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Impact of multiple natural enemies on immature *Drosophila suzukii* in strawberries and blueberries

Justin M. Renkema · Andrew G. S. Cuthbertson

Abstract *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) oviposits in ripening fruit, larvae render crops unmarketable, and significant economic losses can occur. Biological control research has focused on individual natural enemy species against immature *D. suzukii*. Here we combine two predators and an entomopathogenic nematode, expecting species complementarity and increased control of *D. suzukii*. In strawberries, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) plus *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) resulted in fewest *D. suzukii* (81% reduction), and in blueberries, results were similar (60% reduction), although *H. bacteriophora* was not as effective as in strawberries, which was likely due to drier substrate conditions. There was neither strong complementarity nor interference between predators, *O. insidiosus* and *Dalotia coriaria* Kraatz (Coleoptera: Staphylinidae). Inclusion of *O. insidiosus* resulted in 50% fewer *D. suzukii* than combinations without *O. insidiosus*. Control of *D. suzukii* can be improved with multiple natural enemies, and combinations of *O. insidiosus* with other agents (parasitoids, fungal entomopathogens) should be tested.

Keywords Complementarity · *Dalotia coriaria* · Diptera · Drosophilidae · *Heterorhabditis bacteriophora* · *Orius insidiosus*

Introduction

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a vinegar fly invasive to large areas of North and South America and Europe, where it has become a major pest of tender fruit and berry crops. Native to temperate regions of Asia, *D. suzukii* was first recorded in 2008 in California, USA Italy and Spain (Asplen et al. 2014; Calabria et al. 2012; Cini et al. 2012; Deprá et al. 2014; Hauser 2011; Haye et al. 2016). Crop damage is caused when females use large, serrated ovipositors to lay eggs in unripe and ripe fruit, and larvae consume fruit tissue, leading to soft, contaminated and unmarketable fruit (Lee et al. 2011a). Economic losses due to *D. suzukii* have been estimated at $26 and $43 million US$ in susceptible crops in eastern USA and in raspberries in California, respectively (Burrack 2014; Goodhue et al. 2011; Lee et al. 2011b). Due to the potential for rapid population increases prior to and during fruit harvest, frequent insecticide applications are relied on in order to

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control adult flies (Diepenbrock et al. 2016a; Lin et al. 2014; Tochen et al. 2014). However, 92% of the *D. suzukii* population may exist as eggs, larvae or pupae at any given time (Emiljanowicz et al. 2014), thus creating both challenges for effective control using insecticides targeting only adults and opportunities for developing control tactics aimed at immature stages.

A primary reason as to why a biological invader is often successful is due to the lack, or reduced impacts, of natural enemies in the invaded range (the "Enemy Release Hypothesis") (Liu and Stiling 2006). For *D. suzukii*, relatively few generalist larval and pupal *Drosophila* parasitoids in its invaded areas have been documented, and typically they have limited effects on *D. suzukii* populations (Gabarra et al. 2015; Miller et al. 2015; Stacconi et al. 2015) due, at least in part, to the high haemocyte loads in *D. suzukii* (Kacsoh and Schlenke 2012; Poyet et al. 2013). In Japan, *D. suzukii* larvae are parasitized by *Ganaspis xanthopoda* (Ashmead) (Hymenoptera: Fitigidae), *Asobara japonica* Belokobylskij, and *A. tabida* Nees (Hymenoptera: Braconidae) (Mitsui et al. 2007; Mitsui and Kimura 2010), and classical biological control using *Asobara*, *Ganaspis* as well as *Leptopilina* species is also being investigated (Daane et al. 2016). Predation on immature *D. suzukii* in invaded ranges has received less research attention, but Woltz and Lee (2017) recently found a 19–49 and 61–91% decrease in larvae and pupae in fruits and soil, respectively, when they were exposed to predators. Ants and spiders were commonly observed (Woltz and Lee 2017), and in another study *Labidura riparia* Pallas (Dermaptera: Labiduridae) was common and recorded as an active predator of *D. suzukii* larvae and pupae (Gabarra et al. 2015). A significant impact of predators on *D. suzukii* is corroborated by studies that have shown efficacy in the laboratory of the commercially available predators *Orius* spp. (Hemiptera: Anthocoridae) and *Dalotia coriaria* Kraatz (Coleoptera: Staphylinidae) (Cuthbertson et al. 2014a; Renkema et al. 2015; Woltz et al. 2015). Augmentative releases of biological control agents against immature *D. suzukii*, including entomopathogens, particularly nematodes (Cuthbertson et al. 2014b; Cuthbertson and Audsley 2016), may serve to complement existing predation or infection rates by increasing natural enemy diversity.

