Attachment C

Christmas Tree Promotion Board

Final Research Report

CTPB Project Number: 19-01-CAES

Project Title: Exploring Sustainable Management for Armored Scales in Christmas Tree Plantations

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Introduction

Each species of Christmas tree grown in the United States is susceptible to at least one, and sometimes several scale insect pests. Armored scales inject toxic saliva into the underside of the needles while feeding. The resulting severe yellowing and partial defoliation makes infested trees unsalable. Transport of infested trees across state lines is of regulatory concern for locations where these non-native scales have not been found, such as Florida and the Pacific Northwest. Therefore, presence of scales on infested trees has become an impediment to interstate marketing of trees. Because of their ability to reach high populations in Christmas tree plantations, the difficulty in reaching the scales with insecticides (because they may be found in interior vegetation on the undersides of needles), and their genetics (all traits are dominant in the haploid males), insecticide-dependent management programs are likely to fail due to insecticide resistance. Three subject areas to enhance sustainable management of armored scales were studied in this project: (1) testing insect pathogenic fungi for biological control, (2) screening alternatives to the currently favored bee-toxic insecticide dinotefuran for effectiveness against scale insects, and (3) preliminary tests of a banker plant strategy.

Methods

Biological control with insect pathogenic fungi.

Populations of elongate hemlock scale and hemlock scale were visually assessed for the presence of insect pathogenic fungi on eastern hemlock in Shenipsit State Forest and Penwood State Park (Somers and Bloomfield, respectively) in addition to cryptomeria scale on hemlock in Windsor and East Windsor and Fraser fir in East Windsor, CT, during March, 2020. Infected scales were brought to the laboratory, where the fungi were isolated on malt extract agar. Fungal isolates were inoculated under sterile conditions into one-quart mason jars containing moist, double-autoclaved wheat grain. Jars containing inoculated grain were shaken every 1 – 2 weeks to disperse the fungi, allowing full colonization of the grain.

Metarhiziopsis microspora was not observed in the field, and so a pure culture was obtained from the UAMH Centre for Global Microfungal Biodiversity, The Gage Research Institute, Toronto, Ontario, Canada. This isolate had originated from elongate hemlock scales infected with this fungus in Connecticut.

Colletotrichum fioriniae was obtained from numerous elongate hemlock scales and from multiple collection sites. However, it has recently been found to be a pervasive plant pathogen, and so it was not used for field experiments (Duke, 2020).

An unidentified fungus was found apparently infecting cryptomeria scales in East Windsor, CT (Fig. 1). Several fungi were isolated from these scales, with tentative identifications of a Cladosporium spp., a Fusarium spp., and a possible Metarhizium spp. As it was not known which, if any, of these species caused disease in the affected scales, each was grown as pure cultures on wheat grain.

On June 26, 2020, the contents of one jar each of the cultured fungi (*Metarhiziopsis microspora*, *Cladosporium* spp., *Fusarium* spp., and *Metarhizium* spp.), were mixed with deionized distilled water and blended for about 1 minute to generate mycelial fragments. In order to generate a sprayable mixture, the homogenized mixture was coarsely filtered through a 10-mesh screen. A commercial preparation of an insect pathogenic fungus (*Beauveria bassiana*, BotaniGard ES [10 mL in 2 L of spray mixture]) was included in the trial for comparison. The resultant mixture was either applied alone or in combination with 100 g of pure whey isolate (per 2 L of spray mixture), using a Stihl backpack mist blower sprayer (Stihl Model SR450, using flow setting No. 2) on a collaborating grower's farm in Coventry, CT. The spray mixture (2 L) was applied to five trees in a single plot per treatment, at a spray volume equivalent to 150 gallons per acre.

Four heavily scale-infested shoots were clipped from each treatment group of trees on Sept. 23, 2020 and brought to the laboratory. They were examined at 9 - 20× magnification with a dissecting microscope to detect fungal infections.

Chemical control

Two insecticide trials were conducted, one investigating basal bark sprays of systemic insecticides, and one comparing systemic insecticides when applied as foliar sprays to target the crawler stage of elongate hemlock scale.

