Forest Pest Management Cooperative

Research Projects and Final Accomplishments in 2016-2017

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2016 FPMC Members

Forest Investment Associates
Hancock Forest Management, Inc.
Plum Creek Timber Company, Inc.
Weyerhaeuser Company
Anthony Forest Products
Arborgen, LLC
Arborjet, Inc.
International Forestry Company
International Society of Arboriculture – Texas Chapter
USFS, International Programs
US Forest Service/FHP
Texas A&M Forest Service

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2016 – 2017 ACCOMPLISHMENTS

FOREST PEST MANAGEMENT COOPERATIVE UPDATE AND FINAL REPORT

The Texas A&M Forest Service (TFS) initiated the Western Gulf Forest Pest Management Cooperative in March, 1996. The name was condensed to Forest Pest Management Cooperative (FPMC) in 2000. The FPMC reached a milestone in 2016, celebrating its 20-year anniversary. The FPMC has had three coordinators: Dr. Donald Grosman (1996-2012), Dr. Melissa Fisher (2013-2014), and Dr. Ronald Billings (2015 to 2017).

The first two coordinators were headquartered at the Texas A&M Forest Service (TFS) Forest Health laboratory in Lufkin. In February 2015, when Dr. Billings (headquartered in College Station) took over leadership of the FPMC, TFS Regional Forest Health Specialist L. Allen Smith (headquartered in Longview) was assigned duties as temporary Research Supervisor (10%), to oversee the activities of the FPMC staff in Lufkin. In CY 2016, the Lufkin staff consisted of Staff Forester William "Bill" Upton, Research Specialist Larry Spivey, and Staff Assistant Patricia Faries. Charles Jackson also participated as a seasonal worker from 2015 to 2017.

At the end of CY2016, the FPMC faced unsurmountable financial challenges. Due to this and a variety of other factors, the administrative decision was made in January, 2017, to discontinue the FPMC. The present document represents the final FPMC report and summarizes the latest findings of those research projects that were conducted in CY2016 and the first half of CY2017.

Despite a reduced field staff, the FPMC wrapped up five-year growth studies on pine tip moth and continued treatment evaluations for oak wilt, leafcutting ants and southern pine beetle in 2016. Research studies continued with evaluations of emamectin benzoate (TREE-äge) treatments for SPB in Alabama, including new studies to evaluate the duration of emamectin benzoate injections and effectiveness of winter injections for SPB prevention and control. Also, the duration of a commercially-available fungicide (BotaniGard™) for southern pine beetle prevention and control, a study to improve pheromone baits for SPB prediction, evaluations of attractiveness and control efficacy of various commercially available fire ant baits against Texas leafcutting ants, and a new study to evaluate macro- and micro-infusion systems for oak wilt prevention, in conjunction with Dr. David Appel, Texas A&M University, were conducted in 2016.

At the end of CY 2015, three full members – The Campbell Group (member since 2007), Forest Investment Associates (member since 2003), and Rayonier (member since 2008) decided to drop their membership in the FPMC. On the positive side, one new full member (US Forest Service/International Programs) and the Coop's first supporting member (International Society of Arboriculture-Texas Chapter) joined at the beginning of CY 2016. Also, Plum Creek Timber Company was merged with Weyerhaeuser late in 2015, but the decision was made for both companies to pay their 2016 membership dues and maintain their members on the FPMC Executive and Contact teams. In 2016, full members consisted of Plum Creek Timber Company, Hancock Forest Management, Texas A&M Forest Service, USFS/Forest Health Protection, USFS/International Programs, and Weyerhaeuser. Associate members were Anthony Forest Products, Arborgen, Arborjet, and International Forest Company. The International Society of Arboriculture, Texas Chapter was a supporting member.

Other activities of the FPMC for the 18-month period January 1, 2016 to May 31, 2017 include the following:

- Six issues of the quarterly FPMC newsletter *PEST* (*Progress, Education, Science, Technology*) were prepared and distributed to members as a means to keep them abreast of FPMC projects and accomplishments, as well as other forest pest-related topics of interest.
- The annual southern pine beetle prediction survey with pheromone-baited traps was conducted in 19 East Texas counties; results of South-wide SPB prediction surveys carried out by Federal and State cooperators were compiled and displayed on the TFS Forest Health web page.
- At the invitation of full member U. S. Forest Service/International Programs, FPMC Coordinator Ron Billings provided two weeks of technical assistance to Honduras in June and September, 2016 and made recommendations to the Honduran Forest Service to address the worst southern pine beetle outbreak in 50 years.
- A survey of FPMC Executive and Contact team members was conducted to rank various research topics for 2016.
- A large three-panel poster describing the FPMC was made for display at the Texas Tree Conference, and future forestry venues.
- Articles on emerald ash borer and black twig borer were prepared and published in Texas Forestry Association's newsletter *Texas Forestry*.
- Allen Smith attended the North American Forest Insect Work Conference held in Washington, D. C. from May 31 to June 3, 2016, and presented a poster describing the Forest Pest Management Cooperative's first twenty years.
- The FPMC webpage was resurrected using the TFS server and is available at https://fpmc.tamu.edu/.
- FPMC staff members gave presentations on FPMC research projects at the East Texas Forest Entomology Seminar in 2016.
- In 2015, the FPMC conducted a survey of urban foresters and arborists in Texas a means to identify the major forest health problems facing urban trees and forests within Texas and those issues in need of applied research. A poster discussing results of this survey was prepared to be displayed at the 2016 International Society of Arboriculture convention in Fort Worth in August, 2016.
- Research proposals in 2016 were prepared and submitted to capture outside funding for the FPMC. Four of the proposals were funded in 2016 for a total of ca. $130,000.

The 2016 meeting of the FPMC Executive Team was held on August 31-September 1 at the TFS headquarters in College Station. The decision was made by those in attendance at this meeting to increase FPMC annual dues for 2017 to $13,000 for full members and $4,500 for associate and supporting members. The FPMC dues have remained unchanged since 2009 at $10,000 per year for full members and $3,500 per year for both associate and supporting members. In preparation for this meeting, a summary of research projects and accomplishments for the period January 1, 2015 to December 31, 2015 were presented. Also, new research projects underway in CY 2016 and preliminary results were described.

Executive Summary of Research Results in 2016-2017

An executive summary of major findings of FPMC research projects for 2015-2016 is presented below:

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

- The fire ant bait Siesta™ was evaluated using standardized protocols to determine preference and efficacy against leafcutting ants. Neither the commercially-available fire ant bait nor one consisting of a larger pellet proved efficacious in field tests for control of leafcutting ant colonies.
- Preference and efficacy tests of different formulations of Amdro™ fire ant insecticide and Extinguish Plus™ fire ant bait were tested on colonies of Texas leafcutting ants in the fall and winter of 2016-2017. A preference for large pellets of Amdro™Ant Block compared to other formulations of the commercial fire ant bait proved significant.

Pine Tip Moth Trials

- Two Nantucket pine tip moth field trials were monitored for growth of treated and untreated seedlings at the end of the 2016 growing season following various chemical treatments and dosage rates.
- After 5 growing seasons, increases in diameter, height or volume growth of nursery plug injection treatments and soil treatments with PTM™ and/or Insignia™ applied to containerized and bare-root seedlings were largely insignificant.
- A new electronic insect trap, manufactured by Spensa, Inc., showed potential for monitoring Nantucket pine tip moth populations, particularly in a 2-year old loblolly pine plantation. When tip moth populations were high, trap catch numbers in the new Z-traps were comparable to those found in adjacent sticky traps and the data were recorded remotely and fairly accurately on a designated cell phone.

Incorporating Emamectin Benzoate (EB) into a Control Strategy for SPB

- A series of studies conducted in Alabama and Mississippi since 2012 have tested the effectiveness of emamectin benzoate (TREE-äge™) for controlling southern pine beetle (SPB).
- At a rate of 5 ml/in DBH, EB is effective for preventing SPB brood development in attacked trees, but most trees eventually die, presumably from blue-stain infection.
- Except when SPB populations are at high levels, loblolly pines can be injected and baited the same day to induce attacks with similar results.
- Rates of 1.25ml/in and 2.50 ml/inch also prevented SPB brood development in most trials.
- Tree injected with EB in winter at 2.5 ml/inch and 5.0 ml/in and baited with pheromones 4 weeks later served as effective trap trees. Most trees eventually died from blue stain infection but produced little or no brood.

- Trees injected with EB at rates of 2.5ml/in or 5.0 ml/inch in November 2014 and baited 18 months later (April 2016) eventually died presumably from blue stain infection but failed to produce SPB egg galleries or brood.

Ev**aluation of BotaniGard (*Baeuveria bassiana*) for Control of Southern Pine Beetle**

- Exposure tests were conducted in East Texas to evaluate the longevity of spores of the fungus *Baeuveria bassiana* as a potential control method for southern pine beetle. Also, in Alabama, a few standing trees were treated with the same fungus and baited to induce SPB attacks. Results were largely negative for both tests.

Improving the Prediction System for the Southern Pine Beetle

- Field trials on 8 different sites in Louisiana, Mississippi and Alabama in the spring of 2016 of 6 different SPB pheromone baits showed that the combination of frontalin, *endo*-brevicomin and Caribbean pine turpentine deployed from a polyethylene bag was by far the most attractive lure, catching ca. 60% of all SPB in traps. The second most attractive bait combination was fontalin + Sirex lure + *endo*-brevicomin.
- The least attractive lure was frontalin and Sirex bait, used since 2007 as the standard lure used in pheromone traps for predicting SPB outbreaks.
- The bioassays of 5 different lure combinations were evaluated for 10 consecutive weeks in the fall of 2016 at 8 sites, comprised of the same 6 sites used in the spring plus 2 additional sites in North Carolina.
- Results were similar to those of the spring trials. The combination of frontalin + Caribbean turpentine + *endo*-brevicomin was significantly more attractive than the other bait combinations, while the frontalin + Sirex lure + *endo*-brevicomin was less attractive, but caught more SPB than the other treatments. Catches of SPB in traps baited with frontalin + Sirex lure (standard) in fall surveys were low, similar to those utilizing frontalin + Caribbean turpentine deployed from a bottle or polyethylene bag without *endo*-brevicomin.
- The SPB prediction chart was modified, based on results of the spring study and future surveys are likely to utilize the combination of frontalin + Sirex lure + *endo*-brevicomin (displace 4 m from the trap) as the optimal lure.

2016-2017 ACCOMPLISHMENTS

Evaluation of BASF Bait Formulations of Siesta™ for Attraction and Control of the Texas Leafcutting Ant

Initiated and Completed in 2016

Cooperator: BASF

Background

Siesta™ Insecticide Fire Ant Bait, with the active ingredient metaflumizone, delivers fast and long lasting control of native and imported fire ants. Metaflumizone is formulated on corn grit, along with soybean oil, a proven attractant bait for native and imported fire ants. Siesta Insecticide Fire Ant Bait is the only sodium blocker insecticide (SCBI) that does not require metabolism for bioactivation. The specific site of the insecticidal action is not currently known, but it does act on the insect's nervous system, where it blocks the voltage-dependent sodium neuron channel. As a result, these neurons are inactivated, causing the ant to enter a state described by researchers as "relaxed paralysis." The direct effects are that Siesta Insecticide Fire Ant Bait causes the cessation of feeding, increasing levels of immobility, and ultimately ant death. BASF provided a quantity of Siesta™ to be tested in preference and efficacy trails in 2016 as a potential method to control Texas leafcutting ants (TLCA) (*Atta texana*).

Objectives: 1) To determine the attractiveness of the Texas leaf-cutting ant to Siesta™ baits.
2) To determine the efficacy of Siesta™ baits for control of Texas leaf-cutting ants.

Methods

Two types of bait were tested in preference and efficacy trials: 1) the commercial Siesta™ fire ant bait and 2) Siesta bait passed throught the FPMC pelletizer to make a larger pellet, known to be preferred by TLCA (Grosman et al. 2002).

