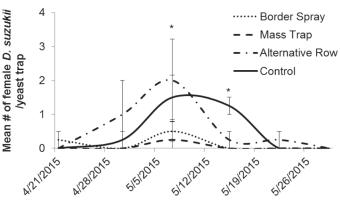


Fig. 6. Mean (± SE) number of female *Drosophila suzukii* captured in yeast + sugar traps placed in a blueberry field in Hawthorne, Florida, USA, blocked into 4 separate treatments: border spray, mass trapping, alternative row spray, and an untreated control. Populations were monitored weekly during a 6-wk period; asterisks indicate those treatments that were significantly different ($P \le 0.05$) during a sample period.

compared with control plots. However, female *D. suzukii* counts in the border spray treatment were not significantly different from the mass trap treatment and alternative row spray treatment plots (Fig. 6). Male abundance was not significantly different between treatments (Fig. 7; $F_{3.72} = 0.61$; P = 0.6092).

Mean number of *D. suzukii* reared from the infested blueberries collected from the field was low (Fig. 8) and did not differ between control strategies ($F_{3,72} = 1.99$; P = 0.1231), over time ($F_{5,72} = 0.95$; P = 0.4541), and there was no treatment × time interaction ($F_{15,72} = 0.85$; P = 0.6178). However, there were more *D. suzukii* adults reared from blueberries collected in the control treatment, followed by fewer reared from berries in the alternative row spray treatment and mass trap treatment. No *D. suzukii* emerged from berries collected in the border spray (Fig. 8).

Discussion

The numbers of adult *D. suzukii* captured in the wind chamber bioassays were similar across bait treatments. These findings suggest that the attraction of this species to fruit-based baits, such as those used in

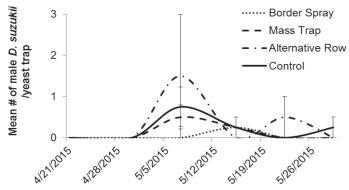


Fig. 7. Mean (\pm SE) number of male *Drosophila suzukii* captured in yeast + sugar traps placed in a blueberry field in Hawthorne, Florida, USA, blocked into 4 separate treatments: border spray, mass trapping, alternative row spray, and an untreated control. Populations were monitored weekly during a 6-wk period. Treatments were not significantly different (P > 0.05).

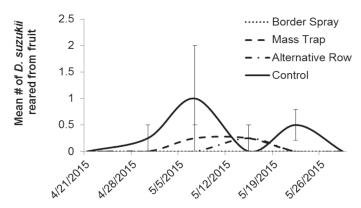


Fig. 8. Mean (± SE) number of *Drosophila suzukii* reared from blueberries collected from a field in Hawthorne, Florida, USA, blocked into 4 separate treatments: border spray, mass trapping, alternative row spray, and an untreated control. Fruit was collected weekly for 6 wk. Treatments were not significantly different (P > 0.05).

our study, was comparable to that of yeast + sugar bait commonly used to monitor *D. suzukii* in the field. Briem et al. (2015) demonstrated that RIGA® AG was highly attractive, with captures of *D. suzukii* comparable to bait mixtures of red wine and apple cider vinegar. Early studies in Japan on *D. suzukii* behavior demonstrated a strong attraction to red grape wine, as well as rice wine and cherry wine (Kanzawa 1935). It has been suggested that adult *D. suzukii* immigrating into farms during the early stages of fruit maturation may use fruit volatiles during host location (Revadi et al. 2012; Abraham et al. 2015). Baits containing fruit and fermentation products have demonstrated varying degrees of success at attracting *D. suzukii* adults (Landolt et al. 2011, 2012; Walsh et al. 2011; Iglesias et al. 2014).

Findings from our field study demonstrating that fewer adult *D. suzukii* were caught in the yeast + sugar bait traps placed in the mass trap and border spray treatments compared with alternative row spray treatments and no treatments (control) was unexpected. However, an understanding of adult *D. suzukii* biology and movement may explain why alternative row sprays may not be an effective tactic to reduce pest migration into a field. *Drosophila suzukii* has been shown to use wild hosts in wooded areas surrounding commercial fruit crops, and potentially could be migrating into fields from field margins as fruit begins to develop (Lee et al. 2015; Liburd et al. 2015; Briem et al. 2016; Klick et al. 2016a; Iglesias & Liburd 2017). Tactics including border sprays and mass trapping, that implement a complete barrier surrounding the field, may be more effective at preventing *D. suzukii* migration into the field.

Border sprays and mass trapping may be more effective strategies when implemented early in the season when pest pressure is relatively low. The benefit of implementing these strategies early in the season would be to prevent the buildup of *D. suzukii* populations, and ultimately reduce the input of insecticides in the field. Reducing the amount of insecticides applied over a growing season can be particularly important in organic production, where the availability of effective insecticides is limited. For instance, the organic grower's standard, Entrust[®] (Spinosad active ingredient), has restrictions on how many times it can be applied throughout the growing season in order to minimize the development of resistance in pest populations.

With regard to the effect of control tactics on berry infestation rates in this study, the average number of *D. suzukii* reared from the blueberries collected from the field was low, and there was a marginal difference between treatments. More *D. suzukii* were reared from blueberries collected in the control treatment, followed by the alternative row

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spray and mass trapping treatments, and no D. suzukii were reared from berries collected in the border spray. These findings suggest that the control tactics implemented had some effect on reducing D. suzukii infestation rates. Even in the alternative row spray treatment, where more adults were captured, there were similarly low rates of berry infestations compared with the border spray and mass trapping treatments. Klick et al. (2016b) hypothesized that within-field movement of D. suzukii can be high, and that flies from untreated rows can move to treated crop rows. This hypothesis could explain why higher adult populations were observed within the field in the alternative row spray treatments, but there were low infestation rates. This discrepancy highlights that the correlation of trapped adult populations with larval infestation rates can be problematic and unreliable (Beers et al. 2011; Hamby et al. 2014). A higher population of D. suzukii would need to be present in order to determine if any significant differences exist between treatments.

In summary, mass trapping and border sprays could be an effective and sustainable alternative to conventional whole planting spraying techniques for preventing adult *D. suzukii* migration into a field. Moreover, the use of the RIGA® AG mass trapping system, with traps placed along the perimeter of the field, could be a sustainable alternative to conventional spraying techniques for controlling *D. suzukii* in blueberries. However, more research needs to be conducted on the recommended spacing between traps. Currently we suggest reducing the spacing to 2 m.

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