

Exclusion Netting Delays and Reduces *Drosophila suzukii* (Diptera: Drosophilidae) Infestation in Raspberries

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Abstract

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a new frugivorous pest of raspberries and other soft fruits in North America, causing infestation of fruit at harvest time. Control of this pest has primarily been through the application of broad-spectrum insecticides to prevent oviposition and larval development, and there is an urgent need for alternative approaches. Over two growing seasons, we compared *D. suzukii* control in a research planting with insecticide and exclusion treatments in a factorial design, monitoring first-, second-, and third-instar *Drosophila* larvae in ripening, ripe, and overripe berries. Each of the two control approaches provided significant reduction of infestation in raspberry fruit, but the combination treatment had the lowest overall abundance of larvae in fruit. This pattern was seen for all larval instars in both years. The combination treatment also delayed the first detected larval infestation by 10 d compared to the untreated plots. Exclusion netting applied to commercial size high tunnels resulted in a significant reduction in overall *D. suzukii* infestation in raspberries, as well as a 3-wk delay in the average first detectable fruit infestation. Raspberry size and quality were not affected by the exclusion treatments, indicating that this approach can be an important component of growers' response to invasion by *D. suzukii* in temperate climates. We discuss the opportunities and limitations for implementing exclusion netting in raspberry production.

Key words: spotted wing *Drosophila*, exclusion netting, integrated pest management, *Rubus idaeus*, high tunnel

Spotted wing *Drosophila*, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), was first detected in the United States in 2008 and has since become an important economic pest of soft fruit production (Bolda et al. 2010, Hauser 2011). *Drosophila suzukii* is equipped with a doubly-serrated ovipositor, allowing it to lay eggs in fresh, undamaged fruit (Lee et al. 2011b). The resulting larvae degrade fruit quality, causing a risk of detectable contamination that can reduce marketability (Goodhue et al. 2011, Walsh et al. 2011). Puncture wounds from oviposition also increase the ability of pathogens to colonize the fruit (Walsh et al. 2011), further reducing marketable yields. Female flies can lay up to 25 eggs per day, depending on host and environmental conditions (Kinjo et al. 2014), making continued and efficacious control important. While *D. suzukii* has a broad host range, the most impacted crops include raspberry, blackberry, blueberry, and cherry (Lee et al. 2011a, Asplen et al. 2015). Raspberry is particularly at risk due to its highly attractive odors and soft epicarp, making oviposition relatively easy for *D. suzukii* (Lee et al. 2011b, Bellamy et al. 2013, Burrack et al. 2015, Abraham et al. 2015). Growers use baited traps to monitor for the presence of *D. suzukii*, with insecticidal protection of crops beginning when the fruit start to ripen and when *D. suzukii* flies have been trapped in the vicinity (Diepenbrock et al. 2016). Since the invasion by *D. suzukii*, insecticide applications in these systems have increased

dramatically (Bruck et al. 2011, Van Timmeren and Isaacs 2013, Diepenbrock et al. 2016). Without repeated treatment of fruit, the high fecundity and short life cycle of *D. suzukii* allow it to rapidly increase in abundance (Wiman et al. 2014). *Drosophila suzukii* is known to use noncrop hosts, often at the borders of crop fields, so immigration into fields is a major source of ovipositing flies during the growing season (Klick et al. 2015, Lee et al. 2015, Pelton et al. 2016). This makes complete control of this pest with insecticides highly challenging and very expensive (Bruck et al. 2011). Few alternative controls exist, limiting the options for organic and sustainable production of these fruit (Bruck et al. 2011).

The invasion of *D. suzukii* into fruit production regions has disrupted previously reliable IPM systems, and long-term restructuring of those programs should include biological, physical, cultural, and chemical control methods (Cini et al. 2012, Asplen et al. 2015, Haye et al. 2016). Physical exclusion has significant potential for use under protected culture such as high tunnels (Lee et al. 2011b). Exclusion netting has shown promise for reducing *D. suzukii* infestation in small-scale plantings of blueberries and raspberries in North America (Link 2014, Cormier et al. 2015, Rogers et al. 2016) and for blueberries in Europe (Kawase et al. 2007, Grassi and Pallaoro 2012). Rogers et al. (2016) found that exclusion netting significantly lowered the number of infested raspberries when

compared to either untreated or insecticide-treated field plots. However, effects on the timing of *D. suzukii* arrival and subsequent population growth in these protected areas will also have important implications for management of this pest. Mini-tunnels covered with netting or plastic reduced infestation by *D. suzukii* (Rogers et al. 2016), but the high levels of control reported in the plastic exclusion treatment was thought to be caused by extreme high temperatures that may not be as likely in a commercial high tunnel. There is limited information on the performance and feasibility of the exclusion approach in commercial production settings, and growers are also interested in combining control approaches to increase the proportion of fruit that meet the marketable standard. To explore the efficacy, feasibility, and limitations of using exclusion combined with insecticides for control of *D. suzukii* in raspberries, we tested these approaches separately and together over two seasons. Fruit were sampled using a method that revealed larval stage, allowing us to compare treatment effects on recent infestations and on larvae most likely to be detected. We also tested the efficacy of exclusion netting for controlling *D. suzukii* adults and larvae in commercial production of high tunnel grown red raspberry and measured its effect on temperature, fruit quality, and the abundance of pest and beneficial arthropods.