Preference Trial

Trials were conducted near Jasper and Colmesneil in East Texas in February, 2016, by placing 5 g portions of different baits (Siesta commercial fire ant bait and Siesta bait modified into larger-sized pellets) into Petri dishes. Each treatment was replicated ten times per trial period. For each trial replicate, one dish of each treatment was distributed at random within the central nest area (but near areas of high activity) or along foraging trails. All dishes within each replicate were retrieved when the dish, containing the most attractive bait, was 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 was noted and means calculated for each treatment. Petri dishes with each of the baits also were placed near imported fire ant mounds to test for differences in preference, based on pellet size.

Efficacy Trial

Experiments were conducted in east Texas; within 100 miles of Lufkin. In this area, 40 Texas leaf-cutting ant colonies were 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 were excluded from this study. Treatments were 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: 12 oz/m^2 of Siesta fire ant bait
2) Treatment 2: 12 oz/m^2 of Siesta fire ant bait in large-sized pellets
3) Treatment 3: 8 – 12 oz/m^2 of Amdro™ Ant Block
4) Treatment 4: untreated colonies

Procedures used to evaluate the effect of treatments on Texas leaf-cutting ant colonies followed those described by Cameron (1990). The number of active entrance/exit mounds was 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 Texas leafcutting ants removed 2.7 times more Siesta large-pellet baits (mean = 1.67 g.) from Petri dishes on average compared to the commercial Siesta fire ant bait (mean = 0.62) bait (mean = 0.80g) (Table 1). However, differences were not significant ($P>0.05$). Four of ten large-pellet bait dishes were taken over by fire ants, which discourage further removal of pellets by leafcutting ants. In preference tests with fire ants, there were no significant differences in weight of baits removed between treatments (mean = 0.57 gm of fire ant bait removed verses 0.38 gm of pelletized baits).

Efficacy Trial

Niether of the Siesta treatments reduced the number of active leafcutting ant mounds significantly, compared to the check after 8 week (Figure 1). At the end of week 8, only the Amdro AntBlock significantly affected ant survival, reducing mean town ant activity by 95%. This reduction was significantly greater than that of the check and the two Siesta baits.

Overall, results of this field trial were disappointing with regard to Siesta. Ant activity was reduced by 40% after 16 weeks by the Siesta pelletized bait and only 31% by the unpelletized, standard Siesta bait. Amdro AntBlock spread across active colonies and PTM injected into the feeder holes remain the best commercially available options for Texas leafcutting ant control.

Table 1: Results of preference tests of Siesta commercial fire ant bait and Siesta bait offered as large pellets to Texas leafcutting ants, East Texas February, 2016.

Trt	Rep	Initial Wt (g)	Post Wt (g)	Diff. Wt (g)	Notes
A Siesta standard	1	5.00	3.49	1.51	
A for TLCA	2	5.00	3.67	1.33	
A	3	5.00	4.68	0.32	
A	4	5.00	4.64	0.36	
A	5	5.00	4.56	0.44	
A	6	5.00	4.82	0.18	
A	7	5.00	4.41	0.59	
A	8	5.00	4.56	0.44	
A	9	5.00	4.64	0.36	
A	10	5.00	4.30	0.70	
	Avg		**4.38**	**0.62**	

Trt	Rep	Initial Wt (g)	Post Wt (g)	Diff. Wt (g)	Notes
B Siesta Pellets	1	5.00	1.94	3.06	
B for TLCA	2	5.00	2.79	2.21	
B	3	5.00	3.08	1.92	
B	4	5.00	4.76	0.24	fire ants
B	5	5.00	4.77	0.23	fire ants
B	6	5.00	3.01	1.99	
B	7	5.00	3.32	1.68	fire ants
B	8	5.00	4.49	0.51	fire ants
B	9	5.00	2.57	2.43	
B	10	5.00	2.60	2.40	
	Avg		**3.33**	**1.67**	

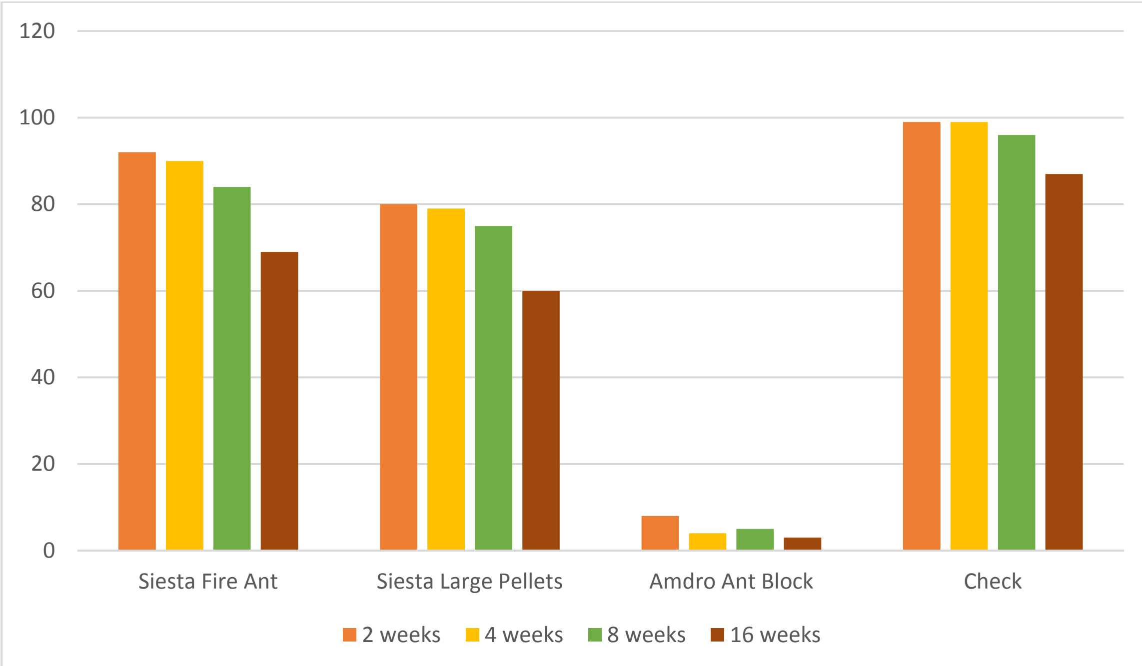

Figure 1: Proportion of active colonies following treatment with Siesta™ commercial fire ant bait and Siesta bait offered as large pellets compared to Amdro Ant Block™ and untreated colonies, East Texas, 2016.

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Evaluation of Amdro™ Formulations and Extinguish Plus™ for Control of Texas Leafcutting Ants

Additional field trials were conducted in the winter of 2017 to evaluate two formulations of Amdro™ fire ant bait and Extinguish Plus™fire ant bait for control of Texas leafcutting ant colonies. Extinguish Plus combines the insecticide hydramethylnon with an insect growth regulator (IGR) to kill fire ant workers and prevent queens from laying fertile eggs. The result is complete fire ant colony control. Whether it would control Texas leafcutting ant colonies remained to be determined.

Objective:

Compare the preference for and effectiveness of Amdro™Ant Block and modified Amdro™, containing the insecticide hydramethylnon, with another commercially available fire ant bait, Extinguish Plus™, for control of colonies of the Texas leafcutting ant.

Methods:

Preference tests were conducted by offering petri dishes filled with 5 gm of each of the three baits within active leafcutting ant colonies and allowing the foraging ants to retrieve the baits. The petri dishes were retrieved after 3 hours and weighed to determine amount of bait the ants carried off. The tests were replicated 5 times at two different sites (in Tyler and Nacogdoches counties, TX) on November 15 and 16, 2016.

Thirty active colonies of Texas leafcutting ants in East Texas were treated on November 16, 2016 – January 3, 2017, with Amdro™ Ant Block, modified Amdro™ and Extinguish Plus™ (10 replicates per treatment) and monitored at intervals of 2, 4, 8, and 16 consecutive weeks. Treatment effectiveness was determined by comparing initial number of active mounds per colony with final number of active mounds. Ten untreated check colonies also were included in the study. The Extinguish Plus bait and the Modified Amdro (a pelletized experimental bait derived from Amdro fire ant bait) were provided by Doug Van Gundy of Central Garden and Pet Company.

Results:

The amounts of the three baits retrieved in 3 hours by leafcutting ants in two different locations is shown in Table 1. The ants showed a slight preference for the modified Amdo bait while the Extinguish Plus bait proved the least attractive.

Table 1: Amounts of bait granules removed in 3 hours of foraging by Texas leafcutting ants in Tyler and Nacogdoches counties.

Treatment	Initial weight (gm)	Final weight (gm)	Difference (gm)
Amdro Ant Block	5	4.13	0.87
Modified Amdro	~~5~~	4.06	0.94
Extinguish Plus	5	4.29	0.71

Results of the efficacy tests are shown in Table 2: The Modified Amdro treatment was associated with the greatest reduction in ant activity, while the two commercially available baits (Amdro Ant Block and Extinguish Plus controlled substantially fewer of the colonies treated. The Modified Amdro had reduced ant activity by 100% on 5 of 10 colonies after 16 weeks, compared to 2 of 10 and 3 of 10 for the Amdro Ant Block and Extinguish Plus treatments. As shown in previous FPMC trials, Texas leafcutting ants are more likely to retrieve and be controlled by a pellet that is larger in size than is commercially available at this time. All of the 10 untreated, check colonies either remained unchanged or increased in number of active mounds throughout the 16-week test period.

Table 2; Percent reduction in active leafcutting ant mounds active following treatment with 3 different insecticide baits during winter 2016, Tyler and Nacogdoches, counties, TX.

Treatment	2 wks	4 wks	8 wks	16 wks
Amdro Ant Block	70.9%	70.1%	65.2%	66.3%
Modified Amdro	87.4%	92.1%	90.5%	85.0%
Extinguish Plus	31.7%	45.4%	50.5%	61.2%

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

Initiated in 2012; Growth monitored through 2016

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). Similarly, at the end of the 2016 growing season, differences in seedling growth among all treatments remained insignificant at $P>0.05$ (Tables 8-9).

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.

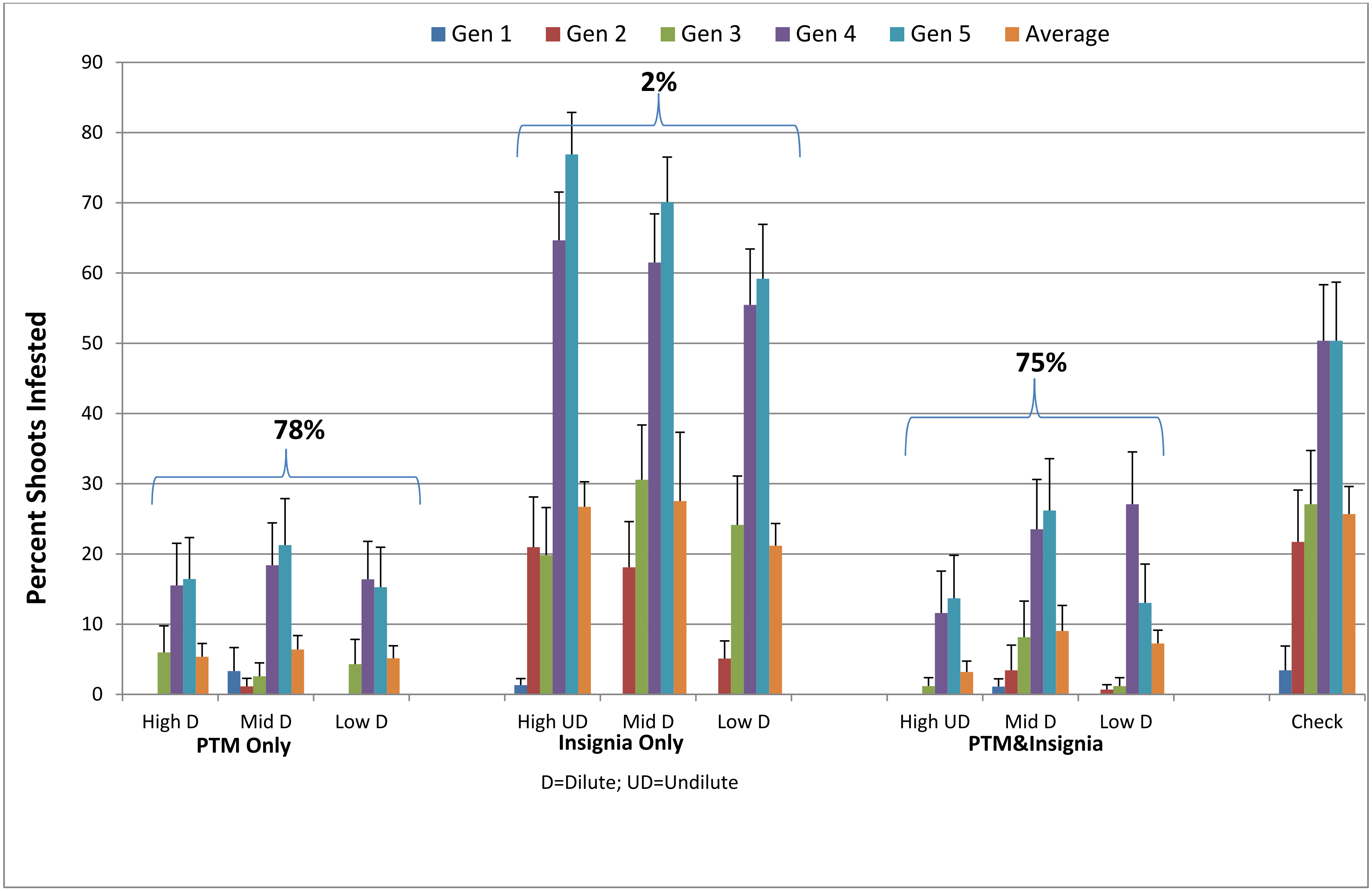

Figure 1. Effect of PTM™ and/or Insignia®SC soil injection dose on tip moth infestation of bareroot loblolly pine at one site in East Texas, 2012.