The commercial biological control agents tested in this study, two predators and an entomopathogenic nematode, have been individually screened against immature stages of *D. suzukii*. *Orius insidiosus* Say is a predatory bug, well-known for its ability to control thrips and other small, soft-bodied pests. When placed with *D. suzukii*-infested blueberries, control was 50 and 12% in containers or cages in the laboratory, respectively, but no control was recorded in outdoor cage experiments (Woltz et al. 2015). *Dalotia coriaria* is used primarily against soil-dwelling fly larvae in greenhouses. Reductions in *D. suzukii* larvae in the laboratory due to *D. coriaria* ranged from 0% in intact blueberries to 10% in Petri dishes to 50% in decomposing raspberries (Cuthbertson et al. 2014a; Renkema et al. 2015; Woltz et al. 2015). *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) is effective against soil-dwelling larvae of beetle pests. Woltz et al. (2015) recorded no *D. suzukii* larval infectivity in diet or blueberries, but Cuthbertson and Audsley (2016) recorded over 90% mortality when drenching larvae in sand. While variability in results among experiments for each species may be due to factors such as predator or pest densities, a consistent reduction of *D. suzukii* immatures may be insured by combining natural enemy species.

The goal of increasing natural enemy richness is higher prey consumption rates as a result of complementary resource use or emergent effects among enemies (Casula et al. 2006; Ives et al. 2005; Sih et al. 1998). Natural enemies complement each other when prey consumption is additive, usually reaching a saturation point, and *per capita* resource use is independent of natural enemy numbers (Pfannenstiel and Yeargan 2002; Straub and Snyder 2008). Emergent effects result from direct or indirect interactions among natural enemies, creating non-additive results (Sih et al. 1998; Snyder and Tylianakis 2012). Positive emergent effects are due to biological synergy, whereby one natural enemy enables a greater level of resource consumption by a second natural enemy (Fodrie et al. 2008; Losey and Denno 1998). Negative effects of multiple natural enemies may be attributable to strong intraguild predation or non-consumptive interference of foraging or other behaviors (Hawes et al. 2013; Michalko and Pekár 2017; Rosenheim et al. 1993). Relative strengths of intra- *versus* inter-specific competition or of trophic links between predator and prey will influence outcomes of using multiple natural enemies, with situation-specific factors such as habitat having important modifying

effects (Finke and Denno 2002; Snyder and Wise 1999).

Our objective was to determine the effect of increasing richness of three natural enemies—*O. insidiosus*, *D. coriaria*, and *H. bacteriophora*—on immature stages of *D. suzukii* developing in strawberries and blueberries. We used a substitutive design, whereby total natural enemy abundance was held constant at varying levels of natural enemy species richness. Using the substitutive design, opportunities for intra-specific interactions declines with species richness, while opportunities for inter-specific interactions increase (Straub and Snyder 2006). Thus, the expectation is that since natural enemy species (likely) exploit resources in different ways, increased species richness will lead to greater resource consumption than simply increasing abundance of a single natural enemy species.