Materials and Methods

Exclusion Netting Combined With Insecticides

A trial was conducted in a raspberry planting (cv. 'Caroline') in 2014 and 2015 at the Trevor Nichols Research Center in Fennville, MI. Plots were established in the planting (1.8 by 1.8 m) and were either covered with 32 by 32 Lumite mesh-covered cage frames (BioQuip Products, Rancho Dominguez, CA) or left uncovered. These plots were either sprayed with insecticides or not, creating a factorial design with four replicates of each treatment in a randomized complete block design. Temperature probes (Hobo Pendant Temperature Data Logger, Onset Computer Corporation, Bourne, MA) were placed inside radiation shields (Spectrum Technologies, Inc., Aurora, IL) and attached to a PVC pole (3.8 cm diameter) in the center of each plot to determine the effect of netting on temperature in the plant canopy. In 2014, netting was applied to the fall raspberry crop in late August, and to reduce the starting infestation level all ripening or ripe fruit were removed from the plants immediately prior to the start of the experiment. In 2015, netting was applied to the summer crop in early July as soon as the fruit began to ripen. Insecticides were applied using a CO₂-powered backpack sprayer operating at 3.5 kgf/cm² in a volume of water equivalent to 1,496 liters per hectare and equipped with a single head boom and a TeeJet 8003VS spray nozzle (TeeJet Technologies, Wheaton, IL). Insecticide-treated plots received four applications of insecticide on 7-d intervals rotating between zeta-cypermethrin (Mustang Maxx, 292 ml/Ha), spinetoram (Delegate WG, 438 ml/Ha), malathion (Malathion 8F, 2,338 ml/Ha), and zeta-cypermethrin (Mustang Maxx, 292 ml/Ha). Fruit were collected immediately before the next application was applied, to provide three (2014) and four (2015) samples during the crop ripening period.

At each sample date, five ripening, ripe, and overripe berries were collected from each plot and assessed for presence of immature *Drosophila* using a modified salt test. This consisted of placing the berries in a one gallon resealable plastic bag and lightly crushing the fruit before adding salt water (237 ml of table salt added to 3.78 L of tap water). After 30 min, the fruit and liquid were poured over a coarse screen to remove the berries and then into a reusable coffee

filter (Medelco 4-Cup Universal Coffee Filter, Medelco Incorporated, Bridgeport, CT) and the retained solids were examined under a stereomicroscope (Olympus SZX10 set at 20× magnification [10× eyepiece lens, 0.5× objective lens], Olympus America, Inc., Center Valley, PA) to facilitate accurate counting of *Drosophila* eggs and larvae. Larvae were classified as small, medium, and large, which correlates approximately with the first, second, and third instar stages.

High Tunnel Exclusion Netting

Fly-proof netting was installed on two high tunnels over raspberry plantings at a commercial, conventionally managed farm in Coloma, MI. Netting was also installed on one high tunnel over raspberry plants at an organic research farm in East Lansing, MI. All three netted tunnels were adjacent to a paired control tunnel that was open at the ends, and all six tunnels were covered by UV-blocking Visqueen Luminance plastic coverings (BPI, Stevenson, UK). To exclude *D. suzukii* from the three tunnels, 80 gram Tek-Knit netting (Berry Protection Solutions Stephentown, New York) was applied to the sides of the tunnels by suspending it using 16-gauge galvanized steel wire attached along its length using zip ties along the interior of the curved roof struts, and with shade clips (FarmTek, Dyersville, IA) that held the netting to the wire. To allow movement of pickers to the outside rows of raspberry, the netting was also secured to the sides of the tunnel using 40mm metal clips (Haygrove Tunnels, Mount Joy, PA) and to the ground using landscaping fabric staples. Netting was applied to the outside frame on the two ends of each tunnel, using a different door design to accommodate different needs for access. The research farm site had two 7.6 by 60 m Haygrove tunnels oriented north-south, with each containing organic summer and fall red raspberries (cv. 'Polka', 'Himbo Top', and 'Joan J'). In the netted tunnel, an access point was installed in the south end using a small door frame with a magnetic closure. At the commercial farm, four 7.6- by 122-m Haygrove tunnels were oriented east-west with three rows of summer and fall red raspberries (cv. 'Prelude') in each. Two of these were netted, and both ends of the tunnels were fitted with two 2.4- by 2.1-m barn style wooden doors covered in netting, which allowed for access by a sprayer (Fig. 1). Netting was installed on the tunnels in early June prior to activity of *D. suzukii*. Bumble bee colonies (Koppert Biological Systems, Howell, MI) were introduced for pollination, using three hives per tunnel at the commercial farm and two hives per tunnel at the research farm. Summer berry harvest occurred in late June through July and fall harvest occurred from the middle of August through early October.