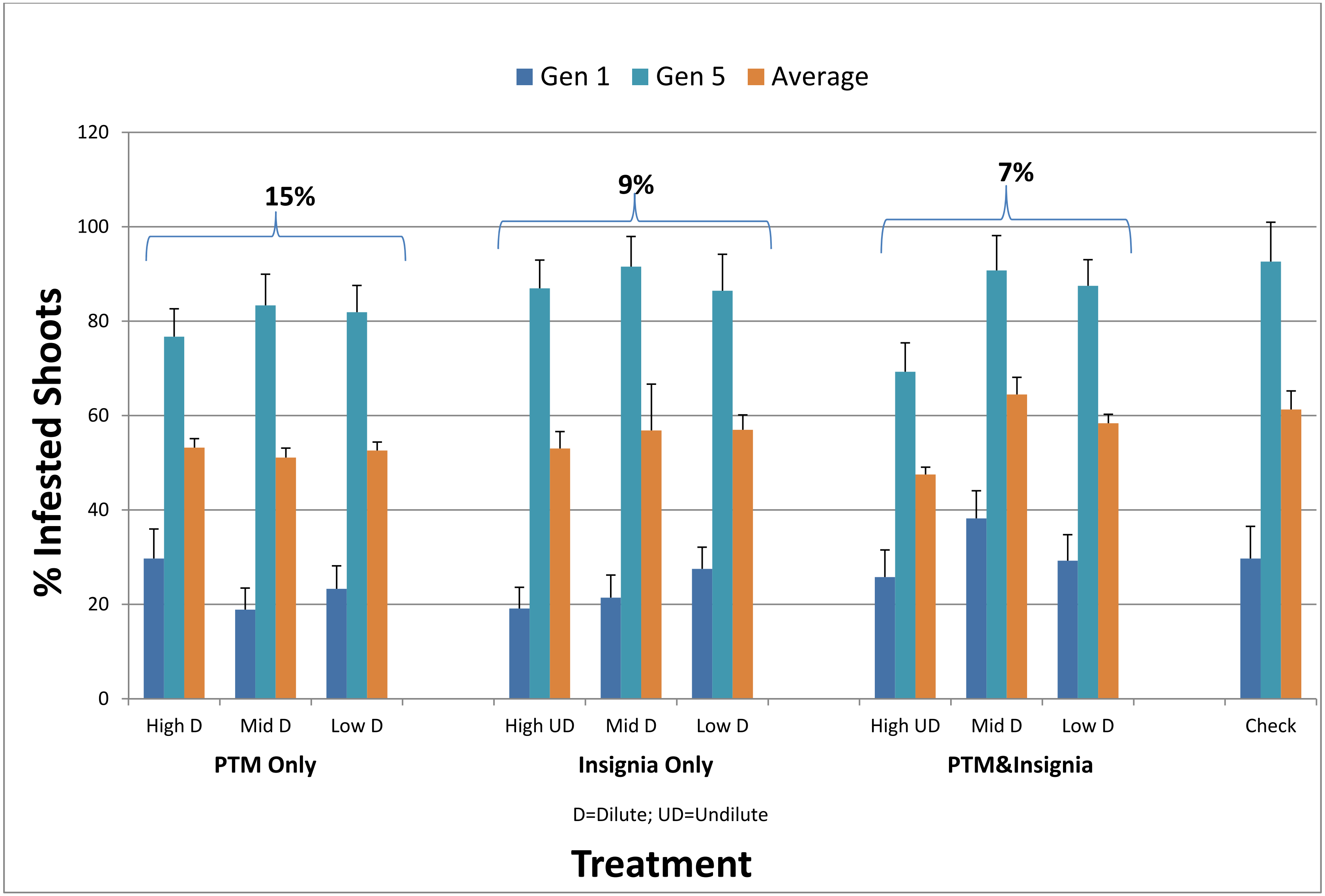

Figure 2. Effect of PTM™ and/or Insignia®SC soil injection dose on tip moth infestation of bareroot loblolly pine at one site in East Texas, 2013

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 (cm³)		
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.

Year	Treatment	Conc.	Dilute or Undilute	N	Height (cm)			Diameter (cm)[a]		Volume (cm^3)	
					Mean Growth 2013 (**Growth Difference (cm or cm3) Compared to Check)**						
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 201 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™ 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 p tip moth control on bareroot seedlings in East Texas using PTM™ and Insignia™ at e of 2014 growing season. Means followed by the same letter are not significantly diffe (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

Table 8: Analysis of variance for DBH Volume for bareroot seedlings in East Texas using PTM and Insignia™ at end of 2016 growing season.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Color	9	5302965788	589218421	0.9357	0.4947
Error	240	1.5114e+11	629741664		
C. Total	249	1.5644e+11			

Likewise when seedling growth from 2014 to 2016 is considered, there are no significant differences among treatments in seedling growth after 5 years (Table 9

Table 9: Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Color	9	3279152061	364350229	0.7243	0.6865
Error	239	1.2023e+11	503070972		
C. Total	248	1.2351e+11			

Table 10. Mean Height (cm), Diameter at Breast Height (DBH) (cm) and Volume (cm3) of 5 year old pine seedlings evaluated over 10 treatments in Etoile, Texas. Trees were planted in 2012. This evaluation occurred after 5 growing seasons.

Treatment	Mean Ht. (cm)	Mean DBH (cm)	Mean Volume (cm^3)
9 Green	629.08	9.95	65192.28
1 Red	628.19	9.96	67324.93
3 Orange	626.82	9.94	65753.02
2 Blue	626.12	9.92	64740.47
6 Red/Wh	620.87	9.30	55642.14
7 Red/Yel	614.60	9.27	57020.45
8 Yellow	614.36	9.77	62090.56
5 White	611.40	9.32	55654.58
4 Pink/Blue	606.88	9.68	59972.58
10 Pink	597.71	9.11	54606.48

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

Initiated in 2012; 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. The following study was conducted to evaluate efficacy of the plug injection system to treat containerized loblolly pine seedlings using the plug injection system and two insecticides (PTM™ and Insignia® SC).

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) alonecombined 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™: 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™ + 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™: 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™: (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™ and/or Insignia®SC at different rates based on the restricted rate of 59g AI/acre/year (PTM™) 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.

Treatment sites in East Texas were measured for the final time in December 2016. Only the growth in seedlings treated with the low PTM bare root soil injection was significantly greater than growth of seedlings treated with either the mid PTM container plug injection or the high rate of Insignia soil injection treatments (Tables 26-27).

Acknowledgments:

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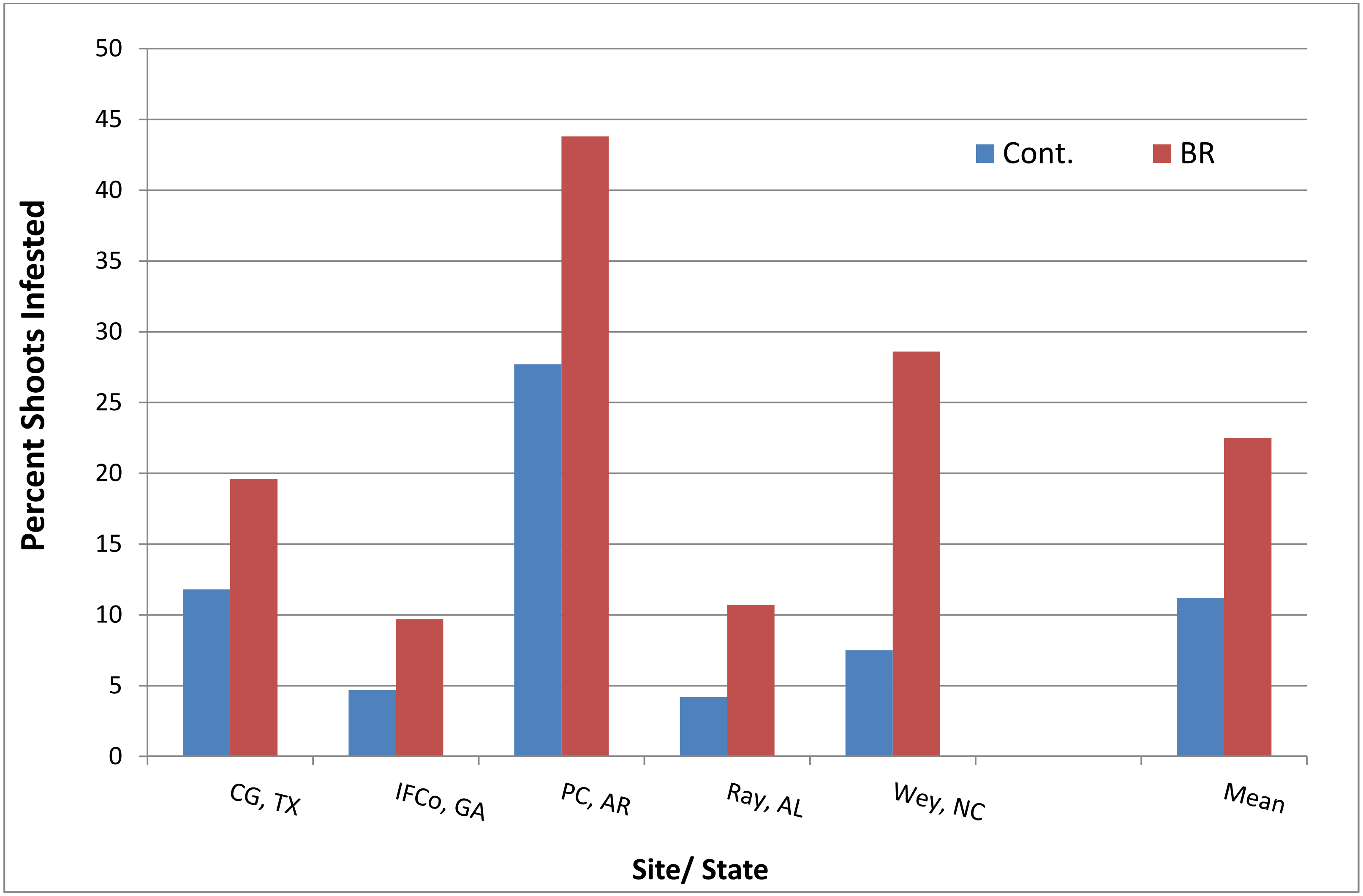


Figure 1. Mean tip moth infestation levels on first year containerized and bareroot loblolly pine on five sites across the southeastern U.S., 2012.

