Forest Pest Management Cooperative

Research Accomplishments in 2014

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Evaluation of Emamectin Benzoate for Protection of Loblolly Pine from Black Turpentine Beetle

Initiated 2012; completed 2014

Background and Initial Study

A study to determine the efficacy of TREE-äge (emamectin benozoate) for protecting loblolly pine from black turpentine beetle was conducted within the Fairchild State Forest, Rusk, TX in 2012-2013. The treatments included:

A. TREE-äge (5.0 ml / inch DBH) treatment applied at ground level
B. TREE-äge (2.5 ml / inch DBH) applied at ground level
C. TREE-äge (2.5 ml / inch DBH) applied at 36 inches above ground
D. Scimitar (lambda-cyhalothrin, Syngenta) spray applied from ground to 10 feet
E. Untreated

Each treatment was applied to 10 randomly-assigned trees in September 2012. Each systemic insecticide treatment (treatments 1, 2, & 3) was injected at the labeled rate after dilution in 1 part water with the Arborjet Tree IV™ microinfusion system (Arborjet, Inc. Woburn, MA) into evenly spaced points (number is calculated by DBH/2). In October 2012 (30 days post-injection), treatment 4 trees (up to 10 ft) were sprayed with scimitar to runoff using a backpack sprayer. Thirty days post treatment, each tree was baited with frontalin and endo-brevicomin lures and turpentine (in amber bottle and wick). The baits were replaced in March, May and July 2013.

The number, height of attack, and success of BTB attacks were evaluated periodically (November, December 2012, May, July and October 2013, May, July and October 2014). Success was determined by the size and composition of the pitch tubes exuding from each BTB attack site. Large pitch tubes containing frass (phloem tissue and beetle waste) and brood emergence were used to indicate success of females alone or with males in colonizing the host. Small, crystalized pitch tubes with little or no frass and no brood emergence were used to indicate failure to successfully colonize host (or attacks by *Ips*). At the termination of the experiment in October 2014 (about 25 months after treatment), final crown ratings were made. An analysis of variance was used to test for differences among injection treatments.

All trees were alive by the end of the first year of this study. Most BTB attacks occurred on the lower bole, within 3 feet of the ground. Significantly fewer and smaller BTB attacks were observed on TREE-äge-treated trees compared to those treated with a bole spray or were left untreated (Figures 1 and 2). The number of attacks did not differ between injection rate and application height. Only two control trees appeared to produce brood based on presence of emergence holes. No emergence holes were observed on any of the injected or sprayed trees.

All three injection treatments, regardless of application rate, were successful in protecting loblolly pine trees from BTB in the first year.

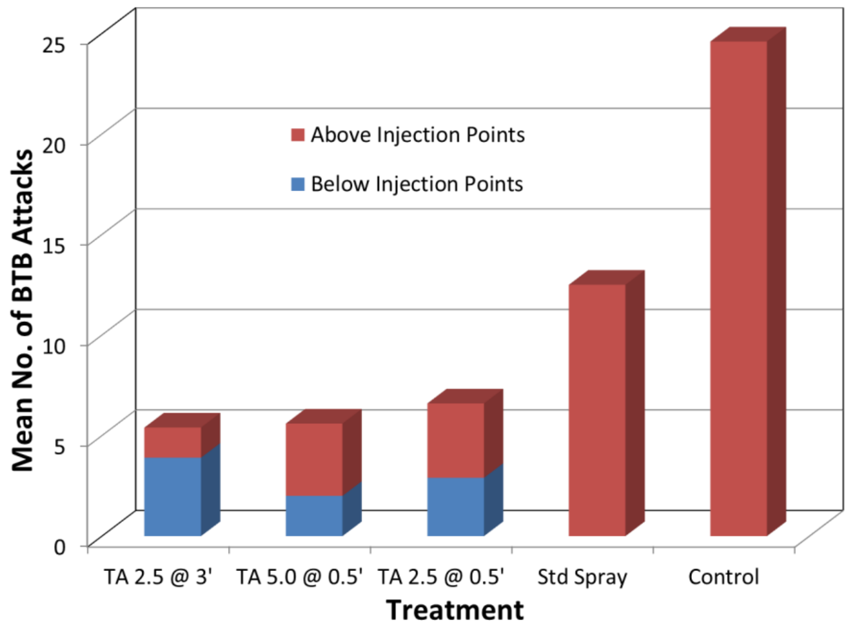

Figure 1: Mean number of black turpentine beetle attacks on loblolly pine within Fairchild State Forest, TX; October 2012 – October 2013. TA = TREE-age; Std = Scimitar

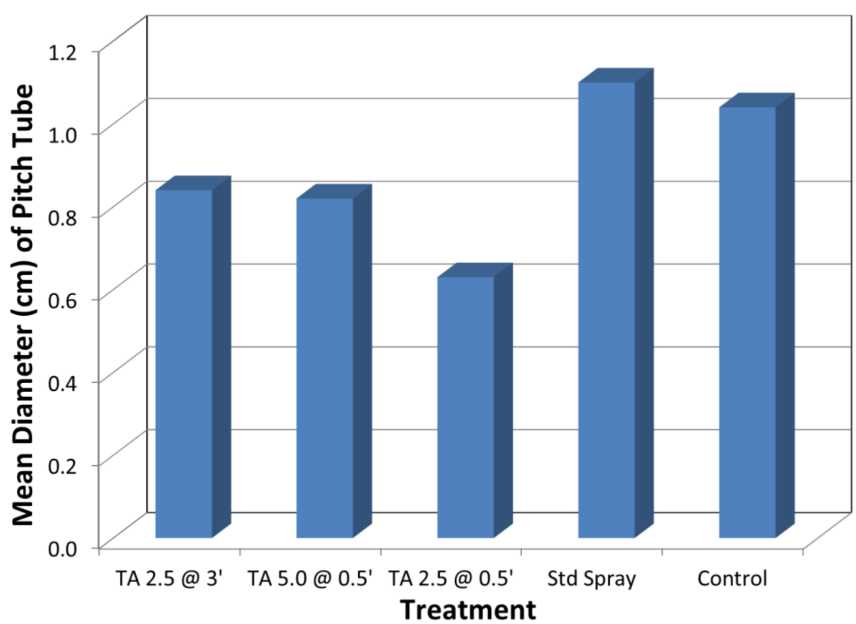

Figure 2: Mean diameter of pitch tubes created by black turpentine beetle adults attacking insecticide-treated and untreated loblolly pine, Fairchild State Forest. TA = TREE-age; Std = Scimitar

Objectives in 2014

Trees injected in September 2012 and control trees were rebaited and monitored in 2014 to determine if TREE-äge (emamectin benzoate) would be effective in protecting loblolly pine from BTB for a second year.

Methods

This study was again conducted within the Fairchild State Forest, Rusk, TX using the thirty loblolly pine trees that were previously treated with TREE-äge (emamectin benzoate) and ten untreated control trees.

A. TREE-äge (5.0 ml / inch DBH) treatment applied at ground level
B. TREE-äge (2.5 ml / inch DBH) applied at ground level
C. TREE-äge (2.5 ml / inch DBH) applied at 36 inches above ground
D. Untreated control

Treatment Efficacy

Trees were baited three times in 2014, in May, July and September. The number, height of attack, and success of BTB attacks were evaluated monthly. At the termination of the experiment in October 2014, final crown ratings were made. An analysis of variance followed by Tukey's HSD was used to test for differences among injection treatments.

Results

All trees were alive by the end of the study, 25 months after injection. Significantly more BTB attacks occurred on the lower bole, within 3 feet of the ground ($p < 0.0001$). Significantly fewer BTB attacks were observed on TREE-äge-treated trees compared to the untreated control trees ($p = 0.0074$, Figure 3). The average number of attacks was not significantly different between injection rate and application height. No emergence holes were observed on any of the trees included in this study.

Conclusions

BTB populations and attack levels were insufficient to cause tree mortality even on untreated control trees. As a result, attack numbers were used to measure treatment efficacy. The injection treatments, regardless of application rate and height, were most effective in limiting BTB attacks. As in previous injection trials with emamectin benzoate/TREE-äge (Grosman and Upton 2006, Grosman et al. 2009, 2010), the attacking BTB adults quickly die upon contact with treated phloem tissue. This prevents the release of pheromones and host volatiles that attract additional beetles, thus reducing the overall numbers of attacks. These trial results indicate that TREE-äge (emamectin benzoate) applied to loblolly pine at as little as 2.5 mL/inch DBH is effective in protecting loblolly pine trees for two full years.

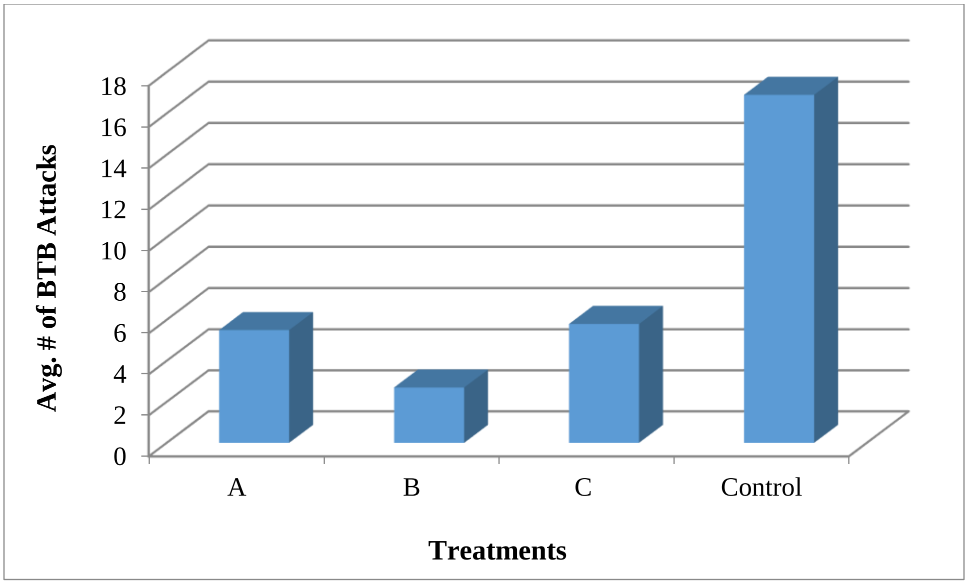

Figure 3: Mean number of black turpentine beetle (BTB) attacks on loblolly pine within Fairchild State Forest, TX; May 2014 – October 2014 (A = 2.5 mL/inch EB @ 3 feet; B = 2.5 mL/inch EB @ ground level; C = 5.0 mL/inch EB @ ground level; Control = untreated).

Literature Cited

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Grosman, D.M., S.R. Clarke, and W.W. Upton. 2009. Efficacy of two systemic insecticides injected into loblolly pine for protection against southern pine bark beetles (Coleoptera: Curculionidae). Journal of Economic Entomology 102: 1062-1069.

Grosman, D.M., and W.W. Upton. 2006. Efficacy of systemic insecticides for protection of loblolly pine against southern pine engraver beetles (Coleoptera: Curculionidae: Scolytinae) and wood borers (Coleoptera: Cerambycidae). Journal of Economic Entomology.

Incorporating Emamectin Benzoate into a Control Strategy for Southern Pine Beetle

Initiated in 2014; On-going in 2015

Justification

The Forest Pest Management Cooperative (FPMC) initiated trials in 2012 in AL and VA to evaluate the ability of emamectin benzoate-treated pines to serve as trap trees for maintaining southern pine beetle (SPB) populations at low levels. It was found that SPB was more likely to attack untreated trees surrounding a central-baited, treated tree compared to treated trees surrounding a central-baited, treated tree. In this study, treated trees were baited 4 weeks after injection of emamectin benzoate. The reduced attack density on injected trees suggests that SPB may have detected emamectin benzoate within the trees injected 4 weeks earlier and therefore preferred to attack nearby untreated trees rather than the baited, injected tree in the center of the plot. For this reason, it is of interest to assess the efficacy of emamectin benzoate for protection of southern yellow pines against SPB by applying injection and baiting treatments at different timings. Perhaps if a tree is baited at the same time it is injected or soon thereafter (two weeks after injection), beetles would not detect the chemical, as the emamectin benzoate will not have had time to move upward from the basal injection points. The bait may attract beetles that then attack the injected tree and may even produce brood, but the brood would not be expected to live.

Funding: FPMC and grant from Syngenta

Location: Talladega National Forest, Alabama

Objectives of 2014 Study

1. Optimize the timing of tree baiting and injections to maximize mass attacks on target trees and minimize development and emergence of brood (trap tree effect)
2. Test for seasonal effects between spring and fall dispersal periods on treatment effectiveness

Methods

Sites chosen for this study were selected based on low to moderate trap catch levels in early spring on the Talladega National Forest, AL. Trap catches of SPB in Virginia in 2014 were too low to justify treatments. Four paired Lindgren twelve-funnel trap sets, one trap in each pair baited with frontalin + Sirex lure (*alpha* and *beta*-pinene) only (standard) and the other baited with frontalin + Sirex lure + *endo*-brevicomin displaced by 4 m (enhanced), were deployed in locations 300 m away from study sites to monitor local beetle populations.

There were two trials (spring and fall) with five treatments (listed below) and six replicates of each treatment. Loblolly pines chosen for experimentation were located in closed-canopy, pine-hardwood stands and were isolated (no other pine within 8 m). When possible, poor quality (form, health, etc.) trees were selected. Treatment applications were timed to coincide with peak spring and fall SPB dispersal periods. TREE-äge™ containing emamectin benzoate was injected into the lower trunk of trees at 5 ml per inch of diameter at breast height (DBH) in trees 12 inches in diameter and 10 ml per inch DBH in trees 12 inches in diameter for three of the five treatments (1, 3, and 4) and at a half rate for one treatment (2). The Tree IV™ microinfusion system (Arborjet, Inc. Woburn, MA) was used to inject TREE-äge™ into 4 injection points (for trees ≤12 inches DBH) or 8 injection points (for trees >12 inches DBH) at a height of 0.3 m above the ground. For each seasonal trial, all trees were baited with species-specific lures (frontalin, Sirex lure, and *endo*-brevicomin). The spring trial was baited for two consecutive 6-week periods, first on April 15^{th,} again on May 28th, and a third, fall baiting on September 23rd, 2014. The fall trial was baited twice, initially on September 2nd and again on October 14th, 2014, to coincide with peak SPB flight periods.

Treatments

1. Inject tree with TREE-äge @ 5 ml/inch DBH and wait four weeks to bait
2. Inject tree with TREE-äge @ 2.5 ml/inch DBH and wait four weeks to bait
3. Inject tree with TREE-äge @ 5 ml/inch DBH and wait two weeks to bait
4. Inject tree with TREE-äge @ 5 ml/inch cm DBH and bait on same day
5. Control: Bait only, not injected

To evaluate the various treatments, the following protocol was followed:

1. Monitor SPB and associated black turpentine beetle (BTB) attack level by visually estimating the number of pitch tubes on entire stem and observing the health of study trees at one to two week intervals following installation of baits and at four to five week intervals thereafter until final evaluation.
2. At the end of the field season (November), each study tree was sampled at heights of 1.5, 4 and 6.5 m by counting brood emergence holes in 20 X 25 cm (500 cm^2) sample windows on northern and southern aspects. Attack success or failure was determined on the basis of study tree survival or mortality.
3. All dead study trees will be felled upon exhibiting complete whole crown needle fading (from green to yellow) and treatment evaluation methodology assessed as described in 2 above. In addition, SPB gallery length and percent cerambycid larval feeding will be measured on the corresponding bark plates (10 X 10 cm = 100 cm^2) to each of the six 20 X 25 cm (500 cm^2) sampling windows.

The average number of SPB and BTB attacks and emergence holes and percent tree mortality will be compared among treatments and between spring and fall seasons.

Results

The study plan initially called for conducting the study in Virginia and Alabama, but in 2014 there were insufficient SPB populations in the Virginia site to bring trees under sufficient attack, so only the Alabama results are presented here.

The overall mean catch of SPB adult beetles per trap per day (b/t/d) for the standard monitoring bait was 7.8; for the enhanced bait (with *endo*-brevicomin) trap catches averaged 12.9 b/t/d. The SPB trapping data was separated into three periods: Spring dispersal (Apr-May), during which the standard bait caught a mean of 18.9 b/t/d and traps baited with *endo*-brevicomin caught 27.7 b/t/d/; summer (June-Aug), in which traps with the standard bait caught a mean of 1.6 b/t/d and those enhanced with *endo*-brevicomin caught 5.9 b/t/d/; and fall dispersal (Sept-Oct), during which the standard bait attracted a mean of 1.0 b/t/d and the *endo*-brevicomin enhanced baits attracted a mean of 2.0 b/t/d (Figure 1). Of note is the significate decline in SPB flight activity from the spring to summer seasons as is typical; but there was a continued decline in fall flight activity, rather than the increase that usually occurs during this season.

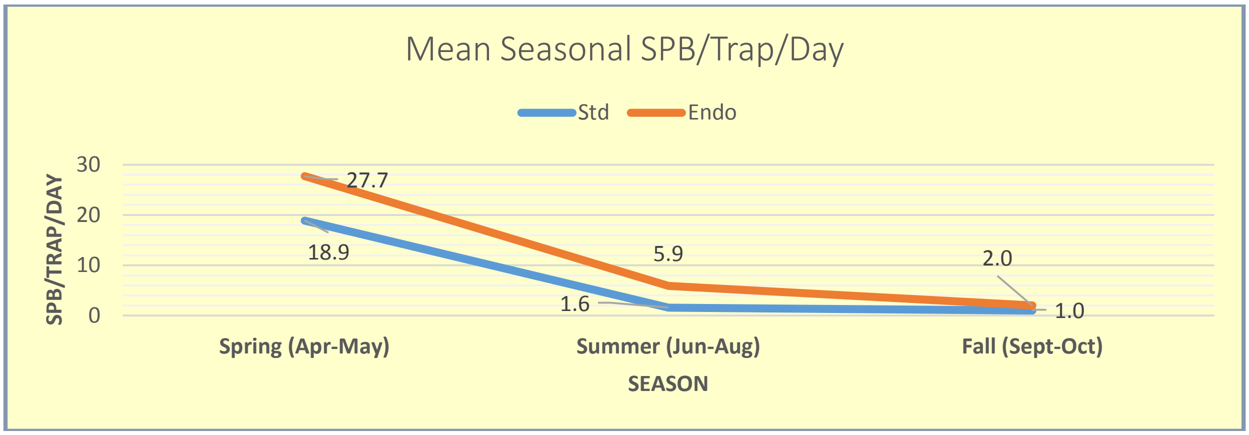

Figure 1. Average number of SPB per trap per day for standard and enhanced (with *endo*-brevicomin) baited traps by season on Oakmulgee Ranger District, AL, 2014.

An analysis of variance showed no significant differences among treatments in the average number of SPB or BTB attacks on the trees treated in the spring (P = 0.325; Figure 2). In contrast, the ANOVA revealed a significant difference at the 0.05 level among treatments initiated during the fall (P = 0.048, Figure 3). During this season, treatments 1 and 2 had SPB attack densities significantly lower than treatments 3, 4 or 5 (check). BTB attacks in both seasons were too low to show any significant differences.

The average numbers of SPB and BTB attacks per 500 cm2 bark samples also yielded no significant differences among treatments (Figures 4 and 5). By November 19th, 2014, six trees had died; two control trees, and one each of the four injection treatments.. Only the two control trees were successfully attacked and subsequently colonized by SPB and BTB as measured by the presence of adult egg gallery and brood development, confirmed by observation and evidenced by emergence holes. Similarly, only the two successfully-attacked control trees exhibited any cerambycid larval feeding (see Figure 8). Infection by beetle-vectored blue-stain fungi was considered the most probable cause of tree mortality for the one tree of each of the injection treatments which died during the course of this study. Blue stain was observed to be heavily present beneath the bark of these dead trees.

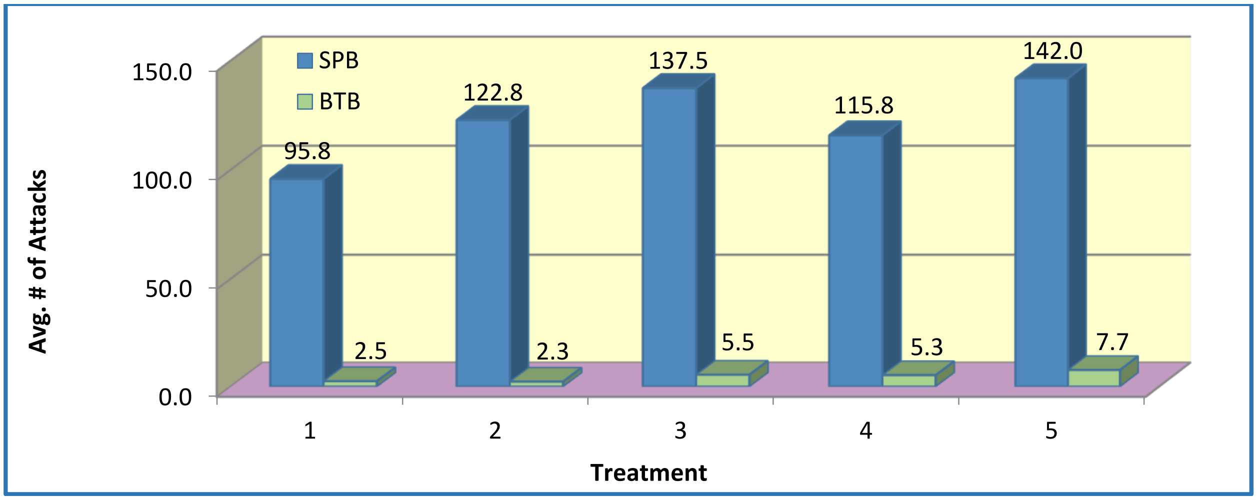

Figure 2. Average number of SPB and BTB attacks found on loblolly pine trees treated with: **1.** 5 ml/ inch DBH TREE-äge and wait four weeks to bait; **2.** 2.5 ml/ inch DBH TREE-äge and wait four weeks to bait; **3.** Inject tree with TREE-äge @ 5 ml/ inch DBH TREE-äge and wait two weeks to bait; **4.** 5 ml/ inch DBH TREE-äge and bait on same day; **5.** Control: Bait only, no injection, in spring 2014.

Fall trial values: ($p = 0.950$ and $p = 0.029$, respectively; Figure 3).

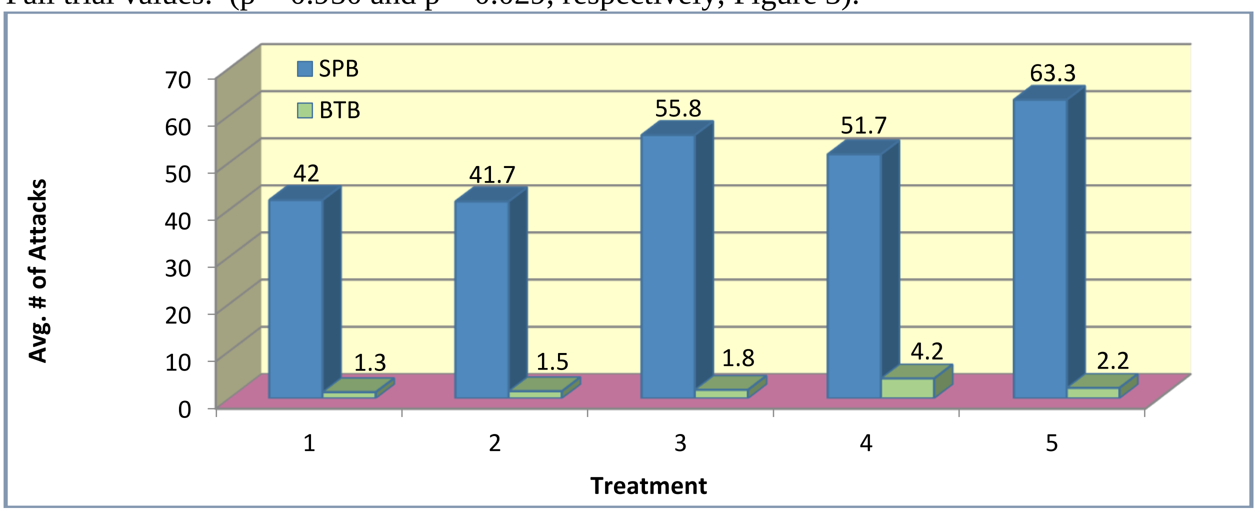

Figure 3. Average number of SPB and BTB attacks found on loblolly pine trees treated with: **1.** 5 ml/ inch DBH TREE-äge and wait four weeks to bait; **2.** 2.5 ml/ inch DBH TREE-äge and wait four weeks to bait; **3.** Inject tree with TREE-äge @ 5 ml/ inch DBH TREE-äge and wait two weeks to bait; **4.** 5 ml/ inch DBH TREE-äge and bait on same day; **5.** Control: Bait only, no injection, in fall 2014.

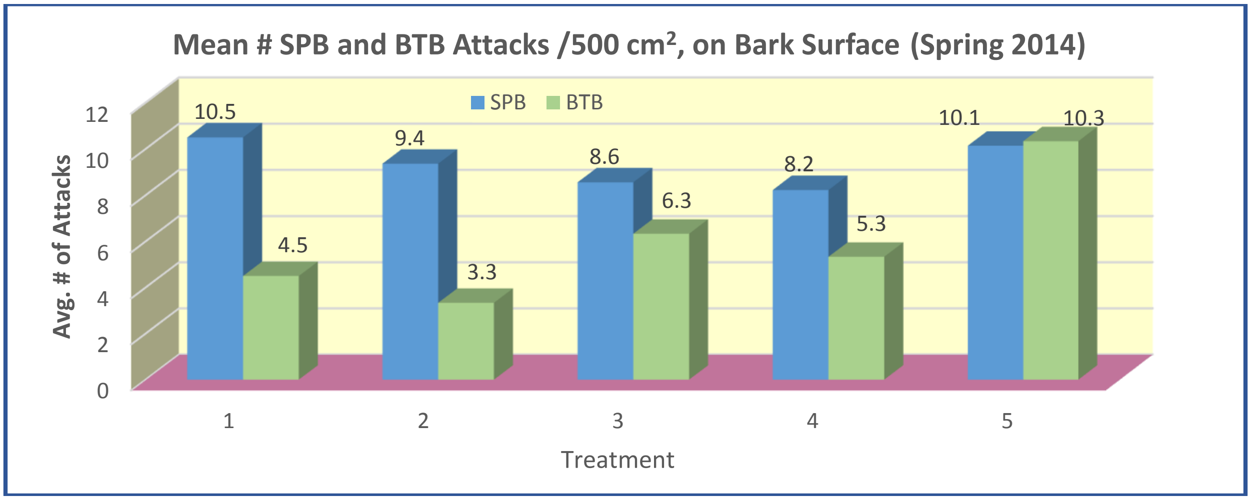

Figure 4. Average number of SPB and BTB attacks per 500 cm2 samples on loblolly pine trees treated with: **1.** 5 ml/ inch DBH TREE-äge and wait four weeks to bait; **2.** 2.5 ml/ inch DBH TREE-äge and wait four weeks to bait; **3.** Inject tree with TREE-äge @ 5 ml/ inch DBH TREE-äge and wait two weeks to bait; **4.** 5 ml/ inch DBH TREE-äge and bait on same day; **5.** Control: Bait only, no injection, in spring 2014.

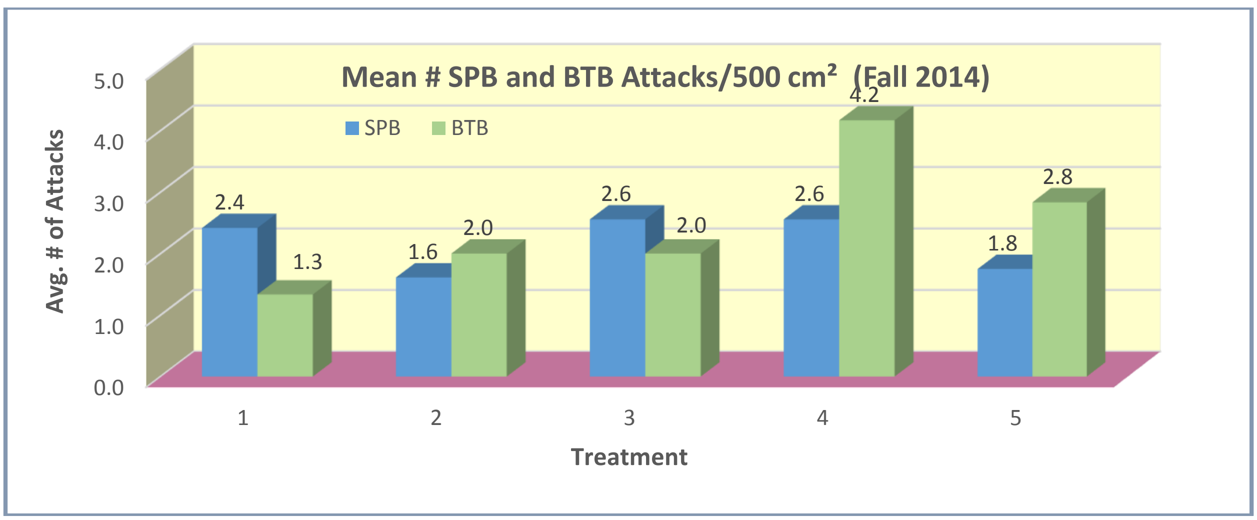

Figure 5. Average number of SPB and BTB attacks per 500 cm2 samples on loblolly pine trees treated with: **1.** 5 ml/ inch DBH TREE-äge and wait four weeks to bait; **2.** 2.5 ml/ inch DBH TREE-äge and wait four weeks to bait; **3.** Inject tree with TREE-äge @ 5 ml/ inch DBH TREE-äge and wait two weeks to bait; **4.** 5 ml/ inch DBH TREE-äge and bait on same day; **5.** Control: Bait only, no injection, in fall 2014.

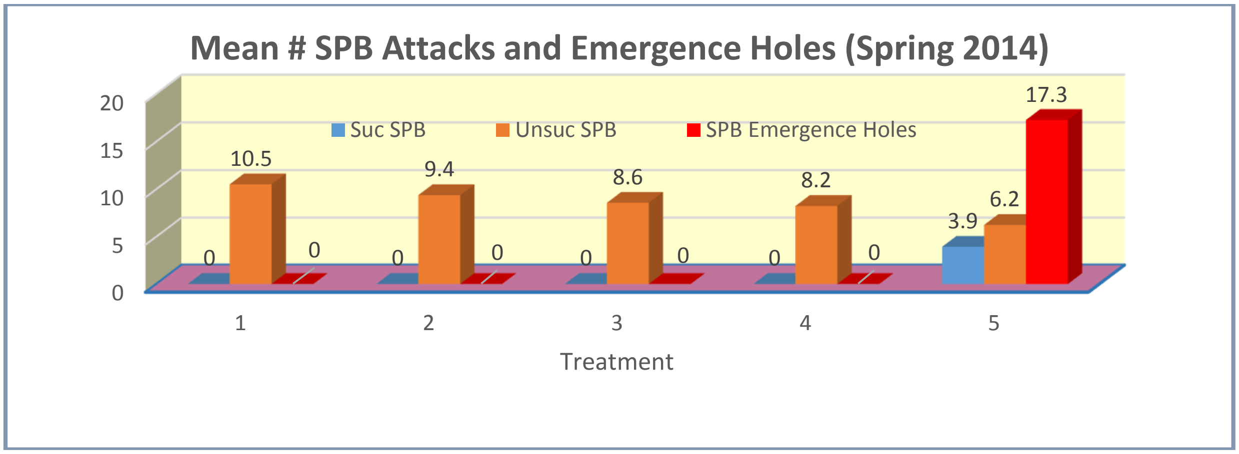

Figure 6. Average number of SPB attacks and emergence holes per 500 cm2 found on loblolly pine trees treated with: **1.** 5 mL/ inch DBH TREE-äge and wait four weeks to bait; **2.** 2.5 mL/ inch DBH TREE-äge and wait four weeks to bait; **3.** Inject tree with TREE-äge @ 5 mL/ inch DBH TREE-äge and wait two weeks to bait; **4.** 5 mL/ inch DBH TREE-äge and bait on same day; **5.** Control: Bait only, no injection, in spring 2014.

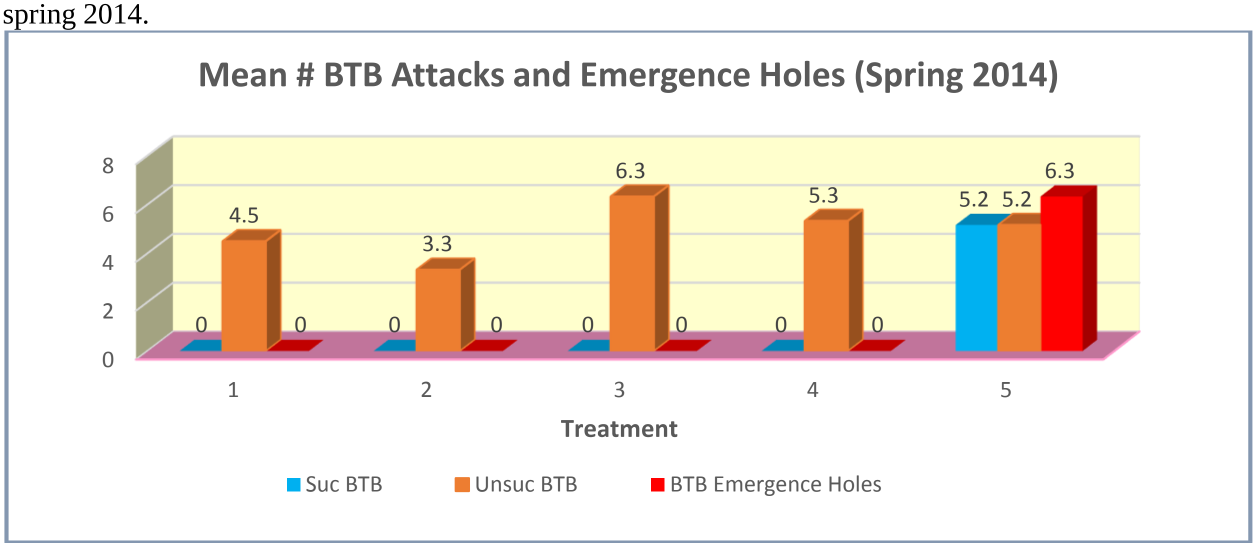

Figure 7. Average number of BTB attacks and emergence holes per 500 cm2 found on loblolly pine trees treated with: **1.** 5 mL/ inch DBH TREE-äge and wait four weeks to bait; **2.** 2.5 mL/ inch DBH TREE-äge and wait four weeks to bait; **3.** Inject tree with TREE-äge @ 5 mL/ inch DBH TREE-äge and wait two weeks to bait; **4.** 5 mL/ inch DBH TREE-äge and bait on same day; **5.** Control: Bait only, no injection, in spring 2014.