Temperature probes (Hobo Pendant Temperature Data Logger, Onset Computer Corporation, Bourne, MA) inside radiation shields were hung in the center of each tunnel, taking readings every hour. Two monitoring traps baited with a yeast and sugar mix (Van Timmeren and Isaacs 2013) were placed 9 m from the end of each tunnel at canopy height and were checked weekly. Traps were made from 32 oz deli cups filled with 150 ml of solution and a yellow sticky insert hung from the top. When ripe fruit were available to harvest, 25 ripe raspberries were sampled every week within 5 m of each yeast-sugar trap and in the center of the tunnel. The weight and diameter of these berries was recorded, and the degrees brix was recorded using a portable refractometer (Model RHB-32ATC, Westover Scientific Inc., Bothell, WA). These fruit were then immersed in a salt solution as described above and the number of *Drosophila* eggs were counted, along with the number of larvae of each stage as described above. At regular intervals during summer and fall harvest, additional ripe fruit were sampled and the flies



Fig. 1. High tunnel raspberry planting with netting added to exclude *D. suzukii*. The barn style wooden doors were installed to allow access for a tractor-pulled sprayer.

were reared to confirm the infestation as *D. suzukii*. All flies emerging from the fruit were identified as *D. suzukii*.

To monitor activity of other arthropods in the tunnels, 14- by 23.5-cm yellow sticky traps (Scentry MultiGuard; Great Lakes IPM, Vestaburg, MI) were suspended above the plant canopy in the middle of each tunnel and replaced weekly. Arthropods captured were identified at least to family and later sorted by functional group. Direct leaf observations were also conducted weekly on 25 randomly selected raspberry leaves in each tunnel. Leaves were evaluated for percent leaf damage and suspected causes of the damage. The numbers and types of arthropods found on the leaves were also recorded.

Statistical Analyses

In the factorial experiment with netting and insecticide treatments, the numbers of first, second, and third instar, and total larvae per gram of fruit were analyzed using a Kruskal–Wallis test followed by a Conover–Inman test for post hoc comparisons. For the high tunnel experiment, fruit quality, temperature, and immature and adult *D. suzukii* data were analyzed using analysis of variance to compare netted and open tunnels, followed by Tukey's Honest Significant Difference for post hoc comparisons. A Student's *t*-test was used to analyze data from the direct leaf observations and yellow sticky traps. Data were analyzed using R (3.2.2., R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

Results

Exclusion Netting Combined With Insecticides

In two growing seasons, there were significantly fewer *Drosophila* larvae present in netted raspberries than in raspberries grown in the open (Table 1). This was most apparent and was statistically significant in the overripe fruit, though the same trend was also found in the ripening and ripe fruit. For all ripeness stages across 2014 and 2015, the open unsprayed plots had the highest number of *D. suzukii* larvae, the plots receiving exclusion netting or insecticide applications were intermediate, and the fewest larvae were found in plots with the combination of insecticide applications and netting. The differences among treatments varied depending on the sampling date, in part because the earlier sampling dates, especially those in early July 2015, had fewer larvae overall (Table 1).

In 2015, netting was installed on summer red raspberry plants before *D. suzukii* activity increased, allowing us to detect the first

infestation in each plot. Average first infestation in berries on the open control plants was July 10 (± 1.0 d), 10 d earlier than the combination netting and insecticide treatment on July 20 (± 1.1 d). The other treatments were intermediate, with average first larval detection for the open insecticide treatment on July 15 (± 1.1 d) and the netted non-insecticide treatment on July 16 (± 1.0 d).

The combination of netting and insecticides resulted in significantly lower abundance of first-, second-, and third-instar *D. suzukii* larvae in berries in 2014 and 2015 compared to the untreated control (Table 2). The insecticide treatment and exclusion treatment alone had intermediate levels of infestation for all instars in both years. While the presence of *Drosophila* larvae was lower in the netted treatments, it never remained at zero. As pest pressure continued to build throughout the 2014 and 2015 growing seasons, we found that netting alone was not sufficient to control *D. suzukii* (Table 1). In contrast, combining netting with insecticide applications resulted in significantly lower infestation of the overripe fruit with *D. suzukii* than with netting alone (Table 1). The trends were similar in ripening and ripe fruit, with lower abundance of larvae in the combined treatment compared with the netting treatment on nine of the ten assessment dates when larvae were detected in the berries.