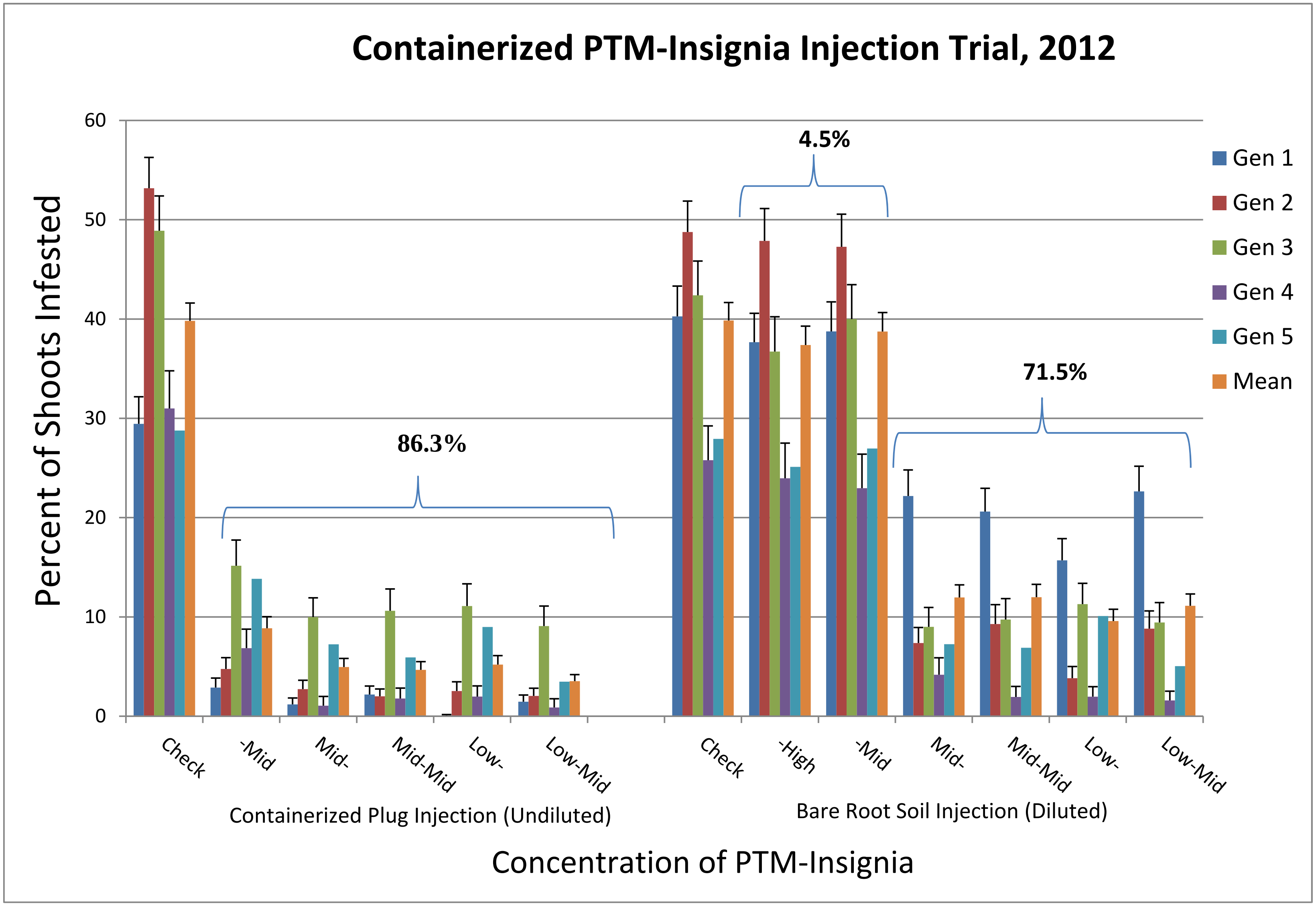


Figure 2. Effect of PTM and/or Insignia SC dose and technique on pine tip moth infestation of containerized or bareroot loblolly pine on five sites across the southeastern United States, 2012.

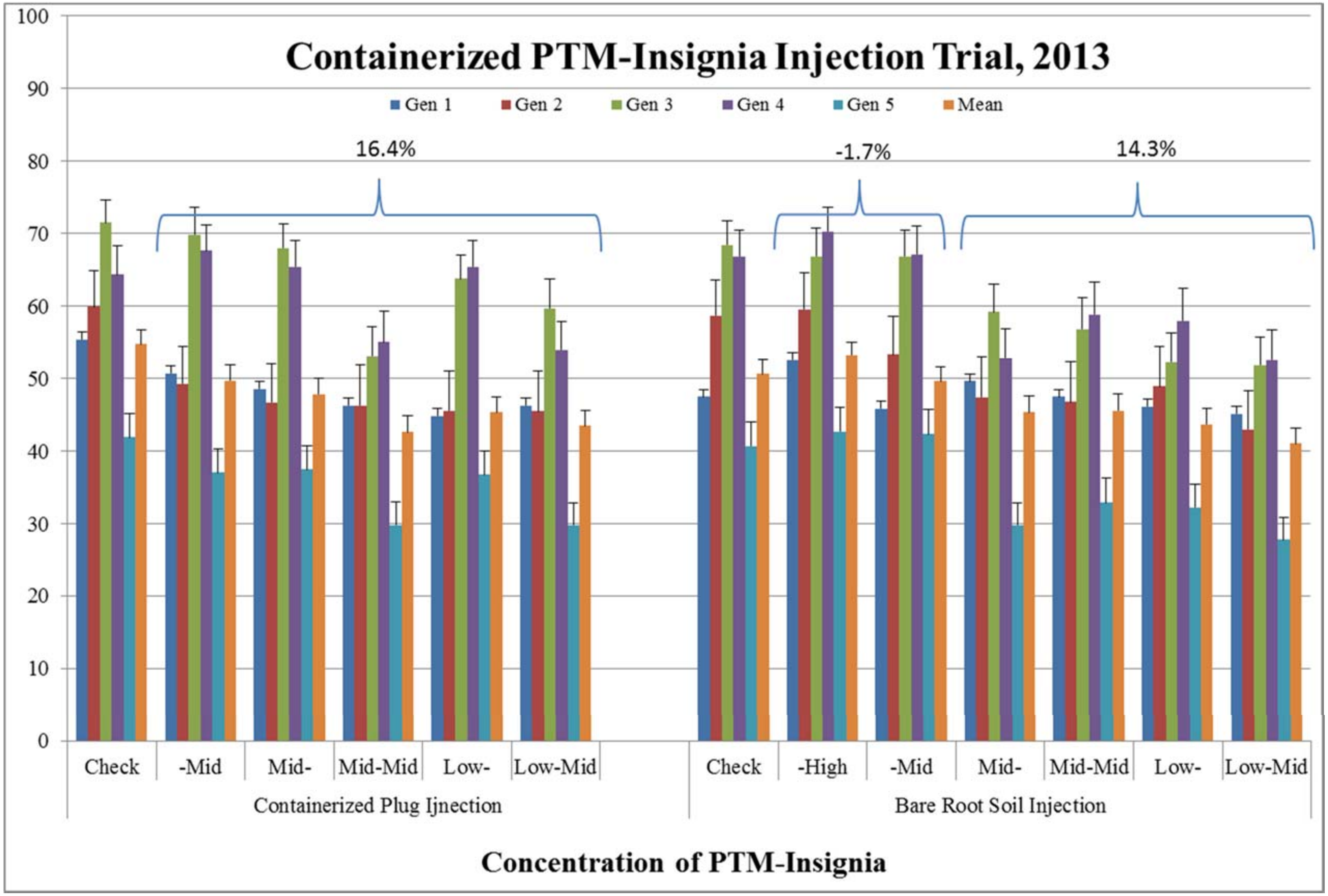


Figure 3. Effect of PTM and/or Insignia SC dose and technique on pine tip moth infestation of containerized or bareroot loblolly pine on five sites across the southeastern United States, 2013.

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 2

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)		Diameter (cm) [a]		Volume (cm^3)		Tree Survival (**Percent Improvement Compared to Check**)	
							Mean End of Season Loblolly Pine Seeding Growth Measurements (**Growth Difference (cm or cm3) 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	

* 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 One way 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 CY2014. Connecting 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 26: Seedling growth at end of CY2016. Results of one-way ANOVA looking at seedling growth based on diameter at breast height by treatment in East Texas.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Color 2	12	1.808e+10	1.5067e+9	2.0410	0.0194*
Error	494	3.6467e+11	738197372		
C. Total	506	3.8275e+11			

Table 27: Seedling growth at end of CY2016. Connecting letters report for seedling growth based on diameter at breast height over 13 treatments in East Texas. Levels not connected by the same letter are significantly different (P<0.05).

Level			Mean
Pink	A		65009.344
Blue&White	A	B	58341.992
Green	A	B	57575.039
Yellow	A	B	54147.331
White	A	B	52582.750
Orange	A	B	51116.496
Red	A	B	51076.481

Level			Mean
Pink&Blue	A	B	50833.995
Blue&Red	A	B	50466.524
Yel&Red	A	B	47483.856
Green&Org	A	B	45765.787
Blue		B	43214.544
Red&White		B	43144.935

Levels not connected by same letter are significantly different.

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

Field Comparison of Two Different Pheromone Traps for Monitoring Pine Tip Moth

Justification: Spensa Technologies (www.spensatech.com) has developed an electronic trap for monitoring pest insects. The Z-Trap (Fig. 1) uses pheromones to attract target pests, which are counted automatically as they enter the trap and subsequently killed by an electronic current produced by "zapper" rods within the trap. The insect counts are transferred remotely and periodically to a cell phone.

Fig. 1: Research Specialist Larry Spivey checks a Spensa Z-trap in Tyler County.

Collaborator: Shannon Pickering, Spensa Technologies, Inc., West Lafayette, IN

Spensa Technologies provided the FPMC with six Z-traps for testing with Nantucket pine tip moth in East Texas. The traps were set out in six young (1-2 year old) pine plantations in Tyler County. Each Z-trap was tested against a standard pheromone-baited sticky trap, baited with the same tip moth pheromone and situated 50-100 feet away. All traps were set in the field on February 16, 2017 and monitored weekly for 6 weeks. Results are shown in Table 1 below.

Table 1: Weekly catches of Nantucket pine tip moth in standard sticky pheromone traps versus Spensa Z-traps, each located 2 feet above ground level in young pine plantations; Tyler Co, TX
.

		Standard Pheromone Trap						Spensa Z-trap					
Date	baited 2/16	1	2	3	4	5	6	1	2	3	4	5	6
2/23/2017		13	7	19	41	5	0	1	0	0	19	1	0
3/2/2017		8	2	56	52	3	6	2	0	5	32	0	0
3/8/2017		3	2	6	12	1	3	3	0	6	32	0	1
3/15/2017		3	0	8	12	0	0	0	0	1	13	0	2
3/22/2017		11	1	8	18	2	8	0	0	3	17	0	0
3/1/2917		22	5	7	17	1	6	0	1	4	20	1	0
		60	17	104	152	12	23	6	1	19	133	2	3

Results show that the Z-trap caught fewer tip moths, compared to the adjacent standard sticky trap. Only Z-trap number 4 caught comparable numbers of tip moths. This was a two-year old loblolly pine plantation. Because of these discouraging results, further testing of the Z-trap for tip moth monitoring is not planned.

Evaluating the Effectiveness of Winter Injections of Emamectin Benzoate for Control of the Southern Pine Beetle

Initiated December 2015; completed in 2016

Funding: $10,284 (Grant from Syngenta, Inc.)

Justification: The southern pine beetle (SPB) (Coleoptera: Curculionidae, Scolytinae) is considered the most destructive insect pest of southern pine forests. Since 1997, no SPB infestations have been detected in Western Gulf states (TX, AR, LA & OK) and very few SPB have been caught in pheromone traps in East Texas since 2001 (11 SPB). Pheromone traps deployed during the spring have proven effective for predicting SPB population increases since 1988 across the South (Billings and Upton 2010). SPB populations in 2012 -2015 were at unprecedented low population levels throughout the South and Northeast, with the exception of southern New Jersey, the Hommochitto and Bienville National Forest and surrounding private lands in Mississippi, and local areas in Alabama and Virginia. A method for effectively dealing with SPB outbreaks in early stages of development is needed. Much is known about SPB biology and seasonal habits (see Coulson and Klepzig 2011). Most new SPB infestations are initiated following long-distance dispersal in the spring (March-May) and to a lesser extent in the fall (October-December). SPB adults, however, may emerge from brood trees, fly, and attack additional trees throughout the winter, whenever ambient temperatures exceed the flight threshold of ca. 59 degrees F.

