Entomology

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Section Editor

Feeding Preference of Southern Pine Sawyer Beetle on Four Species of Pine

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Index Words: choice tests, feeding behavior, *Monochamus titillator*, pine wilt disease

Significance to Industry: Pines (*Pinus* L.) are important to the lumber, conservation, and nursery/landscape industries. Although not native to Kansas, pines are fast growing and adaptable to various growing conditions making pines highly valuable tree species for Kansas. These qualities have led to an increase in the use of Scots (*Pinus sylvestris*) and Austrian (*P. nigra*) pines in Christmas tree and windbreak plantings as well as widespread use in Kansas landscapes. In the last 30 years, Kansas has experienced an increase in the death of trees and due to the spread of pine wilt, a fatal disease caused by the interaction between pinewood nematode (*Bursaphelenchus xylophilus*) and pine sawyer beetle (*Monochamus* spp.) (1). Choice feeding preferences of the southern pine sawyer beetle (SPSB), *Monochamus titillator*, were evaluated using four species of pine as a preliminary investigation to determine the potential of pine wilt tolerant/resistant pine species. The pine species selected for this study consisted of one exotic species; *Pinus sylvestris* (Scots pine) and three native species; *P. ponderosa* (ponderosa pine), *P. strobiformis* (southwestern white pine), and *P. taeda* (loblolly pine). Species were selected based on foresters' perceived disease susceptibility. Scots pine is widely recognized as the most susceptible across the region whereas, native pines are generally considered resistant unless otherwise stressed. The objective of this study was to determine if SPSB feeding preference partially explains tree species susceptibility to pine wilt.

Nature of Work: In April, 2011, trunk sections and main lateral branches from three Scots pines previously confirmed to have died from pine wilt were placed into 162 L (42.8 gal) translucent, polyethylene containers or a screened enclosure prior to adult SPSB emergence. No lateral shoots or needles were placed in emergence containers to prevent beetle feeding upon emergence. SPSB emergence was observed approximately four weeks later in May, 2011. Each morning, newly emerged beetles (less than 24 hrs. old) were placed into 90 L (23.8 gal) translucent polyethylene containers, which served as feeding arenas for the choice experiments. Each feeding arena contained one shoot of current season growth for four pine species (*Pinus sylvestris, P. ponderosa*, *P. strobiformis*, and *P. taeda*). Pine shoots were placed into 250 ml (8.5 oz.) flasks filled with tap water and sealed with parafilm to prevent the beetle from entering the water. There was 7 in (17.8 cm) of shoot length exposed above the parafilm for beetle feeding. One sample, each of the four species, was placed randomly in a quadrant of the feeding arena. Beetles were placed into the center of the feeding arena, sealed with a lid. Feeding arenas were arranged in a randomized complete block design with beetle emergence date as the blocking factor. The choice feeding trial consisted of 11 feeding arena replications. Beetles remained in the arena for 48 hours and were then collected for species identification and sex determination. Shoot samples were assessed for feeding site area and feeding area percent [(feeding site area ÷ total shoot surface area) × 100] using a leaf area scanner system (WinFolia, Regent Instruments Inc., Ottawa, Ontario). Data associated with feeding area and percent feeding data were transformed using a square-root transformation method and analyzed using the least square means approach with the PROC Mixed procedure in SAS (2).

Results and Discussion: Southern pine sawyer beetle, *Monochamus titillator*, was the only species recovered from the emergence containers with roughly 50% male and 50% female specimens. Feeding area and percent feeding data revealed that *P. sylvestris*, *P. ponderosa*, and *P. taeda* were preferred feeding hosts for SPSB while *P. strobiformis* was not preferred (Table 1). SPSB preferred two of the three native species (*P. ponderosa* and *P. taeda*) and the one exotic species (*P. sylvestris*). Based on the results, SPSB feeds on native pine species as well as exotic pine species. The analysis confirmed visual observations for beetle preference, which were used to design a no-choice feeding assay for SPSB with *P. strobiformis* in June 2011 (data not presented). Preliminary results from both the choice and no-choice studies will be used for further evaluations determining pinewood nematode susceptibility of evaluated pines to pine wilt. Further investigations to assess pine sawyer beetle feeding preferences and pinewood nematode pathogenicity may result in identifying pine wilt tolerant/resistant pines or other conifer selections for use as windbreaks, in Christmas tree production, and in landscapes.

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Table 1. Southern pine sawyer beetle, *Monochamus titillator*, preference for four species of pine based on feeding area and percent feeding area.

Pinus Species	Common Name	Mean Total Shoot Area (mm^2)	Mean Feeding Area (mm^2)[z]	Mean % Feeding Area[yx]
P. ponderosa	ponderosa pine	45.1 ± 2.5	6.5 **	15.4 **
P. taeda	loblolly pine	38.5 ± 2.3	7.4 ***	19.8 ***
P. strobiformis	southwestern white pine	38.1 ± 5.1	1.6	4.4
P. sylvestris	Scots pine	39.0 ± 2.3	4.8 *	14.4 *

[z]Least square estimated means for feeding area measured in square millimeters with WinFolia leaf area scanner, Regent Instruments Inc., Ottawa.