The average temperature inside the netted cages was very similar to the outside temperature over the course of the experiment in 2014 (Netted: 21.7 ± 0.5 °C, Open: 21.8 ± 0.5 °C) and in 2015 (Netted: 18.0 ± 0.4 °C, Open: 18.1 ± 0.4 °C). The average maximum temperature was slightly higher inside the netted cages over the course of the experiment in 2014 (Netted: 30.9 ± 0.6 °C, Open: 29.7 ± 0.4 °C), though this same trend was not found for 2015 (Netted: 25.9 ± 0.5 °C, Open: 25.4 ± 0.5 °C).

High Tunnel Exclusion Netting

There were significantly fewer *Drosophila* eggs, larvae, and adults in the netted tunnels than the open tunnels at both sites (Fig. 2). Over the entire season, there was an 82% reduction in *Drosophila* eggs ($F=18.5$; $df=1,16$; $P=0.0002$), a 74% reduction in *Drosophila* larvae ($F=4.7$; $df=1,16$; $P=0.02$), and a 65% reduction in *D. suzukii* adults ($F=30.0$; $df=1,10$; $P=0.0003$) in the netted tunnels (Fig. 2). Over the entire season, there were significantly fewer first-instar larvae found in fruit in the netted tunnels (81.4 ± 67.8 larvae/kg) than the open tunnels (361.6 ± 206.6 larvae/kg) ($F=4.8$; $df=1,16$; $P=0.02$). There were also significantly fewer second instars from fruit in the netted tunnels (45.2 ± 45.2 larvae/kg) compared to the open tunnels (162.7 ± 62.6 larvae/kg) ($F=3.6$; $df=1,16$; $P=0.03$). Third-instar larvae from fruit in the netted tunnels (9.0 ± 9.0 larvae/kg) was reduced compared to the open tunnels (22.6 ± 4.5 larvae/kg), but not significantly so ($F=0.8$; $df=1,16$; $P=0.18$). Furthermore, the netted treatments delayed the arrival of *D. suzukii* adults by 23 d (Fig. 3). The average first catch in the open tunnels was on July 8 (± 12.5 d) and the netted tunnels on July 31 (± 18.7 d). Larval infestation was delayed by 24 d, with the average first larval detection in the open tunnels on August 16 (± 17.6 d) and the netted tunnels on September 3 (± 14.1 d). However, none of the netted treatments maintained zero adult *D. suzukii* captures in the traps or zero larval infestation in the raspberries, and later in the season the infestation built up inside the netted tunnels (Fig. 3). Despite this, the overall level of infestation remained lower in the netted tunnels than in the open tunnels.

Netting of the high tunnels affected other arthropods present in this system, though the composition of the arthropods remained similar between the two treatments. The most abundant pests and natural enemies from the combined sampling using yellow sticky

Table 1. Average number of *Drosophila* larvae per gram of raspberries collected from plots receiving insecticide and netting treatments