A new systemic insecticide (emamectin benzoate) has been developed by the Texas A&M Forest Service (TFS) Forest Pest Management Cooperative (FPMC) and is sold by Syngenta under the trade name Tree-äge™. This insecticide is effective against SPB (Grosman et al 2009, 2010) and has been registered and is now available for pine bark beetle control in forest situations. This is the only insecticide registered for control of SPB in forests. Allee effects (positive density dependence) have been shown to play an important role in the establishment and spread of invasive species. A certain population density is essential before an invasive species can become established and spread in a new environment (and because of Allee effects, many new introductions of invasive plants and animals fail to succeed). Increased interest in recent years is being focused on the potential to exploit Allee effects as a means to manage invasions of exotic species (Tobin et al. 2011).

Field studies conducted by the FPMC from 2012-2015 in Alabama, Virginia and Mississippi have documented the following:

- Loblolly pines injected with 1.25 - 5.0 ml/diameter inch of emamectin benzoate (TREE-äge) are effective as trap trees for absorbing attacking SPB during summer and fall months when SPB occur at low population levels (<2.0 SPB/trap/day).
- Attacked trees containing emamectin benzoate accumulate attack densities comparable to un-injected pines, but no SPB galleries are constructed and no broods emerge from treated trees.
- Pines that are injected and baited simultaneously also are successful trap trees, but only if initial attacks are delayed or occur over a prolonged period (allowing uptake of the insecticide).

Objectives:

- Determine the effectiveness of isolated trap trees injected with emamectin benzoate and baited with SPB pheromones during winter months (December through February).
- Evaluate three dosage levels of emamectin benzoate for effectiveness in a trap-tree tactic applied during winter months.

Cooperators:

Ms. Cindy Ragland	Oakmulgee R.D, Talladega N.F., Brent, AL
Mr. David Cox	Syngenta, Inc., Madera, CA

Study Sites: The study is to be conducted in the Talladega National Forest, Oakmulgee Ranger District in Bibbs and Perry Co., Alabama with SPB attacking loblolly pine, *Pinus taeda*. Isolated loblolly pines (8-15 inches DBH) will be selected for treatments.

Insecticides:

Emamectin benzoate (TREE-äge™, Arborjet Inc.) – an avermectin derivative

Treatments (Winter 2015-2016):

- Loblolly pine tree isolated from other pines by > 30 feet, injected with 1.25 ml/diameter inch of emamectin benzoate in December and baited four weeks after injection (10 trees).
- Loblolly pine tree isolated from other pines by > 30 feet, injected with 2.50 ml/diameter inch of emamectin benzoate in December and baited four weeks after injection (10 trees).
- Loblolly pine tree isolated from other pines by > 30 feet, injected with 5.0 ml/diameter inch of emamectin benzoate in December and baited four weeks after injection (10 trees).
- Baited and uninjected check tree (10 trees).

Treatment Methods and Evaluation:

Two sets of Lindgren funnel traps baited with frontalin + Sirex lure + *endo*-brevicomin (displaced by 4 m) and frontalin + Sirex lure will be deployed in the area 300 m away from injection plots, to monitor local southern pine beetle populations.

Note: Where possible, poor quality (form, health, etc.) trees were selected as trap trees.

TREE-äge™ will be injected at 1.25, 2.50 or 5.0 ml per inch DBH. The Tree IV™ microinfusion system (Arborjet, Inc. Woburn, MA) will be used to inject TREE-äge™ into 4 (for trees <12" DBH) or 8 (for trees ≥12" DBH) points 0.3 m above the ground. The injected trees will be allowed 4 weeks to translocate chemicals prior to being challenged by the application of synthetic pheromone baits.

Treatment evaluation:

- Treated trees will be revisited at intervals of 4, 8, 12 and 24 weeks after baiting to monitor attack level (occurrence of pitch tubes).
- During the winter and spring, 2016, each study tree will be monitored periodically to determine the approximate date of mass attack, based on presence of more than 100 pitch tubes along the bole.

- All dead study trees will be felled when they begin to fade. Bark plates (10 X 10 cm = 100 cm2) will be collected at approximately 1.5, 4.0 and 6.5 m height at northern and southern aspects. SPB gallery length, density of emergence holes, and presence of cerambycid galleries and percent of surface area covered with blue stain will be recorded.
- Ambient temperatures will be monitored at the closest weather station (Tuscaloosa, AL) to determine number of days favorable for SPB flight throughout the winter.

Expected outcome: SPB activity, generation times, long-range dispersal and intensity of attacks are known to be seasonally dependent (Coulson and Klepzig 2011). Field studies conducted by the FPMC to date have been conducted in the summer and fall months. This study will provide insight into the utility of trap trees containing emamectin benzoate for application in winter months, when SPB flight is more sporatic and duration of SPB attack occurs over prolonged periods and at lower levels. The optimal dosage level of emamectin benzoate for use to create trap trees during winter months will be determined.

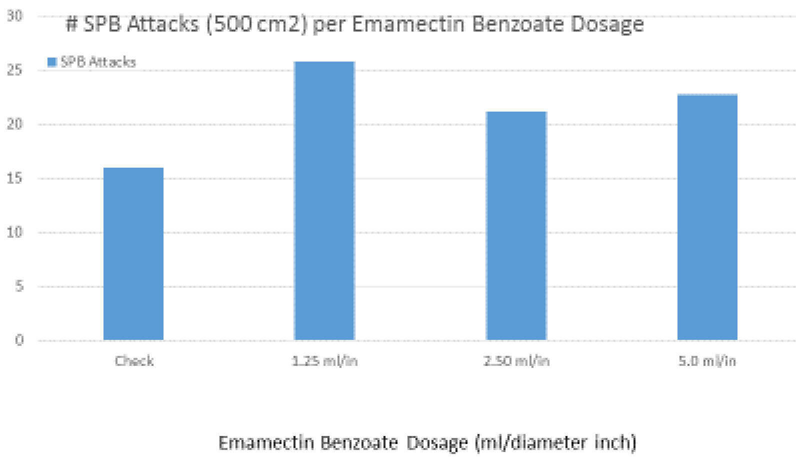
Results:

The study trees were injected during the first week of December, 2015, and baited with SPB pheromones one month later. Attacks were observed on baited trees on February 4 and by March 2, 31 of 40 trees (78%) had more than 100 SPB pitch tubes visible from the ground. By the April 13th visit, 37 of 40 study trees had been mass attacked (>200 pitch tubes/tree).

Comparisons of injection treatments are shown below for density of SPB attacks per 500 cm^2 (Figure 1A), treatment of trees killed (Figure 1 B), SPB egg gallery length per 100 cm^2 (Figure 1C), SPB emergence holes per 100 cm^2 (Figure 1 D), and percent blue stain by treatment are shown below (Figure 1 E). The most effective treatment for protecting loblolly pines from mortality due to SPB attacks was 5.0 ml/diameter inch of emamectinn benzoate. Trees in this treatment exhibited essentially no SPB galleries, no emergence holes and high levels of blue stain infection at all levels sampled. Only 3 of 10 trees had begun to fade as of 20 July (presumably due to blue stain infection). Compared to check trees, those trees treated with lower dosages of emamectin benzoate (2.5 ml and 1.25 ml/diameter inch) showed reduced SPB galleries and emergence, but to a lesser extent than trees treated at the 5.0 ml/in dosage. As of November 9, 2016, 7 of 10 trees injected with 2.5 ml or 5.0 ml/diameter inch, and 8 of 10 trees injected at 1.25 ml/in had faded from successful colonization of blue stain following mass attack of SPB. Among baited check trees, 9 of 10 died following SPB attack and egg gallery density was typical for infested trees..

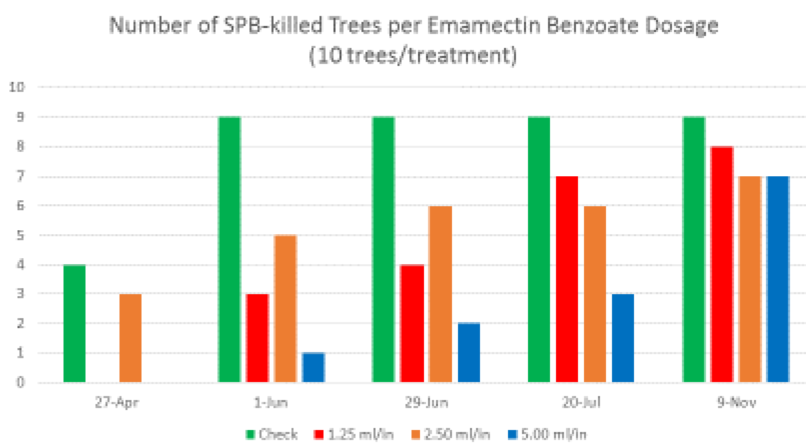
A. SPB Injection: Winter Study 2016

Oakmulgee Ranger District, AL



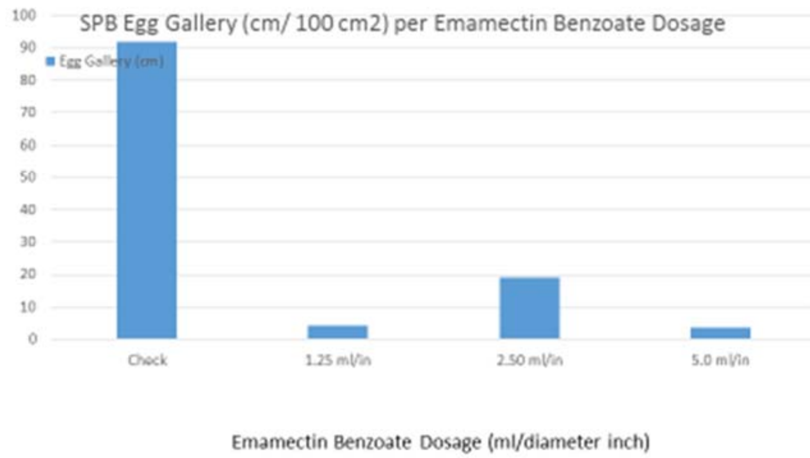
B. SPB Injection: Winter Study 2016

Oakmulgee Ranger District, AL



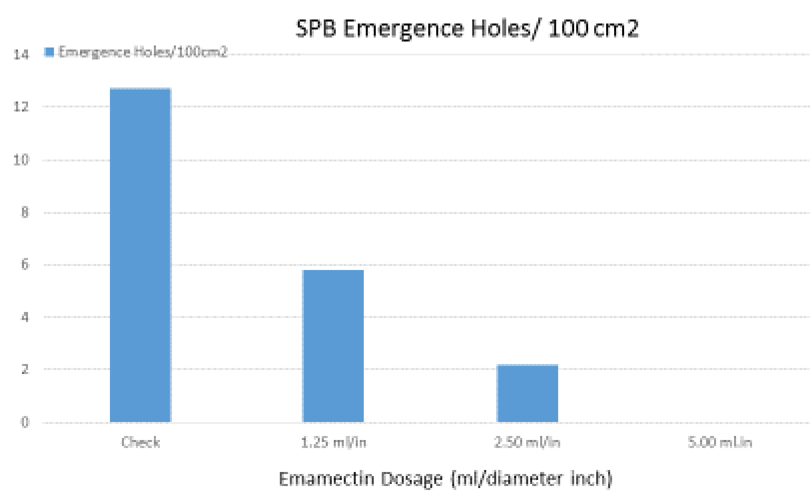
C. SPB Injection: Winter Study 2016

Oakmulgee Ranger District, AL



D. SPB Injection: Winter Study 2016

Oakmulgee Ranger District, AL



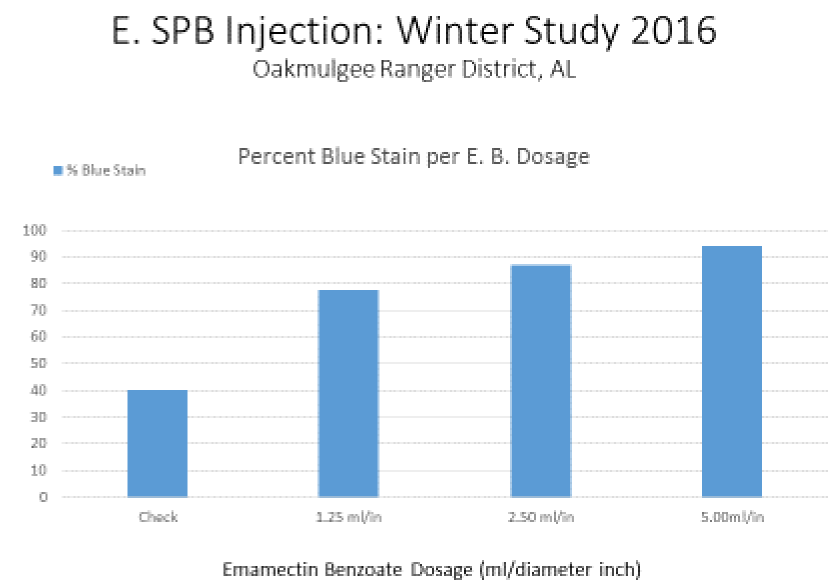


Figure 1: A. Number of SPB attacks/500 cm; B. Number of SPB-killed trees; C. density of emergence holes; D**.** length of SPB egg galleries, and E. percent blue stain per 100 cm^2 in pines injected with increasing dosages of emamectin benzoate during the winter, 2015-2016 and monitored through November 9, 2016; Oakmulgee Ranger District, AL.