[y]Least square estimated means for feeding area percent determined by [(feeding area ÷ total shoot area) × 100]

[x]Asterisks refer to the probability of a greater t value for the estimated least square means: * = $0.10 > P \geq 0.05$; ** = $0.05 > P \geq 0.01$; *** = $P < 0.01$

Assessing the integrated pest management practices of Southeastern U.S. ornamental nursery operations

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Significance to Industry: Growers can compare their current IPM practices with those used by the industry in the Southeast U.S. Improvements in scouting techniques, monitoring devices, and other pest emergence alerts are needed across all segments of nursery growers indicated by this survey. Researchers and Cooperative Extension agents can use the results to direct future research and teaching projects to obtain these goals.

Nature of work: The Southern Nursery IPM working group (SNIPM) formed in 2009 to stimulate regional progress in improving IPM in nursery crop production (1). SNIPM developed and distributed a survey in 2009, based on Sellmer et al. (2) to commercial growers of woody ornamental plants in Georgia, Kentucky, North Carolina, South Carolina, and Tennessee. The principal objectives of the survey were to:

1. Assess the pest management practices currently used by ornamental nursery growers in the Southeastern U.S.
2. Identify critical areas of instructional and outreach needs for education and research related to ornamental nursery IPM.
3. Gain grower input relative to ornamental nursery growers' perceived best methods of receiving information about nursery IPM practices.

Respondents answered questions about monitoring practices for insects, diseases, and weeds; prevention techniques; intervention decisions; concerns about IPM; and educational opportunities. A total of 178 surveys were completed and 124 surveys were analyzed. Survey respondents were categorized into three groups, similarly to Sellmer et al. (2), based on IPM knowledge and pest management practices adopted.

Results and Discussion: Respondents that our analysis clustered into Group 1 used IPM practices more frequently than other groups at various levels of the IPM continuum. For example, they were more likely to employ preventative practices such as sanitizing clippers, and pots, quarantining incoming plants, and scheduled applications of preemergence herbicides over the nursery to reduce pest problems in the future. Likewise, they were more likely to scout pests using a standardized sampling plan and monitor pests using sticky cards and permanent records rather than wait for plant damage to appear before scouting. Moreover, this group submitted more samples to a diagnostic clinic to determine pest identification and used the recommendations of the diagnostic clinic in their decision making process to intervene. When control was necessary, respondents in G1 were more likely than the other groups to select reduced risk pesticides and employ them in a more judicious manner such as spot treating small areas. These practices reflect a greater understanding and appreciation of the potential benefits of IPM for the health of their business, workers, and the environment.

Unfortunately G1 respondents represented just 8 percent of all participants and most growers surveyed fell into Group 2 (32%) or Group 3 (60%). Despite differences in stated action and philosophy, many practices employed by G2 growers overlapped with those classified as G1. Respondents in G2 utilized important components of IPM, for example, phenology of host plants, growing degree days, identifying natural enemies, and keeping records of monitoring, but did so less consistently. Businesses employing respondents in G2 had similar gross sales as those in G1, but employed fewer workers. Even though fewer employees may mean less emphasis on IPM, G2 did believe that IPM practices allowed labor to be used more efficiently at their nursery. This is good news, because G2 was also most receptive to extension training, which, combined with their relatively high appreciation for IPM, makes them the group for which extension agents could affect the most change.

G3 respondents generally used IPM tactics much less often and had a worse impression about anticipated benefits from adopting IPM than those in G1. This contrast was most apparent in prevention, scouting and monitoring techniques and sources to retrieve information. Additionally, businesses employing respondents of G3 had been operating for less time, with fewer employees, and had lower gross revenues compared to G1 and G2. Those in G3, however, shared a similar affinity for information and opportunity for learning as those in G2. The current snapshot suggests that by 2009, growers at many nurseries in the Southeastern U.S. have not adopted several principles of IPM, and that G3 respondents were more likely to be found at businesses that have not been in business as long as nurseries employing G1 and G2 respondents. Therefore, an emphasis by cooperative extension over time may improve knowledge about and adoption of more IPM principles by G3.

Nursery-based IPM involves decision-making processes that coordinate knowledge of pest biology, environmental information, and available technology in combination with biological, cultural, physical, and chemical tools in optimized approaches to reduce economic, health, and environmental risks.[5] Our survey validates our observation that virtually all growers use some nursery IPM components within their growing operation. Many growers, particularly those classified as G3, were unaware that many of the techniques they reported using, did in fact, constitute IPM practices. This highlights the necessity and ease of educating growers about IPM principles. Targeting G3 and G2 respondents with educational programming may provide a high impact yield for IPM education programming.