Materials and methods

Insect and nematode sources

Drosophila suzukii were from a colony kept in ventilated Plexiglas® cages (26 × 26 × 26 cm) under laboratory conditions (21–22 °C, 30–40% RH) at the Gulf Coast Research and Education Center (GCREC) in Balm, Florida, USA since August 2015. The colony was started in 2009 from *D. suzukii* that emerged from orange jasmine fruit, *Murraya paniculata* (L.) Jack, and was periodically supplemented with flies emerging from commercial blueberries and strawberries in Florida. Flies were provided with moist cotton batting as a water source and fresh diet/larval growth media every 3–4 days, consisting of water (4 l), agar (45 g), cornmeal (125 g), white sugar (200 g) and nutritional yeast (70 g) with 1 M propionic acid (17.7 ml) and methyl paraben (3.3 g) dissolved in 95% ethanol (33.3 ml). The mixture was boiled and then cooled before pouring into Petri dishes.

Dalotia coriaria were obtained from Biobest Biological Systems (Westerlo, Belgium) and reared in a growth room (25 °C, 50–60% RH, L:D 16:8) in clear plastic containers (4 l) partially filled with sphagnum peat moss (Miracle Gro® Lawn Products Inc., Marysville, OH, USA), coconut fiber (Eco Earth®, Zoo Med Laboratories Inc., San Luis Obispo, CA,

USA), and vermiculite in a 4:2:1 ratio by volume, respectively. Water (350 ml per container) was mixed with the dry materials, and additional water (15 ml) was added weekly to maintain a consistent moisture level. Beetles were fed crushed, pelletized trout food (3 g per container) (AquaMax® Sportfish 600, Purina, St. Louis, MO, USA) mixed into the substrate weekly. *Dalotia coriaria* were removed from the colony and held individually, without food for 18 h in microcentrifuge tubes (1.5 ml) before the start of the experiments.

Orius insidiosus were obtained from Biobest Biological Systems (Westerlo, Belgium), and *Heterorhabditis bacteriophora* (Nemasys® G) were obtained from BASF Agriculture (Research Triangle Park, NC, USA). Both were held in a refrigerator at 4 °C for approximately 24 h before being utilized in experiments. *Orius insidiosus* were placed individually in microcentrifuge tubes (0.6 ml) 3–4 h before the start of experiments.

Strawberry experiment

Strawberry transplants ('Radiance') were grown for three weeks in a greenhouse in black plastic pots (3.78 l) partially filled with standard growing media and fertilized once with Osmocote® Smart Release® Plant Food (Bloomington, IN, USA). The day prior to the start of the experiment, plants were trimmed to four or five fully expanded trifoliate leaves, and a hand-lens was used to inspect leaves to ensure they were free of mites, thrips or other insects. On the day of the experiment, the surface of the potting media was covered with a layer of sand (100 g) (Quickrete® Play Sand, Jacksonville, FL, USA) that was dried at 240 °C for 24 h and rewetted to 10% moisture by weight. A Plexiglas® cylinder (30 cm high, 14.7 cm inside diameter, 3.0 mm thickness) was placed over the strawberry plants, fitting tightly in the plastic pot, and pressed about 1 cm into the sand to prevent insect escape. White, organdy mesh was glued to the top of the cylinder and over a 3.2 cm diameter hole that was 7 cm from the bottom of the cylinder. Insects were introduced through a 3.2 cm diameter hole, 8.5 cm from the bottom of the cylinder that was plugged with a cork after introductions. During the experiment, arenas (potted strawberry + cylinder) were held in a growth room at 24 °C, 45–50% RH, and L:D 16:8 under fluorescent light.

To obtain infested fruit, fully ripe strawberries ('Radiance') were harvested on 9th February 2016 from research plots at GCREC that had not been sprayed with insecticides. A subsample of strawberries ($n = 6$) were held individually for five days in vented plastic containers to determine the presence of *D. suzukii*, but no *Drosophila* larvae were found. Strawberries used in the experiment ($n = 64$) were rinsed for 15 s in 1% bleach solution and then in distilled water. After drying, they were individually weighed, placed in small, labelled plastic weigh-boats and divided among three Plexiglas® cages (same as those used for *D. suzukii* colony rearing). Each cage had approximately 200, one week old *D. suzukii* flies from the colony at a 1:2, male:female ratio. After 24 h, strawberries were removed from cages and checked under magnification to ensure they contained *D. suzukii* eggs.