Literature Cited:

Billings, R. F., and W. W. Upton. 2010. A methodology for assessing southern pine beetle risk across the southern region using pheromone traps, pp.73–85. *In* J. M. Pye, H. M. Rauscher, Y. Sands, D. C. Lee, and J. S. Beatty (eds.), Advances in threat assessment and their application to forest and rangeland management, vol. 1. PNW-GTR-802, U.S. Department of Agriculture Forest Service, Portland, OR.

Coulson, R. N. and K. D. Klepzig (eds) 2011. Southern Pine Beetle II. *USDA Forest Service, Gen. Tech. Rpt. SRS 140. 512 pp.*

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). J. Econ. Entomol. 102: 1062-1069.

Grosman, D.M., C.J. Fettig, C.L. Jorgensen, and A.S. Munson. 2010. Efficacy of two systemic insecticides for protection of western conifers against *Dendroctonus* bark beetles (Coleoptera: Curculionidae, Scolytinae). W. J. Appl. For. 25: 181-185.

Evaluating the Duration of Emamectin Benzoate Injections for Control of the Southern Pine Beetle

Initiated in 2014 and Completed in 2016

Budget: $9,580 (Grant from Syngenta, Inc.)

Justification: The southern pine beetle (SPB) (Coleoptera: Curculionidae, Scolytinae) is considered the most destructive insect pest of southern pine forests. Since 1997, no SPB infestations have been detected in Western Gulf states (TX, AR, LA & OK) and very few SPB have been caught in pheromone traps in East Texas since 2001 (11 SPB). Pheromone traps deployed during the spring have proven effective for predicting SPB population increases since 1988 across the South (Billings and Upton 2010). SPB populations in 2012 -2015 were at unprecedented low population levels throughout the South and Northeast, with the exception of southern New Jersey, the Homochitto and Bienville National Forest and surrounding private lands in Mississippi, and local areas in Alabama and Virginia. A method for effectively dealing with SPB outbreaks in early stages of development is needed. Much is known about SPB biology and seasonal habits (see Coulson and Klepzig 2011). Most new SPB infestations are initiated following long-distance dispersal in the spring (March-May) and to a lesser extent in the fall (October-December). SPB adults, however, may emerge from brood trees, fly, and attack additional trees throughout the winter, whenever ambient temperatures exceed the flight threshold of ca. 59 degrees F.

A new systemic insecticide (emamectin benzoate) has been developed by the Texas A&M Forest Service (TFS) Forest Pest Management Cooperative (FPMC) and is sold by Syngenta under the trade name TREE-äge™. This insecticide is effective against SPB (Grosman et al 2009, 2010) and has been registered and is now available for pine bark beetle control in forest situations. This is the only insecticide registered for control of SPB in forests. Allee effects (positive density dependence) have been shown to play an important role in the establishment and spread of invasive species. A certain population density is essential before an invasive species can become established and spread in a new environment (and because of Allee effects, many new introductions of invasive plants and animals fail to succeed). Increased interest in recent years is being focused on the potential to exploit Allee effects as a means to manage invasions of exotic species (Tobin et al. 2011).
Field studies conducted by the FPMC from 2012-2015 in Alabama, Virginia and Mississippi have documented the following:

- Loblolly pines injected with 1.25 - 5.0 ml/diameter inch of emamectin benzoate are effective as trap trees for absorbing attacking SPB during summer and fall months when SPB occur at low population levels (<2.0 SPB/trap/day).
- Attacked trees containing emamectin benzoate accumulate attack densities comparable to un-injected pines, but no SPB galleries are constructed and no broods emerge from treated trees.
- Pines that are injected and baited simultaneously also are successful trap trees, but only if initial attacks are delayed or occur over a prolonged period (allowing uptake of the insecticide).

Objectives:

- Determine the duration of isolated trap trees injected with emamectin benzoate and baited with SPB pheromones 18 months post-injection during spring months (April through June).
- Evaluate the duration of two dosage levels (2.5 and 5.0 ml/diameter inch) of emamectin benzoate for effectiveness in a trap-tree tactic.

Cooperators:

Ms. Cindy Ragland	Oakmulgee R.D, Talladega N.F., Brent, AL
Mr. David Cox	Syngenta, Inc., Madera, CA

Study Sites: The study is to be conducted in the Talladega National Forest, Oakmulgee Ranger District in Bibbs and Perry Co., Alabama with SPB attacking loblolly pine, *Pinus taeda*. Isolated loblolly pines from 8 to 14 inches DBH will be selected for treatments.

Insecticides:

Emamectin benzoate (TREE-äge™, Arborjet Inc.) – an avermectin derivative

Treatments (Fall 2014):

- Loblolly pine tree isolated from other pines by > 30 feet, injected with 2.50 ml/diameter inch of emamectin benzoate in November 2014 and baited in April 2016 (6 trees).
- Loblolly pine tree isolated from other pines by > 30 feet, injected with 5.0 ml/diameter inch of emamectin benzoate in November 2014 and baited in April 2016 (18 trees).
- Uninjected check tree (loblolly pine) isolated from other pines by > 30 feet (10-12 trees) to be baited in April 2016 (6 trees).

Treatment Methods and Evaluation:

Two sets of Lindgren funnel traps baited with frontalin + Sirex lure + *endo*-brevicomin (displaced by 4 m) and frontalin + Sirex lure will be deployed in the area 300 m away from injection plots, to monitor local southern pine beetle populations.

Note: Where possible, poor quality (form, health, etc.) trees were selected as trap trees.

TREE-äge™ was injected at 2.50 or 5.0 ml per inch DBH in the fall, 2014. The Tree IV™ microinfusion system (Arborjet, Inc. Woburn, MA) was used to inject TREE-äge™ into 4 (for trees <12” DBH) or 8 (for trees $\geq$12” DBH) points 0.3 m above the ground. The injected trees will be allowed 16 months to translocate chemicals prior to being challenged by the application of synthetic pheromone baits.

Treatment evaluation:

- Treated trees will be revisited at intervals of 4, 8, 12 and 24 weeks after baiting to monitor attack level (occurrence of pitch tubes).
- All study trees with SPB attacks will be felled when they begin to fade. Bark plates (10 X 10 cm = 100 cm2) will be collected at approximately 1.5, 4.0, 6.5 and 17 m height at northern and southern aspects. SPB itch tubes, adult gallery length, density of emergence holes, and presence of blue stain and cerambycid larval galleries will be measured.

Expected outcome: Field studies conducted by the FPMC to date have involved inducing SPB attacks simultaneously with tree injection or 2 and 4 weeks post injection. This study will evaluate the duration of treatment effectiveness by inducing SPB attacks on trees that were injected ca. 18 months earlier. This information will be useful for developing a practical trap-tree control method for SPB populations.

Project Timetable:
CY 2014 - October:

1) Select and inject treatment trees

CY 2016:

2) Bait and monitor trees (April)
3) Collect pheromone traps
4) Rebait injected trees if not mass attacked (June)
5) Sample all study trees (when they begin to fade)
6) Data summary and analyses (October)
7) Progress report (November)

Results

Dead or Alive: All trees in this study were baited with SPB pheromones on March 16, 2016. All but 3 trees injected with 5.0ml/diameter inch were attacked by SPB. By July 20, all 6 check trees had become infested and were felled for bark analysis. On that same date, 5 of 6 trees treated with 2.5 ml emamectin benzoate also had begun to fade and were felled while 11 of 18 trees injected with 5.0 ml/diameter inch also were fading and were felled. By November 20, the final date of observation, 13 of 18 trees injected with 5ml/inch had died and 5 of 6 trees injected with 2.5 ml/in or check trees had died.

Attack density: An analysis of the mean number of SPB attacks per 500 cm^2 revealed that attack density was comparable between injected trees and checks and didn't vary significantly with height on the bole (Table 1).

Egg Galleries: An analysis of egg gallery length per 100 cm^2 of bark sampled at heights of 1.5m, 4.0 m and 6.5 m was conducted. Results (Table 1) revealed that essentially no SPB galleries were established in trees injected with either 2.5 or 5 ml/inch of emamectin benzoate. In contrast, mean gg gallery length per 100 cm^2 exceeded 100 cm in check trees, representing a normal colonization density of SPB.

SPB emergence: No SPB emergence holes were found in trees injected with either 2.5 ml or 5.0 ml/diameter inch, whereas in check trees emergence holes were abundant, indicating good brood survival (Table 2).

% Blue Stain: Mean blue stain infection exceeded 90% in injected trees at the 3 heights sampled, due presumably to the lack of SPB galleries. In contrast, blue stain covered was less than 50% of the bark surface area in check trees where SPB egg galleries were abundant (Table 2).

Table 1: Evaluation of SPB attack density and egg gallery length in bark samples (200 cm2) taken at 3 different heights from trees treated with emamectin benzoate; Oakmulgee Ranger District 2016

Treatment	No. trees	Attacks/500cm2			Egg gallery cm/100 cm2		
		1.5 m	4.0 m	6.5 m	1.5 m	4.0 m	6.5 m
5.0 ml.	13	25.0	30.1	25.6	0.7	0.6	0.1
2.5 ml	5	18.1	18.1	23.2	1.8	0.4	0.3
Check	5	23.5	18.7	16.4	105.6	108.7	121.3

Table 2: Evaluation of SPB emergence and % blue stain in bark samples (200 cm2) taken at 3 different heights from trees treated with emamectin benzoate; Oakmulgee Ranger District 2016.

Treatment	No. trees	SPB emergence holes/100 cm2			% Blue Stain		
		1.5 m	4.0 m	6.5 m	1.5 m	4.0 m	6.5 m
5.0 ml.	13	0	0	0	83.5%	91.2%	83.2%
2.5 ml	5	0	0	0	95.3%	91.4%	90.8%
Check	5	11.0	23.6	23.8	49.0%	40.0%	31.0%

In summary, this study further documented the effectiveness of emamectin benzoate for preventing colonization and brood production by attacking SPB in trees treated with as little as 2.5 ml/diameter inch. The insecticide proved effective even in trees mass attacked 18 months after injection. Unfortunately, most injected trees eventually died, presumably from blue stain infection. Accordingly, tree injections of emamectin benzoate injections offer promise in a trap tree approach to SPB control, but not as a preventative treatment if attack densities are high.

Literature Cited:

Billings, R. F., and W. W. Upton. 2010. A methodology for assessing southern pine beetle risk across the southern region using pheromone traps, pp.73–85. *In* J. M. Pye, H. M. Rauscher, Y. Sands, D. C. Lee, and J. S. Beatty (eds.), Advances in threat assessment and their application to forest and rangeland management, vol. 1. PNW-GTR-802, U.S. Department of Agriculture Forest Service, Portland, OR.