Ripeness	Insecticide treatment		2014				2015			Overall 2014 & 2015
	Netting treatment	Insecticide treatment	27 Aug.	4 Sept.	10 Sept.	9 July	13 July	17 July	23 July	
Ripening	No netting	No insecticide	0.8 ± 0.4a	1.2 ± 0.2a	0.3 ± 0.1a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.4 ± 0.1a	0.3 ± 0.09a
	No netting	Insecticide	0.09 ± 0.03b	0.7 ± 0.3ab	0.3 ± 0.1a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.07 ± 0.07ab	0.1 ± 0.03ab
	Netting	No insecticide	0.05 ± 0.05c	0.2 ± 0.1bc	0.8 ± 0.5a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.09 ± 0.09ab	0.1 ± 0.08b
	Netting	Insecticide	0.03 ± 0.03d	0.08 ± 0.08c	0.1 ± 0.01a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.03 ± 0.03b	0.03 ± 0.01b
		Statistics (Kruskal-Wallis):	H = 11.3 df = 3,10 P = 0.01	H = 10.2 df = 3,12 P = 0.02	H = 2.6 df = 3,12 P = 0.47	H = n/a df = 3,12 P = n/a	H = n/a df = 3,12, P = n/a	H = n/a df = 3,12 P = n/a	H = 8.3 df = 3,12 P = 0.03	H = 13.7 df = 3,12 P = 0.003
Ripe	No netting	No insecticide	3.3 ± 0.6a	2.85 ± 0.6a	3.7 ± 1.1a	0.0 ± 0.0a	0.01 ± 0.01a	0.03 ± 0.03a	0.1 ± 0.08a	1.2 ± 0.2a
	No netting	Insecticide	0.5 ± 0.2b	1.0 ± 0.2b	1.9 ± 0.4a	0.0 ± 0.0a	0.01 ± 0.01 a	0.02 ± 0.02a	0.2 ± 0.1a	0.5 ± 0.1ab
	Netting	No insecticide	1.0 ± 0.6b	0.6 ± 0.2bc	0.7 ± 0.5a	0.0 ± 0.0a	0.02 ± 0.02a	0.06 ± 0.03a	0.2 ± 0.1a	0.4 ± 0.1ab
	Netting	Insecticide	0.08 ± 0.01c	0.2 ± 0.2c	1.0 ± 0.8a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.01 ± 0.01a	0.1 ± 0.09b
		Statistics (Kruskal-Wallis):	H = 11.8 df = 3,12 P = 0.008	H = 11.6 df = 3,12 P = 0.009	H = 5.9 df = 3,12 P = 0.12	H = n/a df = 3,12 P = n/a	H = 1.1 df = 3,12 P = 0.75	H = 3.1 df = 3,12 P = 0.36	H = 4.3 df = 3,12 P = 0.20	H = 7.7 df = 3,12 P = 0.05
Overripe	No netting	No insecticide	5.8 ± 1.5a	6.9 ± 0.8a	5.2 ± 1.3a	0.1 ± 0.07a	0.2 ± 0.07a	1.2 ± 0.1a	7.6 ± 1.8a	3.7 ± 0.6a
	No netting	Insecticide	1.9 ± 0.4a	2.3 ± 0.5b	1.7 ± 0.3b	0.0 ± 0.0a	0.08 ± 0.04ab	0.6 ± 0.2ab	1.9 ± 1.9ab	1.1 ± 0.1b
	Netting	No insecticide	0.8 ± 0.1b	1.6 ± 0.5b	3.3 ± 1.8ab	0.0 ± 0.0a	0.02 ± 0.02ab	0.4 ± 0.2ab	3.6 ± 1.9a	1.3 ± 0.4b
	Netting	Insecticide	0.2 ± 0.05b	0.3 ± 0.2c	0.6 ± 0.2c	0.0 ± 0.0a	0.0 ± 0.0b	0.09 ± 0.06b	0.02 ± 0.02b	0.1 ± 0.04c
		Statistics (Kruskal-Wallis):	H = 12.1 df = 3,12 P = 0.007	H = 12.3 df = 3,12 P = 0.006	H = 9.1 df = 3,12 P = 0.03	H = 5.8 df = 3,12 P = 0.11	H = 7.7 df = 3,12 P = 0.05	H = 8.2 df = 3,12 P = 0.04	H = 10.8 df = 3,12 P = 0.01	H = 29.4 df = 3,12 P < 0.0001

Five ripening, ripe, and overripe berries were collected from each plot weekly and assessed using a modified salt test. Averages with the same letter within each column are not significantly different at $\alpha = 0.05$.

Table 2. Average number of first-, second-, and third-instar *Drosophila* larvae per gram of raspberries in fruit of all ripening stages collected from plots receiving insecticide and netting treatments

Year	Netting treatment	Insecticide treatment	First instar	Second instar	Third instar
2014	No netting	No insecticide	1.8 ± 0.2a	1.1 ± 0.2a	0.3 ± 0.07a
	No netting	Insecticide	0.7 ± 0.1b	0.3 ± 0.05b	0.04 ± 0.01b
	Netting	No insecticide	0.4 ± 0.07c	0.3 ± 0.1bc	0.2 ± 0.1ab
	Netting	Insecticide	0.1 ± 0.02d	0.08 ± 0.03c	0.07 ± 0.04b
	Statistics (Kruskal–Wallis):			H = 60.75 df = 3,12 P < 0.0001	H = 29.01 df = 3,12 P < 0.0001
2015	No netting	No insecticide	0.5 ± 0.1a	0.2 ± 0.1a	0.07 ± 0.03a
	No netting	Insecticide	0.2 ± 0.07ab	0.07 ± 0.02ab	0.03 ± 0.01ab
	Netting	No insecticide	0.3 ± 0.1ab	0.06 ± 0.03ab	0.09 ± 0.04ab
	Netting	Insecticide	0.01 ± 0.006b	0.007 ± 0.005b	0.0 ± 0.0b
	Statistics (Kruskal–Wallis):			H = 13.89 df = 3,12 P = 0.003	H = 13.32 df = 3,12 P = 0.003

Five ripening, ripe, and overripe berries were collected from each plot and assessed using a modified salt test. Averages with the same letter within each column are not significantly different at $\alpha = 0.05$.

