

Laboratory Assays against Adult and Larval Sap Beetles (Coleoptera: Nitidulidae) using Entomopathogenic Nematodes, Microbial-Based Insecticides, and Synthetic Insecticides¹

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Abstract Sap beetles, *Carpophilus* spp. (Coleoptera: Nitidulidae), damage peach fruit leading to the need for efficacious control measures. We assayed different species and strains of entomopathogenic nematodes (*Heterorhabditis bacteriophora* Vs strain, *H. indica* HOM1 strain, *H. megidis* UK211 strain, *Steinernema carpocapsae* All strain, *S. feltiae* Sn strain, and *S. riobrave* 355 strain) against larval *Carpophilus* spp. and insecticides (microbial-based Grandevo[®] and Venerate[™] bioinsecticides, along with the synthetic thiamethoxam, acetamiprid, indoxacarb, β -cyfluthrin, clothianidin, carbaryl, chlorantraniliprole, fenpropathrin, spinetoram, phosmet, malathion, and imidacloprid) against adult *Carpophilus* spp. in the laboratory. All entomopathogenic nematodes assayed caused significantly higher larval mortality than the control. How the insecticides were presented to the adult beetles affected whether beetles were rated as nonfeeding (dead + moribund). Fewer insecticides were active against the adults when applied to filter paper than when applied to a plug of pear that beetles fed upon. Overall, indoxacarb and phosmet provided consistently better control, regardless of the exposure method. These two insecticides, with different modes of action, also have a 14-d preharvest interval when used on peach, making it imperative to detect these pests well before harvest. Chlorantraniliprole and the microbial-based products had no effect on adult beetles regardless of the exposure method.

Key Words *Carpophilus*, peach, stone fruit, *Chromobacterium*, *Burkholderia*

Many species of sap beetles (Coleoptera: Nitidulidae) feed on plant fluids, particularly fermenting fluids (Triplehorn and Johnson 2005). Within this family, many *Carpophilus* spp. are serious pests of fruits and grains both before and after harvest (Whitlaw et al. 1959, Dowd 2000, Bartelt and Hossain 2010). James et al. (2000) report *Carpophilus* spp. attacking ripening peach, nectarine, and apricot fruit in Australia, and several species of sap beetles are commonly found feeding on overripe peach fruit across the southeastern United States (Blaauw et al. 2017).

Sap beetle damage to preharvest peach fruit has been detected recently in the southeastern United States. Members of the *Carpophilus* spp. complex contribute to this problem. Historically, insecticide applications directed toward fruit-attacking

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pests, such as the plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), and the brown stink bug, *Euschistus servus* (Say) (Hemiptera: Pentatomidae), controlled secondary pests in orchards, most likely including *Carpophilus* spp. In recent years, the dependence on organophosphate insecticides for use against the primary fruit pests in southeastern U.S. peach orchards has decreased. This change in preharvest insecticide use patterns may have provided sap beetles an opportunity to exploit peaches well before harvest, thus leading to the observed damage. A similar occurrence was detected in Australia when the use of broad-spectrum insecticides in stone fruits was reduced (James et al. 2000). In fact, changing insecticide use is credited with problems in southeastern U.S. peach production concerning populations of San Jose scale, *Comstockaspis perniciosus* (Comstock) (Hemiptera: Diaspididae), and lesser peachtree borer, *Synanthedon pictipes* Grote & Robinson (Lepidoptera: Sesiidae), building to economically damaging levels in orchards after the use of methyl parathion on peaches was discontinued (Horton et al. 2005). Thus, continued preharvest injury to peach will require managing *Carpophilus* spp. in commercial peach orchards.

Several monitoring and management strategies for *Carpophilus* spp. have been reported in the literature (Bartelt et al. 1994, Bartelt et al. 1995, James et al. 1996, James et al. 2000, Hossain et al. 2006). For example, using pheromones and attractants, James et al. (1996) and Hossain et al. (2006) found a potential way to manage *Carpophilus* spp. attacking peach in Australia. Entomopathogenic nematodes may be another option to decrease *Carpophilus* spp. populations (Vega et al. 1994, Glazer et al. 1999) when mature larvae leave fruit to pupate in the soil (Glazer et al. 2007). In addition, insecticides (both microbial-based and synthetic) have the potential to manage *Carpophilus* spp. populations below economic thresholds. Blumberg (2008) reports that imidacloprid, thiacloprid, and certain pyrethroid insecticides provided effective control of *Carpophilus* spp. attacking date palms. The insect growth regulators diflubenzuron, hexaflumuron, and teflubenzuron were also effective but do not control the adult stage. As such, additional management strategies are needed to help improve the control of *Carpophilus* spp. in southeastern U.S. peaches.