At the start of the experiment, a 6 cm diameter ring was impressed in the surface of the sand adjacent to the base of the strawberry plant using the rim of the plastic cup, and a shallow depression was made in the center of the ring. *Heterorhabditis bacteriophora* were mixed in cool, distilled water according to the manufacturer's recommendations. The highest application rate of 54 000 infective juveniles was equal to the rate tested in Cuthbertson and Audsley (2016) on a per cm² basis (Table 1). Mixtures of *H. bacteriophora* (5 ml per arena) were poured slowly and evenly over the area of the impressed ring. Natural enemy combinations without *H. bacteriophora* received 5 ml of water. An infested strawberry was placed on each depression. A cylinder was placed over each potted strawberry, and required numbers of *D. coriaria* and *O. insidiosus* were tapped out of microcentrifuge tubes into the arenas (Table 1). Each natural enemy combination was replicated eight times. Replicates were arranged in a complete block design in the growth room. A block was eight arenas on two 45 × 35 cm trays, with about 60 cm between blocks.

After six days, cylinders were removed and arenas searched for live *D. coriaria* and *O. insidiosus*. The same cup that was used to make the ring was pressed into the sand to transfer a shallow cylinder of sand and what remained of the strawberry to a plastic container (500 ml). Containers were stored in a refrigerator and assessed for *D. suzukii* larvae and pupae during the following week using a saltwater test. Each sample was stirred vigorously with 300 ml of one part salt to 16 parts water, larvae and pupae that floated were removed, saltwater and debris were poured through a screen, and the screen was searched for pupae and larvae. This process was repeated three times per sample, until no additional larvae or pupae were recovered.

Blueberry experiment

The methods for the blueberry experiment were identical to the strawberry experiment, except for the following. Fully ripe southern highbush blueberries ('Jewel') from research plots at GCREC that had not been sprayed with insecticides were harvested on 17th May 2016 and exposed to laboratory adult *D. suzukii* for 24 h for infestation. Three groups of ten blueberries not exposed to laboratory *D. suzukii* were held for

Table 1 Combinations and numbers of three natural enemy species in 170 cm² arenas assessed against immature stages of *Drosophila suzukii* in strawberries and blueberries

Species richness	*Dalotia coriaria*	*Orius insidiosus*	*Heterorhabditis bacteriophora*	Total
3	6	6	18,000 (6)[a]	18
2	9	9	0	18
2	9	0	27,000 (9)	18
2	0	9	27,000 (9)	18
1	18	0	0	18
1	0	18	0	18
1	0	0	54,000 (18)	18
0	0	0	0	0

[a] Numbers in brackets indicate equivalent number of *D. coriaria* or *O. insidiosus*

five days, but no *Drosophila* larvae were found. Instead of potted strawberries, cylinders were fitted over with Petri dish lids (14.6 cm outer diameter) filled with 280 g of sand at 10% moisture by weight, mounded in the center of the dish to a depth of approximately 2 cm. Ten infested, weighed blueberries were placed in a shallow depression in the impressed ring at the peak of the mound. Arenas were arranged in a completely randomized design in the same growth room as the strawberry experiment.

Data analysis

Initially, multiple linear regression was used to test all possible interactions among natural enemies on numbers of *D. suzukii* recovered. Since none of the interactions were significant ($P > 0.05$) for either strawberry or blueberry, a one-way analysis of variance (ANOVA) was used to compare the different natural enemy combinations. For the strawberry experiment, replications were grouped by blocks, and so a random blocking effect was included in the model. For the blueberry experiment, a completely randomized design was used, so a blocking term was not used in the model. Data were log $(x + 1)$ transformed for the strawberry and log (x) transformed for the blueberry experiment to improve normality and homoscedasticity of error variance. Back-transformed least square means and confidence intervals are displayed. Also using ANOVA, recovery rates of *D. coriaria* and *O. insidiosus* among natural enemy combinations including each predator were compared. Tukey's HSD test was used to separate significantly different means.