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Cottony Cushion Scale Management

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Index words: *Icerya purchasi*, *Nandina* spp., reduced risk insecticides

Significance to Industry: We investigated the efficacy of insecticide applications for control of Cottony Cushion Scale, *Icerya purchasi* Maskell, in nursery crops. This is significant to the industry because scale in general have been identified as one of the most important and hard to control pests of nursery crops (1, 4). We found that all the insecticides tested provided good control of cottony cushion scale adults and nymphs. Demonstrating good efficacy of several products with different modes of action provides growers with options for insecticide rotation programs or to select products that could target multiple pests at once.

Nature of Work: Cottony cushion scale is an important pest of *Nandina* spp., *Euonymus* spp. and other nursery crops. Cottony cushion scale damage plants by feeding on phloem which reduces plant growth and survival. In addition, cottony cushion scale produce copious honeydew and leave cottony white debris on plant leaves and stems. These products further reduce the aesthetic and monetary value of nursery plants. A number of insecticides have been used in citrus production to manage cottony cushion scale including organophosphates, insect growth regulators, and neonicotinoids (2, 3). Very little research has investigated management of cottony cushion scale on ornamentals and no research in ornamentals has tested an assortment of EPA-classified reduced risk insecticides that could be used instead of organophosphates or pyrethroids.

This work was conducted at North Carolina State University using harbor dwarf *Nandina* in 1.5 gallon containers. The experiment had 12 treatments (Table 1) with 6 replicates each. Plants were arranged in a randomized complete block design on weed cloth. On 1 July 2011 cottony cushion scale ovisacs were collected from nearby infestations on *Fatsia* spp. and *Euonymus* spp. plants growing in the campus landscape. Two ovisacs were pinned to the stem of each plant. This procedure was repeated every week for three weeks. On 2 August 2011 we conducted a pre-count by collecting 2 randomly selected, fully expanded leaves from each plant and inspecting them under a dissecting scope to count live adult and nymphal scale. Based on this pre-count, plants were blocked by scale density and randomly assigned to insecticide treatments.

Insecticide applications were made on 4 August 2011. Foliar treatments were applied using a CO_2 powered backpack sprayer fitted with a single spraying Systems D2-33 full-cone nozzle at 60 psi delivering 12.5 gpa. Drenches were applied using 6 oz of formulated solution. Granular products were applied by sprinkling the material evenly over the media surface. TriStar 30SG was reapplied 14 DAT (18 August) and Distance was reapplied 21 DAT (25 August). Post counts were made 7, 14, 28, and 75 DAT using the same procedure as pre-counts. Data were analyzed using ANOVA in ARM v. 8.

Results and Discussion: All products reduced the abundance of cottony cushion scale nymphs on *Nandina* plants in this trial (Table 1). This information provides growers with insecticides from several IRAQ mode of action categories in order to develop insecticides rotations. In addition, the neonicotinoids, insect growth regulators, and other products that are generally less toxic to natural enemies than organophosphates and pyrethroids. In is important to note that although direct toxicity to natural enemies is than that of organophosphates and pyrethroids, insect growth regulators and neonicotinoids can reduce natural enemy abundance and efficacy by reducing host abundance and via sub-lethal or slow acting effects (2, 3, 5).

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Table 1. The average number of cottony cushion scale nymphs per leaf on plants treated with different insecticides. Treatments with different letters within a column are significantly (P<0.05) different.

No.	Name	Rate	Pre-count	7DAT	14DAT	28DAT	75DAT
1	A16901B	5 oz/100 gal	8.8 a	1.5 b	1.0 b	1.2 b	0.0 b
2	Distance	12 oz/100 gal	11.0 a	0.7 b	0.3 b	0.7 b	0.0 b
3	Flagship 25 WG	0.5 g/plant	10.7 a	1.2 b	0.5 b	0.8 b	0.0 b
4	Flagship G	30 g/plant	8.3 a	0.0 b	0.0 b	0.2 b	0.0 b
5	Kontos	3.4 oz/100 gal	8.7 a	1.5 b	1.7 b	1.2 b	0.0 b
6	NNI-0101	18 oz/100 gal	9.8 a	2.8 b	2.2 b	2.5 b	0.0 b
7	Safari 20 SG	24 oz/100 gal	11.0 a	0.3 b	0.2 b	0.0 b	0.0 b
8	Safari 2 G	2.6 g/plant	11.0 a	0.3 b	0.0 b	0.0 b	0.0 b
9	Talus 70 DF	14 oz/100 gal	8.5 a	1.7 b	0.7 b	0.3 b	0.0 b
10	TriStar 30 SG	8 oz/100 gal	9.5 a	0.0 b	0.0 b	0.0 b	0.0 b
11	Horticultural Oil	0.5 oz/gal	9.0 a	3.7 b	3.0 b	2.5 b	0.0 b
12	Untreated	--	9.7 a	14.5 a	16.7 a	12.5 a	1.3 a
		F =	0.8	12.9	16.2	7.9	4.7
		P =	0.643	0.001	0.001	0.001	0.001

Effect of Contact and Systemic Insecticides Used for Scale Pests on Beneficial Insects

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Index Words: natural enemies, nursery crops, ornamental plants, production, sustainability

Significance to the Industry: Systemic insecticides offer nursery crop producers an alternative to broad spectrum, contact insecticides. Systemic insecticides are particularly useful for insects like scales that can be difficult to cover thoroughly with pesticides, especially on densely-branched plants. Identification of insecticides that help conserve beneficial predators and parasites can help growers achieve greater pest control with less insecticide use, thereby reducing production and management costs.