The objective of this laboratory study was to assess the potential to use certain entomopathogenic nematodes against larval *Carpophilus* spp. and microbial-based insecticides or synthetic insecticides against adult *Carpophilus* spp. for future use in peach orchards.

Materials and Methods

Insects. A colony of *Carpophilus* spp. was established from approximately 100 beetles collected from an unidentified pear cultivar, *Pyrus pyrifolia* (Burman f.) Nakai. Beetles were housed in a 19.0 × 13.5 × 9.5-cm plastic container with a vented lid (Pioneer Plastics, Dixon, KY). A moist layer of autoclaved potting soil, 2.5 cm deep, lined the bottom of the container. Halved, pears (*Pyrus communis* L.) purchased in a local store were placed on the soil for food as needed. These containers were kept in an environmental chamber (Model I-36VL, Percival Scientific, Inc., Perry, IA) at 27 ± 1°C and a 14:10 light:dark (L:D)-h photoperiod. Adults fed and oviposited on the pears. Larvae fed on the pears and pupated in the

soil. As the population increased, more containers were used. When adults or late instars were needed for experiments, they were collected from the pear halves.

Entomopathogenic nematodes. For the experiments, entomopathogenic nematodes (*Heterorhabditis bacteriophora* Vs strain, *H. indica* HOM1 strain, *H. megidis* UK211 strain, *Steinernema carpocapsae* All strain, *S. feltiae* Sn strain, and *S. riobrave* 355 strain) were reared on last instar *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) at 25°C according to procedures described in Shapiro-Illan et al. (2016). All of the nematode species tested in our study are currently available commercially. *Galleria mellonella* larvae were obtained from Webster's Waxworms (Webster, WI). After harvesting the nematodes, they were aerated and kept at 13°C for ≤ 2 weeks before being used in experiments.

Microbial-based and synthetic insecticides. Microbial-based insecticides used in this study included Grandevo® and Venerate™ bioinsecticides (Marrone BioInnovations, Davis, CA) (Table 1). Synthetic insecticides used in this study included thiamethoxam (Actara®, Syngenta Crop Protection, LLC, Greensboro, NC), acetamiprid (Assail® 30SG, United Phosphorous, Inc., King of Prussia, PA), indoxacarb (Avaunt®, E.I. du Pont de Nemours and Company, Wilmington, DE), β -cyfluthrin (Baythroid®XL, Bayer CropScience LP, Research Triangle Park, NC), clothianidin (Belay®, Valent U.S.A. Corporation, Walnut Creek, CA), carbaryl (Carbaryl 4L, Loveland Products, Inc., Greeley, CO), chlorantraniliprole (Coragen®, E.I. du Pont de Nemours and Company, Wilmington, DE), fenpropathrin (Danitol® 2.4EC, Valent U.S.A. Corporation, Walnut Creek, CA), spinetoram (Delegate® WG, Dow AgroSciences, LLC, Indianapolis, IN), phosmet (Imidan® 70-W, Gowan Company, Yuma, AZ), malathion (Malathion 5EC, Drexel Chemical Company, Memphis, TN), and imidacloprid (MANA Alias® 4F, Makhteshim Agan of North America, Inc., Raleigh, NC) (Table 1). All treatments were prepared in 50-ml total volume at rates equivalent to using 935.4 l/ha, as commonly used by peach growers using airblast sprayers in southeastern orchards (Table 1).

Assays using entomopathogenic nematodes. Infective juvenile nematodes of the previously described entomopathogenic nematode species were assayed twice in parallel against late instar *Carpophilus* spp. in 30-ml portion cups (Comet, WNA Inc. division of The Waddington Group, Covington, KY). These cups were filled with 10 g of sterile soil classified as a loamy sand (80:16:4 [sand:silt:clay]; pH, 6.1). The moisture content of the soil was brought to field capacity (17%) by adding 1 ml of nematodes in solution and 700 μ l of water. Each nematode species was tested at two rates: 1,000 and 2,000 infective juveniles. A small piece of apple fruit was partially submerged into the soil and five larvae were added. This assay was completed using five replicates of each treatment with five larvae in each treatment. A nontreated control was included. Cups were placed on trays with the treatments randomly arranged within each of the five replicates. Replicates were housed in darkness at 25°C within an environmental incubator. Based on preliminary testing (to determine optimum duration of the assay, unpublished data), the cups were sampled for larvae 7-d postinoculation. Soil was removed from the cup and sifted, and the piece of apple was examined for the presence of any larvae. Larvae were recorded as alive or dead. The entire experiment was repeated once in time (i.e., two full trials were completed).