The *per capita* effects of predators—*D. coriaria* and *O. insidiosus*—were calculated using the equation $\ln(N_{na}/N_p)/P$, where N = final *D. suzukii* numbers, na = natural enemies absent, p = predators present, and P = initial predator density (Straub and Snyder 2008; Wootton 1997). The mean for the no-natural enemies control was used for the N_{na} term. We did not compare the *per capita* effect of *H. bacteriophora* because of the much greater numbers of individuals applied compared to predators. Since *O. insidiosus* had stronger *per capita* effects than *D. coriaria*, we also compared numbers of *D. suzukii* recovered between treatments with or without *O. insidiosus*. Analyses were performed in JMP® Pro 12.0.1 (SAS 2015) at $\alpha = 0.05$.

Results

The average strawberry size was 28.9 ± 0.4 g ($\pm$ SE), with a range of 22.9–34.3 g, and the average weight of ten blueberries was 20.5 ± 0.1 g ($\pm$ SE), with a range of 18.2–22.8 g. Numbers of recovered *D. suzukii* larvae and pupae were affected by the composition of natural enemy species in strawberries ($F_{7,44} = 5.5$, $P < 0.001$) and blueberries ($F_{7,60} = 18.8$, $P < 0.001$). In strawberries, fewest *D. suzukii* were recovered with the combination of *O. insidiosus* and *H. bacteriophora*, and *O. insidiosus* alone also reduced *D. suzukii* numbers to levels below that of the control (Fig. 1). Natural enemy combinations with *D. coriaria* did not have fewer *D. suzukii* than the control (Fig. 1). In blueberries, fewer *D. suzukii* were recovered with *O. insidiosus* alone or any combination of *O. insidiosus* and *H. bacteriophora*
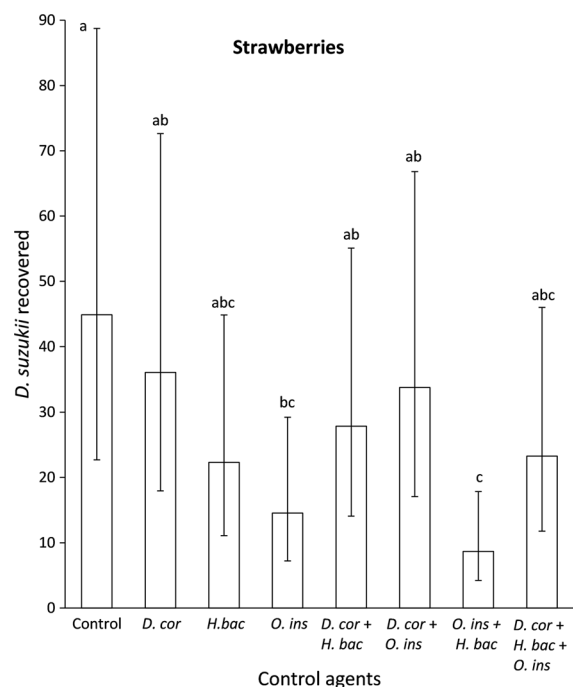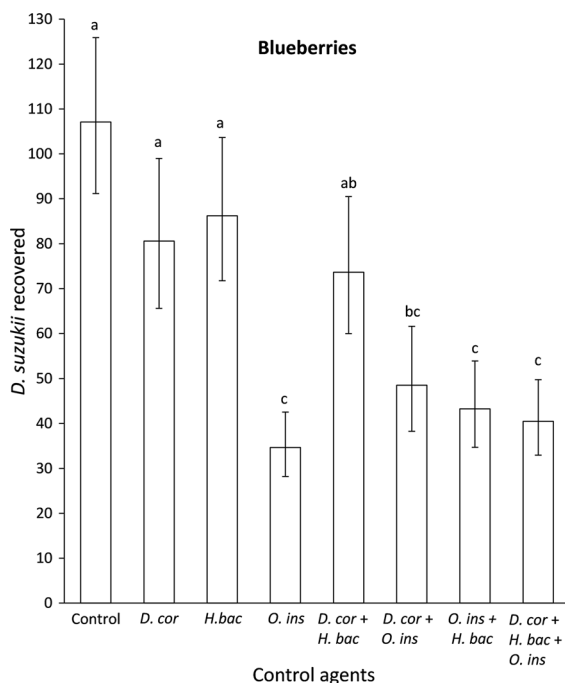