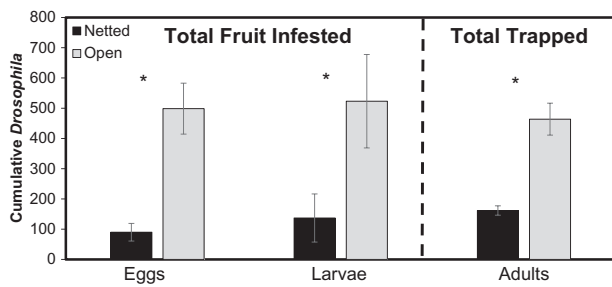


Fig. 2. Cumulative number of *Drosophila* eggs and larvae per kilogram of raspberries (\pm SE) and the cumulative number of *D. suzukii* adults caught each week per trap (\pm SE) in open and netted high tunnels throughout the entire raspberry season. Bars marked with an asterisk denote life stages where treatments were significantly different at $\alpha = 0.05$.

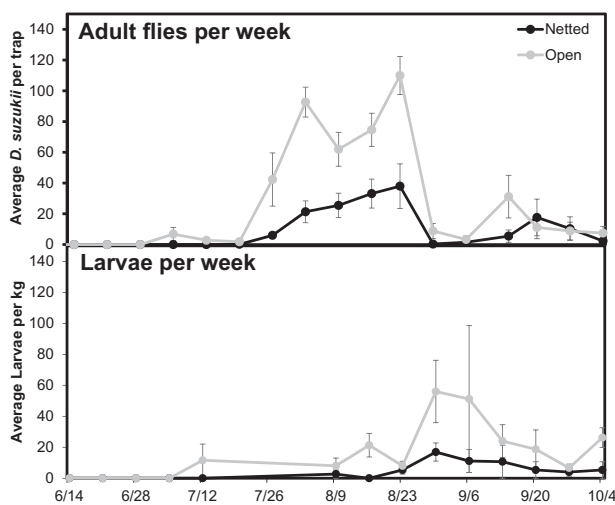


Fig. 3. The number of *D. suzukii* adults caught per trap (\pm SE) per week in open and netted tunnels (top) and the number of *Drosophila* larvae per kilogram of fruit (\pm SE) per week in open and netted tunnels (bottom) throughout the raspberry growing season. Summer berry harvest started in mid-June and ran through mid-July, whereas fall raspberry harvest began in August and ended in late September.

traps and direct leaf observations within the open and netted tunnels are shown in Fig. 4. Examination of the yellow sticky traps revealed that the abundance of pests, natural enemies, and pollinators was reduced in the netted tunnels compared to open tunnels. Pest insects and mites at the commercial farm were reduced by 44% ($t = -7.58$, $P = 0.008$). Natural enemies were reduced at both sites by 48% ($t = -2.88$, $P = 0.02$). Pollinators, excluding the supplemented bumble bees, declined by 77% although this reduction wasn't statistically significant due to variability among sites ($t = -1.78$, $P = 0.07$). From the direct leaf observations, we observed similar reductions of pests (42% decline) and natural enemies (32% decline) but these were highly variable between sites and there was no significant difference between netted and open tunnels ($t = -0.47$, $P > 0.32$; $t = -0.30$, $P > 0.38$, respectively). We found no instances of insect abundance increasing in the netted tunnels, though some insects were more affected by the netting than others. Thrips, mites, and leafhoppers were reduced by greater than 50%, whereas aphids and raspberry beetles were reduced by fewer than 20% compared to populations found in the open tunnels, from both yellow sticky trap and direct leaf observation data. Spiders were the only natural enemy that appeared to be less affected by the netting with an average reduction of 21%. Average percent leaf damage throughout the entire season in the open tunnels ($2.3 \pm 1.3\%$) was slightly higher than the netted tunnels ($2.2 \pm 1.8\%$), but not significantly so ($t = -0.05$, $P = 0.47$).

There was little effect on fruit quality characteristics of raspberries grown under the netted high tunnels. The average weight (Netted: 2.7 ± 0.1 g; Open: 2.5 ± 0.1 g), diameter (Netted: 16.1 ± 0.2 mm; Open: 15.7 ± 0.2 mm), and brix (Netted: $7.5 \pm 0.6^\circ\text{Bx}$; Open: $7.1 \pm 0.2^\circ\text{Bx}$), of the fruit collected from the netted tunnels was not significantly different from the fruit collected from the open tunnels ($F = 2.91$, $df = 1,4$, $P > 0.16$). The temperature in the netted tunnels was not significantly different from the open tunnels (Netted: $21.7 \pm 0.2^\circ\text{C}$, Open: $21.6 \pm 0.3^\circ\text{C}$) ($F = 0.04$; $df = 1,4$; $P = 0.83$). Likewise, the average maximum temperature was similar in the netted tunnels (Netted: $42.9 \pm 0.8^\circ\text{C}$, Open: $42.2 \pm 0.8^\circ\text{C}$) ($F = 0.39$; $df = 1,4$; $P = 0.56$).