Microbial-based and synthetic insecticide assays. Three different approaches were used to assay insecticide treatments against adult *Carpophilus* spp. The

Table 1. Microbial-based and synthetic insecticides used in assays against adult *Carpophilus* spp.

IRAC MoA Classification ^a	Class	Active Ingredient(s)	Trade Name	Rate of Product (ha ⁻¹)	Active Ingredient (L ⁻¹)
1A	Carbamate	Carbaryl	Carbaryl 4L	7.02 l	7.5 ml
1B	Organophosphate	Phosmet	Imidan 70-W	3.36 kg	3.6 g
3A	Pyrethroid	Malathion β-cyfluthrin	Malathion 5EC BaythroidXL	2.34 l 204.62 ml	2.5 ml 220 µl
4A	Neonicotinoid	Fenpropathrin Clothianidin Thiamethoxam	Danitol 2.4EC Belay Actara	804 ml 435 ml 385.29 g	860 µl 470 µl 0.41 g
5	Spinosyn	Imidacloprid Spinetoram ^b	MANA Alias 4F DelegateWG	233.85 ml 490.37 g	250 µl 0.52 g
22A	Oxadiazine	Indoxacarb	Avaunt	420.32 g	0.45 g
28	Diamide	Chlorantraniliprole	Coragen	525.4 g	0.56 g
N/A ^c	N/A	<i>C. subsugae</i> ^c	Grandevo	3.36 kg	3.6 g
N/A	N/A	<i>Burkholderia</i> spp. ^d	Venerate	18.71 l	20 ml

^a IRAC, Insecticide Resistance Action Committee; MoA, mode of action.

^b Chemically modified spinosyns J and L.

^c N/A, not applicable.

^d *Chromobacterium subsugae* strain PRAA4-1 and spent fermentation media.

^e *Burkholderia* spp. strain A396 cells and spent fermentation media.

first was an application of the treatment to filter paper, it was allowed to dry, and then adults were added. The second approach used a plug of peeled pear fruit dipped into the treatment, and it was allowed to dry and placed in a petri dish, and then adults were added. The third was done similarly when a green peach fruit was dipped into the treatment, allowed to dry, and placed in a cup, and then adults added.

The first approach was done using two experiments. For experiment 1, treatments were applied to filter paper (Fisherbrand™ P8 Grade, Fisher Scientific, Pittsburgh, PA) by using an autoloader Potter spray tower (Burkard Scientific, Ltd., Uxbridge, UK) set at 0.35 kg/cm² to deliver 2 ml of treatment, followed by a 5-s settling time. For the second experiment, 250 µl of treatment was pipetted directly onto the filter paper, ensuring that all of the filter paper was wetted. In both trials, the filter paper was allowed to air dry and then placed into the lid of a clean petri dish (6-cm diameter). Five adult *Carpophilus* spp. were added to the petri dish bottom and covered with the lid (housing the filter paper), and then the dish was inverted so the filter paper would be on the bottom. Each treatment was replicated four times, with treatments randomized within replicates. Treatments were held in an environmental chamber at 25 ± 1°C and a 14:10 (L:D)-h photoperiod. Humidity in the environmental chamber was increased by adding food trays filled with water to the bottom of the chamber and using paper towels as wicks. Beetles were examined after 72 h by recording the number that were considered incapable of feeding (i.e., moribund + dead). Moribund beetles were incapable of righting themselves when turned over.

The second approach was conducted using two trials in one experiment. In each trial, a 0.5-cm diameter cork borer was used to remove plugs from a store-bought pear fruit that had been washed. The plugs were then cut into 1-cm-long sections and no peel was ever included. A single section of pear was dipped into a treatment, added to a 30-ml portion cup (Comet, WNA Inc. division of The Waddington Group), and allowed to dry for 1 h. After the pear dried, five adult *Carpophilus* spp. were added to the cup and a lid was placed on each cup. Each treatment was replicated four times in the first trial and three times in the second trial, with treatments randomized within replicates. Treatments were held in an environmental chamber at 25 ± 1°C and a 14:10 (L:D)-h photoperiod, and beetles were examined after 72 h by recording the number that were nonfeeding.