Fig. 1 Least-square mean ($\pm$ 95% CI) number of larval and pupal *Drosophila suzukii* recovered from a strawberry exposed for six days to seven combinations of natural enemies: a rove beetle, *Dalotia coriaria* (*D. cor*), an entomopathogenic nematode, *Heterorhabditis bacteriophora* (*H. bac*), and a minute pirate bug, *Orius insidiosus* (*O. ins*) and a control without natural enemies. Strawberries and natural enemies were confined in 170 cm² arenas with small strawberry plants placed in a controlled environment. Means with the same letters are not significantly different (Tukey's HSD test, $\alpha = 0.05$)

and *D. coriaria* than with *D. coriaria* or *H. bacteriophora* alone or the control (Fig. 2). All recovered larvae and pupae were normally coloured, with none from *H. bacteriophora*-containing combinations having obvious symptoms of nematode infection, such as red or orange colouration.

The recovery rate of *D. coriaria* (strawberries: $F_{3,27} = 0.4$, $P = 0.785$; blueberries: $F_{3,25} = 2.3$, $P = 0.101$) did not vary due to natural enemy combination, nor did the recovery rate of *O. insidiosus* (strawberries: $F_{3,25} = 0.7$, $P = 0.556$; blueberries: $F_{3,24} = 1.0$, $P = 0.419$). Recovery rates were 57–64 and 65–90% for *D. coriaria* and 12–22 and 46–67% for *O. insidiosus* in strawberries and blueberries, respectively (Table 2). *Per capita* impacts of predators on *D. suzukii* recovery were not affected by predator richness on a strawberry ($t_{20} = 0.94$, $P = 0.359$) or on blueberries ($t_{20} = 0.36$, $P = 0.725$),

even though the strength of *D. suzukii* suppression on a strawberry with both predators was about half of that when only one predator was present (Fig. 3). *Per capita* impacts were affected by predator species identity on a strawberry ($t_{12} = 2.23$, $P = 0.046$) and in blueberries ($t_{13} = 6.18$, $P < 0.001$). *Orius insidiosus* was 5.6 and 3.7 more effective than *D. coriaria* on strawberry and blueberries, respectively, at reducing *D. suzukii* (Fig. 3). A species-identity effect occurred for *O. insidiosus* in strawberries ($t_{59} = 2.01$, $P = 0.048$) and blueberries ($t_{66} = 8.48$, $P < 0.001$). Natural enemy combinations with *O. insidiosus* had about half as many *D. suzukii* compared to those without *O. insidiosus* in both strawberries and blueberries (Fig. 4).

Discussion

We expected that increased natural enemy richness would reduce *D. suzukii* to lowest levels, given that complementary resource use by multiple natural enemies has been recorded when multiple life stages of prey are included in experiments (Wilby and Thomas 2002). *Orius insidiosus* forages primarily on fruit using piercing mouthparts to consume eggs and first instars that are on or just below the fruit surface. *D. coriaria* likely forages most effectively once fruit begins to breakdown and some larvae become exposed, and *H. bacteriophora* is most likely to infect pre-pupal larvae as they wander from fruit to pupate. However, we did not find complementarity among the two predators, as the combination of *O. insidiosus* and *D. coriaria* did not produce *per capita* effects different from the average of either. In the strawberry experiment, the combination of *O. insidiosus* and *H. bacteriophora* appeared to be complementary, but *per capita* effects could not be calculated. We suspect that poor performance of *H. bacteriophora* in the blueberry experiment, thus lack of evidence for any complementarity, is due to low soil moisture at the end of the experiment resulting in lower survival and infection rates than in the strawberry experiment (Toledo et al. 2014). Furthermore, a recent study showed that other entomopathogenic nematodes, *Steinernema feltiae* Filipjev and *S. carpocapsae* Weiser, may be more effective biocontrol agents as they infected larval *D. suzukii* at much higher levels than *H. bacteriophora* (Hübner et al. 2017). The
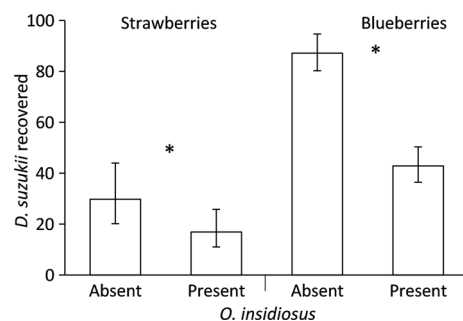