Discussion

Exclusion netting is a non-chemical approach to preventing insect infestation of crops, and in this study we found that netting can

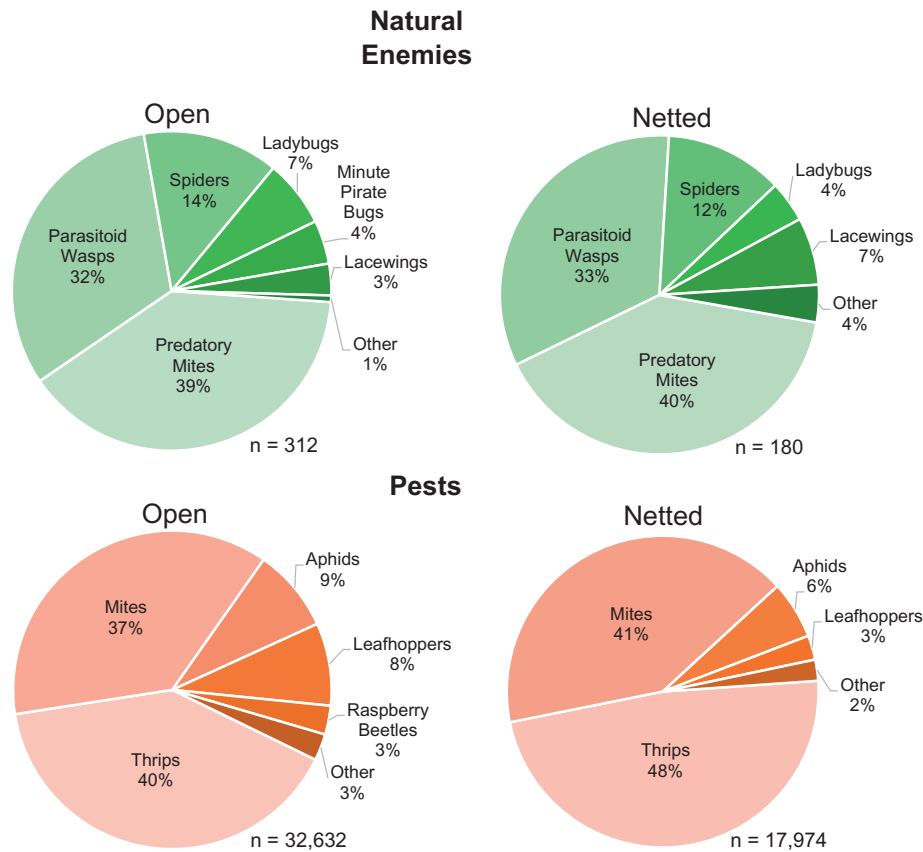


Fig. 4. The composition of the most abundant natural enemies (top) and raspberry pests (bottom) caught on yellow sticky traps and observed on raspberry leaves throughout the season in the open (left) and netted (right) high tunnels. *N* represents the total number of insects and mites observed throughout the season.

significantly reduce and delay *D. suzukii* infestation in red raspberries. This delay may be sufficient to eliminate the threat of *D. suzukii* from the summer crop of raspberries altogether and facilitate the production of insecticide free or organic-certified berries. Delayed fly activity could also prevent up to three weeks of insecticide sprays, lowering pesticide risks to pollinators, reducing the risk of insecticide resistance for *D. suzukii*, and saving growers both time and money. The delay of infestation that we observed was greater in the high tunnels than the smaller netted plots. Previous research has shown that high tunnels without exclusion netting can offer protection from pests compared field-grown raspberries (Demchak 2009, Hanson et al. 2013), so the combination of netting and high tunnels may provide even greater delay. The combined netting and insecticide treatments evaluated in this study also reduced all sizes of *D. suzukii* larvae detected in berries. This is particularly important for third instar larvae, because these are the largest and are most visible to consumers and processors. Larvae that survive to that terminal instar have usually caused collapse of fruit structure and leaking of juices that can be apparent. Preventing *D. suzukii* from reaching third instar stages is important for growers and knowing that combinations of netting and insecticides will complement each other to achieve this provides insights into how best to ensure marketable fruit in regions with *D. suzukii*. These measures could be further complemented by increased raspberry harvest frequency, which has been found to reduce *D. suzukii* infestation in the fruit (Leach et al. unpublished data).