Nature of Work: Insect pests cause significant economic losses to nursery crops. North Carolina's green industry reported $91 million in annual losses due to insect pests and plant diseases (9). Because consumers are expected to have low acceptance of plant damage (6, 7) insecticides are perceived as necessary to control pests throughout nursery crop production cycles (3). Contact insecticides can be broad spectrum, and because they are applied to thoroughly cover the plant and pests, can cause non-target losses of natural enemies that would otherwise provide additional insect control. In addition, some contact insecticides can exacerbate arthropod pests of ornamental plants by causing an outbreak of secondary pests (5, 10).

Systemic insecticides offer selective pest control largely by limiting insecticide exposure to pest insects (i.e., pests feeding on host plants). In a nursery system however, beneficial insects can be exposed to systemic spray and drench residues, as well as poisoning by feeding on pesticide-exposed prey. For example, imidacloprid increased spider mite outbreaks on elm trees by poisoning natural enemies and increasing spider mite fecundity (11). Experiments in various production systems have shown a range of effects of systemic insecticides on beneficial insects. Efforts to control brown planthoppers, *Nilapabata lugens*, using systemic pymetrozine did not effect *Agelena difficilis* spiders, but was moderately toxic to another natural enemy plant bug, *Cyrtorhina lividipennis* (4). Acephate was the least toxic aphicide to predators and parasites in a study of 10 contact and systemic insecticides (1). Bruck et al. (2) found that spirotetramat, a xylem- and phloem-mobile insecticide, had low antagonistic effects with natural enemies. However, imidacloprid was highly toxic to adult and larval 12-spotted ladybird beetle, *Coleomegilla maculata lengi*, a natural enemy of Colorado potato beetle *Leptinotarsa decemlineata* (8). The authors are unaware of research on the effect of systemic insecticide use during nursery production on natural enemies.

The objective of this research was to investigate the comparative effects of systemic and contact insecticides on natural enemies in a nursery production system in order to determine if systemic insecticides offer a more sustainable insecticide option.

Field Experiment: Systemic and contact insecticides were applied to field-grown trees in a nursery planting [systemic: imidacloprid (Marathon® II) and dinotefuran (Safari® 20 SG); contact: bifenthrin (Talstar® Select) and carbaryl (Sevin®SL)] and a water control on April 28, 2011. Insecticides were selected as either commonly used and/or recommended against scale pests of nursery crops. Imidacloprid was applied at 6 ml per dbh, and dinotefuran was applied at 0.126g per dbh (plants were 1/3" dbh), the drench rate for both products. Bifenthrin was applied at 40 fl. oz./per acre and carbaryl was applied at 1 qt. per 100 gallons. Rates for systemics were based on dbh guides on systemic pesticide labels and plants were estimated to be 4 ft^2 for bifenthrin. A test run with water prior to the experiment determined that 500 ml would cover upper and lower surfaces. For all pesticides, 500 ml per plant was sprayed on the upper and lower leaf surfaces using a CO_2 backbpack sprayer. For the systemics, 500ml was also drenched onto the base of the plant and within a 2-ft^2 area around the trunk using a plastic liter bottle with holes drilled in the lid. A total of 1L of product was applied to each systemic treatment plant. Leaves of plants receiving the systemics were sprayed with the backpack sprayer so that they were completely covered with pesticide to ensure that insects would be caged to a leaf with a comparable amount of pesticide residue and to achieve a worst-case scenario of treatment overspray. Prior to pesticide applications, one pitfall trap was installed 18-in. from the base of each plant.

Following pesticide application, cages containing beneficial insects were individually placed around a branch or leaf so that each treated tree had three cages. Each cage contained either 10 adult *Orius* (minute pirate bug), 10 *Aphidius* parasitic wasps, or 10 *Coleomegilla* lady beetles. Each cage had a 10 ml vial with wick of honey-water and glycerol solution (5% v/v) as a food source. Survival of caged beneficial insects was assessed every 48 hr following insecticide application through May 6, 2011. Simultaneously, pitfall trap collections measured presence and type of ground-dwelling arthropods every 48 hr through May 6, 2011, and thereafter on May 13, 2011 and May 24, 2011 (15 and 22 DAT). The experiment was a completely randomized design split-plot with sampling, insecticide is the whole plot (tree), and beneficial insect is the subplot. The experiment was conducted on two-year-old planting *Liriodendron tulipifera* seedlings. Plants selected for the study at the UT Forest in Morgan Co., Tenn. had approx. 1-in caliper diam. and were between 4.0 and 5.5 ft tall. *Aphidius* results are reported for 144 hr after application only.