Fig. 2 Least-square mean (± 95% CI) number of larval and pupal *Drosophila suzukii* recovered from ten blueberries exposed for six days to seven combinations of natural enemies: a rove beetle, *Dalotia coriaria* (*D. cor*), an entomopathogenic nematode, *Heterorhabditis bacteriophora* (*H. bac*), and a minute pirate bug, *Orius insidiosus* (*O. ins*) and a control without natural enemies. Blueberries and natural enemies were confined in 170 cm² arenas placed in a controlled environment. Means with the same letters are not significantly different (Tukey's HSD test, α = 0.05)

Table 2 Proportion (± SE) of *Dalotia coriaria* (*D. cor*) and *Orius insidiosus* (*O. ins*) recovered six days after release in arenas with either a single strawberry or ten blueberries infested with *Drosophila suzukii*

Natural enemies	Initial *n*	Recovery in strawberry		Recovery in blueberries	
		D. coriaria	*O. insidiosus*	*D. coriaria*	*O. insidiosus*
D. coriaria	18	0.57 ± 0.06	–	0.65 ± 0.07	–
O. insidiosus	18	–	0.12 ± 0.05	–	0.46 ± 0.10
D. cor + H. bac	9	0.64 ± 0.05	–	0.86 ± 0.07	–
D. cor + O. ins	9	0.57 ± 0.05	0.17 ± 0.05	0.89 ± 0.09	0.51 ± 0.13
O. ins + H. bac	9	–	0.22 ± 0.05	–	0.67 ± 0.11
D. cor + H. bac + O. ins	6	0.60 ± 0.05	0.19 ± 0.05	0.90 ± 0.07	0.67 ± 0.10

H. bac = Heterorhabditis bacteriophora, an entomopathogenic nematode
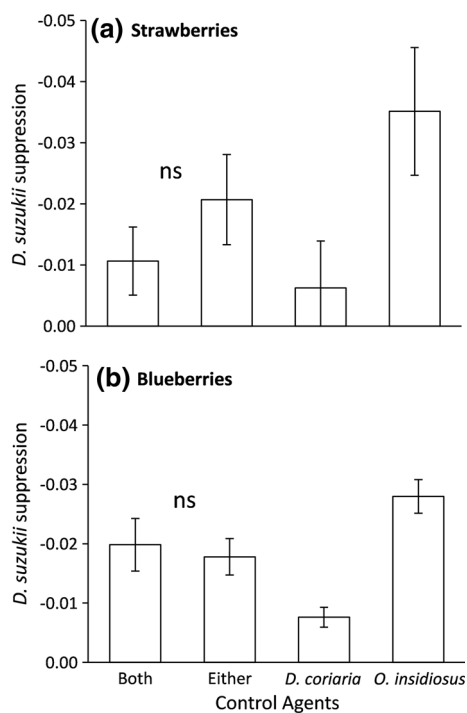
Fig. 3 Mean (± SE) *per capita* predator effects on *Drosophila suzukii* in **a** a single strawberry or **b** ten blueberries for six days in 170 cm² arenas placed in a controlled environment with both or either *Dalotia coriaria* and *Orius insidiosus*. Negative values indicate *D. suzukii* suppression relative to the no natural enemy control. ns denotes no significant differences (*P* > 0.05) between *per capita* effects with both or either predator species