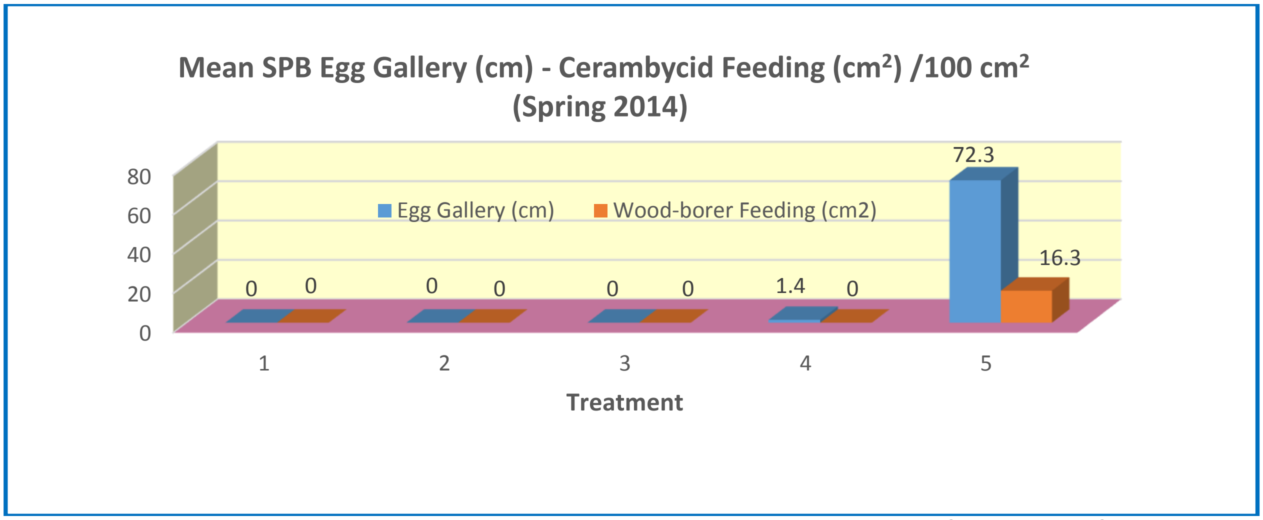

Figure 8. Average length (cm) of SPB egg gallery and cerambycid feeding (cm^2) per $100cm^2$ found on loblolly pine trees treated with: **1.** 5 ml/ inch DBH TREE-äge and wait four weeks to bait; **2.** 2.5 ml/ inch DBH TREE-äge and wait four weeks to bait; **3.** Inject tree with TREE-äge @ 5 ml/ inch DBH TREE-äge and wait two weeks to bait; **4.** 5 ml/ inch DBH TREE-äge and bait on same day; **5.** Control: Bait only, no injection, in spring 2014.

Discussion and Conclusion

The 2012 FPMC study showed that loblolly pines treated with TREE-äge (emamectin benzoate) and baited after 4 weeks tended to have fewer southern pine beetle attacks than untreated, baited trees. This may be because the attacking beetles quickly die upon contact with treated phloem tissue which prevents the release of pheromones and host volatiles that attract additional beetles, thus reducing the overall numbers of attacks. Why this has not been a result in these trials is unknown, but may be advantageous, since there was no survival of broods in treated trees, even those injected at a lower dosage (treatment 2) or on the same day as baiting (treatment 4). An effective trap tree technique should have an attack density similar to a baited, uninjected tree (as in treatments 2, 3 and 4 during the spring and fall trials). Furthermore, injected trees showed no brood survival and emergence. Accordingly, these treatments were considered the most successful treatments. As currently proposed, an additional final evaluation of the 2014 spring and fall trials will occur in March 2015.

The success of treatment 3, in which trees were injected at half the dosage of the other injection treatments, suggests that a lower dose rate may be equally effective, reducing chemical and application costs considerably in operational treatments. Future studies should evaluate the effectiveness of even lower EB dosages (i.e., 1.0 and 2.0 ml/diameter inch). Future development of EB injections as a direct control treatment should consider EB injections at the head of an expanding SPB spot to determine the number of trees to inject and the location of injected trees with reference to trees currently under SPB attack. Also, trials need to be conducted to ascertain if a lower dosage of EB is effective at various distances from the active, expanding head of the spot.

Emamectin Benzoate for Protection of Eastern Red Cedar from *Phloeosinus dentatus* during Outbreak Conditions

Initiated in 2014; monitored through 2015

Justification

A recent outbreak of the cedar bark beetle, *Phloeosinus dentatus* has resulted in extensive mortality of eastern red cedar in the areas of Fayette and Washington counties, TX (Figure 1). Cedar bark beetles are not typically aggressive tree killers, but due to drought and dense stand conditions in central Texas, *Phloeosinus dentatus* populations have had the opportunity to reach epidemic proportions.

Figure 1 (A and B). Extensive mortality of eastern red cedar in Fayette County, TX

Phloeosinus dentatus is a small bark beetle (approximately 3mm in length) with reddish-brown coloration, similar in appearance to *Dendroctonus* spp. (Figure 2). The larvae are cream-colored and have brown head capsules (Figure 3).

Figure 2. *Phloeosinus dentatus* adult

Figure 3. *Phloeosinus dentatus* larvae

Evidence of beetle colonization includes the presence of very fine boring dust in bark crevices and around the base of the trunk. Distinct galleries can be detected by removing a small section of bark (Figure 4). Crowns of infested trees will fade from green to yellow and eventually to red (Figure 5). By the time the entire tree crown is red; beetles likely have matured and are gone (Furniss and Carolin 1977; Leatherman and Lange 1997).


Figure 4. ***Phloeosinus dentatus*** **larval gallery**


Figure 5. Fading eastern red cedar

Management of cedar bark beetle species has typically involved preventative insecticide sprays or soil drenches, to protect high value trees in recreational, historic, and private landowner settings. Many land managers are wary of these methods due to the potential environmental hazards. An alternative method is to inject the insecticide directly into the bole of the tree. This method results in containment of the insecticide within the tree, with minimal impact to the environment. Injections of pesticides and fungicides are an active management solution for many conifer and hardwood species, such as pine and oak, but this method has not been applied to eastern red cedar. It is unknown whether insecticides could be successfully introduced into the bole of an eastern red cedar.
Emamectin benzoate (TREE-äge™) is an insecticide that is often used in tree injection and has been found to be effective against several *Dendroctonus* and *Ips* spp. bark beetles. It is of interest to determine if it would be equally effective in protecting eastern red cedar from *Phloeosinus* spp. bark beetles when applied as a tree injection.

Objectives

1. Determine if emamectin benzoate (TREE-äge™) can be successfully introduced into the bole of eastern red cedar via trunk injection using Arborjet's tree IV system and;
2. Determine if emamectin benzoate (TREE-äge™) is effective in protecting eastern red cedar from attack by *Phloeosinus* spp. bark beetles.

Location: Fayette County, Texas

Pesticide: TREE-äge (emamectin benzoate)

Methods

This study was conducted in Washington and Fayette counties, TX. Two treatments were applied in spring 2014; 33 red cedars were treated with TREE-äge (emamectin benzoate) and 18 were left untreated. Using Arborjet's Tree I.V. system and #4 arbor plugs, trees 14 inches or less in diameter at breast height (DBH) were treated at a rate of 5.0 mL/ inch DBH at four points. Trees greater than or equal to 14 inches DBH were treated at a rate of 10.0 mL/ inch DBH at eight points. The amount of time it took for 12 eastern red cedars to uptake TREE-äge via the

Tree I.V. was recorded. Trees were assessed for evidence of bark beetle attack and mortality following treatment at one and six months.

Results

Trees were treated the last week of April and the first week of May, 2014. Treated trees varied between 5.8 and 19.7 inches DBH. Application was timed during the second day of injections (May) and was found to require an average of 19 min: 59 sec per tree with a range of 1 minute: 56 seconds to 1 hour: 4 minutes: 40 seconds. Although no data on timing was collected the first day, more trees were capable of being injected within the allotted three hour time period (7 more trees) than on the second day. The week prior to the first injections, it had rained 0.39 inches in Round Top, while it did not rain the week prior to the second injections in May. This may suggest that red cedar is able to uptake chemicals much faster than the average 19 min: 59 sec if treated following a recent rain event.

Six weeks after application, exit holes were found on one of 33 treated trees and three of 18 untreated trees. The October 2014 assessment found no change and there has been no tree mortality thus far in either treated or check trees.

Conclusions

Although no timing data was recorded on the first day of injections, it is likely that the average time for red cedar to uptake chemical via the Tree IV system can be shorter than the 19 min: 59 sec average recorded the second day. With the end of drought conditions and no tree mortality in either treated or check trees as of July 2015, this study was discontinued. On can conclude the best treatment to save drought-stressed trees monitored in this study was the return to normal rainfall patterns in 2014 and 2015. Whether systemic insecticides can keep cedar trees alive throughout a prolonged drought remains to be determined.

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Emamectin Benzoate and Propiconazole for Protection of Black Walnut from Walnut Twig Beetle and Thousand Canker Disease

Initiated in 2012; continued in 2015

Status

Continuing, but without additional federal funds. To date, most targets have been met; however the study has been extended due to the efficacy observed. Final progress report to be submitted in October 2015.

Background

Thousand cankers disease was recently discovered in TN, VA and PA, within the native range of black walnut. Protection of individual, high-value walnut trees from insect attack has historically involved applications of liquid formulations of contact insecticides to the tree bole and/or foliage. Recently, an experimental formulation of an injected systemic insecticide, emamectin benzoate (TREE-age™; Arborjet Inc., Woburn, MA), was registered by Syngenta Crop Protection, LLC, Greensboro, NC, with the EPA, and may prove promising for protecting black walnut. In this study, the effectiveness of recommended rates of TREE-age™ alone and combined with the fungicide propiconazole (ALAMO®; Syngenta Crop Protection, LLC Greensboro, NC) are being evaluated for reducing the attack success of walnut twig beetle (WTB) on individual black walnut trees and the progression of the thousand cankers disease fungus introduced during initial phases of tree colonization. Additionally, effects on other walnut pests are being evaluated. The extent of disease infection and the distribution and concentration of emamectin benzoate and propiconazole in xylem, phloem, and nuts were determined.

Objectives

1) To determine the efficacy of emamectin benzoate (TREE-äge™) and the fungicide propiconazole, alone or in combination, for protecting individual walnut trees from attack by walnut twig beetle and other insect pests.

2) To determine if emamectin benzoate, propiconazole or combination treatments can provide preventative and therapeutic control of thousand cankers disease.

3) To provide data on the distribution and concentration of emamectin benzoate in walnut xylem, phloem, and nuts at several points in time after injection.

Methods

<u>Locations, Treatments, and Environmental Conditions</u>
This study was established at three locations: TCD-confirmed location in Sevier Co., TN (about 35°59 N, 83°45 W, elev. 1136 ft) and uninfested locations in Cherokee Co., TX (about 31°45 N, 95°11 W, elev. 429 ft) and Nacogdoches Co., TX (about 31°41 N, 94°26 W, elev. 309 ft). There are as many as four treatments: emamectin benzoate (TREE-äge™) alone injected into trees

(treatment **1**); propiconazole (Alamo®) alone injected into trees (Treatment **2**); TREE-äge™+ Alamo® injected into tree (Treatment **3**); and an untreated control (treatments **4**).

Each treatment was applied to 10-40 randomly-assigned trees per site. Test trees were located in areas with known insect activity, spaced >10 m apart, 13 to 38 cm dbh, and within 100 m of access roads to facilitate the treatment. Each insecticide, fungicide or insecticide + fungicide treatment (treatments 1-3) was injected with the Arborjet Tree IV™ or QUIK-jet™ microinfusion system (Arborjet, Inc. Woburn, MA) into 4-8 evenly spaced points 0.3 m above the ground. Injections occurred in early- (TX) or late-April ((TN). The intent was to bait trees (treated and untreated) in TN with WTB pheromones (provided by Steve Seybold) beginning in June, 2012 and throughout the growing season. However, phytotoxic effects (burned leaves) caused by the treatments made it necessary to delay baiting. All treated trees in treatments 1-3 and the untreated control trees (treatment 4) were baited in June, 2013 and again in September, 2013. WTB populations were monitored throughout the season near the TN location with baited 4-unit Lindgren funnel traps placed at 10 feet on steel conduit poles. Trap catches were recovered every two weeks throughout the season.

In April, 2012 (at the time of treatment) and then four months later(August), the stem and crown of each tree were ranked as to the extent of insect damage. In addition, three small branchs (12" length) were collected from the low, mid and upper crown of several study tree in 2012. The branches were evaluated for the presence of and ranked on the level of WTB (TN) and other insect damage (TX and TN).

Treatment Efficacy

A photograph of the crown of each study tree in TN was taken at the time of treatment. Trees were evaluated for crown condition in May and September 2012 and 2013. The date of appearance of TCD symptoms was recorded. Each walnut crown was given a rating of 0 (healthy), 1 (wilt symptoms comprising < 20% of the crown), 2 (wilt symptoms comprising 20-80% of the crown), 3 (wilt symptoms comprising >80% of the crown) (Mayfield et al. 2008), or 4 (dead tree). At the June and August rating periods, trees with a crown rating of 2 will have wood samples taken from the stem and branches to determine the presence of WTB galleries and *G. morbidia*.

At the termination of the experiment in September 2014 (about 28 months after treatment), the intent was to conduct a final crown ratings. However, an unusually cool summer resulted in premature leaf drop and final ratings will have to be conducted in summer 2015. An analysis of variance will be used to test for differences among injection treatments. A χ^2 (Chi-square) test for homogeneity will be used to test the null hypothesis that the percentage of trees with a crown rating of 2 did not differ between the insecticide-, fungicide- or combination-treated trees and the untreated control group (Mayfield et al. 2008). The null hypothesis will be rejected if more than 20% of the treated trees reached a crown rating of 2. The test will be invalidated if fewer than 60% of the control trees reach a crown rating of 2.

Residue Analyses
Residue levels of emamectin benzoate and propiconazole have been determined in xylem (i.e., to ascertain whether the insecticide was moving within the tree), phloem (i.e., the target tissue where bark beetles feed, etc.) and nuts (that may be consumed). Branch and nut samples were collected June 26, 2012 (treatments 1 - 4), and nuts only September 16, 2012 (treatments 1 - 4) from 3-15 randomly selected trees per treatment (*see* below). Additional tissue samples were collected in September 2013 (treatments 1, 2 and 4). If sufficient concentrations exist in phloem collected in September 2013, we may continue sampling in 2015 if additional funding can be obtained. Samples have been sent to Syngenta, Greensboro, NC for analysis.

Accomplishments and Planned Research Activities

FY2012

1. Study plan	Completed
2. Forest/District contacted, liaison	Completed
3. Field site selection	Completed
4. Trees selected, tagged and treatments assigned	Completed
5. Treatments 1 - 6 applied; monitoring traps installed	Completed
6. Trees baited	Postponed
7. Xylem, phloem & nut samples collected (treatments 1-4)	Completed
8. Nut sampled (treatments 1-4)	Completed
9. Post-treatment assessment of efficacy	Completed
10. Presentation at Bark Beetle Technical Working Group	Completed

FY2013

11. Presentation at East Texas Forest Entomology Seminar	Completed
12. Flight periodicity and environmental parameters monitored	Completed
13. Trees baited (all) and xylem, phloem and nut samples collected (treatments 1-4)	Completed
14. Post-treatment assessment of efficacy	Completed
15. Preliminary data summary	Completed

FY2014

17. Treatments 1 - 6 reapplied	Completed
18. Post-treatment assessment of treatment efficacy	Missed

FY2015

19. Trees baited (all) and xylem, phloem and nut samples collected (treatments 1-4)	June & August 2015
20. Post-treatment assessment of treatment efficacy	August 2015
21. Final report, peer-reviewed publication submitted	December 2015

Results

Texas: Within one week of applications of TREE-age alone and combined with Alamo in April 2012 almost all trees experienced noticeable leaf burn. The combo treatment appeared to show greater phytotoxic symptoms than the insecticide alone.

Very little insect damage (psyliid and defoliator) was observed on any of the walnut trees at the two Texas locations in 2012 and 2013 (Table 1 and 2). As a result of high variability, there was no difference among the treatments.

Table 1: Occurrence and severity of damage caused by insects or injections of sytemic chemicals on Black Walnuts; Power's property, Rusk (Cherokee Co.), TX - 2012

		Psyllid	Tree Condition		
Treatment*	N	20-Jul	13-Apr	10-May	20-Jul
Emamectin benzoate	15	0.67	1.67	1.33	1.80
EB + Propiconizole	15	0.57	3.67	2.30	2.30
Check	14	1.46	1.00	1.00	1.18

Tree Condition: 1 = Excellent, 2 = Good, 3 = Fair, 4 = Poor, 5 = Near Death or Dead

Psyllid Rank: 1 = Isolated; 2 = light; 3 = moderate; 4 = heavy; 5 = extensive

Table 2: Occurrence and severity of damage caused by insects and/or injections of sytemic chemicals on Black Walnuts; Read's property, Martinsville (Nacogdoches Co.), TX - 2012

		Defoliator	Psyllid		Condition		
Treatment*	N	8-Jun	8-Jun	20-Jul	13-Apr	10-May	20-Jul
Emamectin benzoate	10	0.75	1.00	1.90	1.25	1.05	1.05
Check	10	1.90	1.95	2.80	1.00	0.37	0.37

Defoliator and Psyllid Rank: 1 = Isolated; 2 = light; 3 = moderate; 4 = heavy; 5 = extensive

Tree Condition: 1 = Excellent, 2 = Good, 3 = Fair, 4 = Poor, 5 = Near Death or Dead

Tennessee: All products proved to be difficult to inject in April 2012 most likely because the new leaves were about 50% extended. Similar to Texas, all treatments caused phytotoxic symptoms (leaf deformity or burn) on nearly all trees (Table 3). However, the extent of leaf burn was 2x greater on trees treated with the combo treatment compared to trees with either TREE-age or Alamo alone.

Table 3: Occurrence and severity of damage caused by injections of sytemic chemicals on Black Walnuts; Bill France property, Seymour (Sevier Co.), TN - 2012

Treatment*	N	Phytotoxic Symptoms Ranking	Leaf Deformity	Bark Separation
Emamectin benzoate	40	1.09	0.40	0.13
Propiconazole	39	1.06	1.79	0.21
EB + Propiconizole	40	2.33	1.58	0.15
Check	19	0.00	0.37	0.00

Phytotoxicity ranking : 0= no signs; 1 = 20% of crown w burn; 2 = 40%; 3= 60%; 4 = 80%; 5 = 100%

Leaves affected by chemical: 0 = None; 1 =light, 2 = moderate; 3 = severe

Many of the walnut study trees already exhibited signs of decline (flagging and dead branches, thin crown) had died recently. Subsequent branch samples collected in August 2012 showed that 42 – 83% had walnut twig beetle attacks (Table 4) so trees were not baited to attract additional beetles. Although, some larval galleries and canker areas were observed, live WTB adults and larvae and brood emergence holes were not found on any of the branches.

As a result, all trees were baited in June and then again in August 2013. Branches collected in August 2013 were improperly stored so no useable data could be collected. Those branches collected in November 2013, were autopsied and some useful data was collected. There were no differences among the treatments in the number of adult entrance holes, galleries, and adults found in those galleries, and adult gallery length (Table 5). Only the number of holes created by emerging brood adults differed among treatments. Branches from TREE-age treated trees had significantly fewer holes than untreated checks. Analysis of xylem, phloem and nut meat tissue indicates that both emamectin benzoate and propiconazole had been translocated into the crown, though at relatively low levels (Table 7 and 8)

The condition of treated trees improved markedly after treatment indicates that the treatments are beneficial and are allowing them to begin recovering from WTB attack and TCD infection (Table 6).

Conclusions (to date):

Newly emerging black walnut leaf tissue is highly sensitive to TREE-age and Alamo. Trees injected later in the growing season (July), after tissue hardening, did not show any phytotoxic symptoms. Therefore, in the future, walnuts should be treated with TREE-age and/or Alamo, after leaf hardening.

It was expected that TREE-age would be very effective against WTB based on previous success with other bark beetle (southern pine beetle, western pine beetle, mountain pine beetle) and wood boring beetles. However, the concentration of emamectin benzoate was relatively low and WTB are very small and do not appear to score the xylem tissue as they construct their galleries. Therefore, the adult beetles may not be exposed to sufficient chemical to cause direct mortality. In contrast, it appears that brood larvae may be impacted given that there is a reduction in the number of exit holes on TREE-age-treated trees. Study trees were reinjected in September 2014 to increase concentration of chemical. Effects are being monitored in 2015.

Table 4. Occurrence and severity of damage caused by Walnut Twig Beetle/ Thousand Cankers Disease on Black Walnut branches; Seymour(Sevier Co.), TN - 2012

				Number, Length or Area per 100 cm^2 of branch Surface Aea							
Treatment	N	% Branches with WTB	Branch Surface Area	# WTB Attacks	# Egg Galleries	Lgth of Egg Gal (cm)	Adults Present? (N=0, Y=1)	Brood Present? (N=0, Y=1)	Canker Present? (N=0, Y=1)	Canker Area (cm^2)	# Exit Holes
Emamectin benzoate	6	83.3	180.9	1.9	1.2	2.2	0.0	0.0	0.4	3.1	0.0
EB + Propiconizole	7	42.8	186.7	3.6	1.8	2.8	0.0	0.0	0.6	2.8	0.0
Check	8	62.5	178.3	1.1	0.6	0.8	0.0	0.0	0.2	1.4	0.0

Table 5. Occurrence and severity of damage caused by Walnut Twig Beetle/ Thousand Cankers Disease on Black Walnut branches; Seymour(Sevier Co.), TN - 2013

Trt	# of Reps	No. Entry Holes	No. Exit Holes *	No.Adult Galleries	Gallery Length (mm)	No. Adults	No. sporulating	Branch Length (cm)	Branch Width (cm)	Branch area (cm2)	No. Entry Holes/ 100sqcm	No. Exit Holes/ 100sqcm	No. Galleries/ 100sqcm	Gallary Length/ 100sqcm
EB	14	64.14	1.93 A	15.00	92.36	9.86	22.43	90.59	3.19	291.95	22.78	0.58	5.24	31.91
EBP	14	44.93	2.57 AB	10.43	67.36	7.36	10.64	87.45	2.58	228.87	19.54	1.45	4.68	28.95
Check	6	51.33	5.00 B	7.67	40.17	4.83	10.17	84.24	2.66	227.10	22.57	2.78	3.37	17.92

* Means with different letters are significantly different at P= 0.05, Tukeys.

Table 6: Condition of Black Walnuts 4 and 16 months after treatment, Bill France property, Seymore Co., TN - August 2012 & 2013

				# Dead Branches				
Treatment*	N	Branch Flagging (BF)	Thinning Crown (TC)	< 1"	1-3"	> 3"	% Dieback	Tree Condition *
2012								
Emamectin benzoate	40	0.58	1.25	3.63	1.78	0.38	11.25	2.00
Propiconazole	39	1.31	1.74	3.33	1.54	0.49	13.46	2.42
EB + Propiconizole	40	1.21	2.15	3.35	2.28	0.43	13.50	2.53
Check	19	0.58	0.89	2.58	1.79	0.32	8.95	1.92
2013								
Emamectin benzoate	38	0.26	1.84	2.00	1.92	0.45	11.58	1.71
Propiconazole	39	0.51	1.59	2.59	1.67	0.49	10.51	1.72
EB + Propiconizole	40	0.48	1.73	2.38	2.33	0.53	14.25	1.75
Check	19	0.05	2.05	2.11	1.32	0.47	11.05	1.90

BF & TC Rank: 1 = Isolated; 2 = light; 3 = moderate; 4 = heavy; 5 = extensive

Condition: 1 = Excellent, 2 = Good, 3 = Fair, 4 = Poor, 5 = Near Death or Dead

Table 7. Mean Concentration (PPM) of emamectin benzoate (EB) in black walnut xylem, phloem and nut meat tissue 4 and 16 months folowing injection.

	Xylem		Phloem		Nut Meat	
	2012	2013	2012	2013	2012	2013
Emamectin benzoate	12.9710	1.7000	0.0575	0.0178	<0.001	<0.001
EB + Propiconazole	6.4611	1.1045	0.0995	0.0277	<0.001	<0.001
Check	<0.0059	0.0015	<0.0012	0.0013	<0.001	<0.001

Note: LOQ (Limit of qantitation) set at 1 ppb (0.001 ppm); 1 of 4 check samples from xylem and ploem had 0.002 ppm while others below LOQ

Table 8. Mean Concentration (PPM) of propiconazole (P) in black walnut xylem, phloem and nut meat tissue 4 and 16 months folowing injection.

	Xylem		Phloem		Nut Meat	
	2012	2013	2012	2013	2012	2013
Emamectin benzoate	n/a	n/a	n/a	n/a	n/a	n/a
EB + Propiconazole	-----	4.3129	-----	0.2373	<0.050	<0.050
Check	-----	<0.050	-----	<0.050	<0.050	<0.050

Note: LOQ (Limit of qantitation) set at 1 ppb (0.001 ppm); 1 of 4 check samples from xylem and ploem had 0.002 ppm while others below LOQ

Evaluation of Miticides for Control of Conifer Mites on Loblolly Pine: Phase I

Initiated in 2014; completed in 2015

Justification

Conifer mites (family Tetranychidae) attack most species of trees (including conifers) and shrubs. Nursery seedlings and windbreak trees are particularly susceptible because they are often treated with insecticides that kill predators of conifer mites (Cordell et al. 1989). Pine, hemlock, spruce, juniper, fir, and white-cedar are often heavily attacked.

Some trees species are attacked by more than one species of spider mites. The more important species on nursery seedlings are the spruce mite (Oligonychus ununguis), the conifer spider mite (O. coniferarum), and the southern red mite (O. illicis). These mites do best in cool spring and fall weather. Other mites, including the two-spotted spider mite (Tetranychus uriticae) do best in dry, hot summer weather.

Heavy infestations of conifer mites cause reduced seedling and young tree growth, along with foliage yellowing or browning. Although most spider mite attacks do not cause mortality, they may predispose trees to attack by insects and disease or to damage by adverse environmental conditions. Spider mite populations can explode after use of insecticides to control other insects when mite predators are killed as well.

Several miticides (insecticidal/miticidal oils and soaps, Dicofol™, Kelthane™, Avid™, Floramite™, Hexagon™, Sanmite™, and Forbid™) are available for control, but resistance can develop if the applicator relies too heavily on one product (Shetlar 2011). Recently, Arborjet has developed a new formulation of botanical miticide, EcoMite Plus™.

Objectives

Evaluate the potential efficacy of tree injection of TREE-äge™ (emamectin benzoate), and spray applications of EcoMite Plus, for control of secondary conifer mites.

Methods

<u>Locations, Treatments, and Environmental Conditions</u>

This study was conducted at The Campbell Group's Seed Orchard, Jasper, TX (about 30o57 N, 94o09 W, elev. 105 ft). An initial survey was conducted in mid-February 2014 of the general health of five-year-old loblolly pines in a polymix trial containing several families. Each pine will be evaluated for tip moth damage and presence of conifer mites. Twenty trees were randomly selected for treatment. An additional ten trees served as untreated checks.

There were three treatments: TREE-äge (emamectin benzoate) tree injection (treatment 1); Arborjet EcoMite Plus spray (treatment 2); and untreated control (treatment 3).

Each treatment was applied to 10 randomly-assigned trees. Test trees were located in areas with abundant conifer mite and tip moth activity, and spaced >4 m apart. The injection treatment (treatment 1) was injected at the labeled rate (2.5 ml TREE-äge per inch ground line diameter) after dilution in 1 part water with the Arborjet Tree IV™ microinfusion system (Arborjet, Inc. Woburn, MA) into a three points (use #3 Arborplugs) at staggered heights up to 6 inches above the ground. Injections occurred in mid-February 2014. Arborjet spray treatment (2) was applied in late February and again two weeks later.

In February, 2014 (at the time of initial spray treatment) and then 7, 14, 21, 28, 42, 56 days (for Treatment 1 & 2) and 4, 8 and 12 months (for Treatment 1 only) after treatment application, two lower branches were shaken over a white sheet of paper. The conifer mites found on the paper were counted and then identified by Alex Mangini of the USFS, FHP, Region 8. Mite counts at each assessment period were analzyed using MANOVA with repeated measures and time as the response. If signficant, contrasts were used to determine how treatments differed from one another. Prior to analysis, the data was transformed using the equation sqrt (y+0.5); a transformation good for count data when there are a lot of zeroes.

Results

Table one shows the major taxa of mites collected in this study by date of collection and treatment. These counts are rough, as some specimens were lost in processing. Identification of the mites collected in spring 2014 are listed in Table 2. Those collected during the winter and spring of 2015 are listed in Table 3. The spider mite, *Oligonychus milleri* (McGregor, 1950), was most numerous in the samples. This species is widespread in North America and causes yellowing or bronzing of needles on young pines (Jeppson et al. 1975). The other mites collected in the Prostigmata were few in number. Species in the three families Anystidae, Bdellidae and Camerobiidae are mostly predaceous on spider mites and other small arthropods. However, they usually do not occur in sufficient numbers to impact populations of spider mites. Mites in the mesostigmatid family Phytoseiidae are voracious predators of spider mites and occur in large enough numbers to impact spider mite populations. In the samples, three species were identified, with Neoseiulus arenillus (Denmark and Muma, 1967) as the most abundant phytoseiid. All *A. arenillus* were female and almost all contained an egg which indicates rapidly increasing numbers, especially in the final two collections in 2014.

Table 1. Collection information for mite specimens collected at Boyd Lake Seed Orchard during Spring 2014.

Date	Treatment	Rough Counts of Mites* – Major Taxa
02-27-2014	Pre-treatment	10+ each of Prostigmata and Mesostigmata
03-06-2014	Check	10+ each of both groups
	Injection	3 Prostigmata, 1 Mesostigmata
	Spray	3 Mesostigmata
03-13-2014	Check	Not recorded - vial dry
	Injection	No mites present
	Spray	No Prostigmata present, 5 Mesostigmata
03-19-2014	Check	30+ Prostigmata, 8 Mesostigmata
	Injection	3 Mesostigmata
	Spray	8 Mesostigmata, 4 Prostigmata
04-16-2014	Check	30+ each of both groups
	Injection	6 Mesostigmata
	Spray	5 Mesostigmata, 8 Prostigmata

* Counts should not be considered precise; some specimens were lost in processing.

Table 2. Specimen identification for specimens collected at Boyd Lake Seed Orchard Spring 2014.

Major Taxon	Family	Species	Slides Made*
Suborder Prostigmata			
	Tetranychidae	*Oligonychus milleri* (McGregor, 1950)	17
	Anystidae	*Anystis* sp.	7
	Bdellidae	likely Spinibdella sp.	2
	Camerobiidae	*Neophyllobius texanus* (McGregor, 1950)	1
Order Mesostigmata			
	Phytoseiidae	*Amblyseius obtusus* (Koch, 1839)	1
		Neoseiulus arenillus (Denmark & Muma, 1967)	13
		Typhlodromips sp. in the *lugubris* species group	1

*Not all specimens were mounted on microscope slides. Some slide mounts were discarded due to poor quality of specimen or damage to specimen during mounting process.

There was no significant difference in the number of mites found per treatment over time ($p = 0.0609$), but there was a significant difference in the overall number of mites found among the three treatments ($p = 0.0006$) when not accounting for time. Contrasts showed that the TREE-äge and EcoMite Plus treatments were not statistically different from one another ($p = 0.2782$), but were both statistically different from the control ($p = 0.0002$ and $p = 0.0036$, respectively). Overall, significantly more mites were found on the control trees than the TREE-äge and EcoMite Plus treated trees (Figure 1).

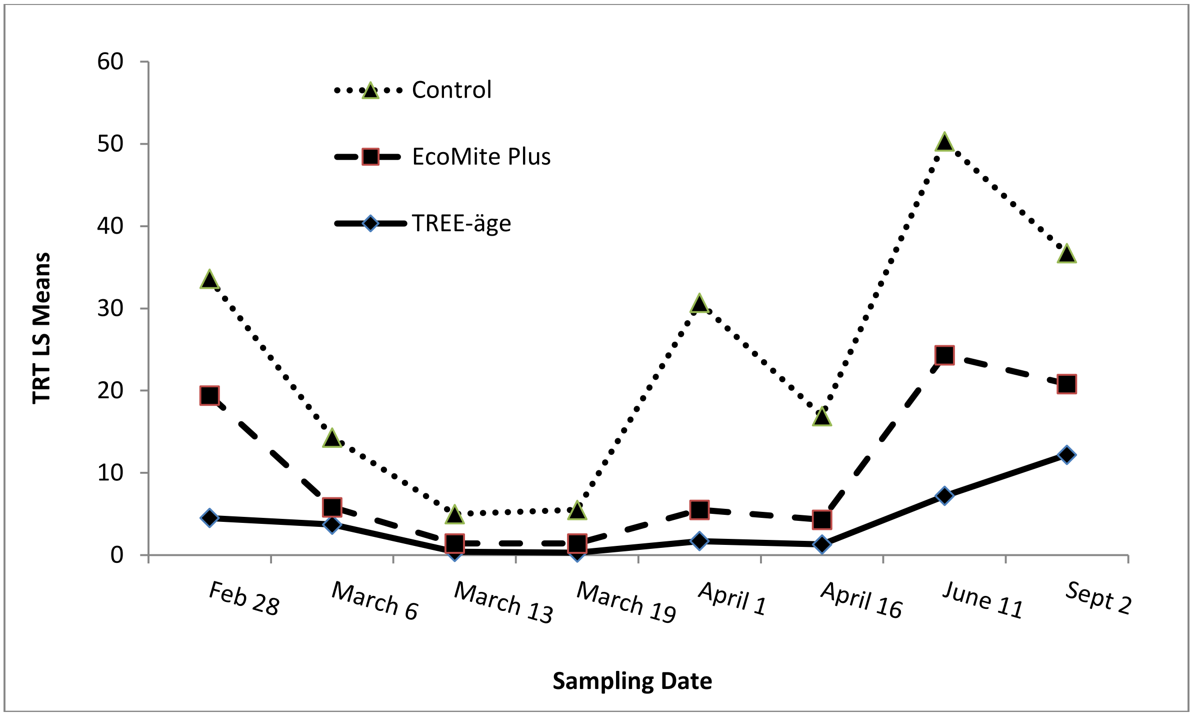

Figure 1. Treatment least square means following analysis (MANOVA) of the number of mites found per sampling date on trees treated with TREE-äge, EcoMite Plus, and untreated (control) trees.