<u>Basal bark spray experiment.</u> This experiment was designed as a 7 × 2 factorial design, with seven insecticide treatments, including the untreated check and a positive control (UpStar [bifenthrin] foliar spray). Five of the insecticide treatments were basal bark sprays applied either in the fall (Sept. 20, 2018) or spring (April 25, 2019), at a cooperating grower's planting in LaGrangeville, NY (Fig. 2). The trees had predominantly elongate hemlock scales and fewer cryptomeria scales. The basal bark spray was applied in 30 mL volume with a CO_2-pressurized research sprayer onto

approximately a 25 cm-wide band around the circumference at the base of each treated tree. There were six single-tree replicates in a randomized complete block design.

Table 1. Insecticide treatments to manage a mixed population of armored scales on Fraser fir in LaGrangeville, NY, 2018 – 2019.

1	Untreated check
2	Safari, basal spray, 0.32 g/tree, fall application
3	TriStar, basal spray, 1.93 mL/tree, fall application
4	TriStar, basal spray, 0.527 mL/tree, fall application
5	Aria, basal spray, 0.168 g/tree, fall application
6	Aria, basal spray, 0.084 g/tree, fall application
7	Upstar Gold, foliar spray, 6.4 mL in 3.8 L water, fall application
8	Untreated check
9	Safari, basal spray, 0.32 g/tree, spring application
10	TriStar, basal spray, 1.93 mL/tree, spring application
11	TriStar, basal spray, 0.527 mL/tree, spring application
12	Aria, basal spray, 0.168 g/tree, spring application
13	Aria, basal spray, 0.084 g/tree, spring application
14	UpStar Gold, foliar spray, 6.4 mL in 3.8 L water, spring application

Data were evaluated by collecting branches on Oct. 1, 2019, from which live scales per shoot were counted in the laboratory under a dissecting microscope.

<u>Crawler spray experiment.</u> Products compared in this trial are given in Table 2. Plots consisted of individual infested 5 – 7-foot-tall Fraser fir trees at a cooperating grower’s farm in Coventry, CT. The planting had exclusively elongate hemlock scales. The experiment was conducted as a seven-replicate completely randomized design. Trees were chosen so that there was always at least one unsprayed buffer tree between any two treated trees. Insecticides were applied as a timed, calibrated spray (70 gallons per acre, based upon the time used to spray each tree) with a backpack mist blower sprayer (Stihl Model SR450, using flow setting No. 2). Trees were sprayed on June 25, 2020, with temperatures about 85 °F, low wind and low relative humidity, and again on July 7, 2020, with temperatures about 75 °F, also with low wind and low relative humidity.

Samples from treated trees were cut on September 23, 2020. Three two-year-old shoots were taken, about 6 – 8 inches in length, equally spaced around the tree, from lower to mid-tree height. An additional shoot was taken from an upright interior shoot at a height of 4 to 5 feet. Samples were held in a plastic bag and refrigerated until the live and dead scales could be counted. Scales were counted between Sept. 24 and Oct. 1, 2020.

Table 2. Insecticide treatments applied as two foliar sprays to manage elongate hemlock scale on Fraser firs, Coventry, CT, 2020. Amounts of insecticides given in the table below were mixed in 1,620 mL; 180 mL was used to spray each tree. A spreader, Silwet L-77 (0.405 mL), was added to all treatments except for the water check.

1. Water check
2. Mainspring, 2.89 mL
3. TriStar, 4.57 mL
4. Aria, 0.74 g
5. Ventigra, 1.266 mL
6. Movento, 1.808 mL
7. Distance, 3.62 mL
8. Safari, 1.665 g
9. Azatin, 3.8 mL
10. Silwet L-77, 0.405 mL

To quantify insecticidal effects, 25 needles at the base of new growth were removed from each of the four shoots from each tree. An insect pin was used to flip the scale test for each scale found, to examine whether the underlying scale was alive (yellow, fleshy) or dead (brown, dry). A total of 400 needles were examined per tree to count live and dead scales.

Banker plants for biological control

Cuttings were taken from *Euonymus japonica* plants at the Connecticut Agricultural Experiment Station headquarters in New Haven, CT, in September, 2019, stuck in potting media after treatment with rooting hormone, and covered with plastic to prevent desiccation while rooting in the research greenhouse at the Valley Laboratory, Windsor, CT. Plants were grown from cuttings in order to avoid working with insecticide-treated plants obtained from nurseries. Successfully rooted cuttings were infested with euonymus scale by placing infested plant material on top of the plants in March, 2020.