The third approach was conducted using two trials of the same experiment, performed concurrently from 16–20 July 2015. Small, green peach fruit (3.3 ± 0.1 cm from pedicel to tip and a circumference of 6.2 ± 0.1 cm) were picked, taken to the laboratory, and each dipped in a treatment. Dipped fruit were placed on filter paper and allowed to dry overnight. Peaches were then placed in a cup (266 ml; Eco Products®, Boulder, CO), five adult *Carpophilus* spp. were added, and a lid was placed on each cup. Each treatment was replicated four times, with treatments randomized within replicates. Treatments were held in an environmental chamber at 25 ± 1°C and a 14:10 (L:D)-h photoperiod, and beetles were examined after 72 h by recording the number that were nonfeeding (i.e., moribund + dead).

Statistical analyses. The cumulative percentage survival of larval *Carpophilus* spp. at 7 d after inoculation with the different entomopathogenic nematodes was arcsine transformed (Zar 1999) and subjected to analysis of variance (ANOVA) by using PROC GLM (SAS 2002). Data from nematode experiments that were

repeated in time were combined, and variation among trials was accounted for as a block effect. Mean separation was done using Tukey's Honestly Significant Difference (HSD) test when $P < 0.05$. When adult *Carpophilus* spp. were assayed using microbial-based and synthetic insecticides, numbers of nonfeeding adults were square-root transformed and subjected to one-way ANOVA for the two different trials when adults were exposed to treated filter paper. When adults were provided a plug of treated pear, numbers of nonfeeding adults were square root transformed for each trial and then subjected to one-way ANOVA. For the assay using treated peach fruit, both trials were combined because no interaction between trials was detected. Numbers of nonfeeding adults were square root transformed and then subjected to one-way ANOVA. For all experiments and trials using microbial-based and synthetic insecticides, if a significant treatment effect was detected ($P < 0.05$), mean separation was completed using Tukey's HSD test (SAS 2014).

Results

Carpophilus spp. larvae were susceptible to all entomopathogenic nematode species and strains tested. Data from both rates of application (1,000 and 2,000 infective juveniles) were combined because there was no treatment \times rate interaction ($P = 0.9359$). Data 7 d after treatment always resulted in significantly lower survival than nontreated larvae ($F = 28.36$; $df = 6, 116$; $P < 0.0001$). Within the entomopathogenic nematodes, survival was lower for *S. riobrave* and *H. megidis* than for *H. bacteriophora* and *H. indica*. Also, survival of larvae treated with *S. carpocapsae* was lower than those treated with *H. bacteriophora* (Fig. 1).

Applying insecticides (microbial-based and synthetic) to filter paper and allowing treatments to dry had a significant effect on the number of *Carpophilus* spp. adults rated as nonfeeding 3 d later when applied by a Potter spray tower ($F = 10.48$; $df = 14, 41$; $P < 0.0001$) and by a micropipette ($F = 8.45$; $df = 14, 42$; $P < 0.0001$). When treatments were applied via the spray tower, a significantly higher number of nonfeeding beetles were recorded from the synthetic insecticides indoxacarb and phosmet than all other treatments except spinetoram (Fig. 2A). In fact, significantly more nonfeeding beetles were also recorded from the spinetoram treatment than from the control and imidacloprid treatments. No other treatments differed significantly from the control, including the microbial-based insecticides. Similarly, when treatments were applied to filter paper via a micropipette, more beetles were recorded as nonfeeding when exposed to phosmet than all other treatments, except indoxacarb and β -cyfluthrin (Fig. 2B). Additionally, except for these three treatments, none of the other treatments were significantly different from the nontreated control. In fact, no mortality was recorded for chlorantranilprole, imidacloprid, or the microbial-based products derived from the metabolites of *C. subsugae* and *Burkholderia* spp.

Dipping pear plugs into the microbial-based and synthetic insecticides, allowing the plugs to dry, and then exposing beetles to them had a significant effect on the number of nonfeeding beetles 3 d later for each of the two trials ($F = 48.34$; $df = 14, 42$; $P < 0.0001$ and $F = 42.45$; $df = 14, 42$; $P < 0.0001$, respectively). During the first