Conclusion

It is likely there was no significant difference found among the treatments over time because generally, the efficacy of pesticides decreases over time, particularly that of spray pesticides such as EcoMite Plus. Therefore, this result is expected. Overall, both TREE-äge and EcoMite Plus resulted in a significant decrease in the number of mites found over a period of 40 days following treatment in comparison with the control trees. The TREE-äge treatment appeared more efficacious than the EcoMite Plus treatment, but differences were not statistically significant.

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Evaluation of Miticides for Control of Conifer Mites on Loblolly Pine: Phase II

Initiated 2014; Continued in 2015

Introduction:

Conifer mites (family Tetranychidae) attack most species of trees (including conifers) and shrubs. Nursery seedlings and windbreak trees are particularly susceptible because they are often treated with insecticides that kill predators of conifermites (Cordell et al. 1989). Pine, hemlock, spruce, juniper, fir, and white-cedar are often heavily attacked.

Some trees species are attacked by more than one species of spider mites. The more important species on nursery seedlings are the spruce mite (Oligonychus ununguis), the conifer spider mite (*O. coniferarum*), and the southern red mite (O. illicis). These mites do best in cool spring and fall weather. Other mites, including the twospotted spider mite (Tetranychus uriticae) do best in dry, hot summer weather.

Heavy infestations of conifer mites cause reduced seedling and young tree growth, along with foliage yellowing or browning. Although most spider mite attacks do not cause mortality, they may predispose trees to attack by insects and disease or to damage by adverse environmental conditions. Spider mite populations can explode after use of insecticides to control other insects when mite predators are killed as well.

Several miticides (insecticidal/miticidal oils and soaps, Dicofol™, Kelthane™, Avid™, Floramite™, Hexagon™, Sanmite™, and Forbid™) are available for control, but resistance can develop if the applicator relies too heavily on one product (Shetlar 2011 In Phase II, the FPMC evaluated emamectin benzoate and Eco-Mite sprays.). Recently, Arborjet has developed several new formulations of miticides that merit field testing.

Objectives:

1) Evaluate the efficacy and duration of tree injection of TREE-age™ (emamectin benzoate), IMA-jet (imidacloprid) and a new chemical (Arborjet's AJT-085), for control of secondary conifer mites.

Methods:

<u>Locations, Treatments, and Environmental Conditions</u>
This study is being conducted at Campbell Global's Boyd Lake Seed Orchard, Jasper, TX (about 30°57 N, 94°09 W, elev. 105 ft). An initial survey was conducted in early September 2014 of the general health of four-year-old loblolly pines in a polymix trial containing several families. Each pine was evaluated for presence of conifer mites. Thirty (30) trees will be randomly selected for treatment. An additional ten trees will serve as untreated checks.

There were four treatments: TREE-age (emamectin benzoate) tree injection (treatment **1**); IMA-jet (imidacloprid) tree injection (treatment **2**); Arborjet product AJT-085 tree injection (treatment

3), and untreated control (treatment **4**).

Each treatment will be applied to 10 randomly-assigned trees. Test trees will be located in areas with abundant tip moth and mite activity, and spaced >4 m apart. Treatment 1 will be injected at the labeled rate (2.5 ml TREE-age per inch ground line diameter) after dilution in 1 part water (=5 ml dilution per inch) while treatments 2 and 3 will be injected undiluted (2ml IMA-jet per inch GLD, 1.25 ml AJT-085 per inch GLD) with the Arborjet Tree IV™ microinfusion system (Arborjet, Inc. Woburn, MA) into a three points (use #3 Arborplugs) at staggered heights up to 6 inches above the ground. Injections will occur in early September 2014 (Trt 1) or early December 2014 (Trt 2 & 3).

On December 8, 2014 (at the time of initial injection treatment) and then 1, 3, 6, 9, and 12 months after treatment application, two lower branches will be shaken over a white sheet of paper. The conifer mites found on the paper will be counted and identified. In addition, the top whorl of each tree will be evaluated for tip moth damage.

Precipitation and temperature data will be obtained from the nearest weather station during the course of this study from 1 September, 2014 to 1 December 2015. A sample of mites collected will be sent to Dr. Alex Mangini, US Forest Service, in Pineville, Louisiana, for identification.

Results to date:

A list of mites collected from study trees is shown in Table 1. The spider mite *Oligonychus milleri* was the most common mite found in all the treatments before and after tree injection. The mean abundance of mites prior to treatment (December 8, 2014) and at intervals following treatment through June 23, 2015 are shown in Table 2. Results to date show that spider mite numbers increased markedly by February 9, 2015 in the IMA-jet and AJT085-treated trees and the check, but not in the TREE-age treated trees. Even by June 23, the spider mite abundance on trees injected with TREE-age more than six months prior had mean numbers of spider mites that were half those of the other treatments. The trees will be continue to be monitored periodically throughout 2015.

Table 1: Collection information – mite specimens collected from December 8, 2014 – June 15, 2015 at Boyd Lake Seed Orchard by Bill Upton. Identifications by Alex Mangini, USDA FS.

Treat	Mites[1] – Major Taxa	Count	Species IDs – Slide Mounts of Selected Specimens
8 December 2014			
Pre-treatment	Tetranychidae(spider mite)	24	*Oligonychus milleri* (McGregor, 1950)
	Phytoseiidae(predator)	9	*Typlhodromips* sp. in *lugubris* sp. grp.
	Anystidae(predator)	1	*Anystis* sp.
	Bdellidae(predator)	1	*Spinibdella* sp.
15 January 2015			
TreeAge	Phytoseiidae	2	*Typlhodromips* sp. in *lugubris* sp. grp.
	Homoptera(scale insects)	2	no slide mounts
ImaJet	Tetranychidae	4	*Oligonychus milleri* (McGregor, 1950)

	Phytoseiidae	4	*Typlhodromips* sp. in *lugubris* sp. grp.
	Homoptera(scale insects)	2	no slide mounts
AJT085	Tetranychidae	7	*Oligonychus milleri* (McGregor, 1950)
	Phytoseiidae	4	*Typlhodromips* sp. in *lugubris* sp. grp.
	Anystidae	1	*Anystis* sp.
Check	Tetranychidae	5	*Oligonychus milleri* (McGregor, 1950)
	Phytoseiidae	4	*Typlhodromips* sp. in *lugubris* sp. grp.
19 February 2015			
TreeAge	Tetranychidae	11	*Oligonychus milleri* (McGregor, 1950)
	Phytoseiidae	1	no slide mounts
ImaJet	Tetranychidae	42	*Oligonychus milleri* (McGregor, 1950)
AJT085	Tetranychidae	32	*Oligonychus milleri* (McGregor, 1950)
	Phytoseiidae	1	*Typlhodromips* sp. in *lugubris* sp. grp.
Check	Tetranychidae	27	*Oligonychus milleri* (McGregor, 1950)
	Phytoseiidae	2	*Neoseiulus arenillus* (Denmark & Muma, 1967) + species to be determined
24 March 2015			
TreeAge	Tetranychidae	1	*Oligonychus milleri* (McGregor, 1950)
	Phytoseiidae	2	no slide mounts
ImaJet	Tetranychidae	23	*Oligonychus milleri* (McGregor, 1950)
	Phytoseiidae	2	no slide mounts
AJT085	Tetranychidae	12	*Oligonychus milleri* (McGregor, 1950)
Check	Tetranychidae	5	*Oligonychus milleri* (McGregor, 1950)
19 June 2015			
TreeAge	Tetranychidae	1	*Oligonychus milleri* (McGregor, 1950)
	Phytoseiidae	5	no slide mounts
	Cunaxidae (predator)	1	further identification not yet determined
	Tarsonemidae (scavenger)	2	further identification not yet determined
	Oribatida (scavenger)	1	further identification not yet determined
ImaJet	Tetranychidae	2	*Oligonychus milleri* (McGregor, 1950)
	Phytoseiidae	24	*Amblyseius obtusus* (Koch, 1839)
	Tarsonemidae	4	no slide mounts
AJT085	Tetranychidae	5	*Oligonychus milleri* (McGregor, 1950)
	Phytoseiidae	20	*Amblyseius obtusus* (Koch, 1839) + *Typlhodromips* sp. in *lugubris* grp.
	Tarsonemidae	2	no slide mounts
Check	Tetranychidae	6	*Oligonychus milleri* (McGregor, 1950)
	Phytoseiidae	9	no slide mounts

[1] Counts should not be considered precise; some specimens were lost in processing.
[2] Only one vial sent so there is no sorting by treatment.

Table 2: Mean numbers of spider mites on treated and check trees in 2015

Treatment	Pre-treatment	Post Treatment			
	12/8/2014	1/15/2015	2/9/2015	3/24/2015	6/23/2015
TREE-age	6.2	1.9	2.3	5.2	9.8
Ima-jet	5.9	3.5	21.2	14	20.1
AJT085	3.9	4.1	17.8	27.5	30.2
Check	4.3	5.6	16.4	18.7	22.1

8. Literature cited:

Cordell, C.E., R.L. Anderson, W.H. Hoffard, T.D. Landis, R.S. Smith Jr., and H.V. Toko. 1987. Forest nursery pests. Agric. Handbook 680. U.S. Dept. Agriculture, Forest Service. 184 p.

Evaluation of Microinjection Systems for Application of Propiconazole to Manage Oak Wilt in Live Oak in Central Texas

Initiated in 2011; Final Evaluation in August 2015

Project funded by the International Society of Arboriculture – Texas Chapter

Justification

Several cultural control techniques (minimize fungal inoculum, timing of branch pruning, prompt removal of infected red oaks, and root disruption/trenching, among others) are available for management of oak wilt, caused by the plant pathogen, Ceratocystis fagacearum (T.W. Brentz) (Koch et al. 2010). However, these techniques are often impractical for treatment of high value individual trees or small groups of trees at risk to infection. Currently, the only published effective treatment available for protecting oaks from oak wilt infection is high volume treatments of the systemic fungicide propiconazole (Alamo®) diluted in water injected at the lower stem or root flare of trees (Appel and Kurdyla 1992, Appel 1995a, b). Applications of propiconazole have been made almost exclusively through the use of macro-infusion systems to deliver 10-20ml Alamo® diluted in 1 liter water per inch of tree DBH. The intent is to saturate the xylem tissue of the root collar with fungicide to prevent movement of the pathogen into the above ground area of the trees. The treatment is often effective in preventing tree death for about 2 years (Blaedow et al. 2010), but is very labor intensive to perform. Arborists are interested to know if propiconazole can be applied at more concentrated levels to live oak using available microinjection/infusion systems and whether these applications are effective in preventing/reducing fungal infection spread within the host.

Objectives:

1) Evaluate ability of various delivery systems to inject propiconazole formulation based on time to prepare/load, install and treat each tree and safety.

2) Evaluate speed and distribution of propiconazole movement based on protection during a 18 month period after injection.

Cooperators

Dr. David Appel	Department of Plant Pathology, Texas A&M University, College Station, TX
Mr. Robert Edmonson	Texas Forest Service, Johnson City, TX
Mr. Gene Gehring	Urban Renewal, Arlington, TX
Mr. Joseph Doccola	Arborjet, Inc., Woburn, MA
Mr. Jim Redicker	Scenic Hills Nursery, Kerrville, TX
Ms. Marianne Waindle	JJ Mauget, Arcadia, CA
Mr. Chip Doolittle	ArborSystems, Omaha, NE
Mr. Shawn Bernick	Rainbow Treecare Scientific Advancements, Minnetonka, MN
Mr. Jerry Pulley	Tree Clinic, Austin, TX
Dr. David Cox	Syngenta Crop Protection, Madera, CA
Mr. Bruce Fairchild	Private landowner near Johnson City, TX
Dr. Robert Conner	Private landowner near Fredericksburg, TX
Mr. David Kuhlken	Private landowner near Stonewall, TX

Methods

The following six injection/infusion systems were evaluated:

Mauget (capsule) System (Mauget; contact: Marianne Waindle) low volume (10 ml/inj pt); low pressure (10 psi)
Pine Infuser System (Rainbow Treecare Scientific Advancements; contact: Shawn Bernick); moderate volume (30 ml/inj pt); moderate pressure (40 psi)
Portle (Direct Inject) System (ArborSystems; contact: Chip Doolittle) – low volume (1 - 10 ml/inj pt); moderate - high pressure determined by applicator (50+ psi)
Chemjet System (Chemjet Trading Pty; contact: Jim Redicker) – low volume (20 ml/inj pt); low - moderate pressure (23 - 37 psi)
Tree IV System (Arborjet, Inc.; contact: Joe Doccola) – moderate volume (50-100 ml/inj pt); moderate pressure (60 psi)
Macro-Infusion System (Rainbow Treecare Scientific Advancements; contact: Shawn Bernick); high volume (200-600 ml/inj pt); low pressure (25 psi)

Information about the systems was requested from each manufacturer. In particular, information was requested on the recommended procedures for installation and injection of trees. Each system was ranked on the following criteria with maximum potential points in parentheses:

1) system cost (5 pts)
2) Can the system be left alone on tree (2 pts) or does the applicator need to manually operate system continuously? (1 pt)
3) Does chemical come prepackaged; can you inject product undiluted (2 pts) or is it necessary to dilute with water? (0 pts)
4) Weather restrictions (moisture, temperature) (2 pts if none)
5) Time and ease to fill system with chemical product (5 pts)
6) Number of injection points required per tree (5 pts)
7) Time and ease to install system on tree (10 pts)
8) Time and ease to inject X amount of product (20 pts)
9) Cumulative time applicator spends at each tree (10 pts)
10) System disposable or time and ease to clean system (4 pts)
11) Potential for chemical exposure (5 pts)
12) Effectiveness of treatment as of 18 months after oak wilt inoculation (30 pts)

This study was conducted within the range of plateau live oak (Quercus fusiformis) at three locations (near Johnson City, Stonewall and Fredericksburg) in central Texas. Non-symptomatic test trees (84), ranging from 14 to 80 cm (6 – 32 in) dbh (diameter at breast height) were selected between root barriers (trenches installed within the past year) and active oak wilt centers. There were four groups of seven study trees (28 total) at each site. On May 17-19, 2011, twelve (12) trees per delivery system were injected with propiconazole (Alamo®, Syngenta) at the label rate (10 ml/inch tree dbh) using each of the six systems described above. Twelve trees are serving as untreated controls. The application procedure used to inject the propiconazole formulation was based on the recommendations of each system manufacturer. The injected trees were allowed 10 weeks to translocate chemicals prior to being challenged with fungal inoculations.

Inoculations were performed using standard procedures (Camilli et al. 2009, Peacock and Fulbright 2009) on three of the four groups of trees at each site. Two *Hypoxylon Canker* isolates were cultured from samples recovered in spring 2011 from infected live oak and Spanish oak (*Q. buckleyi*) in an active oak wilt center in central Texas. The pathogen cultures were serially "plated" on petri plates containing Potato Dextrose Agar. Following 2 weeks of growth, the plates were flooded with 20 ml of sterile distilled water. The surfaces of the plates were scraped with a glass rod, resulting in a suspension of conidia. The conidia were harvested by pouring the water from the plates, combining the aliquots, and quantifying the total suspension with a hemacytometer. The suspension was adjusted to a level of 1 x 106 spores/ml with appropriate dilutions to make a quantity of the inoculum sufficient for the inoculations. On June 28, 2011, three groups of trees (21 total) were selected at each site. Two inoculation points (North and South sides) were located on each tree's roots >23 cm below injection points. At each point, a 14mm-wide wood chisel was used to cut through the bark into the xylem tissue (~ 2 cm deep). A dropper was used to apply 1 ml of conidia suspension into each wound site. Note: due to extreme drought conditions during the initial inoculation, it was be necessary to re-inoculate trees in May, 2012 and third time in June 2012.

The fourth group of trees at each site was evaluated for potential phytotoxic symptoms resulting from the injection of concentrated propiconazole under drought conditions.

A photograph of the crown of each study tree was taken at the time of fungal inoculation. Trees were initially evaluated for crown condition every 4 weeks. The date of oak wilt symptom (veinal chlorosis and necrosis, leaf drop, thinning crown) appearance was recorded and then switch to once every 12 weeks thereafter for 80 weeks (18 months). Each oak crown was given a rating of 0 (healthy), 1 (wilt symptoms comprising up to one-third of the crown), 2 (wilt symptoms comprising greater than one-third of the crown) (Mayfield et al. 2008), or 3 (dead tree). At each rating period, trees with a crown rating of 2 may be felled and wood samples taken from the stem and branches to determine the presence of *Ceratocystis fagacearum*.

At the termination of the experiment in August 2015 (about 32 months after the first pathogen inoculation), final crown ratings will be made. An analysis of variance will be used to test for differences among injection systems. A X2 (Chi-square) test for homogeneity will be used to test the null hypothesis that the percentage of trees with a crown rating of 2 or 3 did not differ between the fungicide-treated trees and the untreated control group (Mayfield et al. 2008). The null hypothesis will be rejected if more than 20% of the fungicide-treated trees reached a crown rating of 2 or 3. The test will be invalidated if fewer than 60% of the control trees reach a crown rating of 2 or 3.

Results

Field evaluations of injection systems were performed May 17, 18 and 19, 2011. Three (Tree IV, Pine Infuser, and Macro-Infusion) of the six systems were found to be capable of injecting the desired amount of propiconazole into all study trees (Table 1). Of the remaining systems,

Table 1: Comparison of six injection system characteristics during operational use in May 2011.

System Evaluated:	Mauget Capsules	Pine Infuser	Tree IV	Chemjet	Portle	Macro-infusion
No. Trees Injected	12	12	12	12	12	12
Mean DBH	12.8	11.9	12.4	12.8	11.7	12.8
Mean Volume Injected (mls)	128.2	237.0	496.7	127.6	117.3	12,625
No. Units used at a time:	12.9	7.9	2	12.6	1	1.4
Time (min) needed to fill system unit with chemical product:	0.0	4.0	3.2	2.6	0.0	3.3
Number of injection points required:	12.9	7.9	6.3	4	23.5	31.4
Time (min) needed to install system on tree:	6.4	7.0	6.1	6.2	11.6	27.8
Time (min) required to inject/infuse X-amount of product:	594.8	80.1	52.7	287.8	17.0	135.4
Cumulative time at tree (min):	6.4	4.3	6.4	6.5	28.6	29.8
Time (min) needed to clean system units	0	4.6	5.9	2.6	3.8	2.5

two (Chemjet and Mauget) were successful on most trees, but each had one tree where chemical remained in a few injectors even after 10 hours post-installation and the third system (Portle) had considerable leakage around most injection points; thus, it was uncertain how much product was injected into each tree.

Based on the time needed to inject product, there was no apparent advantage to injecting undiluted Alamo (Mauget or Portle) than to inject a diluted (Pine Infuser, Tree IV, Chemjet and Macro) solution. However, higher pressure systems (> 40 psi; Portle, Tree IV, and Pine Infuser) were able to push product into the tree faster than were lower pressure systems (Chemjet, Macro-Infusion and Mauget). Although the average injection rate for the Macro-Infusion (84.1 ml/minute) was 89% or more faster compared to that of the Tree IV (9.4 ml/min), Portle (6.9 ml/minute), Pine Infuser (3.0 ml/minute), Chemjet (0.4 ml/minute), and Mauget (0.2 ml/min), the cumulative time spent at a given tree with the Tree IV was 0.5 – 21 minutes shorter than the other systems.

Table 2 compares the six tested injection systems relative to twelve criteria (cost, can it be left alone, prepackaged or mix, weather restrictions, ease/time to fill system, number of injection points, ease/time to install system, ability of system to inject product, cumulative time spent at tree, disposable or ease/time to clean system, potential for chemical exposure, effectiveness of treatment after 18 months). The criteria had a value ranging from 2 to 30 points.

Table 2: Comparison of characteristics of several injection systems that may be compatible with propiconazole (Alamo).

Characteristics (Potential Points)	System: Tree IV		Chemjet		Capsules		Pine Infuser		Portle		Macro-infusion	
Manufacturer	Arborjet		Chemjet Trading		Mauget		Rainbow TreeCare		ArborSystems		Rainbow TreeCare	
Retail Cost to treat 12 study trees = 150" (5)	Equipment ($900) + Plugs ($38) + Chemical ($168) = **$1106**	1	Equipment ($270) + Chemical ($168) = **$438**	5	$3.85 / unit = **$578**	4	Equipment ($656) + Chemical ($168) = **$824**	3	Equipment ($775) + Chemical ($168) = **$943**	2	Equipment ($652) + Chemical ($168) = **$820**	3
Can System be Left Alone on Tree? (2)	Yes	2	Yes	2	Yes	2	Yes	2	No	1	Yes	2
Chemical Prepackaged, Undilute, or Mixed (2)	mixed w/ water	1	mixed w/ water	1	prepackaged	2	mixed w/ water	1	prepackaged	2	mixed w/ high volume water	0
Weather restriction(s) (2)	cold and dry, but less so because of higher pressure	2	cold and dry	1	cold and dry	1	cold and dry	1	cold and dry, but less so because of higher pressure	2	cold and dry	1
Ease / time to fill system with chemical product (5)	3.2 min - need to fill system for each tree	2	2.6 min. - each unit filled separately prior to installation on each tree	3	prepackaged	5	4 min. - each unit needs to be filled separately as it is installed on tree	1	if prepackaged	5	2.7 min. - each unit filled separately prior to installation on each tree	3
No. of injection points required per tree (5)	5.7 points	5	12.6 points	4	12.9 points	4	7.9 points	5	23.5 points	2	31.4 points	1
Ease / time of system installation on tree (10)	install plugs at few pts, but more steps - 6.1 min / tree	7	generally easy, few steps - 6.2 min / tree	10	generally easy, few steps - 6.4 min / tree	10	generally easy, but several steps involved - 7.0 min / tree	6	generally easy, but several injection pts - 11.6 min / tree	6	labor intensive to expose roots and many injection points - 27.8 min / tree	1
Ease and time to inject X amount of product (20)	effectively applied to all trees - 53 min / tree	17	effectively applied almost always - 210 min / tree	8	effectively applied almost always - 255 min / tree	7	effectively applied to all trees - 42 min / tree, but have to monitor pressure	13	application time short (17.4 min / tree), but not easy to get all chemical into tree	10	effectively applied to all trees - 134 min / tree	11
Cumulative time spent at each tree (10)	present at tree only to install and remove - 9 min / tree	10	present at tree only to install and remove - 10 min / tree	10	present at tree only to install and remove - 9.5 min / tree	10	present at tree only to install and remove - 10 min / tree	10	moderate time and must remain at tree - 29 min / tree	1	considerable time for install and removal - 30 min / tree	1
System disposable or ease / time to clean system (4)	need to clean several units at end of day - 5.8 min	3	need to clean several units after each tree - 3 min / tree	2	disposable	4	need to clean several units after each tree - 3.8 min / tree	2	should be easy flush, but chemical was also on outer surface of injector and needles - 11 min	1	need to clean several units, tees and lines at end of day - 10 min	1
Potential for chemical exposure (5)	very little exposure potential	3	little potential for exposure	3	very little exposure potential	5	little potential for exposure	3	frequent leaks from and around needles	1	some potential exposure	2
Effectiveness of treatment as of Dec 18, 2012 (17 month after injection) (30)	good	21	good	18	fair	12	fair	15	good	18	good	18
Total Score (out of 100 possible points)	**74**		**67**		**66**		**62**		**51**		**44**	

Excellent Good Fair Poor Bad

The Tree IV system (Arborjet) accumulated the greatest number of points (74) (Figure 1), so far, based on the fact it was very consistent in its ability to inject propiconazole into live oaks, it can be installed and left alone on a tree, and there is very little chance of chemical exposure. Other attractive features include that it is reusable, it has a large chemical capacity (1000 ml), require few injection points to treat the tree, and is not limited to any great extent by weather restrictions. Some important limitations include that it is fairly expensive system ($900 for 3 units), the need to install plugs and manage spaghetti tubing, the need to mix product with water prior to injection, and the need to measure product and fill the system for each tree.

Arborjet's Tree IV Chemjet

The Chemjet system (Chemjet Trading) was second with 67 points. It has several attractive features including that it is inexpensive, the system can be filled and installed quickly and left alone on the tree, it requires fewer injection points to treat the tree, and it's reusable and easy to clean. Some limitations include that the system requires considerable time (averaged 4+ hrs, but 19 hr for one tree; in this case a few units never emptied completely) to push chemical into the tree, there is some potential for chemical exposure, and it is more limited by weather restrictions than the Tree IV because of lower system pressure.

The Mauget capsules system was third with 66 points. Advantages include the system is prepackaged, low cost per unit, easy to install; does not require constant monitoring, the capsules are disposable (convenience), and showed little potential for chemical exposure. However, Mauget does not normally carry the higher volume (10 ml) of Alamo®, it requires considerable time (averaged near 10 hr, 26 hrs for two trees) to treat trees, and use may be more limited by weather restrictions (cold or dry conditions) than are other higher pressure systems.

Rainbow Treecare's Pine Infuser Mauget's capsules

The Pine Infuser (Rainbow Treecare) system was fourth with 62 points. Advantages include that it requires fewer injection points to treat the tree (compared to the standard Macro), fairly short injection time, it is reusable, and can be left alone on the tree. Limitations include: fairly expensive, there are several steps involved in installation and filling the system, there is some potential for chemical exposure, and it is more limited by weather restrictions than the Tree IV because of lower system pressure.

The Portle System (ArborSystem) was fifth with 51 points. Its attractive features are that the product is prepackaged, the system has a large product capacity (1000 ml), is reusable, and easy to install on the tree. Some important limitations include the need for several more injection points compared to most other systems (more time and effort), the need for the applicator to remain with the system during the injection, there is considerable potential for chemical exposure (particularly when attempting to inject 10 ml per site) because of leakage out of injection points, and a fairly high cost.

Rainbow Treecare's Macro-Infusion

ArborSystems' Direct-Inject Portle

The Macro-Infusion (Rainbow Treecare) system was sixth with 44 points. The system has a large product capacity (13,000 ml), is reusable, can be left alone on a tree, and has been shown to effectively apply product to all trees. However, the overall cost is high (particularly if the operator was to purchase an air spade and compressor), the need to mix large volumes of chemical dilutions, considerable time is required to expose the root flare and install the system, and the need to remove air from the lines during installation. Thus, there is a higher potential for chemical exposure and cleaning the system takes longer compared to other systems evaluated.

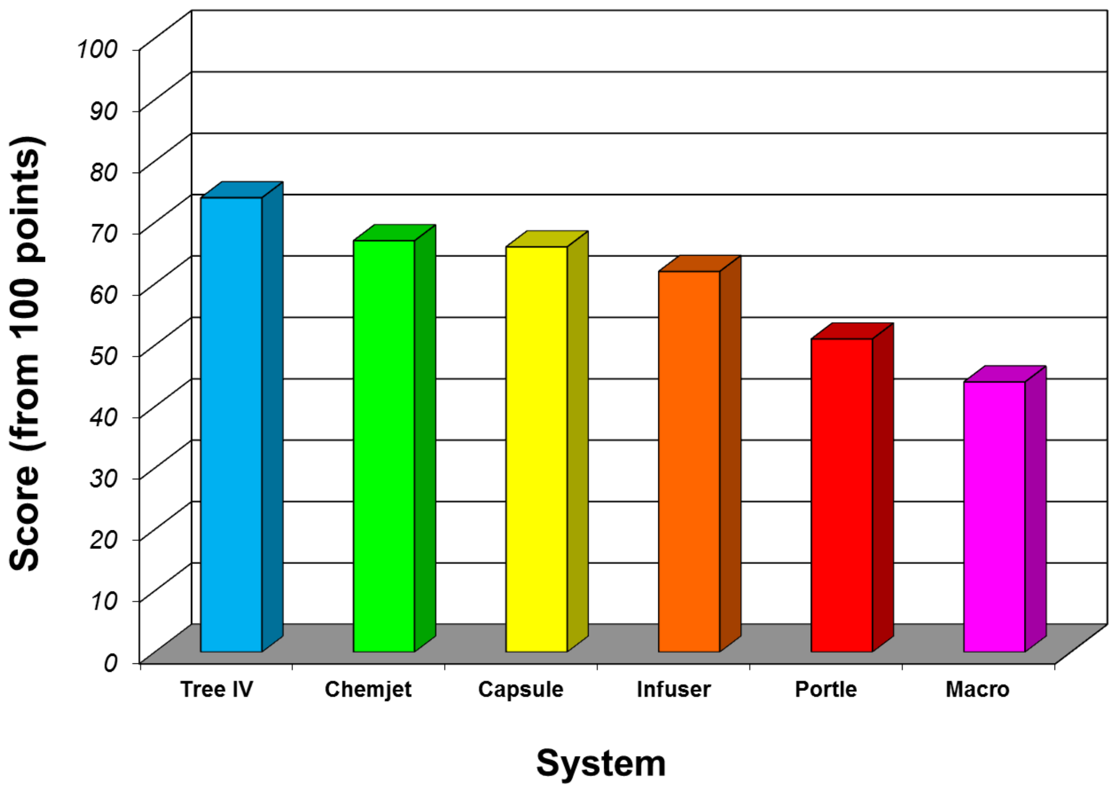

Figure 1. Total score (of 100 points) received by different injection systems.

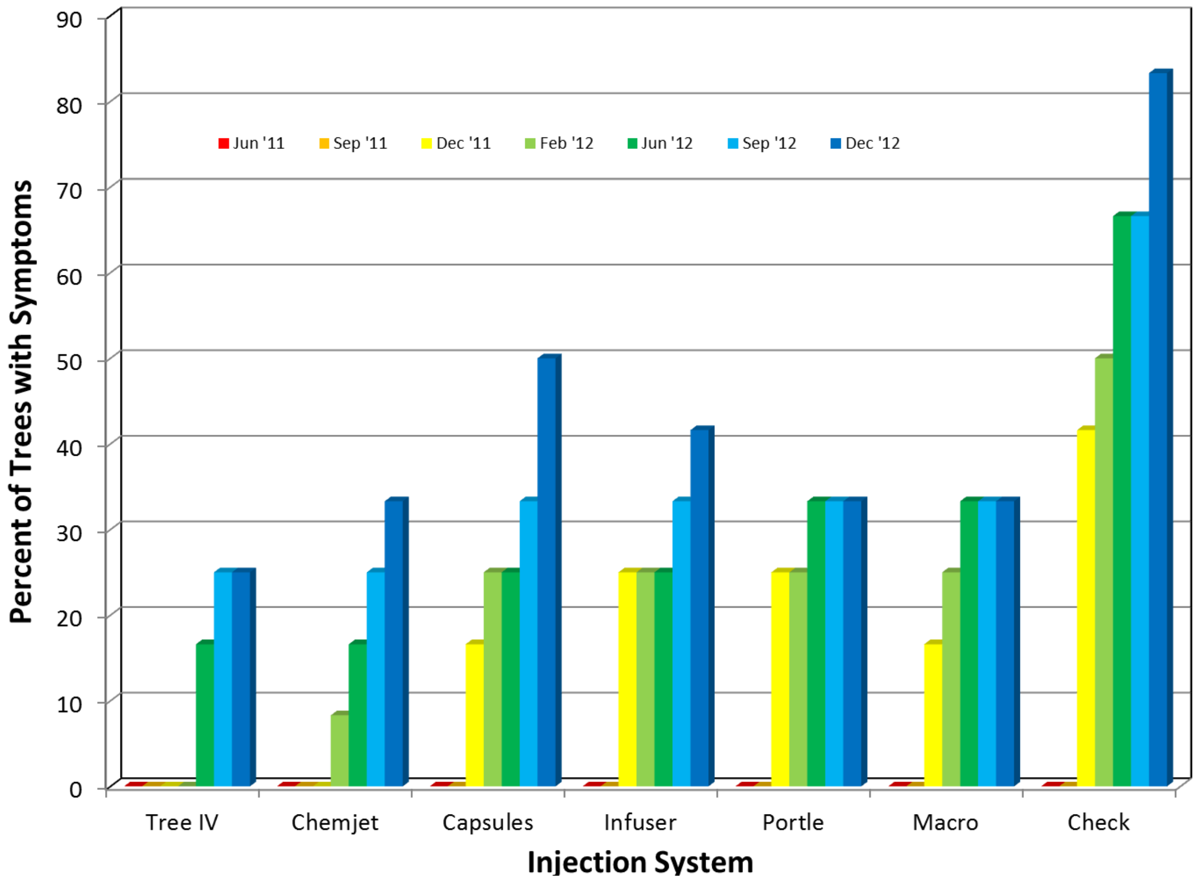

Figure 2. Effect of propiconazole treatments using different injection systems on the occurrence of oak wilt symptoms (veinal necrosis) on live oak in central Texas from June 2011 to December 2012.

Most of the above systems were effective in injecting the desired amount of product into each of 12 trees; the exceptions being one tree each for the Chemjet and Mauget capsules where a few units still held chemical after 19 and 26 hrs, respectively, and the Portle was ineffective at injecting the desired amount as there was considerable leakage. The evaluation of study trees 1, 2, 3, and 4 months after injection revealed that none of the trees exhibited symptoms (veinal necrosis, dieback, mortality) attributable to oak wilt. Note: one oak treated with the Macro-Infusion system appeared to have died, apparently due to extreme drought stress. However, once rain began to fall in October, some of the trees began to exhibit oak wilt symptoms in November and December and February. The positions of newly infected trees relative to the old oak wilt centers suggest that all trees were infected naturally. As of February 2012, the Tree IV system was the only one without symptomatic trees. However, this may be due more to position of treated trees relative to the oak wilt center than due to efficacy of the treatment. Three trees treated via the Macro-Infusion system exhibited oak wilt symptoms by February, but the mean level of defoliation at this time is relatively light (25%) compared to the higher levels (35 – 70%) of defoliation observed on symptomatic trees treated by other systems (Chemjet, Mauget, Pine Infuser, and Portle). This suggests that, so far, the Macro-Infusion treatment is better able to delay fungal infection compared to the other systems.