Results

Biological control with insect pathogenic fungi.

No fungal infections were recovered from the inoculated trees.

Chemical control

The basal bark spray results (Table 3) demonstrated that Safari was the only product applied that significantly reduced the numbers of scales. Treatment timing had no statistically significant effect on scale populations, and so treatment means are averaged across both timings.

Table 3. Mean number of scales per shoot (backtransformed from log[x+1] values)

Treatment	Mean scales per shoot*
Untreated check	19.2 a
Safari	6.9 b
TriStar, low rate	16.1 a
TriStar, high rate	12.8 ab
Aria, low rate	24.9 a
Aria, high rate	16.5 a
Upstar Gold	16.0 a

*Means followed by the same letter are not statistically significantly different, Fisher's protected LSD test, p = 0.05. Each shoot from which scales were counted on new growth had approximately 70 needles per shoot.

The crawler spray experiment (Table 4) found three insecticides (Distance, TriStar, and Ventigra) that were equivalent to or more effective than Safari for suppressing elongate hemlock scale populations. Several interesting discoveries were made with this trial. First, acetamiprid (TriStar) was significantly more effective than any of the other insecticides. TriStar is about 300 times less toxic to honey bees than Safari, and so should be safer to pollinators than Safari if used as a foliar spray. Secondly, of two insect growth regulator insecticides tested, pyriproxyfen (Distance) was effective and azadirachtin (Azatin) was not. Third, of two products affecting the proprioception Ventigra was effective but Aria was not. The bidirectionally systemic insecticide Movento was mediocre, but it was statistically significantly different from the water and Silwet L-77 groups. As it has value for suppressing conifer root aphids, it may be useful for targeting armored scales, either when root aphid management is also required, or in combination with horticultural oil as a rotation material to prevent insecticide resistance.

Table 4. Percent mortality of elongate hemlock scales following two foliar sprays of insecticides. Proportion live scales were transformed using Excel's NORMSINV function, after adding 0.03 to all values. The percent mortality presented below were backtransformed to proportions, and then Abbott's correction for check mortality also applied.

Treatment	Percent mortality*	
Water check	40.6	bc
Silwet L-77	10.8	a
Azatin	56.3	cd
Distance	88.9	f
Mainspring	24.6	ab
Movento	65.2	de
Aria	23.9	ab
Ventigra	79.2	ef
Safari	83.3	f
TriStar	98.0	g

* Means followed by the same letter are not significantly different, Fisher's protected LSD test, $p < 0.05$.

Banker plants for biological control

Euonymus japonica was successfully grown from cuttings, and the plants were successfully infested with euonymus scale.

Discussion

Our studies of insect pathogenic fungi demonstrated that it may be very difficult to work with these organisms to obtain a practical tool for managing armored scales in Christmas tree plantations. Even if a high degree of scale infection by fungi had been found, additional hurdles could prevent commercial development of these fungi. First, an industrial partner would have to be found who would be willing to undertake its registration as a pesticide, possibly through the IR-4 minor use pesticide program. Most new insecticides require an outlay of many millions of dollars, and so a niche biological control biopesticide may not garner enough interest to warrant investment in its commercialization. Secondly, genetic changes in fungal populations are likely when they are cultured outside of hosts. This raises the specter of reduced virulence in a commercialized product, which would jeopardize its usefulness.

Considerable progress was made in finding additional insecticide active ingredients for scale management useful for short-term benefit and for disinfesting plants prior to marketing. Two facts of armored scale biology guarantee that management programs completely relying on chemical control are destined for failure: high populations in the field (increasing the likelihood that favorable mutations for insecticide resistance are present) and a haplodiploid genetic system will lead to rapid evolution of insecticide resistance, if selection with insecticides is not balanced with suppression of the scale populations via other means. Integrated management of armored scales could include (1) improved tree genetics: growing trees that are resistant or tolerant to scale feeding, (2) use of companion plants with nectar, pollen, or guttation resources (Urbaneja-Bernat et al. 2020) to support the hydration, carbohydrate, and protein needs of the natural enemies of scales, (3) use of banker plants, to provide other species of armored scales to bridge any time in which life stages essential to natural enemy development is unavailable within Christmas trees.