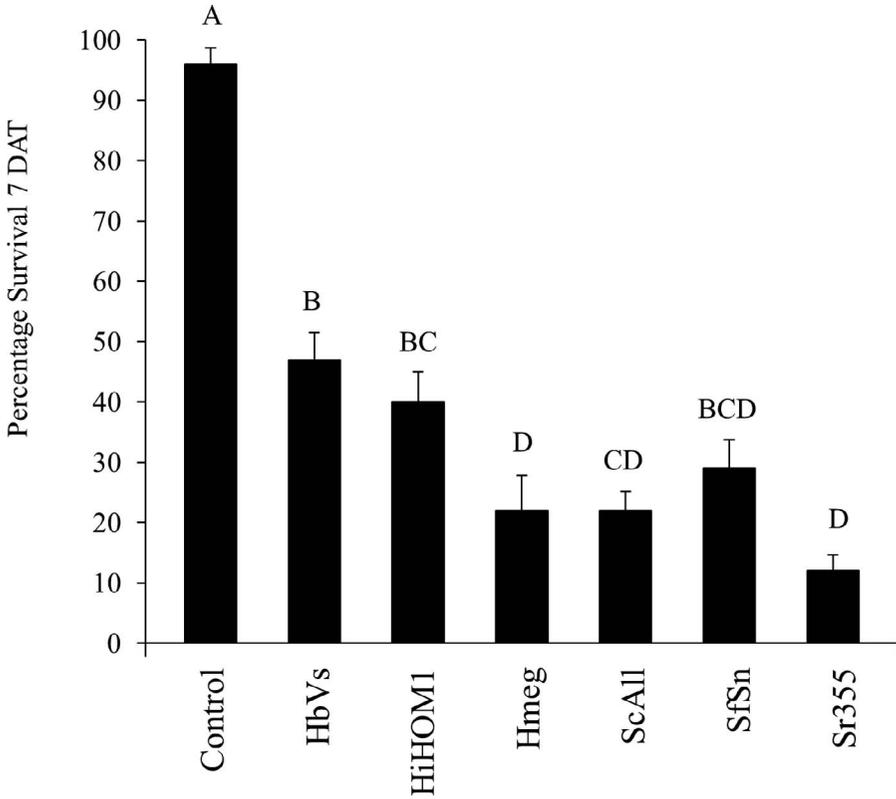


Fig. 1. Percentage survival of late instar *Carpophilus* spp. in soil cups 7 d after treatment with entomopathogenic nematodes. Different letters above columns indicate a significant difference ($P < 0.05$). HBVs, *Heterorhabditis bacteriophora* Vs strain; HiHOM1, *Heterorhabditis indica* HOM1 strain; Hmeg, *Heterorhabditis megidis*; ScAll, *Steinernema carpocapsae* All strain; SfSn, *Steinernema feltiae* Sn strain; Sr355, *Steinernema riobrave* 355 strain.

trial, all treatments resulted in a similar, significantly higher number of nonfeeding beetles than the control except for the similarly lower carbaryl, chlorantraniliprole, *C. subtsugae*, malathion, and *Burkholderia* spp. treatments (Fig. 3A). The synthetic insecticides chlorantraniliprole and malathion, except carbaryl, resulted in a significantly higher number of nonfeeding beetles than either of the microbial-based insecticides. During the second trial, all treatments except chlorantraniliprole, *C. subtsugae*, and *Burkholderia* spp. resulted in a significantly higher number of nonfeeding beetles (Fig. 3B). Again, the synthetic insecticides, except chlorantraniliprole, resulted in a significantly higher number of nonfeeding beetles than either of the microbial-based insecticides.