Fig. 4 Mean (± 95% CI) number of *Drosophila suzukii* recovered from natural enemy communities with (*n* = 30 blueberries and strawberries) or without (*n* = 31 strawberries and 38 blueberries) the predator *Orius insidiosus*. Asterisks denote significant differences (*P* < 0.05) between means for strawberries and blueberries separately

5–6 days between drenching the soil with *H. bacteriopohora* and when *D. suzukii*-third instars exited fruit to pupate and were most susceptible to infection may have meant nematode infective juveniles died

before they were able to access the host. However, Hübner et al. (2017) found entomopathogenic nematodes in decaying fruit, suggesting they can potentially infect all larval stages of *D. suzukii*.

In addition to finding little evidence for natural enemy complementarity, we found no strong evidence for natural enemy interference between the two predators. Studies that evaluate multiple predators often find that interference increases with increasing predator richness (Finke and Denno 2004; Martin et al. 2013). *Per capita* effects were lower, but not significantly, with *O. insidiosus* and *D. coriaria* than the average of either alone on strawberry but not blueberry, suggesting few, if any, interactions between the species. For strong negative interactions to occur, an effective pest predator must be the victim of a strong intraguild predator (Rosenheim et al. 1993; Prasad and Snyder 2006). Since the recovery rates of either

predator were not affected by natural enemy combinations, any interactions may be behavioural and non-consumptive. We did not evaluate *H. bacteriophora* numbers post-experiments, but there is a possibility that predators, particularly *D. coriaria*, foraged on the nematodes and so reduced their potential impact on *D. suzukii* larvae.

These experiments point to the importance of natural enemy identity for prey suppression (Straub and Snyder 2008). Combinations of natural enemies that included *O. insidiosus* consistently resulted in fewer *D. suzukii* compared to those without, meaning that *O. insidiosus* foraging strategies are more effective than those of *D. coriaria* or the infectivity rates of *H. bacteriophora*. While we expected greatest prey consumption by *O. insidiosus* during the first few days of the experiment when eggs and first instars were present, higher survival rates and stronger *D. suzukii* suppression at the end of the blueberry compared to strawberry experiment suggests *O. insidiosus* also forages successfully on older larvae. In blueberries, larvae are likely to be more accessible to *O. insidiosus* probing through the surface than in a larger strawberry where there is more enemy-free space. In addition, there were more than five and about 1.5 recovered *D. suzukii* per gram of blueberry and strawberry, respectively (using control and berry weight means), meaning that *O. insidiosus* was more likely to encounter eggs or larvae in blueberries than in a strawberry.

This study shows that predatory natural enemies and an entomopathogen provide suppression of *D. suzukii* populations, and suggests that further study is warranted to determine their impacts under field conditions and in combination with additional natural enemies, particularly parasitoids. However, significant challenges exist for developing a biological control programme for *D. suzukii*. Of the agents tested, *O. insidiosus* was most effective, but its preferences for other food sources may dilute its effect, particularly if flower thrips are present in crops, like strawberries, where fruit ripening and flowering continuously overlap. Furthermore, achieving a density of 18 *O. insidiosus* per strawberry or ten blueberries may be unrealistic in the field. *Drosophila suzukii* populations build rapidly in alternate host species outside cropped fields whereby adult flies then invade fields and oviposit in ripening fruit (Diepenbrock et al. 2016b; Klick et al. 2015). However, in-field *D. suzukii* population suppression through natural enemy effects on immature life stages may be useful in some situations, particularly organic berry production where *D. suzukii* control options are few and farming practices promote natural enemy abundance and richness (Bengtsson et al. 2005; Hole et al. 2005). Given that we found positive species-identity effects but not natural enemy interference, and that high predation levels can occur in the field (Woltz et al. 2015), conserving and augmenting the "right" kind of natural enemy diversity (Landis et al. 2000) in berry fields should negatively impact *D. suzukii* populations either through sampling effects or, as of yet, unexplored complementarity among natural enemies.

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