Results and Discussion: There was a significant interaction of treatment and insect species for the first two data collection periods, *p*-value <0.0001. On April 30, 48 hr after pesticide application, carbaryl killed more lady beetles than bifenthrin and imidacloprid, which killed more beetles than either dinotefuran or water controls (Table 1). Significantly fewer *Orius* survived when imidacloprid or bifenthrin were applied (Table 2).

On May 2, 2011, after 96 hr post-pesticide applications, carbaryl, bifenthrin and imidacloprid-treated foliage yielded lower numbers of surviving lady beetles than either dinotefuran or water controls (Table 3). Fewer *Orius* were alive on bifenthrin-treated leaves than all other treatments and dinotefuran had a greater number of surviving insects than any other treatment, including water controls (Table 4). Because there were no apparent interactions 144 hr after application, May 4 data were pooled among insects. Fewer insects survived following exposure to imidacloprid, bifenthrin and carbaryl treatments than dinotefuran, which in turn had lower survival than insects exposed to water alone (Table 5).

For pitfall trap data when all beneficial insects were pooled, counts did not differ based on insecticide treatment, with the exception of 4 DAT when dinotefuran treatments yielded more beneficial insects than all other treatments, including controls (Figure 1). The total number of arthropods per pitfall trap varied by treatments except on 15 and 22 DAT (Figure 2). At 2 DAT dinotefuran treatments yielded more arthropods than either imidacloprid, bifenthrin or the controls. At 4 and 6 DAT, bifenthrin yielded fewer insects than all other treatments (except imidacloprid on 6 DAT). By 8 DAT, bifenthrin treatments had fewer arthropods than dinotefuran. There was no difference in number of all spiders in pitfall traps regardless of date (Figure 3). The total number of ants did not differ among treatments except on 4 DAT when dinotefuran treatments yielded more ants than any other treatment (Figure 4).

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Table 1. Number of surviving lady beetles on April 30, 2011 after 48 hr caged to trees sprayed with contact and systemic insecticides.

Insecticide	Lady beetle (ranked)
Imidacloprid	57.7 b
Dinotefuran	98.4 a
Bifenthrin	59.2 b
Carbaryl	18.5 c
Control	107.9 a

Table 2. Number of surviving *Orius* on April 30, 2011 after 48 hr caged to trees sprayed with contact and systemic insecticides.

Insecticide	*Orius* (ranked)
Imidacloprid	29.2 b
Safari	79.4 a
Talstar	38.8 b
Sevin	70.4 a
control	83.4 a

Table 3. Number of surviving lady beetles alive on May 2, 2011 after 96 hr caged to trees sprayed with contact and systemic insecticides.

Insecticide	Lady beetle (ranked)
Imidacloprid	55.6 b
Dinotefuran	67.9 a
Bifenthrin	49.1 b
Carbaryl	20.1 c
Control	66.1 a

Table 4. Number of surviving *Orius* on May 2, 2011 after 96 hr caged to trees sprayed with contact and systemic insecticides.

Insecticide	*Orius* (ranked)
Imidacloprid	24.6 b
Dinotefuran	35.4 a
Bifenthrin	4.9 c
Carbaryl	25.4 b
Control	21.1 b

Table 5. Number of insects surviving on May 4, 2011, after 144 hr caged to trees sprayed with contact and systemic insecticides, lady beetle, *Orius* and *Aphidius* pooled.

Insecticide	All insects (means)
Imidacloprid	4.3 c
Dinotefuran	6.1 b
Bifenthrin	3.1 c
Carbaryl	2.3 c
Control	7.7 a

p-value 0.0008

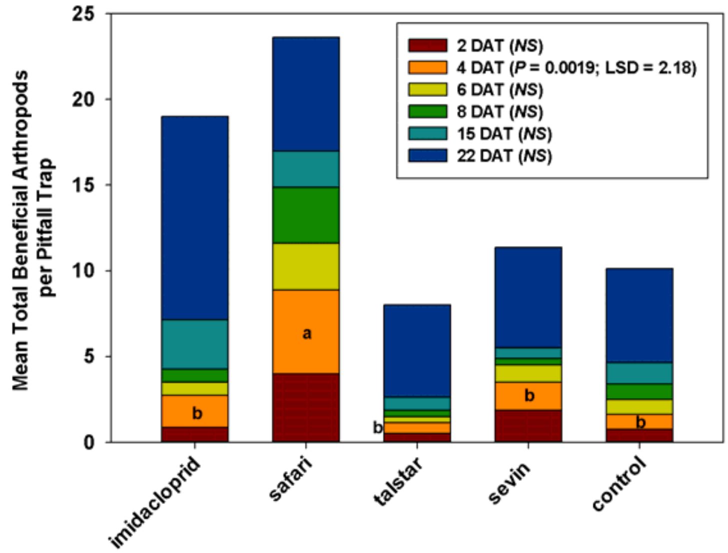

Figure 1. Mean total number of beneficial insects per pitfall trap by date after systemic and contact insecticide application.