Additional evaluations were conducted through the remainder of 2012. By December, 2012, 83% of the untreated trees were exhibiting oak wilt symptoms, while symptoms were observed on 25% (Tree IV) to 50% (capsules) of the treated trees (Figure 2). Tree mortality (where trees have lost >97% of their foliage) was increasing through fall 2012. By December, mortality ranged from 17% (2 of 12 for Tree IV, Chemjet, capsules and macro) to 33% (4 of 12 for Pine Infuser), but the treatments did not differ significantly.

A final evaluation of treated and check trees was made on August 10, 2015. A total of 9 (of 12) check trees had died or were about to die (at least 80% defoliation). Among the 12 injected trees per treatment, mortality or at least 75% defoliation had occurred in five trees treated with the Mauget System; five with the Pine Infuser system; four with the Chemjet system; four with the Portle system; four with the Macro-injection System; and three with the Tree IV system (Figure 3). The presence of the fungicide propiconazole appeared to reduce risk of tree mortality to similar levels, regardless of application system. The two best application systems based on ease of application and efficacy appeared to be the Tree-IV and the Macro-infusion systems. Among surviving trees in these two treatments, the trees treated with the Tree-IV system ranged from 0 to 40% defoliation (mean = 16%) while the surviving trees treated with the Macro-infusion System ranged from 0 to 75% defoliation (mean = 22%) in August 2015. There were no signs of veinal necrosis in any of the study trees still alive in August 2015, and many of the surviving trees seemed to be recovering from previous defoliation and oak wilt infection.

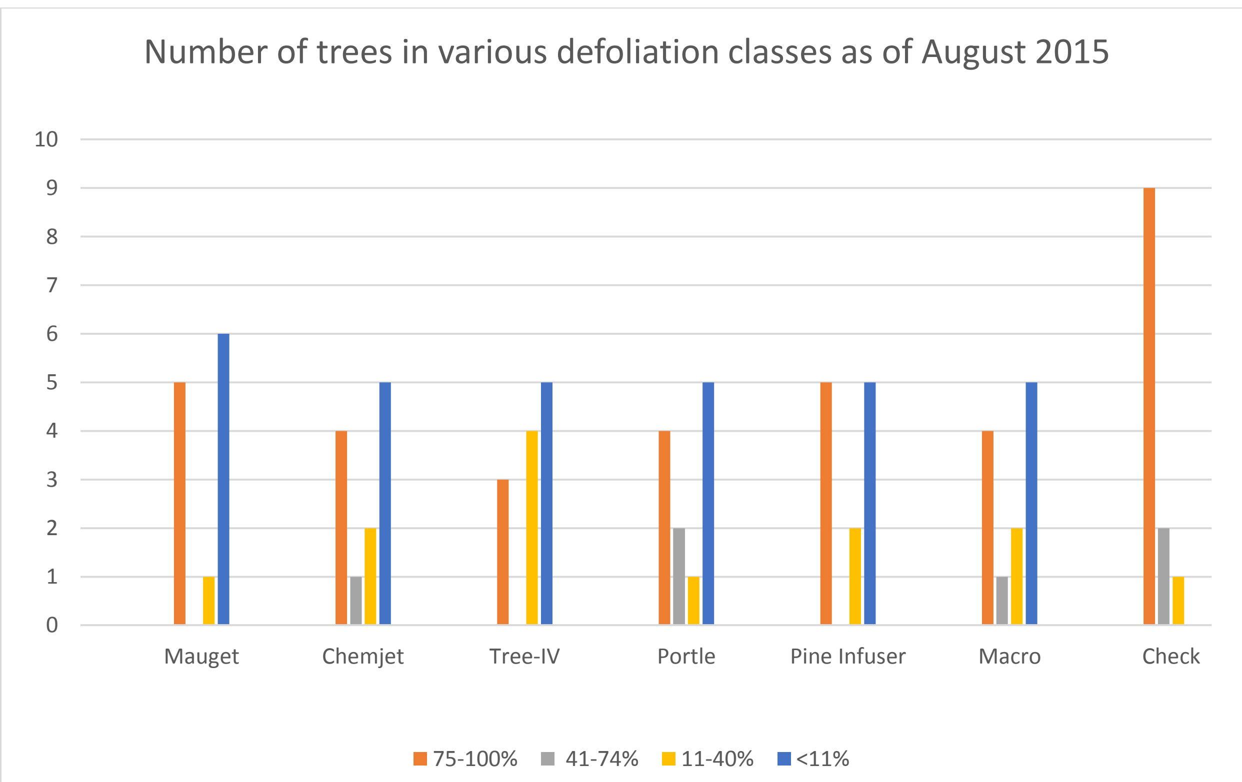

Figure 3: Number of trees (total = 12 replicates/treatment) within various defoliation classes 4 years after inoculation with oak wilt fungus for six different applicator systems as of August 10, 2015. Central Texas.

Conclusions

Two microinjection systems (Tree IV and Pine Infuser) and macro-infusion were found to be operationally effective in the injection of a full dose of propiconazole into live oak. Two other microinjection systems (Mauget capsules and Chemjet) were effective on most (not all) trees. The arborist/tree care provider needs to consider several factors (cost, convenience, injection rate, safety, etc.) before selecting a system to use. These four microinjection systems can be more convenient to use compared to the Macro-Infusion system.

All systems reduced the development of oak wilt symptoms. No treatment was 100% effective for halting oak wilt infection, but the percent of tree mortality and the extent of defoliation were significantly less for treated trees, regardless of the injection system used. The least tree mortality (3 of 12 trees) occurred with the Tree IV system and the Macro-infussion System. More replications are needed to determine if the small differences in tree survival among injection systems is significant.

It is important to note that for two systems, the unit (Mauget capsules) or protocol (Portle) was modified to make them comparable to other systems used in this study (10 ml per inch rate). Mauget capsules normally deliver less product (4 ml or 6 ml of tebuconizole). However, each unit was filled with 10 ml of propiconazole for the study. Nevertheless, they performed well (except for one tree) even under drought conditions. ArborSystems' (Direct-Inject) Portle system was designed to normally deliver up to 2 ml product per injection site. However, it would have required 5X (>100) the number of injection points and considerably increased the time of injection. Thus, we attempted to push the amount per site to 10 ml. Unfortunately, this resulted in considerable leakage around needles at most sites.

The development of new and/or improved injection systems continues with the realization that protection of trees and crops with systemic chemicals is an economically viable option. All participating companies continue to upgrade their systems. Other untested systems, such as Sidewinder□ and Eco-ject (BioForest Technologies) may also prove to be effective options.

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Evaluation of PHOSPHO-jet for Therapeutic Treatment of Oaks Infected with Hypoxylon Canker

Initiated in 2012; completed in 2015

Justification

Hypoxylon canker (HC) is a fungus [*Biscogniauxia atropunctata* var. *atropunctata* (syn. *Hypoxylon atropunctatum*) and other *Hypoxylon* spp.] that causes cankers and death of oak and other hardwood trees (Pase 2012). The disease is common in East and Central Texas and all across the southern United States. Relatively healthy trees are not invaded by the fungus, but the hypoxylon fungus will readily infect the sapwood of a tree that has been damaged, stressed, or weakened. Natural and man-caused factors that can weaken a tree include defoliation by insects or leaf fungi, saturated soil, fill dirt, soil compaction, excavation in the root zone of the tree, removal of top soil under the tree, disease, herbicide injury, drought, heat, nutrient deficiencies, competition or overcrowding, and other factors. The hypoxylon fungus is considered a weak pathogen in that it is not aggressive enough to invade healthy trees.

Hypoxylon canker activity usually increases during and shortly after prolonged droughts. When drought stresses trees, the fungus is able to take advantage of these weakened trees. The moisture content of living wood in live, healthy trees is typically 120% - 160%. It is difficult for HC to develop in wood that has a normal moisture content. However, any of the factors listed above could weaken or stress trees causing the moisture content of the wood to reach levels low enough for the hypoxylon fungus to develop. When this happens, the fungus becomes active in the tree and invades and decays the sapwood causing the tree to die. Once hypoxylon actively infects a tree, the tree will likely die.

An early indication that HC may be invading a tree is a noticeable thinning of the crown. Also, the crown may exhibit branch dieback. As the fungus develops, small sections of bark will slough from the trunk and branches and collect at the base of the tree. Where the bark has sloughed off, tan, olive green, or reddish-brown, powdery spores can be seen. In four to eight weeks, these tan areas will turn dark brown to black and become hard. They have the appearance of solidified tar. After several months, the areas will become a silver-gray color.

Once the fungus invades the tree, the sapwood begins to rapidly decay. Trees that have died from HC and are located in areas where they could fall on structures, roads, fences, powerlines, etc., should be removed as soon as possible.

Probably all oak trees are susceptible to HC. In addition, elm, pecan, hickory, sycamore, maple, beech, and other trees may be infected. The fungus spreads by airborne spores that apparently infect trees of any age by colonizing the inner bark. The fungus is known to be present in many healthy trees and can survive for long periods of time in the inner bark without invading the sapwood. As mentioned earlier, when a tree is weakened or stressed, the fungus may then invade the sapwood and become one of several factors that ultimately kill the tree.

Until recently, there was no known control for HC other than maintaining tree vigor. During drought periods, supplemental watering is recommended, if the tree is near a water source. However, some preliminary evidence suggests that oak trees exhibiting signs of HC may recover after injection with PHOSPHO-jet (salts of phosphorous acid, Arborjet Inc., Woburn, MA) (JB Toorish, personal communication).

Location: Lufkin, TX

Objectives

2) Evaluate the potential efficacy of systemic injections of PHOSHO-jet (salts of phosporous acid) as a therapeutic treatment of oaks against hypoxylon canker;
3) Apply PHOSPHO-jet treatment to assigned trees (September 2012 and May 2014), and
4) Determine the duration of treatment efficacy.

Methods

Locations, Treatments, and Environmental Conditions:This study was conducted within Kit McConnico Park, Lufkin, TX (about 31º22 N, 94º41 W, elev. 249 ft). A survey was conducted in August 2012 (the year following the unprecedented 2011 drought in Texas) of the general health of red oaks along the Kit McConnico Hiking and Biking Trial (5.1 miles in length). Each oak was assigned to one of three health categories: **Healthy**; "healthy", crown with < 20% of crown showing dieback; **Moderate**: evidence of HC infection and 20-80% of crown showing dieback; **Severe**: obvious HC infection and > 80% of crown showing dieback. Ten (10) red oaks from each of the healthy, moderate and severe health categories were randomly selected for PHOSPHO-jet treatment. An additional ten trees from each category served as untreated checks.

There were six treatments: PHOSPHO-jet treatment of healthy tree (treatment **1**); untreated healthy tree (treatment **2**); PHOSPHO-jet treatment of trees with moderate HC infection (treatment **3**); untreated moderate HC tree (treatment **4**); PHOSPHO-jet treatment of tree with severe HC infection (treatment **5**); and untreated severe HC tree (treatment **6**).

In September 2012, each fungicide treatment (treatments 1, 3, & 5) was injected at the labeled rate (5.0 ml PHOSPHO-jet per inch DBH for trees < 24 inch DBH and 7.0 ml per inch DBH for trees ≥24 inch DBH) after dilution in 2 parts water with the Arborjet Tree IV™ or QUIK-jet™ microinfusion system (Arborjet, Inc. Woburn, MA) into evenly spaced points (number is calculated by DBH/2) 0.3 m above the ground was applied to 10 randomly-assigned trees. Test trees were located in areas with abundant HC activity, spaced >10 m apart, 20 to 76 cm dbh, and within 100 m of access trails to facilitate the treatment. In May 2014, trees representing treatments 1, 3 and 5 were reinjected with fungicide at the same rates applied previously.

In September, 2012 (at the time of initial treatment) and then the following spring (May) and fall (September) of 2013 and 2014, the stem and crown of each tree was ranked as to health and the extent of fungal infection, where: 0=Healthy to very light damage (0-20% crown loss), 1=Medium Damage (20%-80% crown loss, 2=Heavy damage (> 80% crown loss), or 3=Dead.

Results

Overall, there was no significant difference in change of condition over time. However, treated trees initially in the poorest of health showed the greatest improvements in health (Table 1). Some severely-infected control trees also improved but not quite to the extent that treated trees did. This improvement is likely due to alleviation of drought conditions. Lightly and moderately infected treated vs. untreated trees showed parallel changes in health over time (Table 1). A final evaluation of treated and check trees was conducted on June 23, 2015. Largely due to abundant rainfall in recent years, all the treated and check trees showed improvement and there were no significant differences in tree condition among treatments. None of the study trees died since the study began. This suggests that hypoxylon-affected oaks may recover and improve in health with the return of sufficient soil moisture, with no need for fungicide injections. Whether Phosho-jet treatments would save drought-stressed trees during a prolonged drought could not be evaluated in this study and remains to be determined.

Table 1. Change in Tree Health 8 and 12 months after Treatment with Phosphojet

		5/1/2013			9/1/2013		
Treatment	Initial Condition	Pct Improved	Pct Stable	Pct Declined	Pct Improved	Pct Stable	Pct Declined
Phosphojet	Healthy (<20%)	0	80	20	10	80	10
	Mod Decline (20-80%	30	70	0	0	80	20
	Sig. Decline (>80%)	80	20	0	10	80	10
	Overall	36.7	56.7	6.7	6.7	80.0	13.3
Untreated	Healthy (<20%)	0	90	10	0	100	0
	Mod Decline (20-80%	20	70	10	0	60	40
	Sig. Decline (>80%)	50	50	0	0	50	50
	Overall	23.3	70.0	6.7	0.0	70.0	30.0

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Efficacy of Emamectin Benzoate for Protecting Loblolly Pine Trees and Logs from Infection by Pine Wood Nematode

Initiated in 2013; completed in 2014

Justification

Export of US-produced softwood lumber exceeded $1 billion in 2011 (Timber Trends, Dec, '11/Jan. '12). However, export of unfinished southern pine logs has been severely restricted due to the potential export with the logs of pine wood nematode (PWN), *Bursaphelenchus xylophilus,* the causal agent of pine wilt disease. The PWN is transmitted (vectored) to conifers by pine sawyer beetles (*Monochamus* spp., Coleoptera: Cerambycidae) either when adult beetles feed on bark and phloem of twigs of susceptible live trees (primary transmission) or when female beetles lay eggs (oviposition) in dying trees or freshly-cut logs (secondary transmission). Bark must be present on tree or log for the adult beetles to oviposit and for the insect larvae to develop (Craighead 1950, Webb 1909). Pines *(Pinus* spp.) appear to be the most susceptible to PWN and at least 27 species in the continental United States and 38 species worldwide (15) have been reported as hosts. Yellow pines (loblolly, shortleaf, slash and longleaf) of the southeastern United States tend to be resistant to the development of pine wilt disease symptoms.

Because there is no cure for pine wilt, management practices have concentrated on preventing the spread of *Bursaphelenchus* and *Monochamus***.** Logs should not be exposed during the June-to-September egg-laying period of *Monochamus***.** If bark is immediately peeled from felled green trees, damage by sawyers is prevented (Webb 1909). A mill certification program (no bark, no grub holes) is strongly supported by the United States and Canada. Based on the biology of *Monochamus***,** this program assumes that if no grub (entrance) holes are visible, no insects in the sawn wood will emerge and transmit the PWN. Furthermore, the European *Monochamus***,** which requires bark for oviposition, will be unable to breed in bark-free wood, eliminating contamination by the PWN (Dwindell 1997).

Phytosanitary certificate requires log shipments to be PWN free. China requires logs to be debarked or fumigated (methyl bromide or phosphine) prior to export. Debarking generally costs a few dollars per ton while fumigation is prohibitively expensive, costing tens of dollars per ton (Hugh McManus, personal communication). Note: The general sampling protocol to obtain phytosanitary certificate: xylem tissue taken using a 2.5" wide drill bit at two points (one third distance) of the ends of each of 29 - 59 logs (number depends on state of harvest).

Data collected in 2012 indicated that 1) PWN is not present in live, standing loblolly pines trees, and 2) cerambycids have the potential to inoculate pine logs with PWN within one day of tree felling. Thus, there is still some risk of PWN infection even when logs are debarked one day after tree felling.

Emamectin benzoate is known to be effective in protecting susceptible pines against PWN for 2 or more years after treatment (Takai et al. 2000, 2001, 2003a, 2003b). Could tree injections of EB in advance of tree harvest serve as a preventative treatment for PWN infection and eliminate the need for fumigation or debarking procedures? Data is needed to confirm the efficacy and duration of emamectin benzoate against PWN and feasibility of treatment of pine trees prior to harvest.

Objectives

Determine the efficacy of emamectin benzoate (TREE-äge™) at two concentrations for protecting loblolly pine from pine wood nematode (PWN).

Cooperators

Hugh McManus	Hancock Forest Management, Shreveport, LA
Wilson Edwards	Weyerhaeuser Company, New Bern, NC

<u>Efficacy of Emamectin Benzoate</u>

In fall 2013, 30 "healthy appearing" loblolly pine trees (25 cm (=10") DBH, ~25 years of age) were selected in an east Texas plantation. In mid-October ten trees were randomly assigned and treated with one of the following treatments:

A = EB (4%) @ 2.5 ml @ **3"** spacing felled 8 month post injection (mid-June)
B = EB (8%) @ 1.25 ml @ **3"** spacing felled 8 month post injection (mid-June)
C = EB (8% (1.25ml) diluted 1:1 with water for final volume of 2.5 ml per point @ 3" spacing felled 8 month post injection)
CHK= Check (untreated) for each Treatment set above (10)

The chemical was allowed 8 months to circulate within each tree prior to felling. Immediately after felling (within an hour), 1.0 m bolts were taken from the main stem of the lower crown (~6 m), and lower bole (0 m). The 80 bolt sections were placed about 1 m apart on discarded, dry pine bolts to maximize surface area available for colonization as well as to discourage predation by ground and litter-inhabiting organisms. A bait blend (ethanol, (-) a-pinene, ipsenol, ipsdienol, and monochamol) was deployed in the harvest area to attract cerambycid beetles. All logs were sampled for PWN 26-30 days after tree felling.

Monitoring *Monochamus* species and PWN occurrence in beetles.
Modified funnel traps were deployed (beginning in early March, 2014) at 2-3 nearby harvest sites. Traps were baited with kairomone blend (ethanol, (-)alpha-pinene, ipsenol, ipsdienol, & monochamol) placed inside the funnels and using a wet cup (Miller et al. 2011, Dave Wakarchuk, personal communication). Traps were monitored once a week from March through September, 2014. Collected cerambycids were identified to species.

Inspecting logs for wood borer and bark beetle colonization
At 28 days after felling, borders of two 10 X 50 cm strips (total = 1000 cm^2) were marked on the bark surface and the number of cerambycid egg niches and bark beetle attacks were counted within each strip.

Just prior to collection of wood samples, two 10 X 50 cm strips (total = 1000 cm^2) of bark were removed from each log and the following assessments were made:

1. Number of live cerambycid larvae present under bark.
2. Cerambycid activity, estimated by overlaying a 100 cm^2 grid over a portion of each bark strip and counting the number of squares overlapping area where cerambycid larvae have fed.
3. Number of oval cerambycid larvae entrance holes into sapwood.
4. Number of successful and unsuccessful *Ips* attacks and number of *Ips* larvae found under the bark.
5. Presence and percent area covered with blue stain.

Sampling logs for pinewood nematodes 28 days after felling
Each log was sampled at four locations: two points within each of the two bark plate areas. A wire brush was used to remove dirt and debris from the sample locations. The first 5 cm from the sample locations was discarded in case of contaminates. A clean container was placed beneath the work site to catch shavings throughout the process. Using a 5.4 cm (2 1/8 in) drill bit, a hole was slowly drilled to the center of the log, reversing and removing the bit from the hole every 3.81 – 5.08 cm (1.5 – 2.0 inches) to collect the shavings. For large diameter trees a utensil was required to remove the final shavings.

All of the material drilled (except the external discard, as recommended on the protocol) from a given log was pooled into a bucket, mixed well, placed in a sealable plastic bag and keep at room temperature. Two hundred grams of the material from each log were sent to Dr. Weimen Ye of the Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture and Consumer Services in Raleigh, NC for nematode extraction and identification.

Identification of nematodes

Nematodes were identified to species at the Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture and Consumer Services in Raleigh, NC using a real-time PCR assay targeting the ITS-1 gene.

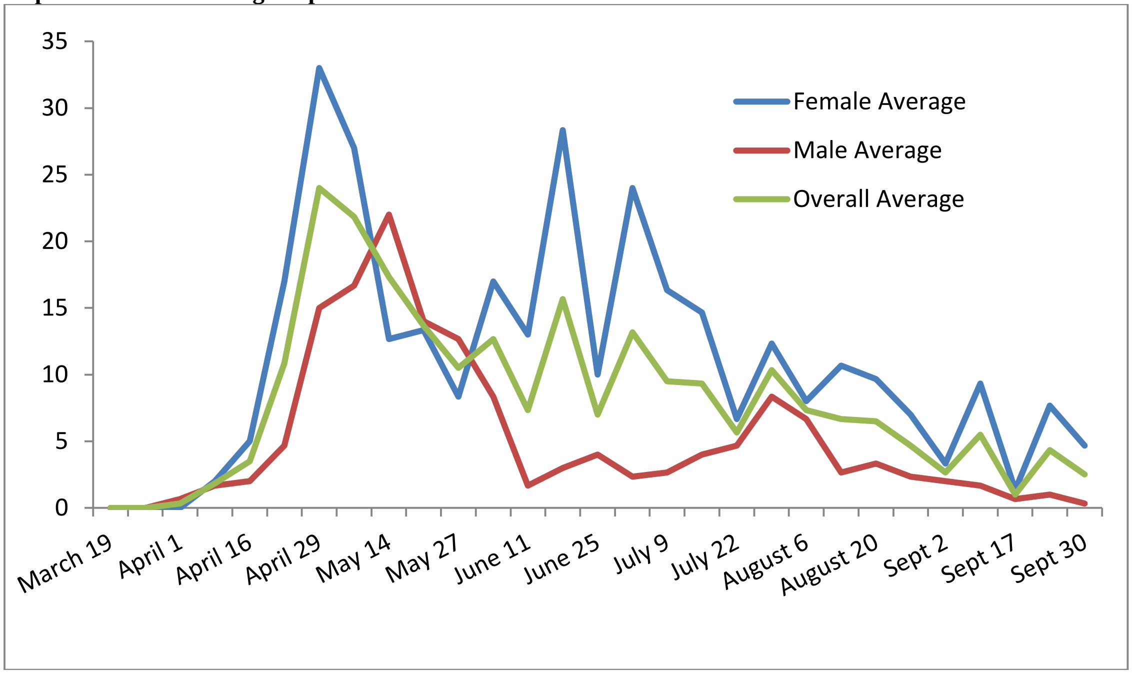
Data Analysis

The number of cerambycid egg niches, bark beetle attacks, nematodes present per log treatment, position on tree, and interval after felling and debarking, were measured to determine the degree of risk of PWN being present in logs destined for export. These data were analyzed using ANOVA followed by Tukey's HSD, if significant differences occurred.

Results

The number of female *Monochamus* woodborer numbers caught in baited traps peaked in April, June, and July, while male woodborer numbers peaked in May (Figure 1). Overall, woodborers were most abundant in the spring and slowly declined in number thereafter. None were caught prior to April 1 (Figure 1).

Figure 1. Average number of female and male *Monochamus* woodborers caught per week in east Texas using funnel traps from March through September 2014.



There was no significant difference between the upper and lower bolts for any of the variables measured except the number of woodborer niches (Table 1). This difference is most likely due to woodborer height of attack preference (i.e., bark thickness) rather than chemical distribution, therefore all data was pooled together per tree for the following analyses.

There was a significant difference among treatments in all variables measured except the number of *Ips* attacks found on the outside of the bark, the number of woodborer niches found on the outside of the bark, and the number of pine wood nematode found. Control trees had significantly more successful and significantly less unsuccessful *Ips* attacks, significantly more *Ips* brood, woodborer larvae, woodborer larvae feeding area (cm^2), and blue stain fungus (%) present compared to treatment trees (Table 1). Excluding the control, there was no significant difference among the different treatments for any parameter measured.

None of the treatments resulted in full protection of loblolly pine from pine wood nematode infection. However, very low numbers of PWN were found in treatments B (emamectin benzoate (8%) @ 1.25 mL @ 3-inch spacing) and C (emamectin benzoate (8%) @ 2.5 mL following dilution 1:1 with water @ 3-inch spacing) (Table 2). Due to the presence of PWN in all treatments, this study was discontinued in 2014.

Conclusions

Given the low number of PWN found in treatments B and C, it is likely that an extended period of distribution time (~ 1 year) would result in full protection of loblolly pine from pine wood nematode infestation. A new study should be conducted focusing on these two treatments, varying the time allowed for chemical distribution and the number of injection points. Logs should be exposed at different seasons to identify those seasons when logs are least likely to be infested by PWN.

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Table 1. Average number of *Ips* attacks found on the surface of bark, successful and unsuccessful attacks and brood found under the bark, number of woodborer niches on the surface of bark, the number of woodborer larvae and their feeding area (cm^2) found under the bark, percent blue stain, and the number of pinewood nematode found in lower and upper bolts of loblolly pine trees that were treated with three different rates of emamectin benzoate (treatments A, B, and C) or untreated (control).

			Ips				Woodborers				
			Surface	Under Bark			Surface	Under Bark			
Treatment	Bolt	n	# Attacks	# Attacks Unsuccessful	# Attacks Successful	Brood	# Niches	# Larva	Feeding Area (cm^2)	% Blue Stain	PWN
A	Lower	10	2	3.9	0.1	0	2.35	0.05	0.2	13.6	12.2
A	Upper	10	2.2	3.7	0	0	5.05	0	0	11.15	48
B	Lower	10	2.65	3.85	1.1	0.25	2.8	0	3.35	20.65	130.6
B	Upper	10	2.45	4.4	0	0	4.25	0	0	12.15	0
C	Lower	10	2.2	3.15	0.7	0.05	2.65	0	0.65	14.85	0.4
C	Upper	10	3.1	6.25	0.05	0.05	5.3	0	0	13.25	0
CHK	Lower	10	2.75	0	3	1	2.15	3.05	62.95	85.6	0
CHK	Upper	10	3.5	0	2.8	1	2.6	2.85	64.95	88.55	0

A = EB (4%) @ 2.5 ml @ 3" spacing felled 8 month post injection

B = EB (8%) @ 1.25 ml @ 3" spacing felled 8 month post injection

C = EB (8% (1.25ml) diluted 1:1 with water for final volume of 2.5 ml per point @ 3" spacing felled 8 month post injection)

Table 2. Number of logs infected with pinewood nematode and total number of pinewood nematode found per treatment of loblolly pine trees with TREE-äge at different rates.

Treatment	No. of Trees with PWN	Total No. of PWN
A	4 of 10	1876
B	3 of 10	32
C	1 of 10	4
Control	3 of 10	338

A = EB (4%) @ 2.5 ml @ 3" spacing felled 8 month post injection

B = EB (8%) @ 1.25 ml @ 3" spacing felled 8 month post injection
C = EB (8% (1.25ml) diluted 1:1 with water for final volume of 2.5 ml per point @ 3" spacing felled 8 month post injection)

Evaluation of Trunk Injections of Plant Growth Regulators for Phytotoxicity and Reduction of Fruit Production in Sweetgum

Initiated in 2013; discontinued in 2014

Justification

American sweetgum (*Liquidambar styraciflua*) can be an excellent landscape or street tree under the right circumstances (Dirr. 1983). The star-shaped leaves are a deep, glossy-green in the summer, and turn a range of colors, golden to red to purple, in the fall. The fruit is a 1 to 1 1/2 inch diameter rounded gumball, brown when mature, and coveted by wreath-makers and artisans for decorative uses. For arborists and landscapers however, the fruit of this species can be messy, unattractive, and a nuisance for maintenance crews. Maintenance of sweetgum would be easier if fruit production could be reduced or eliminated.

Plant growth regulators (PGRs) have been evaluated and used extensively to manage the vegetative growth of trees, shrubs, and grass along utility right of ways, and residential landscapes, etc. Commercial orchardists regularly make use of PGRs to thin fruit crops (Byers et al. 1983, Elfving and Cline 1993). Some of these materials are also registered for use on ornamental trees and shrubs to eliminate or reduce fruit production. Dikegulac-sodium (Atrimmec™, PBI/Gordon and Pinscher™, ArborSystem) spray applications were shown to reduce sweetgum ball production by 57% (Banko & Stefani 1995) and also labeled for suppression of flowers and fruit on ornamental olive, glossy privet, and multiflora rose. Ethephon (Florel™, Monterey) is labeled for home garden tomato ripening, mistletoe shoot removal, and undesirable fruit elimination on a number of ornamental shrubs and trees (including sweetgum and olive). Indol-3-butyric acid (Snipper, Tree Tech) is registered for use on several ornamental shrubs and tree species (including sweetgum and olive) for undesirable fruit elimination. Mefluidide (Embark™, PBI/Gordon) is labeled for suppression of flowers and fruit on ornamental olive. Methyl chlorflurenol (Maintain CF 125™) is labeled for use to eliminate fruit on olive. Timing of chemical spray applications is often critical to obtain optimal fruit reduction. However, injection of chemical treatments may reduce the importance of timing. Pinscher and Snipper are injectable formulations, while the others are labeled for use as foliar sprays. Two trials will be conducted to evaluate the phytotoxic effects and efficacy of injectable formulations of dikegulac sodium, indole-3-buttyric acid, ethephon, medfluidide, and methyl chlorflurenol for elimination of sweetgum fruit in landscape situations.

Objectives

1) Evaluate phytotoxic effects of trunk injections of dikegulac sodium, indole-3-buttyric acid, ethephon, medfluidide, and methyl chlorflurenol on sweetgum;

2) evaluate the efficacy of these five chemicals for elimination of fruit production on sweetgum;

3) determine the longevity of treatments.

Cooperators: Arborjet, Inc., Woburn, MA; Private landowners

Methods

Trial 1: This study was conducted on private land in the East Texas. Individual 2-inch (diameter at ground level) sweetgum trees (66) were selected. One of eleven treatments were randomly assigned to each of six trees. Note: Where possible, healthy (unstressed by drought, insect, or disease, etc.) trees were selected as study trees.

The treatments were:

1) Atrimmec (18.5% dikegulac sodium) at 2.0 ml per inch diameter in 1 point;
2) Atrimmec (18.5% dikegulac sodium) at 6.0 ml per inch diameter in 3 points;
3) Snipper (4% indole-3-buttyric acid) at 2.0 ml per inch diameter in 1 point,
4) Snipper (4% indole-3-buttyric acid) at 6.0 ml per inch diameter in 3 points,
5) Florel (3.9% ethephon) at 2.0 ml per inch diameter in 1 point,
6) Florel (3.9% ethephon) at 6.0 ml per inch diameter in 3 points,
7) Embark 2-S (3.2% medfluidide) at 5.0 ml per inch diameter in 1 point,
8) Embark 2-S (3.2% medfluidide) at 15.0 ml per inch diameter in 3 points,
9) Maintain CF125 (12.5% methyl chlorflurenol) at 5.0 ml per inch diameter in 1 point,
10) Maintain CF125 (12.5% methyl chlorflurenol) at 15.0 ml per inch diameter in 3 points,
11) Control – water at 15 ml per inch diameter in 3 points.

Each treatment was injected using the Arborjet QUIK-jet microinfusion system (Arborjet, Inc. Woburn, MA) and #3 Arborplugs into one or three (staggered heights) injection points starting 3 inches above the ground. The trees were treated in the fall (October) 2013. Three small (pencil thickness) branches were pruned at the time of injection.

Trees were evaluated visually for phytotoxic symptoms (yellowing or browning of leaves, excessive sap flow around injection points or pruning cuts) in February and April. The trees were cut at ground level and 12-inch bolts (containing the injection points) were retained for evaluation. The bolts were sent to Arborjet and examined for presence and length of phytotoxic lesions in the sapwood.

Trial 2: This study is to be conducted on private or forest industry lands in the East Texas, if results of trial 1 are positive. Individual (36-60) sweetgum trees, 8-10" DBH, will be selected. One of six treatments will be randomly assigned to each of 6-10 trees. Note: Where possible, healthy (unstressed by drought, insect, or disease, etc.) fruit-producing trees will be selected as study trees.

The proposed treatments will be:

1) dikegulac sodium (18.5% Atrimmec);
2) indole-3-buttyric acid (4%, Snipper),
3) ethephon (3.9%, Florel),
4) medfluidide (3.2% Embark),
5) methyl chlorflurenol (12.5%, Maintain),

6) untreated control

Note: Rates will be based on results from Trial 1.

Each systemic insecticide treatment will be injected with Arborjet QUIK-jet microinfusion system (Arborjet, Inc. Woburn, MA) at 4 inch interval around each tree's root flare. The trees will be treated prior to flower bloom in mid-March 2014 and again in late-summer (September).

Three branches will be tagged on each study tree. The number of female flowers will be counted on each branch at the time of spring injection. There will be 6-10 trees (replications) for each treatment. Maturing gumballs on tagged branches will be counted in September at the time of the second injection. The number female flowers will be counted on each branch the following spring (2015). Percent reduction in fruit formation will be calculated by the following formula: [(Number of female flowers - number of fruit) / number of fruit] x 100. The data will be subjected to analysis of variance and LSD test following arc sine transformation.

Results

Trial 1

Trees included in treatments 1-3 and 7-11 looked healthy (showed no evidence of phytotoxic effects) as of February 2014 (Table 1). Treatments 4-6 showed evidence of phytotoxic effects through oozing at the injection points, as well as in the area where branches had been cut off in the fall (Table 1).

During the first April 2014 assessment, trees included in treatments 1-2 and 5-8 had no leaves or buds, treatments 3, 4, and 6 looked healthy. Treatments 9 and 10 did leaf out, but the leaves were wilted and brown (Table 1).

Table 1. Three consecutive evaluations of sweetgum injected with 10 different plant growth regulators, in addition to an untreated control treatment. Each assessment shows the number of trees out of six that exhibited the particular characteristics listed. Trees were treated in October 2013, in east Texas.