Importantly, exclusion netting did not have a negative impact on the quality of raspberries harvested from netted tunnels, and the temperature differences caused by exclusion netting were minimal.

The trends for small increases in fruit weight, diameter, and sugar content among berries from the netted treatments could be due to a number of factors, such as increased vigor from loss of other pests or the slight increase in temperature. Cormier et al. (2015) observed similar trends in the weight of blueberries under netted field plots. While the fine mesh netting would block air flow, it also provides shading, which may be responsible for the similarity in temperature despite the enclosure. However, the presence of the netting has the potential to increase the ambient temperature, especially in the later parts of the growing season or in warmer production regions. Extreme temperatures in netted high tunnels is a concern that should be kept in mind for fruit production in regions with different climates. However, there are fan systems and venting options that can be used to minimize the risk of extreme temperatures in high tunnels. The reproductive rate of *D. suzukii* declines as temperatures surpass 28 °C (Tochen et al. 2014), so hotter conditions may also reduce the potential for this pest to cause fruit infestation. Indeed, a recent study using low tunnels found that raspberries grown under plastic covering had much lower infestation rates than those covered with netting, presumably due to the hot microclimate created by the plastic that exceeded the thermal threshold for population growth by *D. suzukii* (Rogers et al. 2016). However, optimum flowering and growth in primocane raspberries occurs at 24–27 °C, dependent on variety (Carew et al. 2003, Sønsteby and Heide 2009), so temperatures higher than this range may have negative implications for plant health and berry yield.

Exclusion netting and screening can have additional pest management benefits by acting as a barrier against other pests including insects and birds (Blua et al. 2005, Dellamano 2006, Qureshi et al.

2007, Simon 2008), thereby providing economic benefits in addition to the reductions of *D. suzukii* noted here. We found that raspberry aphids and raspberry beetles were relatively unaffected by the netting, perhaps because they were already established in the plantings, so these will still require active management in a netted tunnel setting. It is also possible that netting high tunnel plantings from the first year of growth could prevent these pests from becoming established. Still, mobile insects and those with alternative hosts or different overwintering sites may be more affected by the netting than permanent residents that overwinter and complete their life cycle on or near the crop. We therefore recommend that monitoring for pests, including *D. suzukii*, should continue with the implementation of exclusion netting.

We have found that the exclusion netting is an effective way to delay the start of insecticide inputs for *D. suzukii* management, but this does not address grower concerns about netting, including the cost and potential for intensive labor for installation and maintenance (Link 2014). Installation of netting requires a structure for its support, and some producers have adapted a less expensive modified bird netting support system for excluding *D. suzukii* (Pullano 2015). If a structure is already in place, such as a high tunnel or bird netting support, exclusion netting can be a less expensive addition to production costs. In the approach tested here, we calculated that netting the sides and ends of one acre of 122 meter long tunnels would cost approximately \$6,100. This estimate includes the cost of the netting plus its shipping, accessories to secure the netting, bumble bee colonies for supplemental pollination, and labor costs for installation of the netting. This cost could be amortized across the lifespan of the netting, which is projected to be seven years (Tek-Knit Industries). Supplemental pollinators are necessary for raspberries which produce in both the summer and fall. For other crops such as blueberries that bloom before *D. suzukii* is active, netting could be installed after pollination. Furthermore, we expect that labor costs would be reduced with practice in its installation. Further experience is needed with this approach to determine whether reduced insecticide applications or the potential increase in fruit quality and sale price would cover the cost of netting. Nevertheless, netting provides additional insurance for growers to have a marketable crop in years or regions where *D. suzukii* is a pest of concern.

Future research should focus on economic analyses of netting application and the possibility of insecticide-treated netting. While *D. suzukii* adults were not observed resting on the netting in this study, *D. suzukii* attraction is primarily driven by odor (Keeseey et al. 2015, Revadi et al. 2015), making encounters with netting likely. Repellents are also being developed for *D. suzukii* (Pham and Ray 2015, Wallingford et al. 2015, Renkema et al. 2016) and their application in combination with netting could be explored.

This pest has rapidly disrupted established IPM programs in small fruit and berry production around the world. To rebuild these programs, multiple non-chemical management approaches must be explored including the search for classical biological control agents (Guerrieri et al. 2016, Daane et al. 2016), pest monitoring (Burrack et al. 2015), removal of noncrop hosts (Klick et al. 2015, Lee et al. 2015; Briem et al. 2016, Pelton et al. 2016), and use of exclusion netting. This will provide growers across a range of production systems with diverse options to manage *D. suzukii*, and it can also serve as an example for invasive pest challenges that we may face in the future.

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