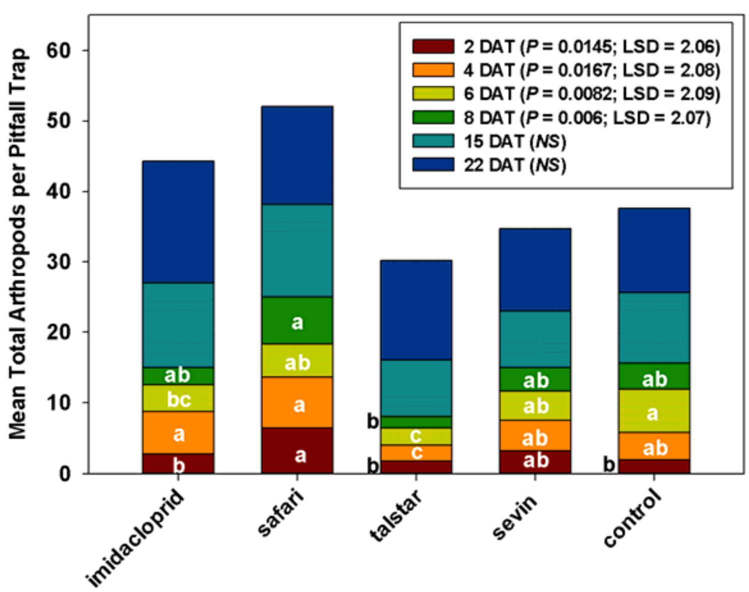

Figure 2. Mean total number of arthropods per pitfall trap by date after systemic or contact insecticide application

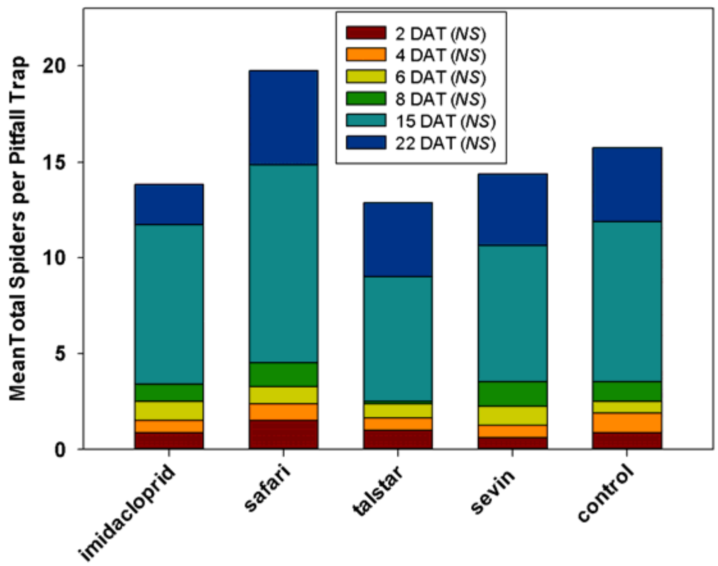

Figure 3. Mean total number of spiders per pitfall trap by date after systemic or contact insecticide application

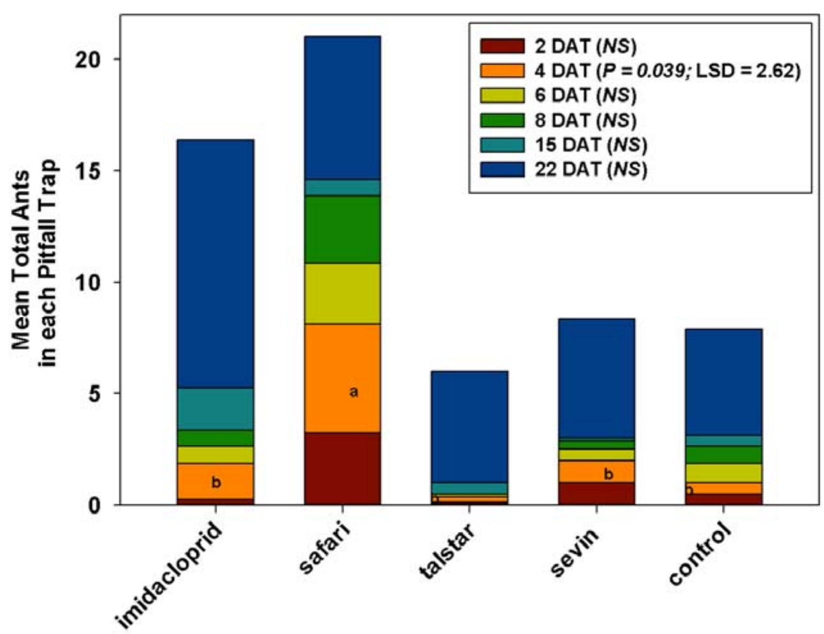

Figure 4. Mean total number of ants per pitfall trap by date after systemic or contact insecticide application

Volatile chemicals associated with host plants of the strawberry rootworm

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Significance to Industry: The strawberry rootworm (SRW), *Paria fragariae* Wilcox (Coleoptera: Chrysomelidae: Eumolpinae), is a primary threat to profitable production of azaleas and other containerized ornamental crops at nurseries throughout the Southeast (6). Due to its cryptic nature, detection of *P. fragariae* populations often occurs after plant foliage has been damaged. Properly timed early-season insecticide applications, when aided by a monitoring program utilizing sweep nets or trapping stations (12), can be critical to reducing potentially devastating late-season outbreaks (3).