Treatment		February 11, 2014		April 10, 2014	April 23, 2014			
1	Dikegulac sodium (18.5% Atrimmec) @ 2.0 mL per inch diameter	6/6: healthy		6/6: no leaves or buds	4/6: no leaves, buds still alive	1/6: deformed leaves, oozing around injection pts.	1/6: dead	
2	Dikegulac sodium (18.5% Atrimmec) @ 6.0 mL per inch diameter	6/6: healthy		6/6: no leaves or buds	3/6: no leaves, buds still alive	3/6: dead		
3	Indole-3-buttyric acid (4% Snipper) @ 2.0 mL per inch diameter	6/6: healthy		6/6: healthy	5/6: healthy	1/6: herbicide damaged		
4	Indole-3-buttyric acid (4% Snipper) @ 6.0 mL per inch diameter	5/6 healthy	1/6: oozing injection point	6/6: healthy	3/6: healthy	2/6: small leaves, oozing around injection pts.	1/6: herbicide damaged	
5	Ethephone (3.9%, Florel) @ 2.0 mL per inch diameter	6/6 oozing injection points		6/6: no leaves or buds	3/6: healthy	1/6: oozing around injection pts.	1/6: top of crown dead	1/6: dead crown, epicormic shoots
6	Ethephone (3.9%, Florel) @ 6.0 mL per inch diameter	6/6 oozing injection points; sap around cut branches		6/6: no leaves or buds	5/6: dead	1/6: lower half of crown alive		
7	Medfluidide (3.2%, Embark) @ 5.0 mL per inch diameter	6/6: healthy		6/6: no leaves or buds	3/6: no leaves, buds still alive	2/6: herbicide damage	1/6: Dead leaves	
8	Medfluidide (3.2%, Embark) @ 15.0 mL per inch diameter	6/6: healthy		6/6: no leaves or buds	3/6: dead	2/6: herbicide damage	1/6: no leaves, buds still alive	
9	Methyl chlorflurenol (12.5%, Maintain) @ 5.0 mL per inch	6/6: healthy		6/6: leafed out, but leaves wilted and turned brown	6/6: deformed leaves			
10	Methyl chlorflurenol (12.5%, Maintain) @ 15.0 mL per inch	6/6: healthy		6/6: leafed out, but leaves wilted and turned brown	6/6: deformed and dead leaves			
11	Untreated Control	6/6: healthy		6/6: healthy	6/6: healthy			

Varying phytotoxic effects were found among the different treatments during the second assessment in April 2014 (Table 1):

Treatment 1: Atrimmec (18.5% dikegulac sodium) at 2.0 ml per inch diameter in 1 point
Many of the trees had no leaves, but still had live buds, although the buds appeared small (Image 1). In one case, the buds had burst, but the leaves were deformed (Image 2).

Image 1

Image 2

Treatment 2: Atrimmec (18.5% dikegulac sodium) at 6.0 ml per inch diameter in 3 points
None of the trees included in treatment two leafed out. Some still had green buds (Image 3), while others appeared dead (Image 4).

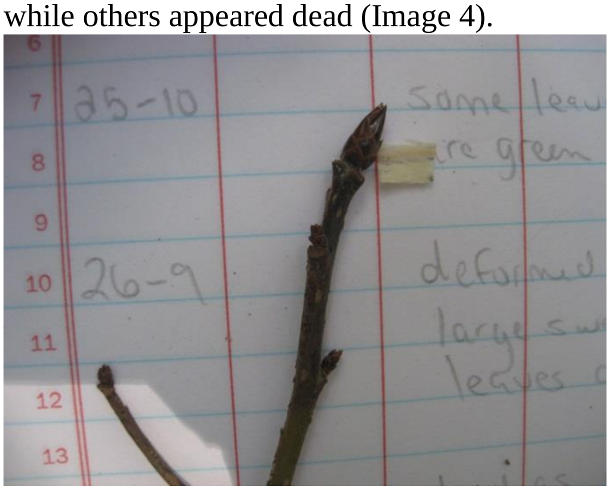

Image 3

Image 4

Treatment 3: Snipper (4% indole-3-buttyric acid) at 2.0 ml per inch diameter in 1 point
All of the trees included in treatment 3 looked healthy (Image 5), except one tree which was located at the side of the road and most likely herbicide damaged (Image 6).


Image 5


Image 6

Treatment 4: Snipper (4% indole-3-buttyric acid) at 6.0 ml per inch diameter in 3 points
While a couple of the trees included in treatment 4 looked healthy, one may have obtained herbicide damage and some had very small leaves that appeared to be dying (Image 7). There was also incidence of oozing at the injection points (Image 8).

Image 7


Image 8

Treatment 5: Florel (3.9% ethephon) at 2.0 ml per inch diameter in 1 point
Three trees included in treatment 5 looked healthy, while 3 others showed evidence of phytotoxic effects through oozing around the injection points, dead crowns, and epicormic shoots.

Treatment 6: *Florel (3.9% ethephon) at 6.0 ml per inch diameter in 3 points*
Trees included in treatment 6 showed extreme phytotoxic effects; some trees had died, while others had no leaves in the crown but epicormic shoots located along the bole (Image 9).

Image 9

Treatment 7: *Embark 2-S (3.2% medfluidide) at 5.0 ml per inch diameter in 1 point*
Trees included in treatment 7 either never leafed out (Image 10) or did break bud, but produced deformed leaves that appeared dead (Image 11). Two trees included in this treatment may have been herbicide damaged.

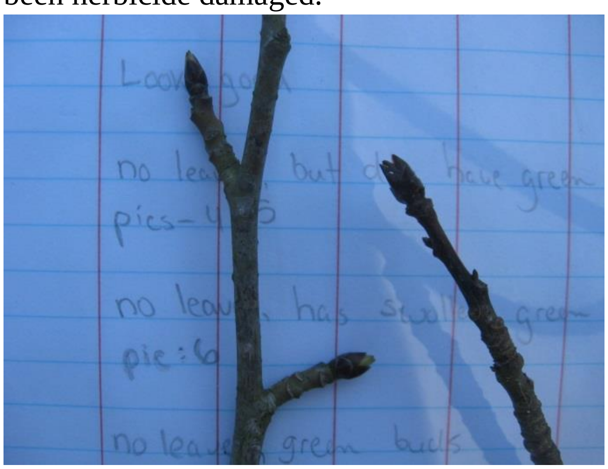

Image 10

Image 11

Treatment 8: *Embark 2-S (3.2% medfluidide) at 15.0 ml per inch diameter in 3 points*
Most trees included in this treatment were dead, while a few, although not leafed out, retained some live buds (Image 12).

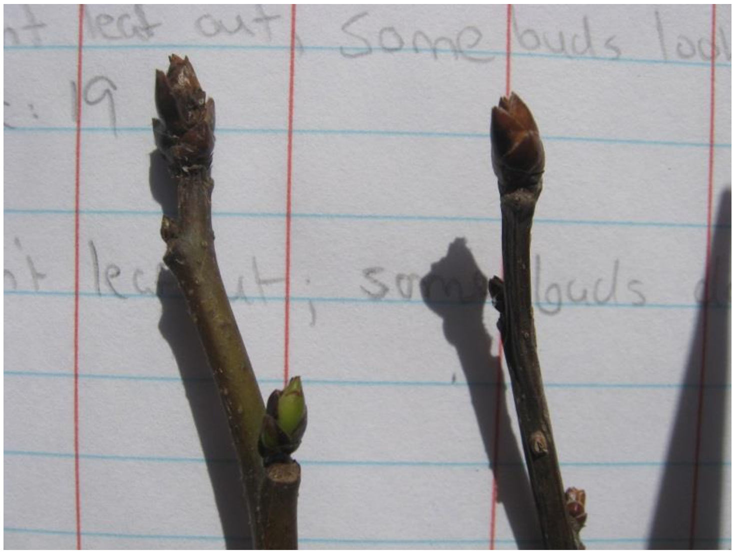

Image 12

Treatment 9: *Maintain CF125 (12.5% methyl chlorflurenol) at 5.0 ml per inch diameter in 1 point*

All six tree included in treatment 9 had deformed leaves (Image 13).

Image 13

Treatment 10: *Maintain CF125 (12.5% methyl chlorflurenol) at 15.0 ml per inch diameter in 3 points*

All trees included in treatment ten had deformed leaves (Image 14).

Image 14

Treatment 11: Untreated Control

All six control trees looked healthy (Image 15).

Image 15

Conclusions

As a result of the numerous and severe phytotoxic effects that developed after injection treatments of the ten plant growth regulators, this study was discontinued in 2014. Trial 2 is on hold until more mild formulations of these chemicals are developed.

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Evaluation of Bayer Bait Formulations for Attraction and Control of the Texas Leaf-cutting Ant

Initiated and Completed in 2014

Cooperator: Bayer CropScience

Background

Bayer CropScience provided a new experimental bait to be tested in preference and efficacy trails in 2014 as a potential method to control Texas leafcutting ants (*Atta texana*).

Objectives: 1) To determine the attractiveness of the Texas leaf-cutting ant to Bayer experimental baits.
2) To determine the efficacy of Bayer experimental baits for control of Texas leaf-cutting ants.
3) To determine effect of active ingredient rate on ant preference and treatment efficacy.

Methods

Preference Trial
As needed, trials will be conducted by placing 5 g portions of different baits (Experimental bait, corn blank, and Amdro Ant Block) into Petri dishes. Each treatment will be replicated ten times per trial period. For each trial replicate, one dish of each treatment will be distributed at random within the central nest area (but near areas of high activity) or along foraging trails. All dishes within each replicate will be retrieved when the dish, containing the most attractive bait, is nearly empty or at the end of the test period (approximately 3 hours). The amount (weight) of bait removed by ants from each Petri dish will be noted and means calculated for each treatment.

Efficacy Trial
Experiments were conducted in east Texas; within 100 miles of Lufkin. In this area, 50 Texas leaf-cutting ant colonies will be selected. Those colonies larger than 30 m by 30 m, smaller than 3m by 3 m, adjacent to each other (within 100 m), and/or lacking a distinct central nest area will be excluded from this study. Treatments will then be randomly assigned to the selected ant nests with 10 replicates per treatment.

The central nest area (CNA) is defined as the above-ground portion of the nest, characterized by a concentration of entrance/exit mounds, surrounded by loose soil excavated by the ants (Cameron 1989). Scattered, peripheral entrance/exit and foraging mounds will not be included in the central nest area. Application rates will be based on the area (length X width) of the central nest. The treatments may include:

Application rates were based on the area (length X width) of the central nest. The treatments included:

1) Treatment 1: Untreated

2) Treatment 2: Bayer experimental bait: Low rate (5 g/m^2)
3) Treatment 3: Bayer experimental bait: Medium rate (10 g/m^2)
4) Treatment 4: Bayer experimental bait: High rate (12.5 g/m^2)
5) Treatment 5: Amdro™Ant Block: 10 g/m^2

Procedures used to evaluate the effect of treatments on Texas leaf-cutting ant colonies will follow those described by Cameron (1990). The number of active entrance/exit mounds will be counted prior to treatment and periodically following treatment at 1, 2, 8, and 16 weeks. Ten untreated colonies will be included as controls and monitored to account for possible seasonal changes in ant activity. For each colony, the percent of initial activity will be calculated as the current number of active mounds at each post-treatment control divided by the initial number of active mounds. Differences in mean percent of initial activity among treatments will be tested for significance. Also, the percent of colonies totally inactive will be calculated for each treatment at each post-treatment evaluation. Data will be analyzed with ANOVA and Student's T test using JMP Pro 11.

Results

Preference Trial
The ants removed slightly more Amdro bait from Petri dishes on average (mean = 1.74g) compared to the experimental bait (mean = 0.80g). However, differences were not significant ($p = 0.113$).

Efficacy Trial
None of the treatments reduced the number of active leafcutting ant mounds significantly, compared to the check after one week (Table 2). At the end of week 2, only the Amdro AntBlock affected ant survival, reducing mean town ant activity by 72.5%. This reduction was significantly greater than that of the check and the three bait dosages (Table 3). At week 4, efficacy of the high rate of Bayer bait had increased, yielding a reduction in ant activity of 40%, compared to ca. 70% for the Amdro AntBlock. Although these two treatments were not significantly different at this point in time (Table 4), the high rate of Bayer bait was not significantly different from the check or lower dosages of Bayer bait, either. Efficacy failed to improve at 8 weeks (Table 5) and there was evidence the ants were recuperating from the pesticide applications.

Overall, results of this field trial were disappointing, compared to efficacy of PTM (fipronil) and other baits tested in previous years for control of leafcutting ants. Only 20% of the treated ant colonies were totally inactive at 8 weeks, following treatment with Amdro, and 10% or less were inactive at 8 weeks following application of the Bayer experimental baits (Figure 1).

Table 1. Results of one-way ANOVA looking at mean percent reduction in leafcutting ant activity over four time periods following treatment with Bayer experimental bait (3 application rates), Amdro AntBlock and a check. October-December 2014.

Time	Rsquare	Prob>F
Week 1	0.12073	0.2035
Week 2	0.480273	<.001*
Week 4	0.352009	0.0005*
Week 8	0.306577	0.0021*

Table 2. Week 1. Mean percent reduction of Texas leafcutting ant mounds following application of 3 dosages of Bayer experimental bait, compared to Amdro™ AntBlock and a check. Connecting letters report comparing mean percent mound reduction by treatment.

Treatment		Mean
High Bait	A	18.8
Medium Bait	A	14.4
Low Bait	A	14.0
Amdro	A	7.9
Check	A	0.0

Table 3. Week 2. Mean percent reduction of Texas leafcutting ant mounds following application of 3 dosages of Bayer experimental bait, compared to Amdro™ AntBlock and a check. Connecting letters report comparing mean percent mound reduction by treatment.

Treatment		Mean
Amdro	A	72.5
High Bait	B	31.0
Medium Bait	B	24.1
Low Bait	B	21.4
Check	B	0.00

Table 4. Week 4. Mean percent reduction of Texas leafcutting ant mounds following application of 3 dosages of Bayer experimental bait, compared to Amdro™ AntBlock and a check. Connecting letters report comparing mean percent mound reduction by treatment.

Treatment		Mean
Amdro	A	69.5
High Bait	AB	40.2
Medium Bait	B	19.5
Low Bait	B	19.1
Check	B	-0.7

Table 5. Week 8. Mean percent reduction of Texas leafcutting ant mounds following application of 3 dosages of Bayer experimental bait, compared to Amdro™ AntBlock and a check. Connecting letters report comparing mean percent mound reduction by treatment.

Treatment		Mean
Amdro	A	58.7
High Bait	AB	35.7
Medium Bait	AB	13.8
Low Bait	B	7.3
Check	B	-5.7

These results were obtained by comparing the percent mound reduction means using the Tukey-Kramer HSD (Honestly Significant Difference) test.

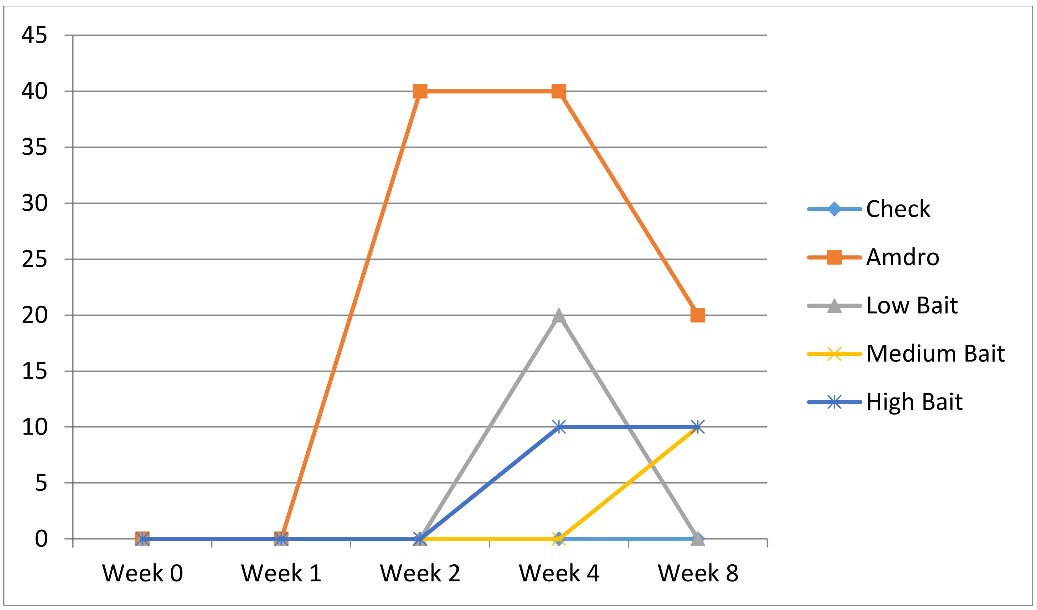

Figure 1. Percent inactive colonies over four time periods following application of Bayer experimental bait (5, 10 and 12.5gm/m2 and Amdro™ AntBlock. East Texas, September – December 2014.

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Pine Tip Moth Trials: Evaluation of PTM^{TM} Treatments for Containerized Pine Seedlings

Initiated in 2011; Final growth measurements in December 2015

Objectives

1. Evaluate techniques for application of PTM^{TM} (fipronil) to containerized seedlings in the nursery or planting site
2. Evaluate efficacy of PTM^{TM} (fipronil) applied to containerized and bareroot seedlings for reducing pine tip moth infestation levels
3. Determine the duration of chemical activity

Methods

One family of loblolly pine containerized seedlings was selected by Cellfor

Treatments:

1. PTM^{TM}: High concentration/ undiluted plug injection [5.6mL PTM undiluted/ seedling (110 TPA rate)]: Injection into container seedling plug just prior to shipping
2. PTM^{TM}: High concentration/ diluted soil injection [5.6mL PTM in 9.4mL water (15mL total volume)/seedling]: Soil injection next to transplanted container plug just after planting
3. PTM^{TM}: High concentration/ diluted soil injection [5.6mL PTM in 9.4mL water (15mL total volume)/ seedling]: Soil injection next to transplanted bareroot just after planting
4. PTM^{TM}: Mid-concentration/ undiluted plug injection [1.4mL PTM undiluted/ seedling (435 TPA rate)]: Injection into container seedling plug just prior to shipping
5. PTM^{TM}: Mid-Concentration/ diluted plug injection [1.4mL PTM in 1.7mL water (3mL total volume)/seedling]: Injection into container seedling plug just prior to shipping
6. PTM^{TM}: Mid-concentration/ diluted soil injection [1.4mL PTM in 13.6mL water (15mL total volume)/seedling]: Soil injection next to transplanted container plug just after planting
7. PTM^{TM}: Mid-concentration/ diluted soil injection [1.4mL PTM in 13.6mL water (15mL total volume)/seedling]: (Standard 1) Soil injection next to transplanted bareroot just after planting.
8. PTM^{TM}: Low-concentration/undiluted plug injection [1mL PTM undiluted/seedling (600 TPA rate)]: Injection into container seedling plug just prior to shipping
9. PTM^{TM}: Low-concentration/ diluted plug injection [1mL PTM in 2mL water (3mL total volume/seedling)]: Injection into container seedling plug just prior to shipping
10. PTM^{TM}: Low-concentration/ diluted soil injection [1mL PTM in 14mL water (15mL total volume)/seedling]: Soil injection next to transplanted container plug just after planting
11. PTM^{TM}: Low-concentration/diluted soil injection [1mL PTM in 14mL water (15mL total volume)/seedling]: (Standard 2) Soil injection next to transplanted bareroot just after planting
12. Containerized Control (untreated)
13. Bareroot Control (untreated)

Containerized seedlings were individually treated using a small syringe on site just prior to planting. The seedlings were treated at different rates based on the restricted rate of 59g AI/acre/year and the number of trees planted per acre (TPA). At 110 TPA = 0.537g AI/seedling (a rate being considered by some forest industries for treatment of high-valued "crop" trees); at 435 TPA = 0.136g AI/seedling (a tree density currently being used by Weyerhaeuser Co.); and 600 TPA = 0.1g AI/seedling (a tree density used by several forest industries).

Ten recently harvested tracts were selected in fall 2010 across the southeastern U.S. (TX, LA, AR, MS, GA, FL, and NC) based on uniformity of soil, drainage, and topography.

- TX: Rayonier (Leach), Weyerhaeuser (Fontenot), Hancock (Bounds)
- LA: Campbell Group (Stansfield)
- AR: ArborGen (Bryant)
- MS: Cellfor (Muir)
- GA: Rayonier (Wilson, Petre)
- FL: Rayonier (Wilson, Petre)
- NC: NC Forest Service (West), Weyerhaeuser (Edwards)

All study sites had been intensively site prepared, i.e., subsoil, bedding and/or herbicide. A 1-acre (approximate) area within each site was selected. A triple Latin square design was established with single tree plots (13 rows X 13 treatments) serving as blocks, i.e., each treatment was randomly selected for placement along each row (bed). Thirty-nine (39) rows were established on each site. Seedlings were planted at 8-foot spacing along each row. Individual tree locations were marked with different colored pin flags prior to tree planting. Herbicide to control broadleaf competitors was applied over the area in the spring to ensure that the seedlings remained exposed to tip moth attack throughout the year.

Damage and Tree Measurements

Tip moth damage was evaluated after each tip moth generation (3-4 weeks after peak moth flight) by 1). Identifying if the tree is infested or not, 2). If infested, the proportion of tips infested on the top whorl and terminal was/will be calculated; and 3). Separately, the terminal was identified as infested or not. Observations also were be made as to the occurrence and extent of damage caused by other insects, i.e., coneworm, aphids, sawfly, etc. All study trees were measured for height & diameter (at ground level) at the beginning of the study (when seedlings were planted). Measurements also were be taken when tree growth has stopped in mid- to late November for at least the first 2 years of the study. Tree form will be evaluated at the end of year 3. Form ranking of the seedling or tree will be categorized as follows: 0 = no forks; 1 = one fork; 2 = two to four forks; 3 = five or more forks. A fork is defined as a node with one or more laterals larger than one half the diameter of the main stem (Berisford and Kulman 1967).

Results:

In 2011, tip moth populations were variable across the South; with relatively low damage levels on checks in TX (5% on container & 11% on bareroot) to ~30% on all seedlings in GA (Figure 1, Table 1). PTM injected into container seedling plugs before planting reduced overall tip moth damage by 92% compared to untreated checks. This was 4% and 13% better than protection provided by PTM applied to container and bareroot seedlings, respectively, after planting (Figure 2). Nearly all PTM treatments (9 of 11) significantly improved height, diameter, and volume of seedlings, compared to the check (Table 2). Mean volume improvement for plugs treated prior to planting was 42% compared to checks. This was 12% higher than volume increase observed on post-plant treated seedlings. In addition, most PTM treatments (8 of 11) significantly improved survival compared to untreated checks. Mean survival of pre-plant treated seedlings was 6.7% better than checks. This was double the improvement (3.4%) in survival observed on post-plant treated seedlings.

In 2012, tip moth populations were again variable, with low damage levels on checks in FL (5% on container & 10% on bareroot) to 58% on bareroot seedlings in LA (Figure 3, Table 3). PTM applied to containers after planting reduced overall tip moth damage by 43% compared to untreated checks. This was only 5% and 7% better than protection provided by PTM injected into container seedling plugs before planting and PTM applied to bareroot seedlings after planting, respectively (Figure 4). Almost all PTM treatments significantly improved height, diameter, and volume (Table 4). Only the containerized high-dilution and bareroot high-dilution treatments applied to the soil after planting did not show significant improvement in diameter growth. The bareroot high dilution treatment applied to the soil after planting did not show significant improvement in volume either (Table 4). Mean volume improvement for plugs treated prior to planting was increased by 39% compared to checks. This was 16% higher than volume increase observed on post-plant treated seedlings. None of the PTM treatments significantly improved survival compared to untreated checks. Mean survival of pre-plant treated seedlings was 9.2% better than checks, and that of post-plant treated seedlings; 5.2%.

In 2013, only tree growth was measured. All treatments resulted in significant growth increases compared to the controls except treatments **3** (Bareroot; high concentration, dilute, soil injection) and **10** (Containerized; low concentration, dilute, soil injection) (Table 5). Mean percent improvement in volume compared to the control for containerized, plug injected treatments was 31%, for containerized soil injected treatments; 25%, and for bareroot treatments; 38%. No measurements were taken in 2014; the study trees will be measured for growth for the final time at the end of the 2015 growing season.

Acknowledgments

Thanks go to Arborgen, The Campbell Group, Hancock, NC Forest Service, Rayonier, and Weyerhaeuser for providing research sites and Cellfor and Plum Creek for providing seedlings. We also thank Jim Bean, BASF, for providing financial support and PTM^{TM} product for the project.

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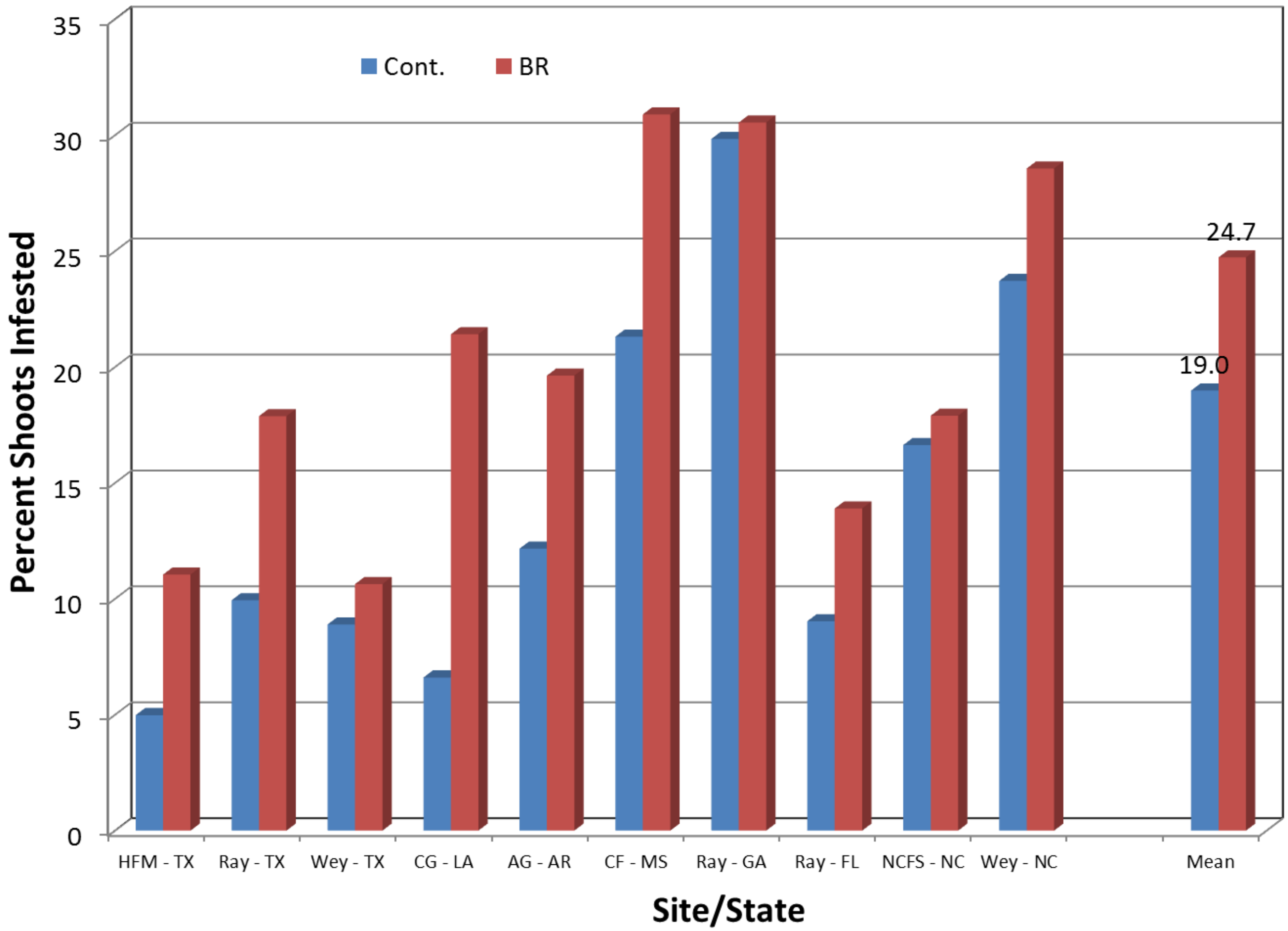

Figure 1. Mean tip moth infestation levels on first year containerized and bareroot loblolly pine on ten sites across the southeastern United States, 2011.

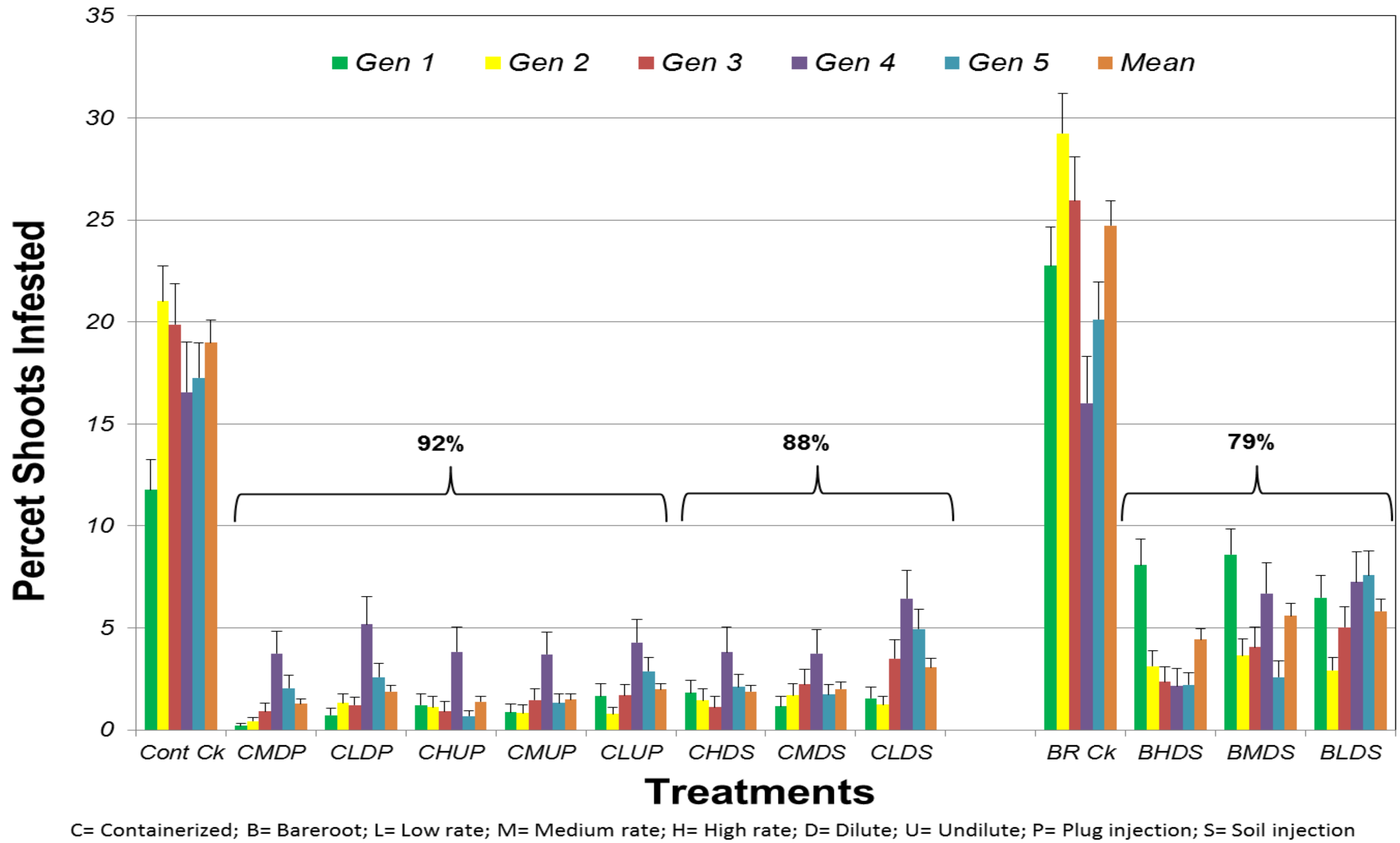

Figure 2. Effect of PTM™ plug and soil injection dose on tip moth infestation of containerized or bareroot loblolly pine on ten sites across the southeastern United States, 2011.

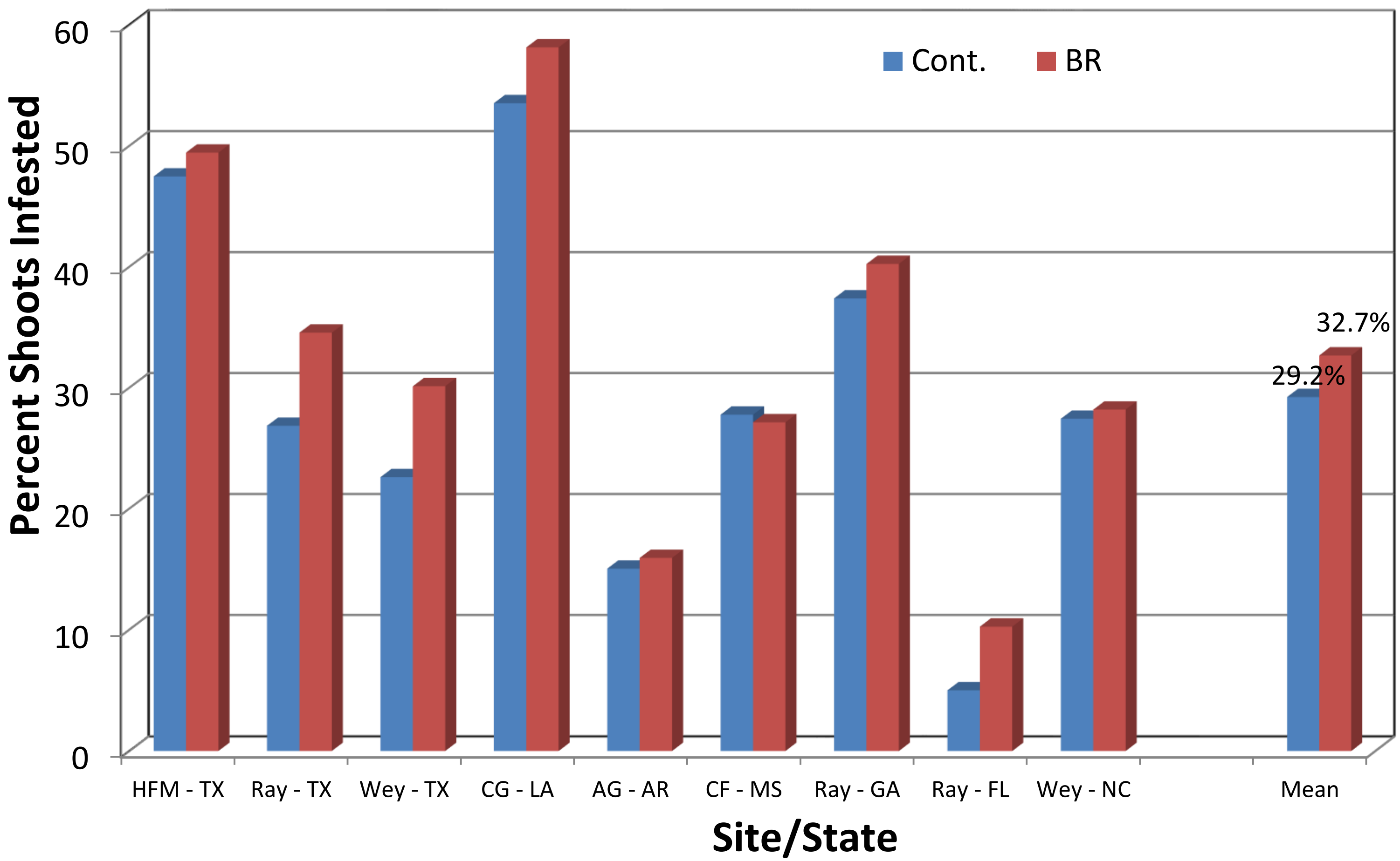

Figure 3. Mean tip moth infestation levels on first year containerized and bareroot loblolly pine on ten sites across the southeastern United States, 2012.