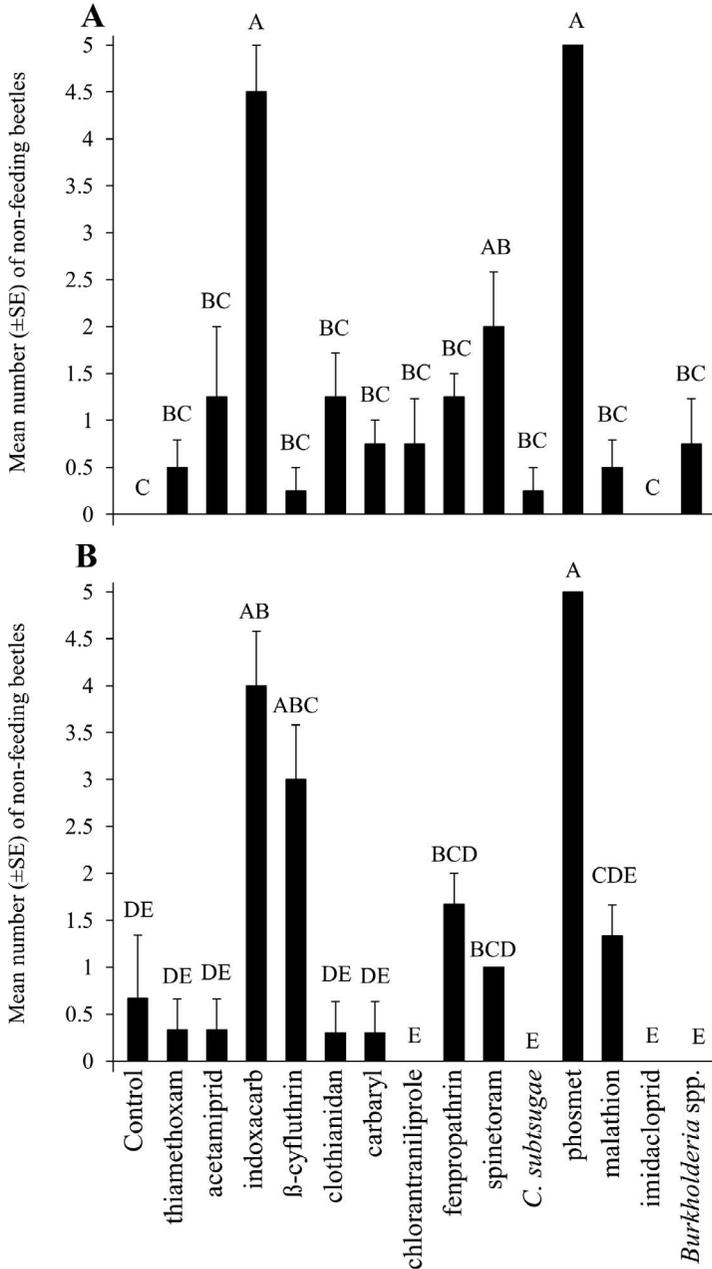


Fig. 2. Mean number of nonfeeding (dead + moribund) adult *Carphophilus* spp. after a 72-h exposure period to insecticide-treated filter paper using a (A) Potter spray tower or (B) micropipette to apply the treatment. Five beetles were used per treatment in each replicate. Different letters above columns indicate a significant difference between treatments ($P < 0.05$).

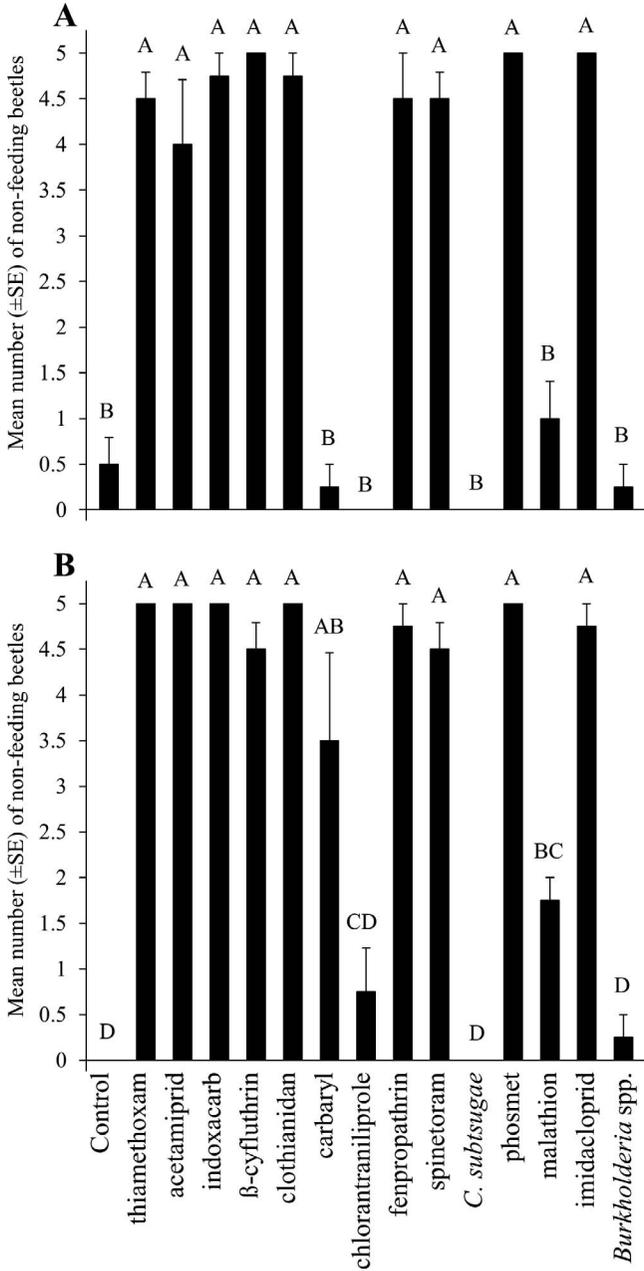


Fig. 3. Mean number of nonfeeding (dead + moribund) adult *Carpophilus* spp. after a 72-h exposure period to an insecticide-treated pear plug. (A) Trial 1, (B) Trial 2. Five beetles were used per treatment in each replicate. Within each trial, different letters above columns indicate a significant difference between treatments ($P < 0.05$).