Host plant volatiles have been widely reported to be attractive to chrysomelids, including the Chrysomeline Colorado potato beetle (11), Alticine flea beetles (4), and most notably the Galerucine cucumber beetles and corn rootworms (7, 10). While the use of plant volatiles in an IPM program can be particularly helpful with population monitoring efforts (8), it can also be part of a direct control measure. These control measures can be in the form of insect traps, commonly available for Japanese beetles (BICONET, Brentwood, TN); as part of a trap-crop strategy (11); or with biocontrol, whereby natural enemies cue in on plant volatiles that are naturally produced in response to herbivory (9).

The aim of this research is to identify volatile chemicals from a range of host plants utilized by *P. fragariae*. Our hypothesis is that these plants may have in common the compounds that assist *P. fragariae* with host recognition. If one or more of these volatile chemicals prove to have a strong-enough attraction, they could potentially be used to augment pest monitoring and control efforts at nurseries. Here we discuss our findings for plant volatiles identified from four host plants and two non-host plants of *P. fragariae*.

Nature of Work: Volatiles associated with four host plants: azalea [*Rhododendron indicum* (L.)], *Fragaria* (*Fragaria* x *ananassa* Duchesne), Virginia sweetspire (*Itea virginica* L.) and Chinese fringe flower (*Loropetalum chinense* Oliv.); and two non-host plants: Cleyera (*C. japonica* Thunb.) and Indian hawthorn [(*Rhaphiolepis indica*) L.] were identified using micro extraction. Stems from each plant roughly 12 cm in length were placed in 1-dram glass vials (Fisher Scientific, Pittsburgh, Pennsylvania, USA) filled with distilled water, the tops then sealed with Parafilm (Pechiney Plastic Packaging, Chicago, IL, USA). Falcon Petri dishes (Becton Dickinson Labware, Franklin Lakes, NJ, USA), 150 x 15 mm, were modified by melting a 0.6 mm hole through the side of both top and bottom dish, enabling the insertion of a rubber septa through the closed Petri dish. One vial with plant stem was placed into the modified Petri dish on one piece of 12.5 cm filter paper (Fisher Scientific, Pittsburgh, Pennsylvania, USA) and sealed with Parafilm, allowing plant volatiles to accumulate within.

Plant volatiles were extracted from each dish using solid-phase micro extraction (SPME) and identified by comparison of GC retention times and mass spectra to those of the standard chemicals. A SPME fiber (Supelco, St. Louis, MO) was inserted through the rubber septa into the sealed Petri dish and left for 30 minutes, and analyzed by GC-MS using an Hewlett-Packard 6890 Series GC System with a 5973 Mass Selective detector. Each plant species was tested using five treatments: a plant stem on its own; a plant stem with varying numbers of *P. fragariae* adults for analysis of the effects of natural herbivory; a plant stem with a simulated-herbivory test, where 10 holes were punched from the leaves using a 0.635 cm hole punch (McGill, Inc., Marengo, IL); *P. fragariae* with no plant stem; and no plant stem or *P. fragariae*, just a dish with a sealed vial of water and a filter paper.

Results and Discussion: There were no notable peaks identified from either control dish: beetles with no plant stems, and no beetles or plant stems. From the beetle control dish, we did however identify heptenone and geranyl acetone, both of which have been reported as beetle pheromone components (2, 5). These findings could prove useful in development of a lure for *P. fragariae*, especially when combined in formulation with a plant volatile (5).

From the plant stem tests (Fig. 1), we identified several leading volatiles, including caryophyllene, ocimene, allo-ocimene and amorphene. We also found that limonene was a volatile component of azalea, *Itea* and *Fragaria* (Table 1). *Cleyera* was a notable exception, producing alpha-pinene and kaurene-15 and -16.

After combining *P. fragariae* with the plant stems, we observed interesting changes in some of the plant volatile profiles (Table 2). In addition to caryophyllene, we also identified farnesene, ocimene and allo-ocimene from azalea. While the *Itea* profile did not change much, the addition of beetles onto *Fragaria* produced a whole range of new volatiles grouped closely together around the retention time of 4.6 to 5.0. *Loropetalum* also changed with the addition of beetles, as both ocimene and allo-ocimene were then identified.

The addition of beetles to the Petri dishes did not bring about as dramatic an effect as we had anticipated, so we attempted to simulate herbivory on the plants by punching 10 holes from the leaves of each cutting. From this series of tests we observed initially large peaks for hexanyl acetate and hexanol in each plant species, compounds that had not been present in any of our previous tests (Table 3). Over time, however, these peaks decreased in volume and the volatile profiles returned to the state we observed without holes punched. With azalea, for example, as levels of hexenol and hexanyl acetate decreased in tests run every 30 minutes, levels of caryophyllene and ocimene showed corresponding increases. This observation is validated by the findings of Agelopoulos et al. (1), whereby large amounts of hexenal and hexenol were produced within five minutes of damaging bean leaves. After ten minutes these compounds reached very low levels, and other volatiles like caryophyllene, farnesene and germacrene became predominent.