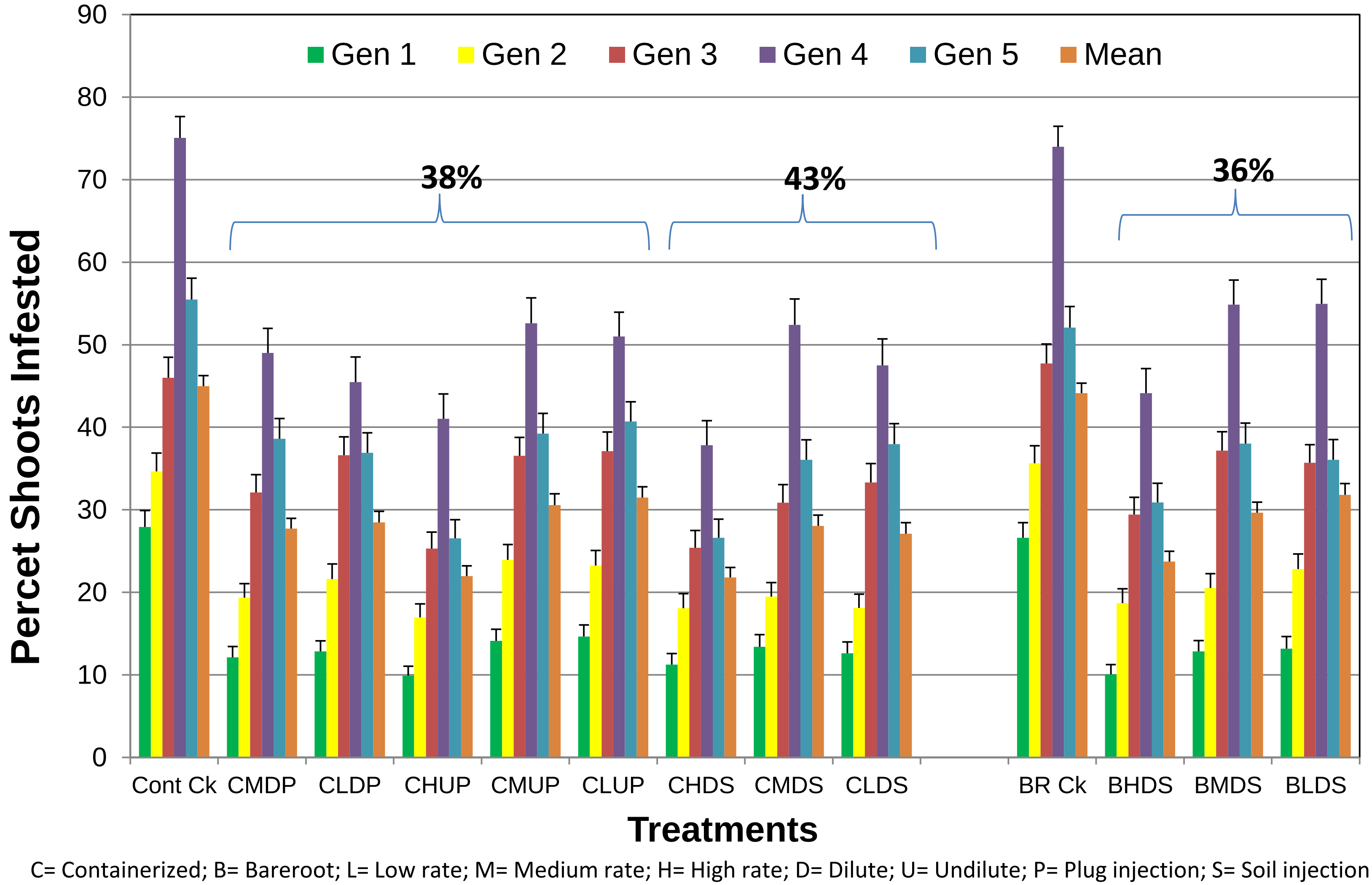

Figure 4. Effect of PTM™ plug and soil injection dose on tip moth infestation of containerized or bareroot loblolly pine on ten sites across the southeastern United States, 2012.

Table 1. Effect of PTM dose and technique on pine tip moth infestation of containerized and bareroot loblolly pine shoots (top whorl) on ten sites across the sotheastern United States, 2011.

Treatment						(**Pct. Reduction Compared to Check**)																	
Year	Cont. or BR	Conc.	Dilute or Undilute	Inj. Loc.	N	Gen 1 (10 sites)			Gen 2 (10 Sites)			Gen 3 (8 Sites)			Gen 4 (6 Sites)			Gen 5 or Last (10 Sites)			Overall Mean		
2011	Cont.	Med	Dilute	Plug	390	0.2	**98**	*	0.4	**98**	*	0.9	**95**	*	3.8	**77**	*	2.1	**88**	*	1.3	**93**	*
	Cont.	Low	Dilute	Plug	390	0.7	**94**	*	1.3	**94**	*	1.2	**94**	*	5.2	**69**	*	2.6	**85**	*	1.9	**90**	*
	Cont.	High	Undilute	Plug	390	1.2	**89**	*	1.1	**95**	*	0.9	**95**	*	3.8	**77**	*	0.7	**96**	*	1.4	**93**	*
	Cont.	Med	Undilute	Plug	390	1.3	**89**	*	0.8	**96**	*	1.5	**93**	*	3.7	**78**	*	1.3	**92**	*	1.5	**92**	*
	Cont.	Low	Undilute	Plug	390	1.6	**86**	*	0.8	**96**	*	1.7	**92**	*	4.3	**74**	*	2.9	**83**	*	2.0	**90**	*
	Cont.	High	Dilute	Soil	390	1.8	**84**	*	1.5	**93**	*	1.1	**94**	*	3.8	**77**	*	2.1	**88**	*	1.9	**90**	*
	Cont.	Med	Dilute	Soil	390	1.2	**90**	*	1.7	**92**	*	2.2	**89**	*	3.8	**77**	*	1.7	**90**	*	2.0	**89**	*
	Cont.	Low	Dilute	Soil	390	1.6	**87**	*	1.2	**94**	*	3.5	**83**	*	6.4	**61**	*	5.0	**71**	*	3.0	**84**	*
	Cont.				390	11.6			21.1			19.9			16.5			17.3			19.0		
	BR	High	Dilute	Soil	390	8.5	**63**	*	2.9	**90**	*	2.4	**91**	*	2.2	**87**	*	2.2	**89**	*	4.4	**82**	*
	BR	Med	Dilute	Soil	390	8.6	**63**	*	3.6	**87**	*	4.0	**84**	*	6.7	**58**	*	3.3	**84**	*	5.6	**77**	*
	BR	Low	Dilute	Soil	390	6.5	**72**	*	3.0	**90**	*	5.0	**81**	*	7.2	**55**	*	7.6	**62**	*	5.8	**76**	*
	BR				390	22.8			29.0			25.9			16.0			20.1			24.7		

***** Means followed by an asterik are significantly different from checks at the 5% level based on Fisher's Protected LSD.

= treatment reduced damage by 75% or better compared to check.

Table 2. Effect of PTM dose and technique on containerized and bareroot loblolly pine growth on ten sites across the southeastern United States, 2011.

	Treatment					Mean End of Season Loblolly Pine Seeding Growth Measurements (**Growth Difference (cm or cm3) Compared to Check)**									Mean Percent Tree Survival (**Percent Improvement Compared to Check**)		
Year	Cont. or BR	Conc.	Dilute or Undilute	Inj. Loc.	N	Height (cm)			Diameter (cm) [a]			Volume (cm^3)					
2011	Cont.	Med	Dilute	Plug	369	52.2	*	**7.0**	1.04	*	**0.12**	91.9	*	**28.2**	94	*	**7**
	Cont.	Low	Dilute	Plug	367	50.7	*	**5.5**	1.00	*	**0.09**	88.6	*	**24.9**	94	*	**6**
	Cont.	High	Undilute	Plug	371	50.0	*	**4.8**	0.98	*	**0.07**	86.1	*	**22.4**	95	*	**7**
	Cont.	Med	Undilute	Plug	360	52.8	*	**7.6**	1.03	*	**0.12**	95.5	*	**31.8**	92	*	**5**
	Cont.	Low	Undilute	Plug	374	51.9	*	**6.7**	1.02	*	**0.11**	91.7	*	**28.0**	96	*	**8**
	Cont.	High	Dilute	Soil	356	47.3		**2.1**	0.95		**0.03**	77.9		**14.2**	91	*	**4**
	Cont.	Med	Dilute	Soil	352	49.6	*	**4.4**	0.98	*	**0.07**	83.5	*	**19.8**	90		**2**
	Cont.	Low	Dilute	Soil	353	49.8	*	**4.6**	0.98	*	**0.06**	87.6	*	**23.9**	91		**3**
	Cont.				342	45.2			0.91			63.7			88		
	BR	High	Dilute	Soil	362	53.6		**3.2**	1.01		**0.04**	95.7		**24.1**	93		**3**
	BR	Med	Dilute	Soil	371	57.2	*	**6.8**	1.07	*	**0.10**	112.1	*	**40.4**	96	*	**5**
	BR	Low	Dilute	Soil	367	58.2	*	**7.8**	1.08	*	**0.11**	118.4	*	**46.7**	94	*	**4**
	BR				352	50.4			0.97			71.7			90		

[a] Ground Line Diameter.

***** Means followed by an asterisk are significantly different from checks at the 5% level based on Fisher's Protected LSD.

Table 3. Effect of PTM dose and technique on pine tip moth infestation of containerized and bareroot loblolly pine shoots (top whorl) on nine sites across the southeastern United States, 2012 (Est. 2011).

	Treatment					Mean Percent Top Whorl Shoots Infested by Tip Moth (**Pct. Reduction Compared to Check**)																	
Year	Cont. or BR	Conc.	or Undilute	Inj. Loc.	N	Gen 1 (10 sites)			Gen 2 (9 Sites)			Gen 3 (8 Sites)			Gen 4 (6 Sites)			Gen 5 or Last (9 Sites)			Overall Mean		
2012	Cont.	Med	Dilute	Plug	390	12.0	**57**	*	19.4	**44**	*	32.1	**30**	*	49.0	**35**	*	38.6	**30**	*	27.7	**38**	*
	Cont.	Low	Dilute	Plug	390	12.5	**55**	*	21.6	**38**	*	36.6	**20**	*	45.5	**39**	*	36.9	**33**	*	28.5	**37**	*
	Cont.	High	Undilute	Plug	390	10.4	**62**	*	17.0	**51**	*	25.3	**45**	*	41.0	**45**	*	26.5	**52**	*	22.0	**51**	*
	Cont.	Med	Undilute	Plug	390	14.2	**49**	*	23.9	**31**	*	36.5	**21**	*	52.6	**30**	*	39.2	**29**	*	30.6	**32**	*
	Cont.	Low	Undilute	Plug	390	11.0	**60**	*	23.3	**33**	*	39.1	**15**	*	51.0	**32**	*	40.7	**27**	*	31.5	**30**	*
	Cont.	High	Dilute	Soil	390	11.0	**60**	*	18.0	**48**	*	25.4	**45**	*	37.8	**50**	*	26.6	**52**	*	21.8	**52**	*
	Cont.	Med	Dilute	Soil	390	13.8	**50**	*	19.4	**44**	*	30.9	**33**	*	52.4	**30**	*	36.0	**35**	*	28.0	**38**	*
	Cont.	Low	Dilute	Soil	390	13.6	**51**	*	18.1	**48**	*	33.3	**28**	*	47.5	**37**	*	38.0	**32**	*	27.1	**40**	*
	Cont.				390	27.7			34.7			46.0			75.1			55.5			45.0		
	BR	High	Dilute	Soil	390	10.0	**61**	*	18.7	**48**	*	29.4	**38**	*	44.1	**40**	*	30.9	**41**	*	23.7	**46**	*
	BR	Med	Dilute	Soil	390	13.5	**48**	*	20.5	**42**	*	37.2	**22**	*	54.8	**26**	*	38.0	**27**	*	29.7	**33**	*
	BR	Low	Dilute	Soil	390	16.2	**37**	*	22.8	**36**	*	35.7	**25**	*	54.9	**26**	*	41.4	**21**	*	31.8	**28**	*
	BR				390	25.9			35.6			47.7			74.0			52.1			44.2		

***** Means followed by an asterisk are significantly different from checks at the 5% level based on Fisher's Protected LSD.

= treatment reduced damage by 75% or better compared to check.

Table 4. Effect of PTM dose and technique on containerized and bareroot loblolly pine growth on nine sites across the southeastern United States, 2012 (Est 2011). GLD = ground line diameter

	Treatment					Mean End of Season Loblolly Pine Seeding Growth Measurements (**Growth Difference (cm or cm3) Compared to Check)**										Mean Percent Tree Survival (**Percent Improvement Compared to Check**)	
Year	Cont. or BR	Conc.	Dilute or Undilute	Inj. Loc.	N	Height (cm)			GLD (cm)			Volume (cm^3)					
2012	Cont.	Med	Dilute	Plug	327	128.3	*	**19.8**	2.96	*	**0.44**	1882.9		**544.0**	*	93	**9**
	Cont.	Low	Dilute	Plug	327	125.0	*	**16.5**	2.86	*	**0.34**	1843.3		**504.4**	*	93	**9**
	Cont.	High	Undilute	Plug	326	127.7	*	**19.3**	2.88	*	**0.36**	1884.0		**545.1**	*	93	**9**
	Cont.	Med	Undilute	Plug	321	127.6	*	**19.1**	2.95	*	**0.43**	2015.4		**676.5**	*	91	**7**
	Cont.	Low	Undilute	Plug	335	124.3	*	**15.8**	2.84	*	**0.32**	1694.9		**355.9**	*	95	**11**
	Cont.	High	Dilute	Soil	314	117.7	*	**9.2**	2.70		**0.18**	1634.6		**295.6**	*	89	**5**
	Cont.	Med	Dilute	Soil	311	120.8	*	**12.3**	2.70	*	**0.18**	1631.4		**292.4**	*	89	**5**
	Cont.	Low	Dilute	Soil	309	119.7	*	**11.2**	2.71	*	**0.19**	1669.3		**330.3**	*	88	**4**
	Cont.				295	108.5			2.52			1339.0				84	
	BR	High	Dilute	Soil	321	129.3	*	**7.9**	2.86		**0.12**	1882.9		**261.0**		91	**4**
	BR	Med	Dilute	Soil	327	136.4	*	**15.0**	3.05	*	**0.31**	2266.5	*	**644.6**	*	93	**6**
	BR	Low	Dilute	Soil	330	136.6	*	**15.2**	3.06	*	**0.32**	2246.8	*	**624.9**	*	94	**7**
	BR				306	121.4			2.74			1621.9				87	

***** Means followed by an asterisk are significantly different from checks at the 5% level based on Fisher's Protected LSD.

Table 5. Effect of PTM dose and technique on containerized and bareroot loblolly pine growth on six (6) sites across the southeastern United States, 2013 (Est 2011). GLD = ground line diameter

	Treatment					Mean End of Season Loblolly Pine Seeding Growth Measurements (**Growth Difference (cm or cm3) Compared to Check)**									Mean Percent Tree Survival (**Percent Improvement Compared to Check**)	
Year	Cont. or BR	Conc.	Dilute or Undilute	Inj. Loc.	N	Height (cm)			GLD (cm)			Volume (cm^3)				
2013 3rd Yr	Cont.	Med	Dilute	Plug	216	238.6	*	**29.0**	4.92	*	**0.61**	7278.8	*	**1562.1**	62	**8**
	Cont.	Low	Dilute	Plug	215	235.9	*	**26.3**	4.84	*	**0.53**	7350.0	*	**1633.4**	61	**7**
	Cont.	High	Undilute	Plug	212	240.4	*	**30.8**	4.97	*	**0.66**	7858.9	*	**2142.3**	60	**7**
	Cont.	Med	Undilute	Plug	208	239.7	*	**30.1**	5.00	*	**0.69**	7997.7	*	**2281.1**	59	**5**
	Cont.	Low	Undilute	Plug	223	232.4	*	**22.8**	4.82	*	**0.51**	6944.8	*	**1228.2**	64	**10**
	Cont.	High	Dilute	Soil	206	229.6	*	**20.0**	4.73	*	**0.42**	7153.8	*	**1437.2**	59	**5**
	Cont.	Med	Dilute	Soil	200	229.8	*	**20.2**	4.76	*	**0.45**	7206.5	*	**1489.9**	57	**3**
	Cont.	Low	Dilute	Soil	201	222.4		**12.8**	4.60		**0.29**	7027.9		**1311.2**	57	**3**
	Cont.				189	209.6			4.31			5716.6			54	
	BR	High	Dilute	Soil	208	245.1		**16.3**	4.94		**0.30**	8011.3		**1552.3**	59	**4**
	BR	Med	Dilute	Soil	212	253.1	*	**24.3**	5.20	*	**0.56**	9180.4	*	**2721.3**	60	**5**
	BR	Low	Dilute	Soil	211	256.2	*	**27.4**	5.22	*	**0.58**	9428.8	*	**2969.8**	60	**5**
	BR				194	228.8			4.64			6459.0			55	

***** Means followed by an asterisk are significantly different from checks at the 5% level based on Fisher's Protected LSD.

Pine Tip Moth Trials: Evaluation of Plug Injection System for Application of PTM™ and Insignia®SC for Containerized Pine Seedlings

Initiated in 2012; to be monitored through 2016

With support from the Forest Pest Management Cooperative, a novel system for injecting insecticides into containerized seedlings at the nursery was developed by Stewart Boots, S&K Designs in 2011.

Objectives

1. Evaluate the new plug injection system for application of PTM™ (fipronil) to containerized seedlings in the nursery
2. Evaluate efficacy of PTM™ (fipronil) and Insignia®SC (pyraclostrobin) alone or combined and applied to containerized and bare-root seedlings for reducing pine tip moth infestation levels and improving seedling health
3. Determine the duration of chemical activity

Methods

One family of loblolly pine containerized and bare-root seedlings were provided by IFCo and Plum Creek.

Treatments:

1. Insignia®SC: Mid-concentration / undiluted plug injection [4.9mL Insignia undiluted/seedling (435 TPA rate)]: Injection into container seedling plug just prior to shipping.
2. PTM™: Mid-concentration/ undiluted plug injection [1.4mL PTM undiluted/ seedling (435 TPA rate)]: Injection into container seedling plug just prior to shipping
3. PTM™ + Insignia®SC: Mid-concentration/ undiluted plug injection [1.4mL PTM + 4.9mL Insignia (6.3mL total volume)/ seedling]: Injection into container seedling plug just prior to shipping.
4. PTM™: Low concentration/ undiluted plug injection [1mL PTM undiluted/ seedling (600 TPA rate)]: Injection into container seedling plug just prior to shipping
5. PTM™: (Low) + Insignia®SC (Mid) Concentration/ Diluted plug injection [1mL PTM + 4.9mL Insignia (5.9mL total volume)/ seedling]: Injection into container seedling plug just prior to shipping
6. Insignia®SC: high concentration/ diluted soil injection [13mL Insignia in 17mL water (30mL total volume)/ seedling]: Soil injection at two points next to transplanted bareroot just after planting
7. Insignia®SC: Mid-concentration/ diluted soil injection [4.9mL Insignia in 25.1mL water (30mL total volume)/ seedling]: Soil injection at two points next to transplanted bareroot just after planting

8. PTM^{TM}: Mid-concentration/ diluted soil injection [1.4mL PTM in 28.6mL water (30mL total volume)/ seedling]: Soil injection at two points next to transplanted bareroot just after planting
9. PTM^{TM} + Insignia®SC: Mid-concentration/ diluted soil injection [1.4mL PTM + 4.9mL Insignia in 23.7mL water (30mL total volume)/ seedling]: Soil injection at two points next to transplanted bareroot just after planting
10. PTM^{TM}: Low-concentration/ diluted soil injection [1mL PTM in 29mL water (30mL total volume)/ seedling]: Soil injection next to transplanted bareroot just after planting
11. PTM^{TM}: (Low) + Insignia®SC (Mid) Concentration/ diluted soil injection [1mL PTM + 4.9mL Insignia in 25.5mL water (30mL total volume)/ seedling]: Soil injection next to transplanted bareroot just after planting
12. Containerized Control (untreated)
13. Bareroot Control (untreated)

Containerized seedlings were individually treated at the nursery prior to planting using a plug injection system developed by Stewart Boots, S&K Designs. The seedlings were treated with PTM^{TM} and/or Insignia®SC at different rates based on the restricted rate of 59g AI/acre/year (PTM^{TM}) or 530g AI/acre/year (Headline®) and the number of trees planted per acre (TPA). For example, fipronil was applied at 110 trees per acre (TPA) = 0.537g AI/seedling (a rate being considered by some forest industries for treatment of high-valued "crop" trees); at 435 TPA = 0.136g AI/seedling (a tree density currently being used by Weyerhaeuser Co.); and 600 TPA = 0.1g AI/seedling (a tree density used by several forest industries).

Five recently harvested tracts were selected in fall 2011 across the southeastern United States (in TX, AR, AL, GA, and NC) based on uniformity of soil, drainage, and topography.

- TX: Campbell Group (Stansfield)
- AR: Plum Creek (Fristoe)
- AL: Rayonier (Leach)
- GA: International Forestry Co. (Bell)
- NC: Weyerhaeuser (Edwards)

All stands were intensively site prepared, i.e., subsoil, bedding, and/or herbicide. A 1-acre (approximate) area within each site was selected. A triple Latin square design was established with single tree plots (13 rows X 13 treatments) serving as blocks, i.e., each treatment was randomly selected for placement along each row (bed). Thirty-nine rows were established on each site. Seedlings were planted at 8-foot spacing along each row. Individual tree locations were marked with different color pin flags prior to tree planting.

The plot corners were marked with PVC pipe and metal tags. If necessary, herbicide was applied over the area in the spring to ensure that the seedlings would remain exposed to tip moth attack throughout the year.

Damage and Tree Measurements

Tip moth damage was evaluated after each tip moth generation (3-4 weeks after peak moth flight) by 1). Identifying if the tree was infested or not, 2). If infested, the proportion of tips infested on the top whorl and terminal were calculated; and 3). Separately, the terminal was identified as infested or not. Observations were also made as to the occurrence and extent of damage caused by other insects, i.e., coneworm, aphids, sawfly, etc. Measurements of tree health were collected periodically and/or at the end of each growing season. Tree health measurements included tree height and diameter; crown diameter, density and color (vigor): number and length of shoots in top whorl, and tree survival. All study trees were measured for height and diameter at ground line at the beginning of the study (when seedlings were planted). Measurements were taken when tree growth stopped in mid- to late November.

Results

In 2012, pine tip moth populations were variable across the South, with low damage levels in AL and GA (average of 4.2% and 4.7% on containerized seedlings, respectively) and higher damage levels in AR (43.8% on bare root seedlings) (Figure 1). All PTM and/or Insignia treatments of containerized seedling plugs significantly reduced overall tip moth damage (mean reduction/ all treatments: 86.3%) compared to the untreated control (Figure 2, Table 1). For bareroot seedlings, all treatments that used PTM significantly reduced overall tip moth damage (mean reduction/ all treatments: 71.5%) compared to the untreated control, while the two bareroot treatments using Insignia only did not significantly reduce tip moth damage (Figure 2, Table 1).

There was a significant difference in mean percent pine tip moth infestation among the treatments (ANOVA, $p < 0.0001$; Table 3). Treatments 2 (Containerized: PTM, mid-concentration), 3 (Containerized: PTM and Insignia, mid-concentration), and 5 (Containerized: PTM, low-concentration & Insignia, mid-concentration) were found to have significantly lower mean percent infestations compared with the other treatments (Table 3).

Only treatments 2 (containerized: PTM, mid-concentration), 4 (containerized: PTM, low-concentration), and 8 (bareroot: PTM mid-concentration) were found to result in significantly improved height, diameter, and volume compared with the controls (Table 4). Percent tree survival was slightly increased compared with controls in the case of two containerized seedling treatments, while four of the bareroot seedling treatments showed a decrease in percent tree survival compared with the control (Table 4).

In 2013, all treatments showed a significant reduction in percent tip moth infestation compared to the control except the two Insignia-only treatments (6 and 7) and treatment 9 (PTM™ + Insignia®SC: Mid-concentration/ diluted soil injection/ bareroot) (Figure 13). Containerized treatments reduced tip moth damage by 16.4% on average; bareroot by 14.3%. Insignia-only treatments resulted in increased infestation compared to the control (-1.7%), although this was not significant.

Treatment 2 (PTM™: Mid-concentration/ containerized), treatment 4 (PTM™: Low concentration/ containerized), and treatment 10 (PTM™: Low-concentration/ bareroot) were the only three treatments that showed significant increases in volume compared with the control (Table 5). The two Insignia-only treatments (6 and 7) showed significant decreases in volume growth compared with the control (Table 5).

In 2014, seedling growth measurements were taken in 2014 on only two sites (Texas and North Carolina). By the end of the 2014 growing season, there were no significant differences ($P > 0.05$) among any of the treatments in DBH (cm3) or ground-level growth (cm3), when all sites were combined (Tables 6-13). On the Texas site, there was no significant difference in growth measured at DBH (Tables 14-17), but there was at ground level between two treatments (Table 18-21). With regard to diameter at ground level, by 2014, the high dose Insignia soil injection treatment exhibited significantly less growth compared to the low dose PTM bare root treatment.

When analyzed separately, 2014 growth data for North Carolina showed no significant differences in growth at DBH (Tables 22-25). No measurements were taken at ground level at the North Carolina site in 2014. All available treatment sites will be measured for growth parameters for the final time at the end of the 2016 growing season.

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Thanks go to ArborGen LLC and BASF for providing Insignia and PTM product. Thanks to: The Campbell Group, International Forestry Co., Plum Creek, Rayonier, and Weyerhaeuser for providing research sites. IFco and Plum Creek provided seedlings.

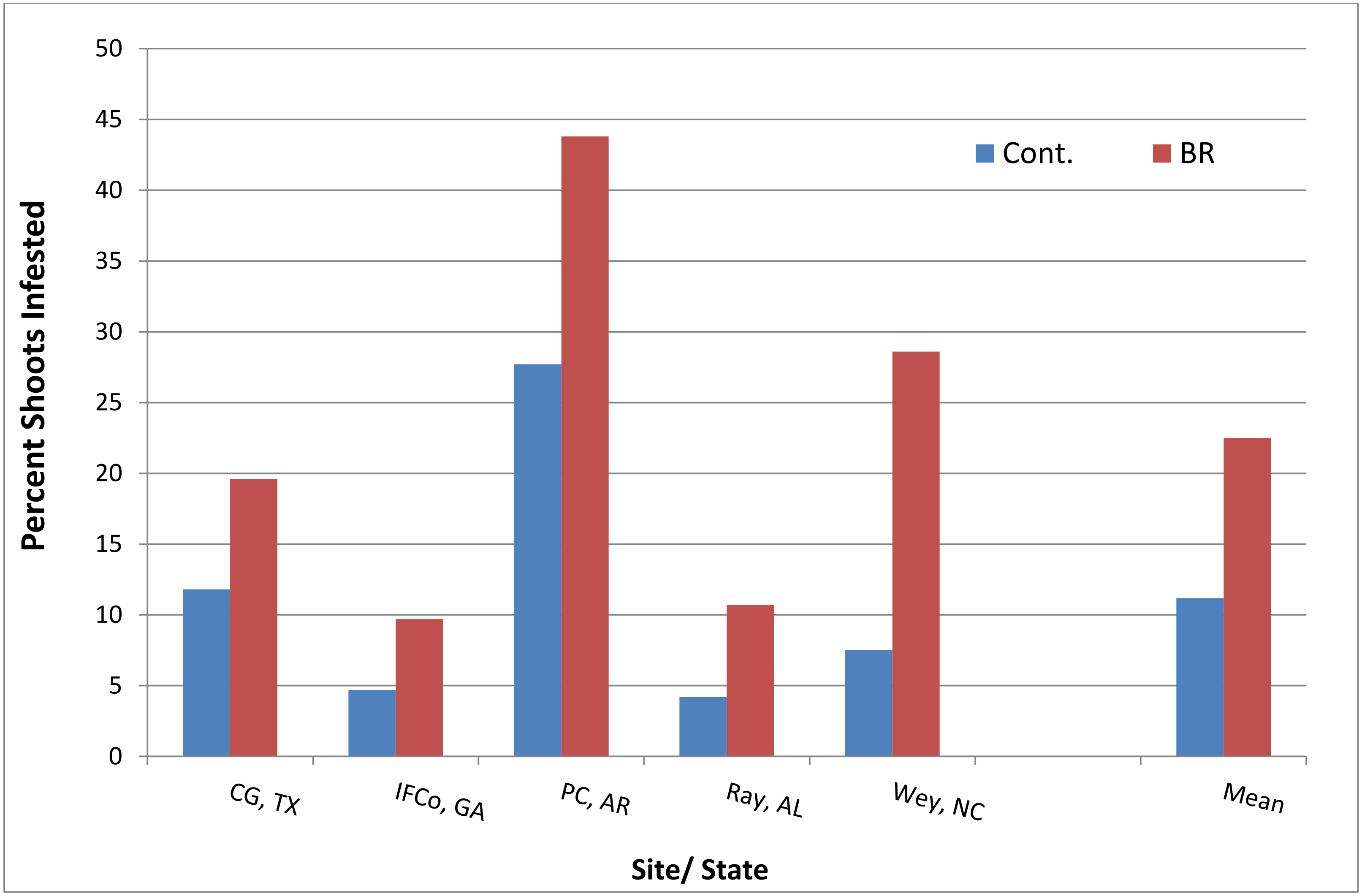


Figure 1. Mean tip moth infestation levels on first year containerized and bareroot loblolly pine on five sites across the southeastern U.S., 2012.

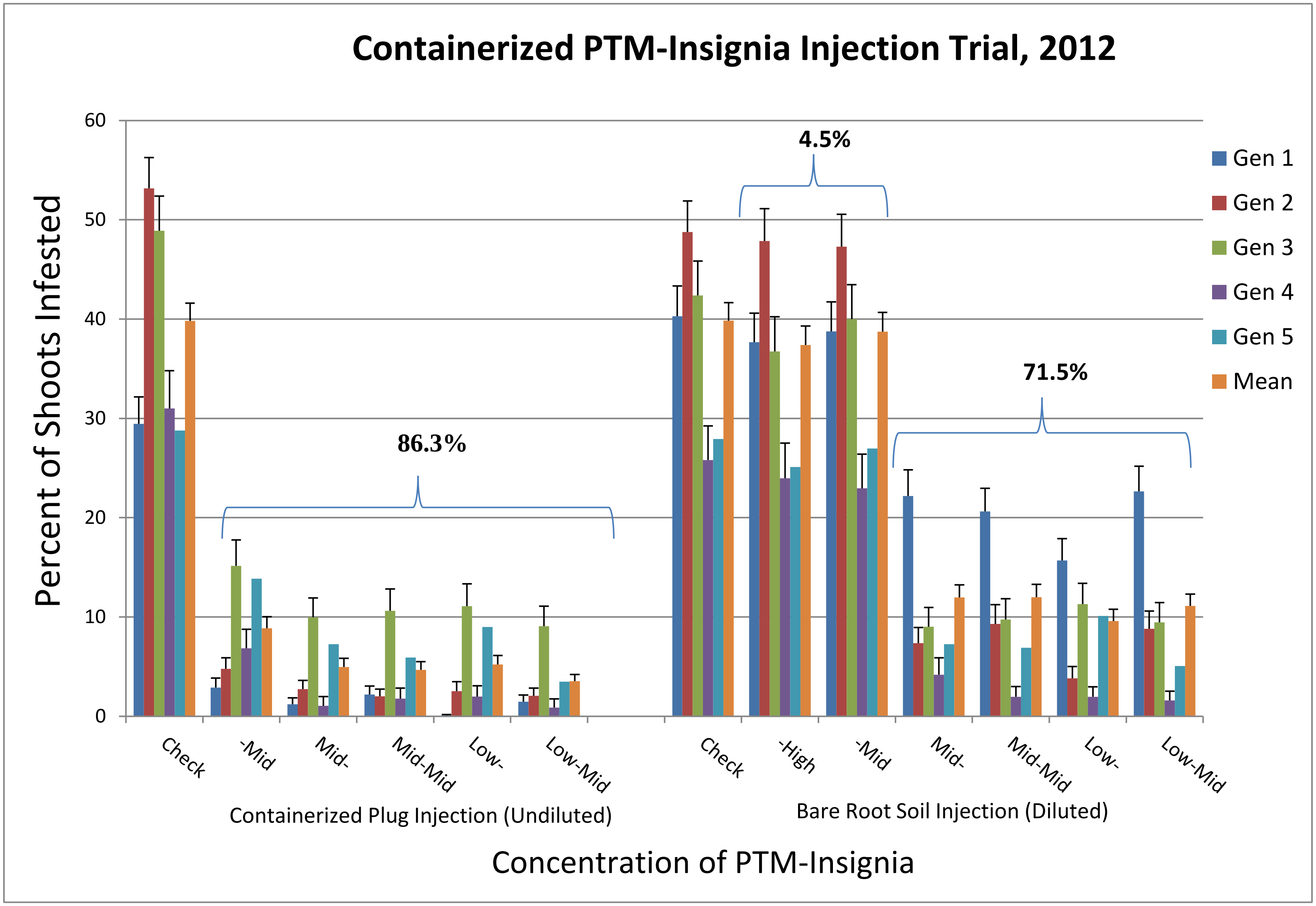


Figure 12. Effect of PTM and/or Insignia SC dose and technique on pine tip moth infestation of containerized or bareroot loblolly pine on five sites across the southeastern United States, 2012.