Coulson, R. N. and K. D. Klepzig (eds) 2011. Southern Pine Beetle II. *USDA Forest Service, Gen. Tech. Rpt. SRS 140. 512 pp.*

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). J. Econ. Entomol. 102: 1062-1069.

Grosman, D.M., C.J. Fettig, C.L. Jorgensen, and A.S. Munson. 2010. Efficacy of two systemic insecticides for protection of western conifers against *Dendroctonus* bark beetles (Coleoptera: Curculionidae, Scolytinae). W. J. Appl. For. 25: 181-185.

Final Conclusions Related to Emamectin Benzoate for Southern Pine Beetle

The FPMC has conducted evaluations of emamectin benzoate (EB) for prevention and control of southern pine beetle since 2007. Overall conclusions from these studies are as follows:

- Emamectin benzoate injections as low as 1.25 ml per diameter inch are successful for preventing brood development of SPB in baited trees, although 5 gm/inch provides more consistent results.
- A combination of EB and the fungicide propiconizole provides increased protection up to 3 years, but eventually most injected trees succumb to blue stain infection following mass attack.
- Loblolly pines injected in winter months and baited simultaneously are effective in preventing SPB brood production, even though levels of mass attacks are comparable to those of baited, non-injected trees.
- EB was found to be effective for preventing SPB brood development even when trees were mass attacked 18 months after injection with 2.5 or 5 gm/diameter inch. Unfortunately, most injected trees eventually died, presumably from blue stain infection.
- Results of various studies suggest EB has potential in SPB prevention programs for concentrating and eliminating low populations of SPB during endemic years as a means to maintain beetle populations below the Allee threshold needed to initiate multiple-tree spots.
- In suppression programs, injection of uninfested trees at the active front of expanding SPB infestations may prove effective, but remains to be tested.

Improving the Prediction System for the Southern Pine Beetle

Special Technology Development Project Number: R8-2016-1
Starting Date: February 15, 2016
Expected Completion Date: February 14, 2018
Grant: $50,000 from USDA Forest Service, Forest Health Protection

Brief Description of Project:

- **FY 2016**: Conduct a replicated, statistically-designed bioassay to compare the relative attractiveness to southern pine beetle (SPB) and clerid predators of traps baited with frontalin and 1) one commercial Sirex lure (*alpha*- and *beta*-pinene); and 2) two Sirex lures to double the release rate; 3) steam-distilled pine turpentine released from amber bottle with wick; 4) pine turpentine released from sealed polyethylene pouch; and 5) one Sirex lure with *endo*-brevicomin during the spring and fall dispersal periods of SPB.
- **FY 2017**: Repeat the comparison bioassay in various locations throughout the South (FL, VA, SC, GA, MS) in fall and spring, using the three most attractive lure combinations from the 2016 bioassays. Modify the standard SPB prediction chart (Appendix 4) and trapping protocol based on the most effective bait.
- **FY 2018 (If federal funding is provided)**: Implement and validate the modified prediction chart across the southern U.S.

Project Objectives:

- To answer the questions: Is a higher elution rate of host volatiles and/or presence of *endo*-brevicomin an important factor for making low SPB populations visible? Is pine turpentine more attractive than the commercial Sirex lure as a host component for SPB traps? Are fall surveys useful for making SPB predictions?
- Modify the traditional SPB prediction chart to reflect comparative attractiveness of single or double Sirex lures/trap.
- Develop, implement and validate a revised South-wide protocol for improved prediction of SPB infestation trends.

Justification:

The southern pine beetle (SPB), *Dendroctonus frontalis* Zimm. (Coleoptera: Curculionidae) is one of the most serious insect pests of loblolly pine in the southeastern United States (Thatcher et al. 1980, Coulson and Klepzig, 2011). In recent years, SPB has reached outbreak levels at both the northern (New Jersey, New York) and southern (Honduras) extremes of its range (Billings 2015). In 2012-2015, local outbreaks of SPB also have occurred on national forests in Mississippi and Alabama. The SPB prediction system developed by the Texas A&M Forest Service in the mid-1980s (Billings 1988, Billings and Upton 2010) and implemented across the South proved to correctly predict SPB outbreaks or declines over 70% of the time during those years when steam-distilled pine turpentine was used as the host component in trap lures (1986-2007). Since 2008, the turpentine has been replaced with the commercial Sirex lure, comprised primarily of *alpha*-pinene. The change was adopted to maintain consistency in the host lure and for ease of deployment. Recent SPB outbreaks in Mississippi from 2012-2015 failed to be forecasted by the SPB prediction system, perhaps due to low trap catches in traps deployed with Sirex lures in these areas. These results suggest the Sirex lure is a poor substitute for pine turpentine (the host compound used to develop the prediction model in the 1980s), possibly due to a low release rate (ca. 2.5 g/day compared to ca. 6 g/day for bottle and wick (see: www.fs.fed.us/foresthealth/technology/elutionrate/). High release rates of host volatiles are known to substantially increase responses of SPB and associated predators (Billings 1985). The SPB male-produced compound e*ndo*-brevicomin (Vité et al. 1985), placed 4-16 m distance from a baited trap, also increases attraction to SPB (Sullivan et al. 2007, Sullivan and Mori 2009) and may have utility in improving the prediction system. It is urgent that the prediction model is modified to increase its ability to detect increasing SPB outbreaks early in their development, prior to the next large-scale SPB event. The current study builds on previous research studies that led to development and implementation of the only bark beetle prediction system in the nation and complements other current studies (Appendix 3).

Scope of Application: The results would be relevant throughout the range of the pest. The current network of Federal and State cooperators, in place since 1986, would put any modifications in SPB prediction to immediate use. Initiation of a fall prediction survey using pheromone traps also would be useful to these cooperators to extend the time between early alert and SPB detection flights. An effective SPB prediction system would be useful throughout the extensive range of SPB.

Measures of Success:

- **Expected outcomes:** A more attractive bait combination for SPB will yield a more effective means to detect SPB outbreaks in early stages of development.
- **Products and Due Dates**: Identification of most attractive bait combination (Dec. 2016); revised protocol for predicting SPB outbreaks (December 2017); implementation and validation of new protocol across SPB range in southeastern U.S. (October 2018)
- **Benefits:** Improved ability to forecast SPB outbreaks early in their development and more efficacious SPB management strategy.

Technology Transfer: State and federal cooperators involved in the annual SPB prediction survey in at least 13 states in the southeastern U. S. are available to immediately implement changes in protocols for SPB prediction that result from this study. In addition to these cooperators, the improved prediction model would be immediately applicable to northeastern states as the SPB population extends its range north. Forest pest specialists in Mexico and Central America have been working in collaboration for many years with the principal investigator and are anxiously awaiting an effective early alert system for SPB.

Research Basis: The SPB Prediction System, in operation across the South since 1986 (Billings 1988, Billings and Upton 2010), will be improved with results from this project.

Methods:

SPB Prediction System Performance: Using procedures described in Billings and Upton 2010, historical data from the South-wide SPB Prediction System will be analyzed to compare relative accuracy of SPB predictions when pine turpentine eluted from bottles was used (1988-2007) to more recent years when the commercial Sirex lure was exclusively used on pheromone-baited traps (2008-2015). Predictions based on SPB and clerid catches across the South for years when the Sirex lure was used will be compared to actual numbers of SPB infestations detected at the end of the year for each county or National Forest Ranger District trapped, as was previously done for years when pine turpentine was used as the host factor in traps (Billings and Upton 2010). This proposal is one step towards improving the SPB prediction system and will complement other on-going studies. For example, Mississippi State University and USFS FHP are developing a degree-day model for the SPB survey to ascertain when best to deploy traps and how long to leave them in the field for predictive purposes. Also, the University of Georgia and US Forest Service/SRS have a collaborative study to determine if average body size of SPB collected in survey traps can be used to enhance prediction of the onset and the decline of SPB outbreaks.

<u>Host volatile bioassays</u>: Replicated field bioassays, conducted in the spring and fall (2016) using Lindgen funnel traps, compared the attractiveness of frontalin plus the following: 1) one Sirex lure deployed from sealed polyethylene pouch (standard lure or check); 2) two Sirex lures per trap; 3) steam-distilled Caribbean pine turpentine, deployed from a 240 ml amber bottle and wick (as per Billings 1988); 4) turpentine used in treatment 3, deployed from sealed polyethylene pouch used for Sirex lure, and 5) one Sirex lure + *endo*-brevicomin displaced by 4 m. After the first 5 weeks, treatment 1 was replaced by steam-distilled Caribbean pine turpentine (from Synergy Semiochemicals) deployed from polyethylene pouch plus *endo*-brevicomin displaced by 4 m. Traps were situated at least 200 m apart and at least 15m from live pines in mixed pine-hardwood stands (or pure hardwood stands adjacent to pine stands) and were placed on metal poles or from hardwood trees at a standard height of 2 m above ground. Insects were collected every 5-7 days for 10 consecutive weeks beginning in mid-February and mid-October of 2016.

Lures were rotated in a Latin-square design following collection of insects at each trap location to eliminate positional effects (every treatment was tested twice at every trap location). Lures were replaced with fresh lures every 5 weeks (or sooner if needed). An analysis of variance was used to document the significance of observed differences in trap catches of SPB and clerids among treatments. The pine and hardwood basal area, mean diameter at breast height and mean tree height was documented for each trap location and subsequently correlated with trap catches of SPB and clerids. The bioassay was replicated eight times by conducting the bioassay on at two (2) different sites each season on each of three (3) or more National Forests or adjacent private lands in Mississippi (Homochitto, Bienville N.F.), Alabama (Oakmulgee R.D.) and Louisiana (Sicily Island) where SPB were present. John Riggins or a student from his lab (Mississippi State University) and USFS personnel from Pineville, LA (Jim Meeker or technicians) and Lufkin, TX (Steve Clarke) assisted in making trap collections and counting SPB and clerids.

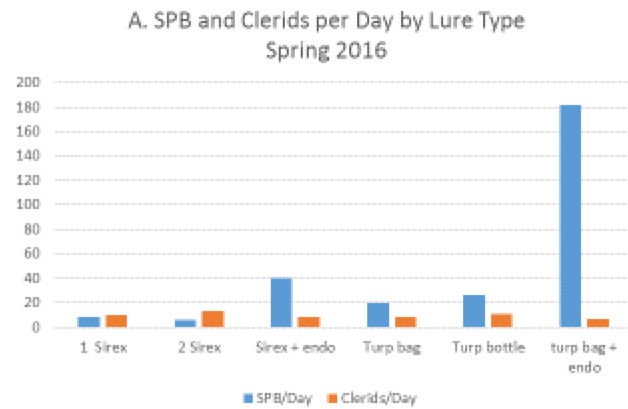
A second bioassay was conducted by the principal investigator and cooperators was conducted in the fall (2016) on the same 6 sites in LA, MS, and AL, plus 2 additional sites in North Carolina. Five different lure combinations were tested: 1) frontalin + Sirex lure; 2) frontalin + Sirex lure + endo-brevicomin; 3) frontalin + Caribbean turpentine deployed from an amber bottle with wick; 4) frontalin + Caribbean turpentine deployed from a polyethylene bag; and 5) frontalin + Caribbean turpentine deployed from a polyethylene bag + endo-brevicomin. Traps were monitored for 10 consecutive weeks, with baits replaced at the beginning of week 5. Results of trap catches for SPB and clerids were subjected to an Analysis of Variance to confirm significance of observed differences in lure attractiveness.