Known host plants for *P. fragariae* had similar volatiles identified from our tests. Ocimene was present in the beetle and hole-punch tests for azalea, *Itea*, *Fragaria* and *Loropetalum*. Despite the fact that we did not find hexanol or hexanyl acetate in the plant-with-beetle tests, it is possible that these compounds are produced by plants in response to herbivory, judging from their presence in all of our hole-punch plants. *P. fragariae* may not feed aggressively enough in our artificial laboratory setting to elicit this type of plant response. In any case, hexanol and hexanyl acetate were present in all of our damaged plant species, host and non-host alike. It was also interesting to observe that aside from these two compounds, *Cleyera* and *Rhaphiolepis* shared none of the plant volatiles in common with the other species. In fact, we identified four unique volatiles from *Cleyera*, suggesting very different plant chemistries.

We plan to determine any attraction for these plant volatiles by *P. fragariae*, using olfactometry and electro-antennagram bioassays. With this knowledge we can then develop a kairomone lure for SRW, making monitoring and control of this destructive pest easier and more cost effective at Southern nurseries.

Table 1. Plant volatiles identified from six plants, using SPME and GCMS.

Volatiles of Plants	**Azalea**	***Itea***	***Fragaria***	***Cleyera***
Caryophyllene	X	-	-	-
Ocimene	-	X	-	-
Allo-Ocimene	-	X	-	-
Limonene	X	X	X	-
Amorphene	-	X	-	-
Alpha-Pinene	-	-	-	X
Kaurene-15	-	-	-	X
Kaurene-16	-	-	-	X

Table 2. Plant volatiles identified from six plants with *P. fragariae*, using SPME and GCMS.

Volatiles of Plants with Beetles	**Azalea**	***Itea***	***Fragaria***	***Loropetalum***	***Cleyera***
Caryophyllene	X	-	-	-	-
Farnesene	X	-	-	-	-
Ocimene	X	X	X	X	-
Allo-Ocimene	X	X	-	X	-
Amorphene	-	-	X	-	-
Copaene	-	-	X	-	-
Cubebene	-	-	X	-	-
Germacrene	-	-	X	-	-
Muurolene	-	-	X	-	-
Cadinene	-	-	X	-	-
Palmitic Acid	-	-	X	-	-
Alpha-Pinene	-	-	X	-	X
Kaurene-15	-	-	-	-	X
Kaurene-16	-	-	-	-	X

Table 3. Plant volatiles identified from six plants with holes punched, using SPME and GCMS.

Volatiles of Plants with Holes	**Azalea**	***Itea***	***Fragaria***	***Loropetalum***	***Cleyera***	***Rhaphiolepis***
Caryophyllene	X	-	-	-	-	-
Ocimene	X	X	X	X	-	-
Allo-Ocimene	X	X	-	-	-	-
Hexanol	X	X	X	X	X	X
Hexanyl Acetate	X	X	X	X	X	X
Copaene	-	-	X	-	-	-
Cubebene	-	-	X	-	-	-
Germacrene	-	-	X	-	-	-
Muurolene	-	-	X	-	-	-
Cadinene	-	-	X	-	-	-
Palmitic Acid	-	-	X	-	-	-
Alpha-Pinene	-	-	-	-	X	-
Kaurene-15	-	-	-	-	X	-
Kaurene-16	-	-	-	-	X	-
Nonatriene	-	-	-	-	X	-

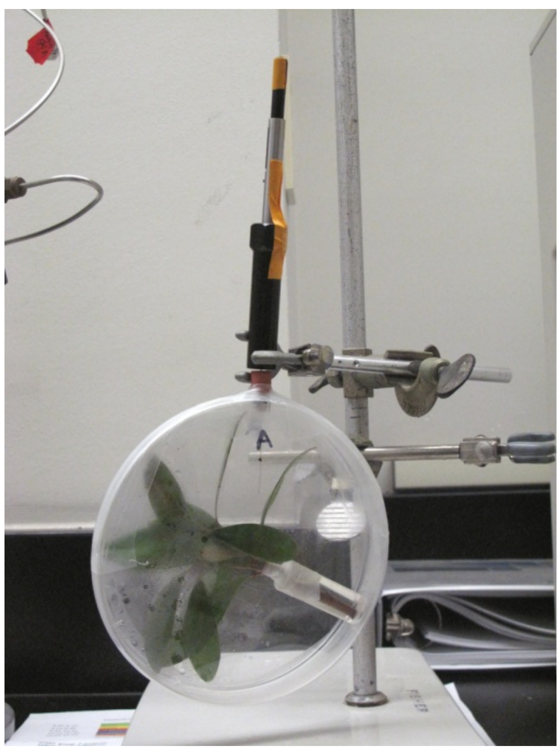

Figure 1. Modified Petri dish for headspace extraction of volatile chemicals using SPME.

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