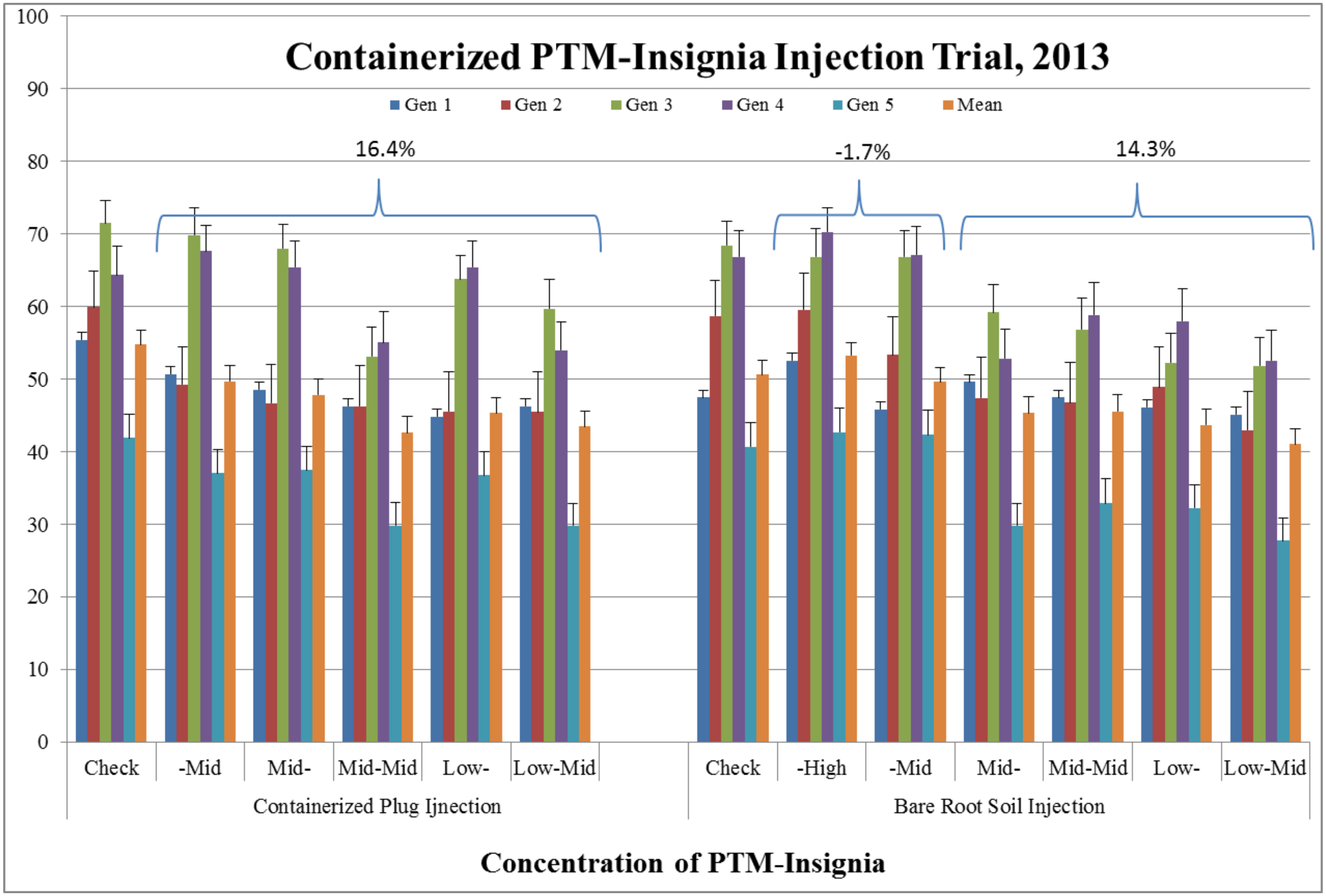


Figure 13. Effect of PTM and/or Insignia SC dose and technique on pine tip moth infestation of containerized or bareroot loblolly pine on five sites across the southeastern United States, 2013.

Table 1. Effect of PTM and/or Insignia SC dose and technique on pine tip moth infestation of containerized and bareroot loblolly pine shoots (top whorl) on five sites across the southeastern United States, 2012.

Year	Cont. or BR	Conc. PTM	Conc. Insignia	Dilute or Undilute	Inj. Loc.	N	Gen 1 (5 sites)			Gen 2 (5 Sites)			Gen 3 (4 Sites)			Gen 4 (3 Sites)			Gen 5 or Last (5 Sites)			Overall Mean		
2012	Cont.		Mid	U	Plug	189	2.9	**90**	*	4.8	**91**	*	15.2	**69**	*	6.9	**78**	*	13.8	**52**	*	8.9	**78**	*
	Cont.	Mid		U	Plug	195	1.2	**96**	*	2.7	**95**	*	10.0	**80**	*	1.1	**97**	*	7.2	**75**	*	5.0	**88**	*
	Cont.	Mid	Mid	U	Plug	190	2.2	**93**	*	2.0	**96**	*	10.6	**78**	*	7.8	**75**	*	5.9	**79**	*	4.7	**88**	*
	Cont.	Low		U	Plug	192	0.1	**100**	*	2.5	**95**	*	11.1	**77**	*	2.0	**94**	*	9.0	**69**	*	5.2	**87**	*
	Cont.	Low	Mid	U	Plug	189	1.5	**95**	*	2.0	**96**	*	9.1	**81**	*	0.9	**97**	*	3.5	**88**	*	3.5	**91**	*
	Cont					190	29.4			53.2			48.9			31.0			28.8			39.8		
	BR		High	D	Soil	178	37.7	**6**		47.9	**2**		36.7	**13**		24.0	**7**		25.1	**10**		37.4	**6**	
	BR		Mid	D	Soil	183	38.8	**4**		47.3	**3**		40.0	**6**		23.0	**11**		27.0	**3**		38.7	**3**	
	BR	Mid		D	Soil	185	22.2	**45**	*	7.4	**85**	*	9.0	**79**	*	4.2	**84**	*	7.3	**74**	*	12.0	**70**	*
	BR	Mid	Mid	D	Soil	182	20.6	**49**	*	9.3	**81**	*	9.7	**77**	*	1.9	**92**	*	6.9	**75**	*	12.0	**70**	*
	BR	Low		D	Soil	190	15.7	**61**	*	3.8	**92**	*	11.3	**73**	*	2.0	**92**	*	10.1	**64**	*	9.6	**76**	*
	BR	Low	Mid	D	Soil	191	22.6	**44**	*	8.8	**82**	*	9.4	**78**	*	1.6	**94**	*	5.0	**82**	*	11.1	**72**	*
	BR					188	40.3			48.8			42.4			25.8			27.9			39.9		

***** Means followed by an asterisk are significantly different from checks at the 5% level based on Fisher's Protected LSD.

Table 21. Effect of PTM and/or Insignia SC dose and technique on pine tip moth infestation of containerized and bareroot loblolly pine shoots (top whorl) on five sites across the sotheastern United States, 2013.

	Treatment					Mean Percent Top Whorl Shoots Infested by Tip Moth (**Pct. Reduction Compared to Check**)																	
Year	Cont. or BR	Conc. PTM	Conc. Insignia	Dilute or Undilute	Inj. Loc.	N	Gen 1 (4 sites[1])		N	Gen 2 (2 Sites[2])		N	Gen 3 (2 Sites[3])		N	Gen 4 (2 Sites[4])		N	Last Gen (4 Sites[5])		N	Overall Mean	
2013	Cont.		Mid	U	Plug	165	50.7	**8**	75	49.3	**18**	76	69.9	**2**	76	67.6	**-5**	151	37.1	**12**	189	49.7	**9** *
	Cont.	Mid		U	Plug	168	48.7	**12**	78	46.7	**22**	78	68.0	**5**	78	65.4	**-2**	156	37.5	**11**	195	47.8	**13** *
	Cont.	Mid	Mid	U	Plug	166	46.2	**17**	75	46.2	**23**	78	53.1	**26** *	76	55.0	**14**	151	29.8	**29** *	190	42.6	**22** *
	Cont.	Low		U	Plug	167	44.9	**19**	75	45.6	**24**	78	63.8	**11**	77	65.4	**-2**	152	36.8	**12**	192	45.4	**17** *
	Cont.	Low	Mid	U	Plug	163	46.3	**16**	74	45.5	**24**	75	59.7	**16**	77	54.0	**16** *	151	29.8	**29** *	187	43.5	**21** *
	Cont					163	55.4		74	59.9		76	71.5		77	64.4		151	41.9		190	54.8	
	BR		High	D	Soil	158	52.5	**-11**	64	59.6	**-2**	77	66.9	**2**	74	70.3	**-5**	138	42.7	**-5**	177	53.3	**-5**
	BR		Mid	D	Soil	159	45.9	**3**	68	53.4	**9**	76	66.9	**2**	72	67.2	**0**	140	42.4	**-4**	180	49.6	**2**
	BR	Mid		D	Soil	162	49.6	**-4**	73	47.4	**19**	75	59.2	**13**	73	52.9	**21** *	146	29.8	**27** *	185	45.3	**10** *
	BR	Mid	Mid	D	Soil	161	47.5	**0**	69	46.7	**20**	75	56.8	**17**	74	58.9	**12**	143	32.9	**19**	182	45.6	**10**
	BR	Low		D	Soil	163	46.1	**3**	75	48.9	**17**	77	52.3	**24** *	77	58.0	**13**	152	32.2	**21**	190	43.7	**14** *
	BR	Low	Mid	D	Soil	164	45.1	**5**	75	43.0	**27**	77	51.9	**24** *	75	52.6	**21** *	150	27.8	**32** *	190	41.0	**19** *
	BR					162	47.5		73	58.7		77	68.4		73	66.9		146	40.7		187	50.6	

1: CG-TX, PC-AR, Ray-AL, Wey-NC
2: IFCO-GA, Wey-NC
3: PC-AR, Ray-AL
4: CG-TX, Ray- AL
5: Last Gen, CG-TX (G4), IFCO-GA (G3), Ray-AL (G4), Wey-NC (G3)
***** Means followed by an asterisk are significantly different from checks at the 5% level based on Fisher's Protected LSD.

Table 3. Mean percent pine tip moth infestation of containerized and bareroot loblolly pine seedlings treated with varying concentrations of PTM and Insignia in 2012. Levels not connected by the same letter are significantly different (Student's T).

Treatment #	Containerized (Cont.) or Bareroot (BR)	PTM Concentration	Insignia Concentration				Mean % Infestation
13	BR	X	X	A			39.85
12	Cont.	X	X	A			39.81
7	BR	X	Mid	A			38.74
6	BR	X	High	A			37.38
9	BR	Mid	Mid		B		11.99
8	BR	Mid	X		B		11.97
11	BR	Low	Mid		B		11.12
10	BR	Low	X		B		9.59
1	Cont.	X	Mid		B	C	8.86
4	Cont.	Low	X			C	D	5.20
2	Cont.	Mid	X				D	4.95
3	Cont.	Mid	Mid				D	4.67
5	Cont.	Low	Mid				D	3.53

Table 4. Effect of PTM and/or Insignia SC dose and technique on containerized and bareroot loblolly pine growth on five sites across the southeastern U.S., 2012.

Year	Treatment: Cont. or BR	Treatment: Conc. PTM	Treatment: Conc. Insignia	Treatment: Dilute or Undilute	Treatment: Inj. Loc.	N	Height (cm)	Height: **Growth Difference Compared to Check**	Diameter (cm) [a]	Diameter: **Growth Difference Compared to Check**	Volume (cm^3)	Volume: **Growth Difference Compared to Check**	Tree Survival	**Percent Improvement Compared to Check**
2012	Cont.		Mid	U	Plug	189	75.28	**2.64**	1.44	**-0**	229.61	**6.07**	97	**0**
	Cont.	Mid		U	Plug	195	86.66 *	**14**	1.73 *	**0.28**	389.76 *	**166**	100	**3**
	Cont.	Mid	Mid	U	Plug	190	77.95 *	**5.31**	1.45	**0**	245.52	**22**	97	**0**
	Cont.	Low		U	Plug	192	86.10 *	**13.5**	1.70 *	**0.25**	364.41 *	**141**	98	**1**
	Cont.	Low	Mid	U	Plug	189	75.96	**3.33**	1.40	**-0**	222.97	**-0.6**	97	**0**
	Cont					190	72.64		1.45		223.54		97	
	BR		High	D	Soil	178	67.00	**-7**	1.38	**-0.1**	184.03	**-98**	91	**-5**
	BR		Mid	D	Soil	183	69.66	**-4.4**	1.40	**-0.1**	203.24	**-79**	94	**-3**
	BR	Mid		D	Soil	185	85.03 *	**11**	1.66 *	**0.14**	347.25 *	**65.1**	95	**-1**
	BR	Mid	Mid	D	Soil	182	77.39 *	**3.34**	1.48	**-0**	251.94	**-30**	93	**-3**
	BR	Low		D	Soil	190	93.62 *	**19.6**	1.83 *	**0.31**	444.07	**162**	97	**1**
	BR	Low	Mid	D	Soil	191	85.00	**11**	1.60 *	**0.09**	318.14 *	**36**	98	**2**
	BR					188	74.05		1.51		282.1		96	

Column group headings: Mean End of Season Loblolly Pine Seeding Growth Measurements (**Growth Difference (cm or cm3) Compared to Check)**; Tree Survival (**Percent Improvement Compared to Check**)

* Means followed by an asterisk are significantly different from checks at the 5% level based on Fisher's Protected LSD.

Table 5. Effect of PTM and/or Insignia SC dose and technique on containerized and bareroot loblolly pine growth on five sites across the southeastern U.S., 2013.

	Treatment						Mean End of Season Loblolly Pine Seeding Growth Measurements (**Growth Difference (cm or cm3) Compared to Check**)						Tree Survival (**Percent Improvement Compared to Check**)	
Year	Cont. or BR	Conc. PTM	Conc. Insignia	Dilute or Undilute	Inj. Loc.	N	Height (cm)		Diameter (cm)[a]		Volume (cm^3)			
2013	Cont.		Mid	U	Plug	148	145.29	**8.2**	3.04	**0.2**	1839.16	**209.0**	76	**0**
	Cont.	Mid		U	Plug	156	156.15 *	**19.1**	3.47 *	**0.6**	2763.88 *	**1133.7**	80	**4**
	Cont.	Mid	Mid	U	Plug	151	149.37 *	**12.3**	3.14 *	**0.3**	2232.86	**602.7**	77	**1**
	Cont.	Low		U	Plug	152	157.95 *	**20.9**	3.45 *	**0.6**	2640.01 *	**1009.8**	78	**2**
	Cont.	Low	Mid	U	Plug	189	146.12	**9.0**	2.99 *	**0.1**	1959.90	**329.7**	97	**0**
	Cont					149	137.09		2.85		1630.18		76	
	BR		High	D	Soil	142	139.23 *	**-14.0**	2.87	**-0.4**	1562.28 *	**-558.2**	73	**-3**
	BR		Mid	D	Soil	149	139.85 *	**-13.4**	2.85	**-0.4**	1565.48 *	**-555.0**	76	**1**
	BR	Mid		D	Soil	146	166.50 *	**13.3**	3.51 *	**0.3**	2637.73	**517.3**	75	**-1**
	BR	Mid	Mid	D	Soil	151	156.12	**2.9**	3.21 *	**0.0**	2216.58	**96.1**	77	**2**
	BR	Low		D	Soil	150	174.99 *	**21.7**	3.82 *	**0.6**	3311.18 *	**1190.7**	77	**2**
	BR	Low	Mid	D	Soil	191	166.31 *	**13.1**	3.45 *	**0.2**	2574.79	**454.3**	98	**23**
	BR					147	153.25		3.23		2120.48		75	

***** Means followed by an asterisk are significantly different from checks at the 5% level based on Fisher's Protected LSD.

Table 6. Seedling growth at end of CY2014. Summary of fit of mean growth (cm^3) at breast height over 13 treatments on all sites.

Rsquare	0.017309
Adj Rsquare	0.004697
Root Mean Square Error	1683.415
Mean of Response	1378.448
Observations (or Sum Wgts)	948

Table 7. Results of one-way ANOVA looking at mean growth (cm^3) at breast height by treatment on all sites in 2014.

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**	**Prob > F**
Color	12	46671056.5	3889255	1.3724	0.1731
Error	935	2649684551	2833887		
C. Total	947	2696355607			

Table 8. Seedling growth at end of CY2014. Means for Oneway ANOVA of mean growth (cm^3) at breast height over 13 treatments on all sites in 2014.

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Blue	76	1363.8	193.10	984.8	1742.7
CheckBR	70	1102.6	201.21	707.8	1497.5
Blue&White	74	1563.8	195.69	1179.8	1947.9
Green	74	1455.7	195.69	1071.6	1839.7
CheckCon	69	1162.0	202.66	764.3	1559.7
Orange	75	1496.3	194.38	1114.8	1877.7
Pink	76	1620.6	193.10	1241.7	1999.6
Pink&Blue	75	1363.5	194.38	982.0	1745.0
Red	74	1671.0	195.69	1287.0	2055.1
Red&White	69	812.7	202.66	415.0	1210.4
White	77	1393.4	191.84	1016.9	1769.9
Yel&Red	64	1335.0	210.43	922.0	1747.9
Yellow	75	1496.1	194.38	1114.6	1877.6

Table 9. Seedling growth at end of CY2014. Connecting letters report for mean growth (cm^3) at breast height over 13 treatments on all sites. Levels not connected by the same letter are significantly different ($P<0.05$).

Level		Mean
Red	A	1671.0
Pink	A	1620.6
Blue&White	A	1563.8
Orange	A	1496.3
Yellow	A	1496.1
Green	A	1455.7
White	A	1393.4
Blue	A	1363.8
Pink&Blue	A	1363.5
Yel&Red	A	1335.0
CheckCon	A	1162.0
CheckBR	A	1102.6
Red&White	A	812.7

Table 10. Seedling growth at end of CY2014. Summary of fit of mean growth (cm^3) based on diameter at ground level over 13 treatments on all sites.

Rsquare	0.00759
Adj Rsquare	-0.00491
Root Mean Square Error	5359.743
Mean of Response	1760.935
Observations (or Sum Wgts)	966

Table 11. Seedling growth at end of CY2014. Results of one-way ANOVA looking at mean growth (cm^3) based on diameter at ground level by treatment on all sites.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Color	12	209366163	17447180	0.6073	0.8373
Error	953	2.7377e+10	28726848		
C. Total	965	2.7586e+10			

Table 12. Seedling growth at end of CY2014. Means for Oneway ANOVA of mean growth (cm^3) based on diameter at ground level over 13 treatments on all sites.

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Blue	78	1070.6	606.87	-120	2261.6
CheckBR	71	2122.9	636.08	875	3371.2
Blue&White	74	2320.5	623.06	1098	3543.2
Green	75	2159.0	618.89	944	3373.6
CheckCon	72	1711.6	631.65	472	2951.2
Orange	76	1276.8	614.80	70	2483.4
Pink	76	2222.8	614.80	1016	3429.3
Pink&Blue	76	1269.0	614.80	63	2475.6
Red	76	1925.2	614.80	719	3131.7
Red&White	72	1450.2	631.65	211	2689.7
White	77	1179.6	610.80	-19	2378.2
Yel&Red	68	2529.6	649.96	1254	3805.1
Yellow	75	1786.2	618.89	572	3000.8

Table 13. Seedling growth at end of CY2014. Connecting letters report for mean growth (cm^3) based on diameter at ground level over 13 treatments on all sites. Levels not connected by the same letter are significantly different ($P<0.05$).

Level		Mean
Yel&Red	A	2529.6
Blue&White	A	2320.5
Pink	A	2222.8
Green	A	2159.0
CheckBR	A	2122.9
Red	A	1925.2
Yellow	A	1786.2
CheckCon	A	1711.6
Red&White	A	1450.2
Orange	A	1276.8
Pink&Blue	A	1269.0
White	A	1179.6
Blue	A	1070.6

Table 14. Seedling growth at end of CY2014. Summary of fit of mean growth (cm^3) based on diameter at breast height over 13 treatments in Texas.

Rsquare	0.040284
Adj Rsquare	0.015194
Root Mean Square Error	1587.407
Mean of Response	2050.545
Observations (or Sum Wgts)	472

Table 15. Seedling growth at end of CY2014. Results of one-way ANOVA looking at mean growth (cm^3) based on diameter at breast height by treatment in Texas.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Color	12	48549371.3	4045781	1.6056	0.0867
Error	459	1156616530	2519862		
C. Total	471	1205165901			

Table 16. Seedling growth at end of CY2014. Means for Oneway ANOVA of mean growth (cm^3) based on diameter at breast height over 13 treatments in Texas.

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Blue	37	1910.9	260.97	1398.1	2423.8
CheckBR	35	1731.8	268.32	1204.6	2259.1
Blue&White	37	2355.6	260.97	1842.7	2868.4
Green	37	2239.3	260.97	1726.4	2752.1
CheckCon	37	1612.0	260.97	1099.2	2124.9
Orange	36	2076.5	264.57	1556.6	2596.4
Pink	38	2678.1	257.51	2172.0	3184.1
Pink&Blue	37	2086.2	260.97	1573.3	2599.0
Red	36	2314.9	264.57	1795.0	2834.9
Red&White	35	1493.2	268.32	965.9	2020.5
White	38	1778.2	257.51	1272.2	2284.2
Yel&Red	33	2214.4	276.33	1671.4	2757.4
Yellow	36	2136.8	264.57	1616.9	2656.7

Table 17. Seedling growth at end of CY2014. Connecting letters report for mean growth (cm^3) based on diameter at breast height over 13 treatments in Texas. Levels not connected by the same letter are significantly different ($P<0.05$).

Level		Mean
Pink	A	2678.1
Blue&White	A	2355.6
Red	A	2315.0
Green	A	2239.3
Yel&Red	A	2214.4
Yellow	A	2136.8
Pink&Blue	A	2086.2
Orange	A	2076.5
Blue	A	1910.9
White	A	1778.2
CheckBR	A	1731.9
CheckCon	A	1612.0
Red&White	A	1493.2

Table 18. Seedling growth at end of CY2014. Summary of fit of mean growth (cm^3) based on diameter at ground level over 13 treatments in Texas.

Rsquare	0.046233
Adj Rsquare	0.021725
Root Mean Square Error	3795.773
Mean of Response	6240.249
Observations (or Sum Wgts)	480

Table 19. Seedling growth at end of CY2014. Results of one-way ANOVA looking at mean GLD growth (cm^3) by treatment in Texas.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Color	12	326160257	27180021	1.8865	0.0339*
Error	467	6728484888	14407891		
C. Total	479	7054645145			

Table 20. Seedling growth at end of CY2014. Means for Oneway ANOVA of growth based on mean diameter at ground level over 13 treatments in Texas.

Level	**Number**	**Mean**	**Std Error**	**Lower 95%**	**Upper 95%**
Blue	39	5851.9	607.81	4657.6	7046.3
CheckBR	35	5955.1	641.60	4694.4	7215.9
Blue&White	37	7111.4	624.02	5885.2	8337.7
Green	38	6741.2	615.76	5531.2	7951.2
CheckCon	38	5246.4	615.76	4036.4	6456.4
Orange	37	5911.3	624.02	4685.1	7137.5
Pink	38	7939.8	615.76	6729.8	9149.8
Pink&Blue	37	6031.7	624.02	4805.4	7257.9
Red	37	6890.4	624.02	5664.2	8116.6
Red&White	36	4705. 5	632.63	3462.3	5948.6
White	38	5551.4	615.76	4341.4	6761.4
Yel&Red	34	6594.3	650.97	5315.1	7873.5
Yellow	36	6580.8	632.63	5337.6	7823.9

Table 21. Seedling growth at end of CY2014. Connecting letters report for seedling growth based on diameter at ground level over 13 treatments in Texas. Levels not connected by the same letter are significantly different ($P<0.05$).

Level		**Mean**
Pink	A	7939.8
Blue&White	AB	7111.4
Red	AB	6890.4
Green	AB	6741.2
Yel&Red	AB	6594.3
Yellow	AB	6580.8
Pink&Blue	AB	6031.7
CheckBR	AB	5955.1
Orange	AB	5911.3
Blue	AB	5851.9
White	AB	5551.4
CheckCon	AB	5246.4
Red&White	B	4705.5

Table 22. Seedling growth at end of CY2014. Summary of fit of seedling growth based on diameter at breast height over 13 treatments in North Carolina.

Rsquare	0.029438
Adj Rsquare	0.004284
Root Mean Square Error	1495.251
Mean of Response	711.9982
Observations (or Sum Wgts)	476

Table 23. Seedling growth at end of CY2014. Results of one-way ANOVA looking at seedling growth based on diameter at breast height by treatment in North Carolina.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Color	12	31397938.8	2616495	1.1703	0.3018
Error	463	1035164489	2235776		
C. Total	475	1066562428			

Table 24. Seedling growth at end of CY2014. Means for Oneway ANOVA of seedling growth based on diameter at breast height over 13 treatments in North Carolina.

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Blue	39	844.7	239.43	374.2	1315.2
CheckBR	35	473.4	252.74	-23.2	970.1
Blue&White	37	772.1	245.82	289.0	1255.1
Green	37	672.1	245.82	189.0	1155.1
CheckCon	32	641.7	264.33	122.3	1161.1
Orange	39	960.6	239.43	490.1	1431.1
Pink	38	563.2	242.56	86.5	1039.9
Pink&Blue	38	659.8	242.56	183.2	1136.5
Red	38	1061.0	242.56	584.4	1537.7
Red&White	34	112.1	256.43	-391.8	616.1
White	39	1018.4	239.43	547.9	1488.9
Yel&Red	31	398.8	268.56	-129.0	926.5
Yellow	39	904.6	239.43	434.1	1375.2

Table 25. Seedling growth at end of CY2014Connecting letters report for seedling growth based on diameter at breast height over 13 treatments in North Carolina. Levels not connected by the same letter are significantly different (P<0.05).

Level		**Mean**
Red	A	1061.0
White	A	1018.4
Orange	A	960.6
Yellow	A	904.7
Blue	A	844.8
Blue&White	A	772.1
Green	A	672.1
Pink&Blue	A	659.8
CheckCon	A	641.7
Pink	A	563.2
CheckBR	A	473.4
Yel&Red	A	398.8
Red&White	A	112.2

Table 30: Code for treatments

Treatments and Plot Design Example

Code	Treatment	Color
A	Mid UD Insignia container plug injection	red
B	Mid UD PTM container plug injection	blue
C	Mid UD PTM + Mid Insignia container plug injection	orange
D	Low UD PTM container plug injection	pink/blue
E	Low UD PTM + Mid Insignia container plug injection	white
F	High D Insignia bareroot soil injection	red/white
G	Mid D Insignia bareroot soil injection	yellow/red
H	Mid D PTM bareroot soil injection	yellow
I	Mid D PTM + Insignia bareroot soil injection	green
J	Low D PTM bareroot soil injection	pink
K	Low D PTM + Mid Insignia bareroot soil injection	blue/white
L	Check (containerized)	green/orange
M	Check (bareroot))	blue/red

UD = undilute; D = dilute

Pine Tip Moth Trials: Evaluation of PTM™ and Insignia®SC Rate for Bareroot Pine Seedlings in Texas

Initiated in 2012; Growth monitored through 2014

Objectives:

1. Evaluate the efficacy of PTM™ (fipronil) and Insignia®SC (pyraclostrobin), alone or in combination, applied to bareroot seedlings at different rates for reducing pine tip moth infestation levels and improving seedling health
2. Determine the duration of chemical activity

Study site: Hancock Forest Management's Rocky Mt. Cemetery site in Etoile, TX

Methods

Bareroot seedlings were provided by Hancock Forest Management.

Treatments:

1. PTM™: high concentration/ diluted soil injection [5.6mL PTM (110 TPA rate) in 24.4mL water (30mL total volume)/ seedling]: soil injection at two points next to transplanted bareroot just after planting
2. PTM™: mid-concentration/ diluted soil injection [1.4mL PTM (435 TPA rate) in 28.6mL water (30mL total volume)/ seedling]: soil injection at two points next to transplanted bareroot just after planting.
3. PTM™: low-concentration/ diluted soil injection [1.0mL PTM (600 TPA rate) in 29.0mL water (30mL total volume/ seedling]: soil injection at two points next to transplanted bareroot just after planting.
4. Insignia®SC: high concentration/ undiluted soil injection [51.6mL Insignia (110 TPA rate) undiluted/ seedling]: soil injection at four points next to transplanted bareroot just after planting.
5. Insignia®SC: mid-concentration/ diluted soil injection [13.1mL Insignia (435 TPA rate) in 11.9mL water (30mL total volume)/seedling]: Soil injection at two points next to transplanted bareroot just after planting.
6. Insignia®SC: low-concentration/ diluted soil injection [9.5mL Insignia (600 TPA rate) in 20.5mL water (30mL total volume)/ seedling]: soil injection at two points next to transplanted bareroot just after planting.
7. PTM™ + Insignia®SC: high concentration/ undiluted soil injection [5.6mL PTM + 51.6mL Insignia (57.2mL total volume)/ seedling]: soil injection at four points next to transplanted bareroot just after planting.
8. PTM™ + Insignia®SC: mid-concentration/ diluted soil injection [1.4mL PTM + 13.1mL Insignia in 15.5mL water (30mL total volume)/seedling]: soil injection at two points next to transplanted bareroot just after planting.

9. PTM™ + Insignia®SC: low-concentration/ diluted soil injection [1.0mL PTM + 9.5mL Insignia in 19.5mL water (30mL total volume)/ seedling]: soil injection at two points next to transplanted bareroot just after planting.
10. Bareroot control (untreated)

Bareroot seedlings were individually treated after planting using a PTM injection probe system developed by Sammy Keziah (formerly with Enviroquip). The seedlings were treated with PTM™ and/or Insignia®SC at different rates based on the restricted rate of 59g AI/acre/year (PTM™) or 1,416g AI/acre/year (Insignia®) and the number of trees planted per acre (TPA). For example, fipronil was applied to 110 TPA = 0.537g AI/seedling (a rate being considered by some forest industries for treatment of high-valued crop trees); at 435 TPA = 0.136g AI/ seedling (a tree density currently being used by Weyerhaeuser Co.); and 600 TPA = 0.1g AI/seedling (a tree density used by several forest industries).

One recently hand planted tract was selected in January 2012 in TX based on uniformity of soil, drainage, and topography. The harvested tract was intensively site prepared, i.e., subsoil, bedding and/ or herbicide were used. A half-acre (approximate) area was selected. A triple Latin square design was established with single tree plots (10 rows X 10 treatments) serving as blocks, i.e., each treatment was randomly selected for placement along each row (bed). Thirty rows were established on each site. Seedlings were planted at 6 foot spacing's along each row. Individual tree locations were marked with different color pin flags prior to tree planting. The plot corners were marked with PVC pipe and metal tags.

Damage and Tree Measurements:

Tip moth damage was evaluated after each tip moth generation (3-4 weeks after peak moth flight) by 1). Identifying if the tree is infested or not, 2). If infested, the proportion of tips infested on the top whorl and terminal were calculated; and 3). Separately, the terminal was identified as infested or not. Observations were made as to the occurrence and extent of damage caused by other insects, i.e., coneworm, aphids, sawfly, etc. Measurements of tree health were collected at the end of each growing season. Tree health measurements included height and diameter; crown diameter, density and color (vigor); number and length of shoots in the top whorl, and tree survival. All study trees were measured for height and diameter at ground line at the beginning of the study. Measurements were also taken when tree growth stopped in mid- to late November.

Results:

In 2012, all PTM and PTM + Insignia treatments significantly reduced percent tip moth infestation compared to the control (by 78% and 75% respectively) (Table 1, Figure 1). Insignia treatments alone resulted in an overall reduction in pine tip moth infestation by only 2%. None of the treatments resulted in a significant improvement in diameter (Table 2). All three PTM treatments and the PTM + Insignia low concentration treatment resulted in a significant improvement in height. Volume was only significantly improved in the case of the low and high concentration PTM treatments (Table 2).

In 2013, measurements of tip moth infestation were only taken after the first and last tip moth generation. There was no significant difference in the overall mean tip moth infestation between the control and any of the treatments (Table 3, Figure 2). The only significant difference in percent tip moth infestation was during the fifth generation; the high-rate PTM & Insignia treatment resulted in a 25% reduction in tip moth infestation. The PTM only and PTM and Insignia low and high-rate treatments resulted in a significant increase in height compared with the control (Table 4). There was no significant difference in the diameter or overall growth (volume) of trees from any of the treatments compared with the control.

At the end of the 2014 growing season, the treated and check seedlings were again measured for growth. A one-way ANOVA was used to analyze the mean volume (cm^3) growth of 10 treatments. Results revealed no significant differences in growth among any of the treatments (Tables 5-7). Future growth measurements are not planned.

Acknowledgments:

Many thanks to Hancock Forest Management for providing a research site and seedlings for this study. Thanks also to Ken Smith and Mike Curry for their contributions.