Sustainable management of armored scales in Christmas tree plantations needs to rely upon application of basic integrated pest management principles:

(1) Any insecticides used in Christmas tree plantations must selectively target the pest and not chemically exclude natural enemies. This includes applications of pesticides to target other pests in the plantation, such as spider mites, adelgids, or balsam twig aphids, as well as insecticides used to suppress scale populations. Broad-spectrum products, such as pyrethroids, are only suitable for targeted spot-treatments, rather than as full foliar sprays. Examples of such uses would be a root dip at the time of planting, or as a stump spray of pine and spruce stumps to manage pales weevils. Foliar sprays of the newly found alternatives to dinotefuran for managing scale insects, namely acetamiprid, pyriproxyfen, afidopyropen, and possibly spirotetramat provide options that should have less impact on natural enemies than dinotefuran.

(2) Non-selective systemic insecticides (e.g., dinotefuran, imidacloprid) need to be applied via basal bark sprays to limit the negative impact to beneficial insects. Use of systemic basal bark sprays to young trees can be effective and economical.

(3) Fields destined for interstate sales of trees need to be started clean. Interplanting transplants among larger trees immediately starts the smaller trees on the wrong course, by guaranteeing infestation soon after planting. Clearcutting of fields before replanting, removing any vegetation harboring scales (basal whorl of branches attached to stumps, or any brush cut while harvesting trees), and planting clean nursery stock are critical for growing trees free of armored scales. Although trees will become infested as they grow (probably from scale crawlers blowing in, or being carried by birds), delaying one or two years of scale development in a field delays and slows the infestation process.

(4) Changes in ground cover management need to be implemented to guarantee that floral resources are available to support populations of natural enemies. Fields in which biocontrol has accidentally occurred appear to have high populations of broadleaved flowering plants that may be providing essential resources such as water,

carbohydrates, and protein through nectar, pollen, or guttation fluids to the various predators and parasitoids. Integration of “companion plants” within Christmas tree plantations will heighten the need for all insecticides, miticides, and fungicides used in a plantation to be compatible with pollinator health. Banker plants should probably be planted around the perimeter of fields, to prevent losing armored scales on banker plants when applying foliar sprays within the Christmas tree planting. Further field tests will be required to establish whether presenting euonymus scale-infested plants next to blocks of Christmas trees infested with armored scales will enhance the persistence and effectiveness of natural enemies of armored scales.

Horticultural oil is a useful tool that acts through suffocation, against which scales should not be able to evolve resistance. As a non-residual insecticide, horticultural oil is recognized as having special value for integrated management of armored scales on trees that tolerate oil application. Because oil can kill scales that may have incipient resistance traits that would allow their survival when treated with any of the other insecticides, future research should investigate combinations of horticultural oil with the four insecticide alternatives to dinotefuran. By combining a contact acting insecticide (oil) with a systemic, it may be possible to suppress armored scales more economically with one well-timed foliar spray. Field tests of such a management program should quantify the resulting parasitization of scales, so that the product and timing with the best compatibility with biological control can be promoted.

References

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Summary of Research Report for Public Release by CTPB

Armored scales are insects that affect the marketability of Christmas trees by causing yellow spots to form on needles, needle loss, and their shipment across state lines on infested trees or greens used in wreaths may be prohibited. In a field test, Dr. Cowles found three insecticides that, when used as foliar sprays targeting newly hatched scales, were as or more effective than the industry standard (dinotefuran). All of these alternatives are less toxic to pollinators than dinotefuran and are likely to be more compatible with beneficial predatory and parasitic insects that feed on armored scales.

Fig. 1. Several insect pathogenic fungi can infect and kill armored scale pests that feed on Christmas trees. These cryptomeria scales on Fraser firs probably were killed by the fungus that has produced chamois-like growth over the scales.

Fig. 2. Dr. Cowles applying systemic insecticides as a basal bark spray to the base of Fraser firs infested with elongate hemlock scales. The only systemic insecticide found to be effective at suppressing scale insects when applied in this manner was dinotefuran.