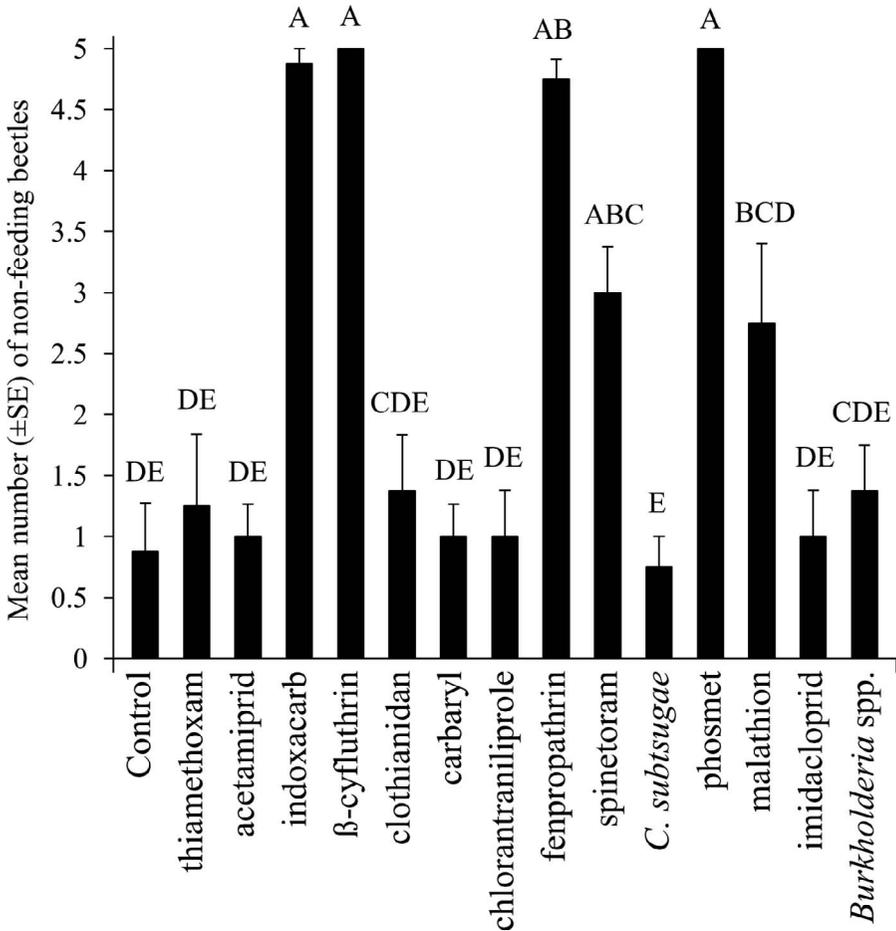


Fig. 4. Mean number of nonfeeding (dead + moribund) adult *Carpophilus* spp. after a 72-h exposure period to a green peach previously treated with one of the insecticide treatments. Five beetles were used per treatment in each replicate. Different letters above columns indicate a significant difference between treatments ($P < 0.05$).

Dipping green peach fruit into the microbial-based and synthetic insecticides, allowing the fruits to dry, and then exposing beetles to the fruit had a significant effect on the number of nonfeeding beetles 3 d later ($F = 17.48$; $df = 14, 87$; $P < 0.0001$). The indoxacarb, β -cyfluthrin, fenpropathrin, and phosmet treatments resulted in a significantly higher number of nonfeeding beetles than all other treatments except for spinetoram (Fig. 4). In fact, only these four treatments resulted in numbers of nonfeeding beetles that were significantly higher than the control.