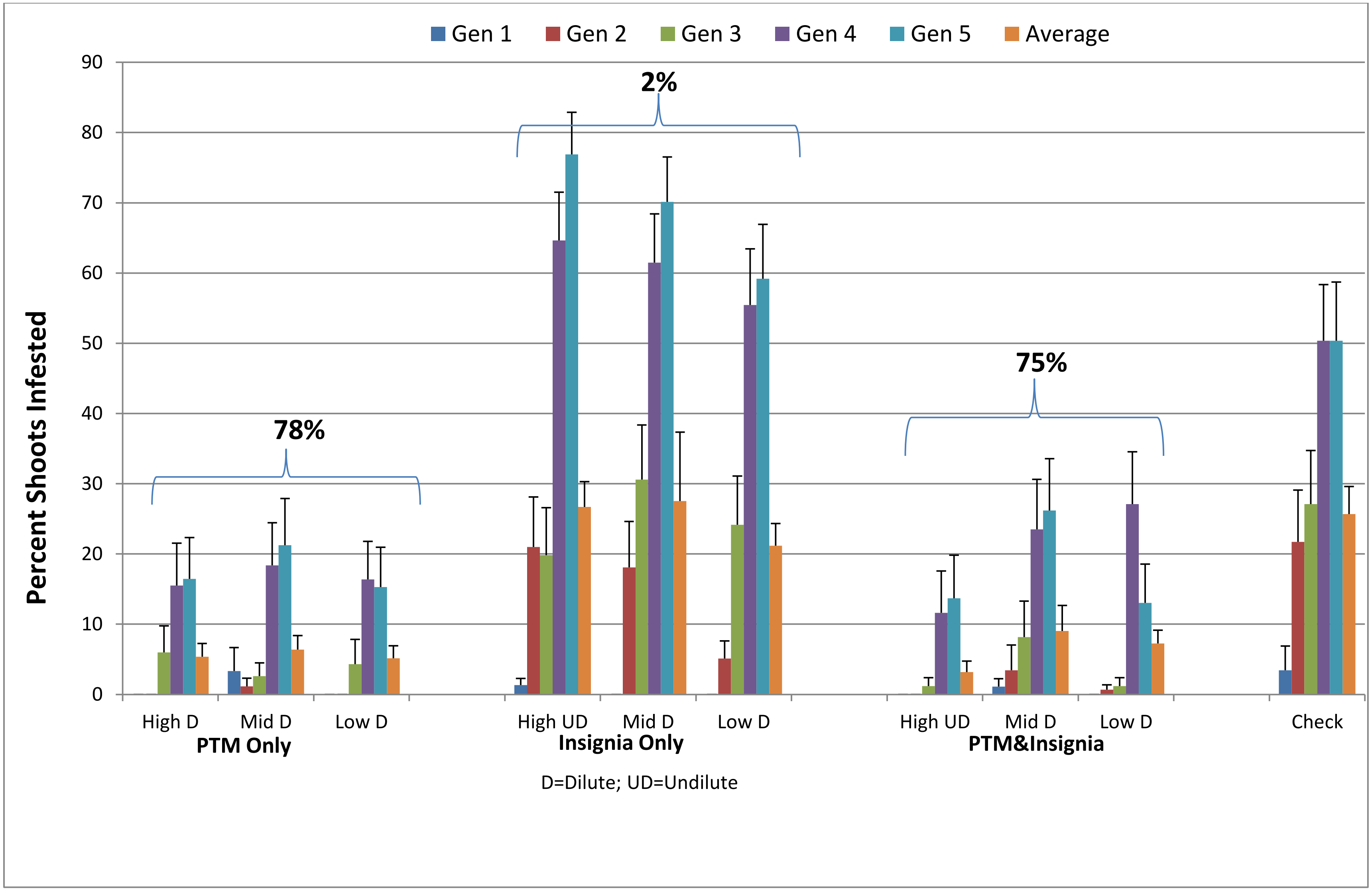


Figure 1. Effect of PTM[TM] and/or Insignia®SC soil injection dose on tip moth infestation of bareroot loblolly pine at one site in East Texas, 2012.

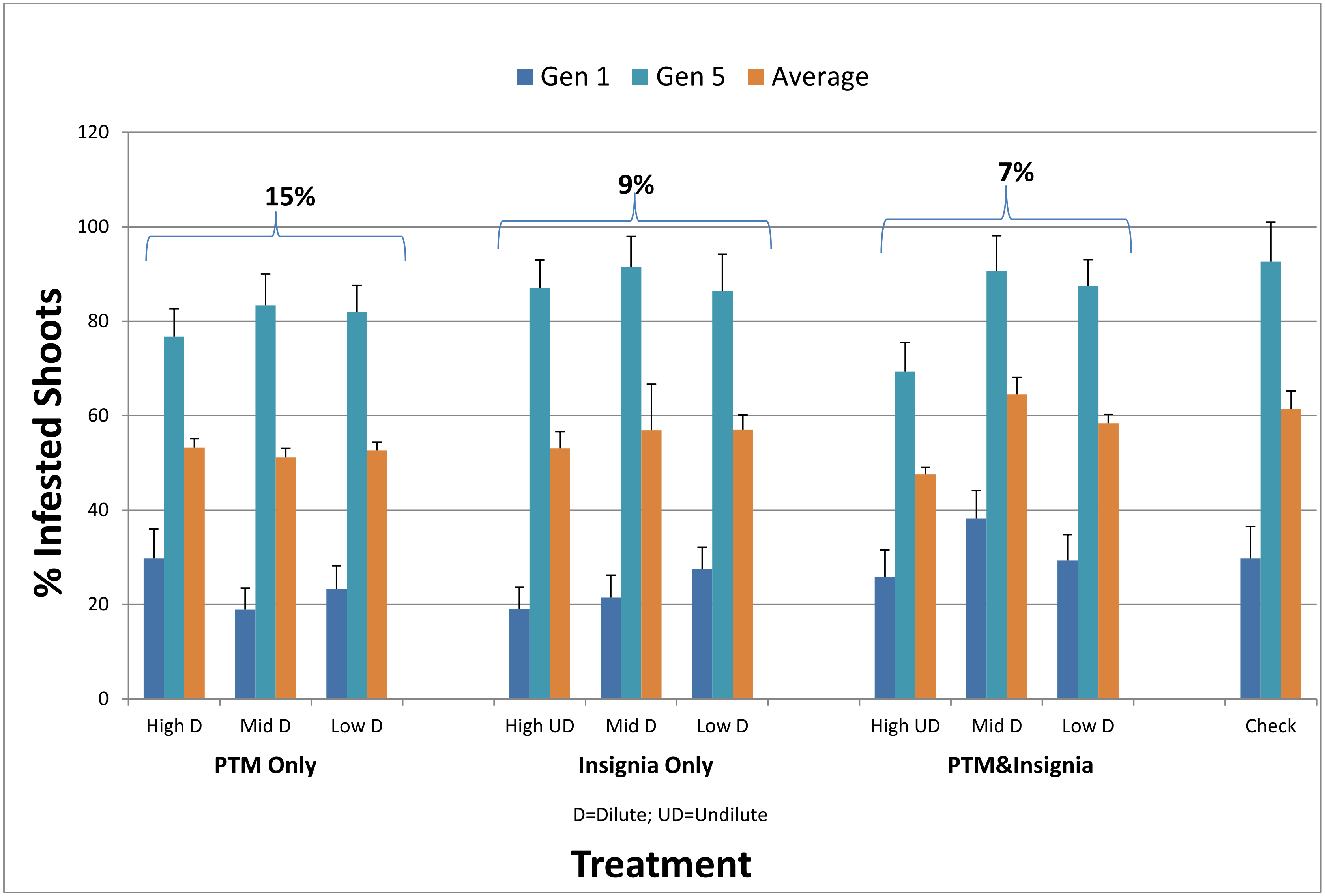


Figure 2. Effect of PTM™ and/or Insignia®SC soil injection dose on tip moth infestation of bareroot loblolly pine at one site in East Texas, 2013

Table 1. Effect of PTM and/or Insignia SC dose and technique on pine tip moth infestation of containerized and bareroot loblolly pine shoots (top whorl) on five sites across the southeastern United States, 2012.

							Mean Percent Top Whorl Shoots Infested by Tip Moth (**Pct. Reduction Compared to Check**)																
Year	Treatment #	Conc. PTM	Conc. Insignia	Dilute or Undilute	# of inj. Pts.	N	Gen 1		Gen 2			Gen 3			Gen 4			Gen 5 or Last			Overall Mean		
2012	1	High	X	dilute	2	30	0.0	**100**	0.0	**100**	*	6.0	**78**	*	15.5	**69**	*	16.4	**67**	*	5.4	**79**	*
	2	Mid	X	dilute	2	30	3.33	**3**	1.1	**95**	*	2.6	**90**	*	18.4	**63**	*	21.3	**58**	*	6.4	**75**	*
	3	Low	X	dilute	2	30	0.0	**100**	0.0	**100**	*	4.2	**85**	*	16.4	**67**	*	15.3	**70**	*	5.1	**80**	*
	4	X	High	Undilute	4	30	1.3	**61**	21.0	**3**		19.8	**27**		64.7	**-28**		76.9	**-53**	*	26.7	**-4**	
	5	X	Mid	Dilute	2	30	0.0	**100**	18.1	**17**		30.6	**-13**		61.5	**-22**		70.1	**-39**	*	27.5	**-7**	
	6	X	Low	Dilue	2	30	0.0	**100**	5.1	**76**	*	24.1	**11**		55.5	**-10**		59.2	**-18**		21.2	**18**	
	7	High	High	Undilute	4	30	0.0	**100**	0.0	**100**	*	1.2	**96**	*	11.6	**77**	*	13.7	**73**	*	3.2	**88**	*
	8	Mid	Mid	Dilute	2	30	1.1	**68**	3.4	**84**	*	7.9	**71**	*	23.5	**53**	*	26.2	**48**	*	9.0	**65**	*
	9	Low	Low	Dilute	2	30	0.0	**100**	0.7	**97**	*	1.2	**96**	*	27.1	**46**	*	13.0	**74**	*	7.2	**72**	*
	10	X	X	X	X	30	3.4		21.7			27.1			50.4			50.4			25.7		

***** Means followed by an asterisk are significantly different from checks at the 5% level based on Fisher's Protected LSD.

Table 2. Effect of PTM™ and/or Insignia SC™ dose on bareroot loblolly pine growth on one site in East Texas, 2012.

Treatment					Mean End of Season Loblolly Pine Seeding Growth Measurements (**Growth Difference (cm or cm3) Compared to Check)**							
Year	Treatment	Conc.	Dilute or Undilute	N	Height (cm)			Diameter (cm)[a]		Volume (cm^3)		
2012	PTM Only	High	Dilute	29	63.8	*	**14.9**	1.32	**0.2**	130.5	*	**46.1**
	PTM Only	Mid	Dilute	29	58.0	*	**9.1**	1.18	**0.0**	93.0		**8.7**
	PTM Only	Low	Dilute	30	61.8	*	**13.0**	1.29	**0.1**	123.9	*	**39.5**
	Insignia Only	High	Undilute	29	54.4		**5.6**	1.13	**0.0**	84.1		**-0.3**
	Insignia Only	Mid	Dilute	29	50.2		**1.4**	1.11	**-0.1**	72.2		**-12.2**
	Insignia Only	Low	Dilute	29	53.4		**4.6**	1.12	**-0.1**	78.3		**-6.1**
	PTM&Insignia	High	Undilute	28	57.0		**8.2**	1.12	**0.0**	97.6		**13.2**
	PTM&Insignia	Mid	Dilute	28	58.0		**9.1**	1.21	**0.0**	115.7		**31.3**
	PTM&Insignia	Low	Dilute	28	61.5	*	**12.7**	1.29	**0.1**	127.2		**42.8**
	Untreated			28	48.8			1.17		84.4		

[a] Ground Line Diameter.

***** Means followed by an asterisk are significantly different from checks at the 5% level based on Fisher's Protected LSD.

Table 3. Effect of PTM and/or Insignia SC dose and technique on pine tip moth infestation of containerized and bareroot loblolly pine shoots (top whorl) on five sites across the southeastern United States, 2013.

							Mean Percent Top Whorl Shoots Infested by Tip Moth **(Pct. Reduction Compared to Check)**						
Year	Treatment #	Conc. PTM	Conc. Insignia	Dilute or Undilute	# of inj. Pts.	N	Gen 1		Gen 5 or Last			Overall Mean	
2013	1	High	X	Dilute	2	30	29.72	**0**	76.72	**17**		53.22	**13**
	2	Mid	X	Dilute	2	30	18.89	**36**	83.33	**10**		51.11	**16**
	3	Low	X	Dilute	2	30	23.29	**22**	81.89	**12**		52.59	**14**
	4	X	High	Undilute	4	30	19.11	**36**	86.95	**6**		53.03	**13**
	5	X	Mid	Dilute	2	30	21.41	**28**	91.55	**1**		56.61	**8**
	6	X	Low	Dilute	2	30	27.51	**7**	86.44	**7**		56.97	**7**
	7	High	High	Undilute	4	30	25.77	**13**	69.29	**25**	*	47.53	**22**
	8	Mid	Mid	Dilute	2	30	38.21	**-29**	90.74	**2**		64.48	**-5**
	9	Low	Low	Dilute	2	30	29.26	**2**	87.50	**6**		58.38	**5**
	10	X	X	X	X	30	29.71		92.62			61.21	

* Means followed by an asterisk are significantly different from checks at the 5% level based on Fisher's Protected LSD.

Table 4. Effect of PTM™ and/or Insignia SC™ dose on bareroot loblolly pine growth on one site in East Texas, 2013.

Treatment					Mean Growth 2013 (**Growth Difference (cm or cm3) Compared to Check)**						
Year	Treatment	Conc.	Dilute or Undilute	N	Height (cm)			Diameter (cm)[a]		Volume (cm^3)	
2013	PTM Only	High	Dilute	29	160.1	*	**26.6**	2.96	**0.3**	1540.0	**380.5**
	PTM Only	Mid	Dilute	29	147.1		**13.6**	2.69	**0.0**	1227.9	**68.4**
	PTM Only	Low	Dilute	30	154.8	*	**21.3**	3.12	**0.4**	1699.5	**540.0**
	Insignia Only	High	Undilute	29	141.7		**8.2**	2.70	**0.0**	1243.7	**84.2**
	Insignia Only	Mid	Dilute	28	140.2		**6.7**	2.69	**0.0**	1103.6	**-55.9**
	Insignia Only	Low	Dilute	29	138.6		**5.1**	2.78	**0.1**	1175.4	**15.9**
	PTM&Insignia	High	Undilute	28	150.6	*	**17.1**	2.76	**0.1**	1433.3	**273.8**
	PTM&Insignia	Mid	Dilute	27	148.3		**14.8**	2.85	**0.2**	1441.0	**281.5**
	PTM&Insignia	Low	Dilute	28	157.6	*	**24.1**	2.98	**0.3**	1522.7	**363.2**
	Untreated			28	133.5			2.69		1159.5	

[a] Ground Line Diameter.

***** Means followed by an asterisk are significantly different from checks at the 5% level based on Fisher's Protected LSD.

Table 5. Summary of fit of mean volume (cm^3) growth over 10 treatments for pine tip moth control on bareroot seedlings in East Texas using PTM™ and Insignia™ at end of 2014 growing season.

Rsquare	0.042744
Adj Rsquare	0.011416
Root Mean Square Error	0.508168
Mean of Response	3.482591
Observations (or Sum Wgts)	285

Table 6. Results of one-way ANOVA looking at mean volume growth (cm^3) by treatment for pine tip moth control on bareroot seedlings in East Texas using PTM™ and Insignia™ at end of 2014 growing season.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	9	3.171010	0.352334	1.3644	0.2042
Error	275	71.014681	0.258235		
C. Total	284	74.185691			

Table 7. Means for Oneway ANOVA of mean volume growth (cm^3) over 10 treatments for pine tip moth control on bareroot seedlings in East Texas using PTM™ and Insignia™ at end of 2014 growing season. Means followed by the same letter are not significantly different (P>0.05).

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
PTM Med	29	3.45 A	0.0944	3.2648	3.6363
P&I Low	28	3.59 A	0.096	3.4059	3.7840
PTM Low	30	3.63 A	0.093	3.4499	3.8152
Insig High	29	3.47 A	0.094	3.2848	3.6563
Check	28	3.32 A	0.096	3.1274	3.5056
Insig Low	29	3.34 A	0.094	3.1542	3.5257
P&I High	28	3.49 A	0.096	3.2975	3.6756
PTM High	29	3.62 A	0.094	3.4353	3.8069
Insig Med	28	3.40 A	0.096	3.2136	3.5917
P&I Med	27	3.50 A	0.098	3.3099	3.6949

Pine Tip Moth Trials: Effects of Cold Storage Time on Efficacy of Fipronil Injection Treatments on Containerized Loblolly Pine Seedlings

Initiated in Winter 2012

Cooperators

Wayne Bell	International Forest Company (No data received from GA)
Jim Bean	BASF, Research Triangle Park, NC

Objectives: 1) Evaluate the effects of cold storage times on containerized seedling survival and 2) efficacy of PTM (fipronil) for reducing pine tip moth infestation levels.

Justification

Several trials (2003 - 2011) have shown that fipronil applied to bare root and containerized seedlings before or after planting is highly effective in reducing tip moth damage for 2+ years. EPA approved the registration and use of PTM insecticide for tip moth control only as a soil injection treatment at or post plant. Recently, a plug injection system was developed that would allow treatment of container seedlings in the nursery prior to shipment to the field. Container seedlings, once package in shipping boxes, are often stored temporarily in coolers. A trial will be established to determine if cold storage of PTM-treated seedlings will affect survival and/or treatment efficacy against tip moth.

Methods

One family of loblolly pine bareroot seedlings will be selected (from IFCo).

Treatments**:**

A = PTM + Storage (4wk) - Injected with PTM (1.4 ml) and placed in cold storage 4 weeks prior to planting.
B = PTM + Storage (2 wk) - Injected with PTM (1.4 ml) and placed in cold storage 2 weeks prior to planting.
C = PTM + Storage (1 wk) – Injected with PTM (1.4 ml) and placed in cold storage 1 week prior to planting.
D = PTM only – Injected w PTM and no storage
E = Storage (4 wk) only – Seedlings placed in cold storage 4 weeks prior to planting
F = Storage (2 wk) only – Seedlings placed in cold storage 2 weeks prior to planting
G = Storage (1 wk) only – Seedlings placed in cold storage 1 week prior to planting
H = Check- no PTM & no storage

Note: If possible, Trt **A** seedlings (150 for each site; 300 total) should be treated first (Nov. 12) and Trt **A** & **E** seedlings placed in cold storage; Trt **B** seedlings would be treated on Nov. 26 and Trt **B** & **F** seedlings placed in cold storage; Trt **C** seedlings would be treated on Dec. 3 and Trts **C** & **G** seedlings placed in cold storage; and Trt **D** seedlings would be treated on Dec. 10 and Trt **A, B, C, E, F,** and **G** seedlings would be taken out of cold storage. All seedlings, including checks (**D** & **H**), could be planted on Dec. 10 or 11. The TX seedlings would be shipped immediately. Containerized seedlings will be individually treated at the IFCo nursery prior to planting using the plug injection system developed by Stewart Boots, S&K Designs. The seedlings will be treated with PTM™ at 1.4 ml per seedling (435 tpa) based on the restricted rate of 59 g AI/acre/year (PTM™).

Square 1

row/column	1	2	3	4	5	6	7	8
A	B	A	G	H	C	F	E	D
B	G	H	C	F	D	A	B	E
C	A	E	B	C	F	H	D	G
D	D	C	F	G	E	B	H	A
E	C	F	D	A	H	E	G	B
F	F	D	H	E	B	G	A	C
G	E	B	A	D	G	C	F	H
H	H	G	E	B	A	D	C	F

Square 2

	1	2	3	4	5	6	7	8
A	G	E	C	H	B	D	F	A
B	H	F	E	D	A	B	G	C
C	E	G	H	B	D	A	C	F
D	F	A	D	G	C	H	B	E
E	B	C	G	A	H	F	E	D
F	A	D	B	C	F	E	H	G
G	C	B	A	F	E	G	D	H
H	D	H	F	E	G	C	A	B

Square 3

	1	2	3	4	5	6	7	8
A	A	B	C	D	H	E	G	F
B	D	F	H	C	B	A	E	G
C	F	A	B	E	G	H	C	D
D	H	E	G	A	F	D	B	C
E	B	H	E	G	C	F	D	A
F	G	C	D	H	A	B	F	E
G	C	D	A	F	E	G	H	B
H	E	G	F	B	D	C	A	H

Square 4

	1	2	3	4	5	6	7	8
A	B	A	G	C	D	E	H	F
B	H	F	A	D	E	B	C	G
C	G	B	C	A	F	D	E	H
D	A	G	E	F	H	C	D	B
E	F	D	B	E	C	H	G	A
F	E	H	D	G	B	A	F	C
G	C	E	F	H	A	G	B	E
H	D	C	H	B	G	F	A	D

A = PTM + 4 week storage
B = PTM + 2 week storage
C = PTM + 1 week storage
D = PTM only (no storage)
E = 4 week storage only
F = 2 week storage only
G = 1 week storage only
H = Check (untreated)

Two recently harvested tracts will be selected; one in east Texas and one near Moultrie, GA (No growth data have been received from GA for this study for the end of the 2014 growing season.)
A 1 acre (approximate) area within each site will be selected. A quadruple Latin square design will be established with single tree plots (8 rows X 8 treatments) serving as blocks, i.e., each treatment will be randomly selected for placement along each row (bed). Thirty-two (32) rows will be established on each site. Seedlings will be planted at 8 foot spacing along each row. Individual tree locations will be marked with different color pin flags prior to tree planting.

The plot corners should be marked with PVC pipe and the individual trees with different color pin flags and tags. It may be necessary to apply herbicide over the area in the spring to ensure that the seedlings remain exposed to tip moth attack throughout the year.

Damage and Tree Measurements

Tip moth damage was evaluated by determining percent of trees infested, percent of infested shoots in the top whorl and percent terminals infested about 4 weeks after peak moth flight at each generation. All study trees were measured (height & diameter @ 6 inches) at the beginning of the study (just after seedlings were planted) and in mid- to late November after growth had stopped.

Results

Standard least squares analysis was conducted on the tip moth infestation and growth data. Three effects were tested: 1. Treatment (PTM-treated or untreated), 2. Storage time, and 3. Treatment x Storage time (crossed). Treatment x Storage time combinations showed a significant effect on percent tip moth infestation in generations 2 and 3. PTM-treated seedlings were found to have significantly ($p < 0.0001$) decreased tip moth infestations compared with untreated seedlings in all four generations. Storage time only showed a significant difference in tip moth infestation among treatments in generation 2.

A Student's T test was conducted to determine how the treatment x storage time combinations differed. Although not significant, treatment A (PTM-treated/ 4 week storage time) resulted in lower percent tip moth infestation than the other treatment x storage combinations (Table 1). The greatest difference in percent tip moth infestation was found between PTM-treated and untreated seedlings. Very little difference in percent tip moth infestation was found among the storage time treatments.

There was no significant difference in diameter or volume for any of the effects tested using standard least squares analysis. Height was significantly different for the PTM-treated vs. untreated trees ($p < 0.0001$). A student's T test was conducted on the treatment x storage combinations to determine how the treatments differed. Treatment B (PTM treated/ 2 week storage time) had the greatest growth increase compared with all other treatments, although this was not significant (Table 2).

Conclusions:

First year data show that storage time does not have an overall significant effect on percent tip moth infestation or seedling growth. Trees treated with PTM have significantly reduced tip moth infestation in all generations and also show a significant increase in height compared with the untreated trees.

Table 1. Mean percent top whorl shoots infested by tip moth per treatment in 2013 at two sites (GA & TX). Levels not connected by the same letter in each generation are significantly different**.**

					Mean Percent Top Whorl Shoots Infested by Tip Moth														
Year	Treatment	PTM Rate (ml)	Storage Period (weeks)	n	Gen 1 (GA & TX)		n	Gen 2 (GA only)		n	Gen 3 (GA only)		n	Gen 4 or Last (GA & TX)		n	Overall Mean	
	A	1.4 ml	4	64	1.26	**C**	32	0	**D**	32	0.78	**C**	53	0.79	**B**	64	0.71	**C**
2013	B	1.4 ml	2	64	0	**C**	32	2.34	**D**	32	1.04	**C**	57	4.3	**B**	64	2.34	**C**
YR1	C	1.4 ml	1	65	2.31	**C**	32	1.04	**D**	32	0	**C**	56	8.93	**B**	65	5.13	**C**
	D	1.4 ml	0	64	0.52	**C**	32	1.56	**D**	32	0	**C**	57	6.29	**B**	64	3.13	**C**
	E	None	4	61	32.70	**AB**	32	90.89	**A**	32	55.75	**B**	51	62.84	**A**	61	48.79	**A**
	F	None	2	62	21.42	**B**	32	78.59	**B**	32	58.92	**B**	50	59.91	**A**	62	40.12	**B**
	G	None	1	62	31.75	**B**	31	74.66	**B**	31	60.99	**B**	57	56.08	**A**	62	48.64	**A**
	H	None	0	63	31.93	**A**	32	63.49	**C**	32	79.22	**A**	58	63.98	**A**	63	49.03	**A**

Table 2. Mean height, diameter (GLD), volume, and growth (difference in volume from 2012-2013) of loblolly pine trees per treatment at two sites (GA & TX) in 2013. Levels not connected by the same letter in each generation are significantly different**.**

					Growth Measurements							
Year	Treatment	PTM Rate (ml)	Storage Period (weeks)	n	Height (cm)		GLD (cm)		Volume (cm^3)		Growth (cm^3)	
	A	1.4 ml	4	53	60.92	**A**	1.45	**A**	208.21	**AB**	183.42	**B**
2013	B	1.4 ml	2	57	61.39	**A**	1.77	**A**	587.77	**A**	567.42	**A**
YR 1	C	1.4 ml	1	56	59.66	**A**	1.45	**A**	206.7	**AB**	182.66	**B**
	D	1.4 ml	0	57	64.84	**A**	1.46	**A**	251.73	**AB**	229.05	**AB**
	E	None	4	51	50.90	**C**	1.51	**A**	188.86	**B**	162.94	**B**
	F	None	2	50	52.72	**BC**	1.58	**A**	259.63	**AB**	236.12	**AB**
	G	None	1	57	52.70	**BC**	1.54	**A**	241.43	**AB**	219.73	**AB**
	H	None	0	58	58.26	**AB**	1.43	**A**	180.36	**B**	146.76	**B**

Pine Tip Moth Trials: Optimal Timing for a Single Spray Application of Mimic for Control of Pine Tip Moth

Initiated in 2014; completed in 2015

Justification

Pine tip moth (*Rhyacionia* spp.) is an important pine insect in the eastern and southern U.S. Its preferred hosts are loblolly (*Pinus taeda* L), shortleaf (*Pinus echinata* Mill.) and Virginia (*Pinus virginiana* Mill.) pine. Larvae feed on buds and new shoots, causing serious damage to young pines, particularly in seed orchards, nurseries, and Christmas tree plantations. Repeated attacks may result in limited growth, stem deformation, loss in wood quality, bushy appearance, reduced cone crop, a lower aesthetic value, and even mortality. Tip moth damage is most severe on seedlings and saplings usually under 5 years of age and less than 7m in height (Sun et al., 2000). A long term study showed that growth differences as a result of tip moth management are maintained (Cade and Hedden, 1987), therefore treatments to control tip moth are often warranted. Unfortunately, treating can be very costly, particularly since pine tip moth has several generations a season (two to five, depending upon the geographic location). For this reason, it is of interest to determine which generation should be sprayed for optimal control, if only one spray treatment can be economically applied.

Objectives

1. Determine which tip moth generation should be sprayed if only one spray treatment can be economically applied.
2. Evaluate several insecticides to determine which provides greatest efficacy.

Cooperators: Plum Creek Timber Company, Weyerhaeuser

Study site locations: Louisiana, Arkansas, North Carolina

Insecticides: Mimic^{TM} 2LV (Valent BioSciences)

Methods

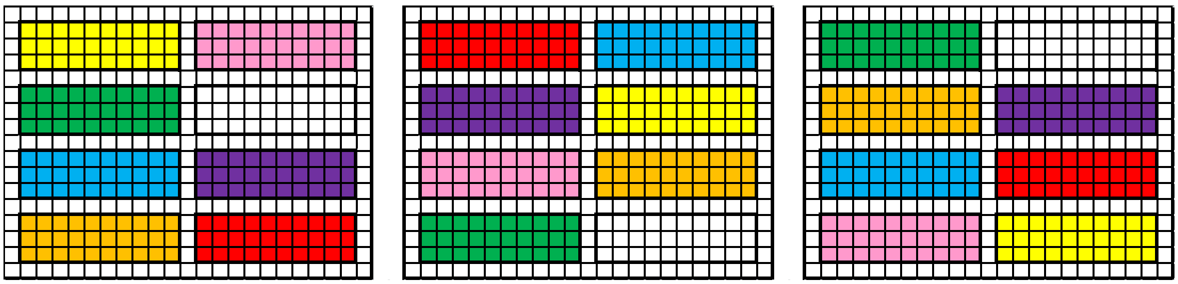
In February 2014, three first-year loblolly pine plantations were selected, two in Louisiana and one in North Carolina. In addition, three second-year pine plantations were selected in Arkansas.

Experimental Design/ Statistical Analysis

A nested block design will be utilized. The first blocking factor will be site (LA, AR, NC) and the second will be subplot (within site). There were up to seven treatments (number of treatments depends on number of tip moth generations at the site) . Only one insecticide (Mimic^{TM} 2LV) was used on all sites.

The seven treatments were randomly assigned to subplots at each site. The subplots will contain 30 trees each (example below).

1. Spray for generation 1 (red)
2. Spray for generation 2 (pink)
3. Spray for generation 3 (white)
4. Spray for generation 4 (purple)
5. Spray for generation 5 (yellow)
6. Spray all generations (orange)
7. Spray no generations (green)



Site 1. North Carolina **Site 2. Louisiana** **Site 3. Arkansas**

Spray timing depended upon the specific location of each site, but approximate timing of spray applications is shown in Table 1.

Table 1. Spray timing applications [after (Fettig et al., 2003)]

	Generation 1	Generation 2	Generation 3	Generation 4	Generation 5
Arkansas	April	June	July/August	September	
Louisiana	March/April	May/June	July	August/September	
North Carolina	April	June/July	August/Sept.		

Tip moth damage was evaluated on all seedlings located in each subplot after each tip moth generation (3-4 weeks after peak moth flight) by 1). identifying if the tree was infested or not, 2). if infested, the proportion of tips infested on the top whorl and terminal will be calculated; and 3). separately, the terminal will be identified as infested or not. Trees will be measured for height and diameter (at 15 cm or 6 in) at initial planting and in the fall of each year. All data will be analyzed using ANOVA. If significant differences are found, Student's T will be used to determine where the differences lie.

Results

The study was carried out in 2014 within three second-year pine plantations in Arkansas belonging to Plum Creek Timber Company and two one-year old pine plantations in Louisiana belonging to Weyerhaeuser. In addition, Weyerhaeuser personnel treated one additional one-year plantation in North Carolina. In Arkansas and Louisiana, pine tip moth exhibited four generations in 2014, while only three generations were observed in North Carolina. Thus, treatment 5 (spray generation five) was not applied in Arkansas or Louisiana and treatments 4 (spray generation four) and treatment 5 (spray generation five) were not applied in North Carolina. A portion of the seedlings within Arkansas and Louisiana study plots also suffered needle damage and perhaps growth loss from unanticipated applications of herbicides.

Arkansas Sites: Results for mean volume (cm^3) growth of three 2nd-year pine seedlings in the Arkansas study sites at the end of the 2014 growing season are listed in Table 2. Table 3 shows results of an analysis of variance among treatments.

Table 2. Mean volume (cm^3) growth by treatment for 6 treatments in Arkansas.

Treatment	**No.**	**Mean**	**Std Error**	**Lower 95%**	**Upper 95%**
Spray Gen 1	148	321.4	24.07	274.1	368.6
Spray Gen 2	149	143.7	23.99	96.6	190.8
Spray Gen 3	149	231.9	23.99	184.8	279.0
Spray Gen 4	149	182.3	23.99	135.2	229.4
Spray All G.	150	245.9	23.91	198.9	292.8
Check	150	169.7	23.91	122.7	216.6

Table 3. One-way ANOVA of mean volume growth connecting letters report for mean volume growth (cm^3) over 6 treatments in Arkansas. Levels not connected by the same letter are significantly different at $P < 0.05$.

Treatment		**Mean**
Spray Gen 1	A	321.4
Spray All G.	AB	245.9
Spray Gen 3	ABC	231.9
Spray Gen 4	BC	182.3
Check	BC	169.7
Spray Gen 2	C	143.7

In this study, spraying only generation 1 resulted in significantly greater seedling growth, compared to the check (no sprays). Also, there were no significant differences between the check and spraying only generation 2, generation 3 or generation 4, or all generations.

Louisiana Sites: For reasons that remain unclear, results for trials within two one-year pine plantations in Louisiana were not the same as those observed in Arkansas (Table 4). Although seedlings sprayed to control only generation four yielded the highest mean growth, there were no significant differences between all the spray treatments and the check, with the exception of spraying generation 1 (Table 5). In sharp contrast to the Arkansas results, spraying only generation one yielded the lowest seedling growth, but the mean was only significantly different from the mean growth observed when generation 4 only was sprayed.

Table 4. Means for one-way ANOVA of mean volume growth (cm3) for 6 treatments in Louisiana.

Treatment	No.	Mean	Std Error	Lower 95%	Upper 95%
Spray Gen 1	93	5.0	1.36	2.36	7.70
Spray Gen 2	95	9.8	1.34	7.17	12.45
Spray Gen 3	93	8.8	1.36	6.18	11.51
Spray Gen 4	99	10.5	1.32	7.87	13.04
Spray All G.	96	8.9	1.34	6.25	11.50
Check	95	6.9	1.34	4.26	9.54

Table 5. Connecting letters report for mean volume growth (cm^3) over 6 treatments in Louisiana. Levels not connected by the same letter are significantly different at $P < 0.05$.

Treatment		Mean
Spray Gen 4	A	10.5
Spray Gen 2	AB	9.8
Spray All Gen	AB	8.9
Spray Gen 3	AB	8.8
Check	AB	6.9
Spray Gen 1	B	5.0

North Carolina Sites: When the treatments were applied in North Carolina to a one-year old plantation, no significant differences were observed between the check and all spray treatments, presumably due to low tip moth pressure in 2014 on these sites (Tables 6 and 7).

Table 6. Means for one-way ANOVA of mean volume growth (cm^3) for 6 treatments in North Carolina.

Treatment	No.	Mean	Std Error	Lower 95%	Upper 95%
Spray Gen 1	55	8.7	2.02	4.70	12.67
Spray Gen 2	53	9.6	2.06	5.57	13.68
Spray Gen 3	45	10.9	2.24	6.50	15.30
Spray All G.	63	7.0	1.89	3.32	10.76
Check	53	8.6	2.06	4.56	12.67

Table 7. Connecting letters report for mean volume growth (cm^3) over 6 treatments in North Carolina. Levels not connected by the same letter are significantly different at $P < 0.05$.

Treatment		Mean
Spray Gen 3	A	10.9
Spray Gen 2	A	9.6
Spray Gen 1	A	8.7
Check	A	8.6
Spray All G.	A	7.0

Conclusions

Due to variable results and unanticipated effects from herbicide applications in most study sites, no management recommendations concerning a single-generation tip moth spray can be made from this study. Whether this study merits replication in the future is a decision to be made by the FPMC Executive Committee.

Literature Cited

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