Discussion

The results of this study show that different options are available for the management of larval and adult sap beetles. All entomopathogenic nematodes assayed in the current study were capable of decreasing the survival of late instar *Carpophilus* spp. in soil cups. This is consistent with the findings of Vega et al. (1994) and Glazer et al. (1999, 2007) that showed that larval *C. hemipterus* and *C. humeralis* are susceptible to entomopathogenic nematodes. We tested three of the nematode species used by Vega et al. (1994), and in both studies, virulence was highest for *S. riobrave*. In contrast, we showed virulence for *S. feltiae* and *S. carpocapsae*, whereas Vega et al. (1994) demonstrated that those species were pathogenic to *Carpophilus* spp.

Although *Carpophilus* spp. larvae typically feed within fruits, these fruits may occur on a plant above ground and may not be amenable to applications of entomopathogenic nematodes due to the nematodes' sensitivity to ultraviolet radiation and desiccation (Shapiro-Ilan et al. 2018). Additionally, larvae can occur within fallen fruits on the ground that likely provide refuge from soil-dwelling entomopathogenic nematodes. However, when larvae leave fruits to pupate in the soil, our results indicate that they are susceptible, and even highly susceptible, to all species and strains of the nematodes we tested, with *S. riobrave* and *H. megidis* appearing to show the most promise for control. Although soil-applied nematodes would be directed against the pest after crop damage had occurred, such an approach could be used to reduce numbers of emerging adults. Similarly, insect growth regulators have provided control of larval *Carpophilus* spp. (Blumberg 2008), but these were not included in the current study.

Our results from assaying adult *Carpophilus* spp. with microbial-based and synthetic insecticides revealed that exposure of adult beetles to the same treatment by different methods (i.e., on a dry surface or likely ingested) affected beetle survival. Indoxacarb and phosmet provided the highest and most consistent effect against the beetles when treatments were applied to filter paper. Indoxacarb and phosmet stood out again, as did β -cyfluthrin and fenpropathrin, when treatments were applied to green peach fruit. No obvious evidence of feeding on the fruit during the assay was detected. Differences in the surfaces of the filter paper and peach fruit possibly led to differences in the numbers of nonfeeding beetles between these two exposure methods. Peach fruit are generally pubescent, and this characteristic may have elevated the treatment above the fruit skin, allowing for more contact with beetles. If so, the nonpubescent, glabrous skin of nectarines could be expected to have similar results as with the filter paper. However, pear plugs dipped into the treatments were likely fed upon by the beetles and this exposure method led to even more treatments having significantly higher numbers of nonfeeding beetles. The insecticides thiamethoxam, acetamiprid, clothianidin, spinetoram, and imidacloprid went from little activity on dry surfaces to high activity against the beetles when they were exposed to the treated pear plug. It is likely that providing the beetles with a treated food source played a large role in this observed difference. Blumberg (2008) reports that the pyrethroids λ -cyhalothrin and bifenthrin and the neonicotinoids imidacloprid and thiacloprid were effective in controlling sap beetles attacking date palm. Nault and Speese (2000) found a significant reduction in the

number of adult *Carpophilus* spp. (but not larvae) attacking sweet corn (*Zea mays* L.) when treated with λ -cyhalothrin and β -cyfluthrin. The microbial-based insecticides, along with chlorantraniliprole and to a lesser extent carbaryl and malathion, did not have a significant effect on the sap beetles regardless of the exposure method. Miller and Williams (1983) achieved similarly poor results by using carbaryl against *C. hemipterus* when figs were dipped in treatments, similar to what was done with pear in the current study, and then exposed to beetles. In that same study, the authors report high mortality of *C. hemipterus* exposed to malathion that is in contrast to the results of the current study. Our results with malathion also contrast with those of Blumberg (2008) who states that malathion has provided the most satisfactory control of nitidulids attacking date palms in Israel.

Our results concerning the high level of activity of phosmet on dry surfaces against adult sap beetles strongly suggests that the decreased use of this insecticide in southeastern U.S. peach orchards can contribute to sap beetle damage, as previously documented in Australia (James et al. 2000). Although clothianidin has good activity against plum curculio and is used instead of phosmet at some points during the season, it is not likely to control adult sap beetles that contact it on dry surfaces. The greatest exposure of adult beetles to insecticides in peach orchards would be on surfaces such as bark, foliage, and fruit when applied as dilute applications via airblast orchard sprayers. Once inside a fruit, beetles would not be exposed to prior or future insecticide applications, unless they moved out of the fruit. This indicates that the efficacy of insecticides used to manage sap beetle adults should be considered when applied to surfaces that the beetle will contact but not feed upon. Additionally, treating the orchard floor with entomopathogenic nematodes prior to when larvae leave fruit to pupate in the soil could alleviate populations building up in orchards. Field trials confirming these laboratory results are needed.

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