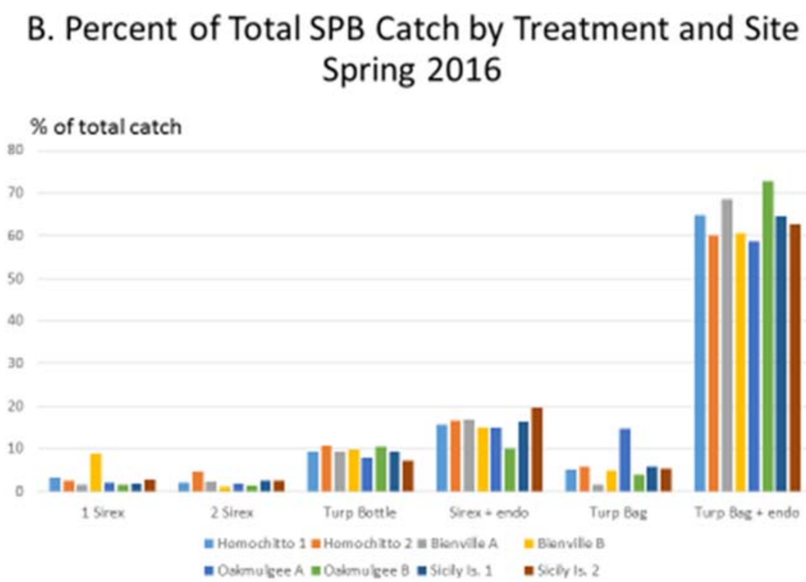
In selected counties or National Forest Ranger Districts in MS, FL, AL, GA, SC, and MD in FY2017 as part of the SPB Prediction Survey, traps baited with frontalin and 3 of the host lures from the 2016 bioassays will be tested. The following treatments will be compared in the spring of 2017, when redbuds begin to bloom (February): 1) frontalin + one Sirex lure (standard); 2) frontalin + one Caribbean turpentine bag; 3) frontalin + Sirex lureplus endo-brevicomen, displaced 4 m from the trap. Mean trap catches of SPB and clerids per treatment will be compared to number of SPB spots detected by the end of the year in each county or Ranger District trapped (Billings and Upton 2010). Elution rates (gm/day) and mean daily temperatures will be monitored for each lure and elution device tested. The chemical composition of the pine turpentine will be determined by chemical analysis. Results from these studies will be used to modify and improve the accuracy the standard SPB prediction chart (Appendix 4b) that was developed using pine turpentine as a host volatile (Billings 1988). The modified chart will be implemented by all State and Federal cooperators involved in the SPB Prediction Survey in FY 2018 and results validated by year-end SPB detection records available in the SPB Portal.

Spring Bioassay Results:

The spring bioassays were conducted from February 19 to April 20 (10 weeks) on 8 sites, two each in Louisiana, Mississippi, and Alabama. Results are shown in Figure 19. The treatment containing two bags of Sirex lures was replaced after 5 weeks with a combination treatment containing frontalin, Caribbean turpentine deployed from a polyethylene bag, and *endo*-brevicomin (displaced 4 m from the trap). Clearly the most attractive treatment in all eight sites was the combination containing frontalin, turpentine bag, and *endo*-brevicomin (Figure 1 A). This treatment caught approximately 60% of all the southern pine beetles trapped per site, consistently more than the combination of Sirex lure + frontalin + *endo*-brevicomin (Figure 1 B). Interestingly, most attractive treatment caught the fewest clerids (96% SPB) (Figure 1 C).

The least attractive treatment was the frontalin + Sirex lure, which has been the standard lure used in SPB prediction surveys since 2007, possibly explaining why SPB pheromone traps have failed to detecting pending outbreaks in recent years. The same 5 treatment test (utilizing the turpentine bag + frontalin + *endo*-brevicomin in place of the 2 bags of Sirex lure) will be repeated in the same locations in the fall of 2016. Results will be used to improve the SPB prediction system.


A. SPB and Clerids per Day by Lure Type
Spring 2016
200
180
160
140
120
100
80
60
40
20
0
1 Sirex
2 Sirex
Sirex + endo
Turp bag
Turp bottle
turp bag + endo
SPB/Day
Clerids/Day


B. Percent of Total SPB Catch by Treatment and Site
Spring 2016
% of total catch
80
70
60
50
40
30
20
10
0
1 Sirex
2 Sirex
Turp Bottle
Sirex + endo
Turp Bag
Turp Bag + endo
Homochitto 1
Homochitto 2
Bienville A
Bienville B
Oakmulgee A
Oakmulgee B
Sicily Is. 1
Sicily Is. 2

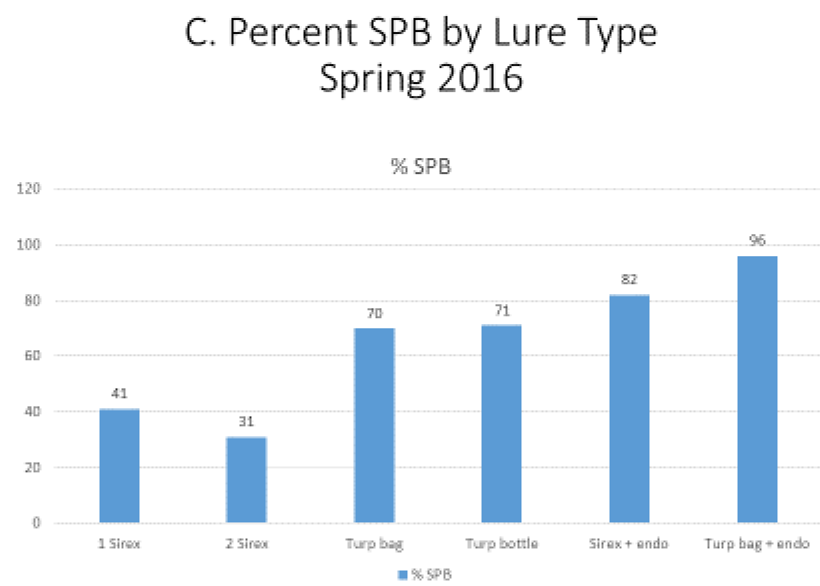


Figure 1: Results of spring bioassays to test the attractiveness of different pheromone lures for southern pine beetle and clerids; **A:** Numbers of SPB (*Dendroctonus frontalis*) and clerids (*Thanasimus dubius*) caught by lure type for all 8 locations combined; **B:** Percent of total catch of SPB by trap location and lure type; **C:** Percent SPB (SPB/SPB + clerids) by lure type for all 8 locations combined. February-April 2016.

Results of the Fall Bioassay

As shown in figures 1 D and 1 E, the most attractive lure combination for SPB in fall bioassays at all locations was frontalin+ Caribbean turpentine + *endo*-brevicomin. This lure combination captured more than 50% of all the SPB in the five treatments combined. The second most attractive treatment was the one containinf frontalin, Sirex lure and endo-brevicomin, which caught significantly fewer SPB than the the Caribbean pine turpentine and endo-brevicomin, but significantly more than the other three treatments with no endo-brevicomin. The number of SPB caught in the other three treatments without endo-brevicomin was comaparable, regardless of treatment.

With respect to clerids, the fewest numbers were caught in traps baited with frontalin + Caribbean turpentine and *endo*-brevicomin, but differences among treatments were not as great as those of SPB. The ratio known as % SPB (# SPBx100% /(#SPB + # clerids) was greatest for the turpentine + *endo*-brevicomin treatment and lowest for the three treatments with no *endo*-brevicomin (Fig. 1 F). Overall, the results were similar to those documented for the spring.

Figure D. Mean number of SPB (*Dendroctonus frontalis*) and clerids (*Thanasimus dubius*) captures per day by lure type, all sites combined; Fall 2016 (8 replicates)

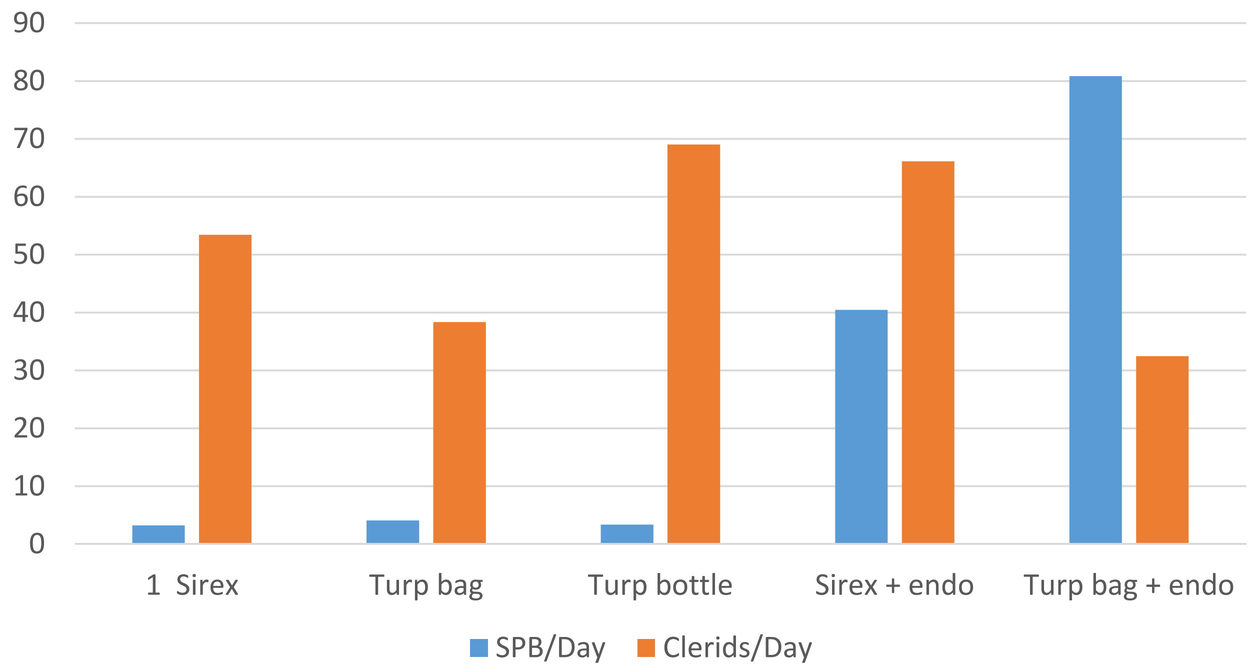


Figure E. Percent of total SPB catch by treatment and site, Fall 2016

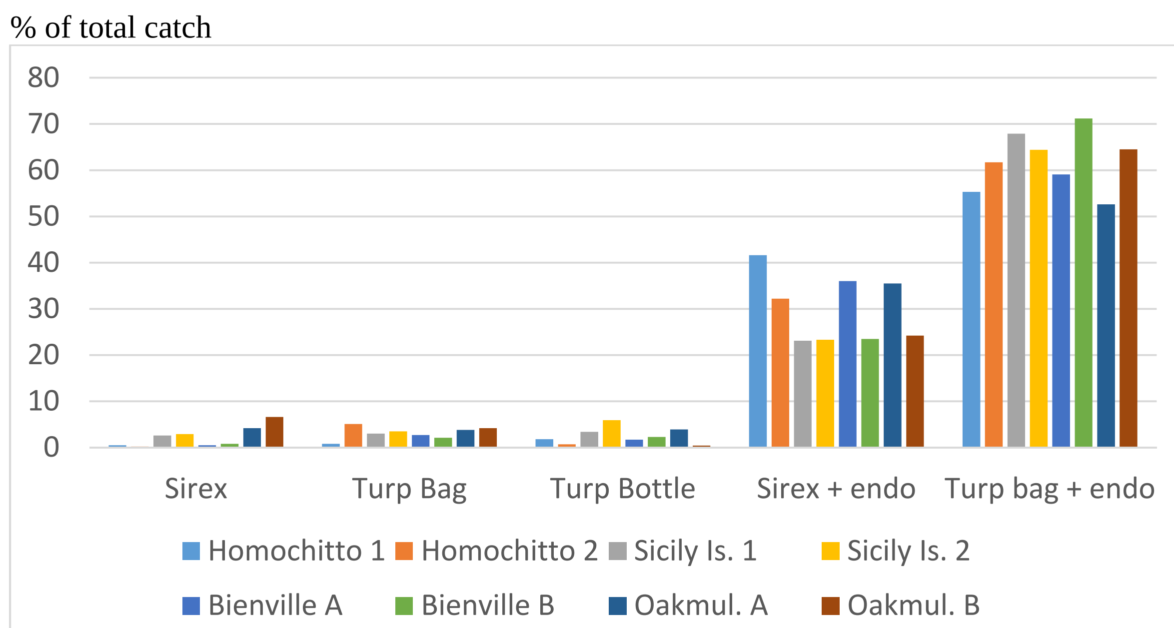


Figure F. Percent SPB by lure type, Fall 2016

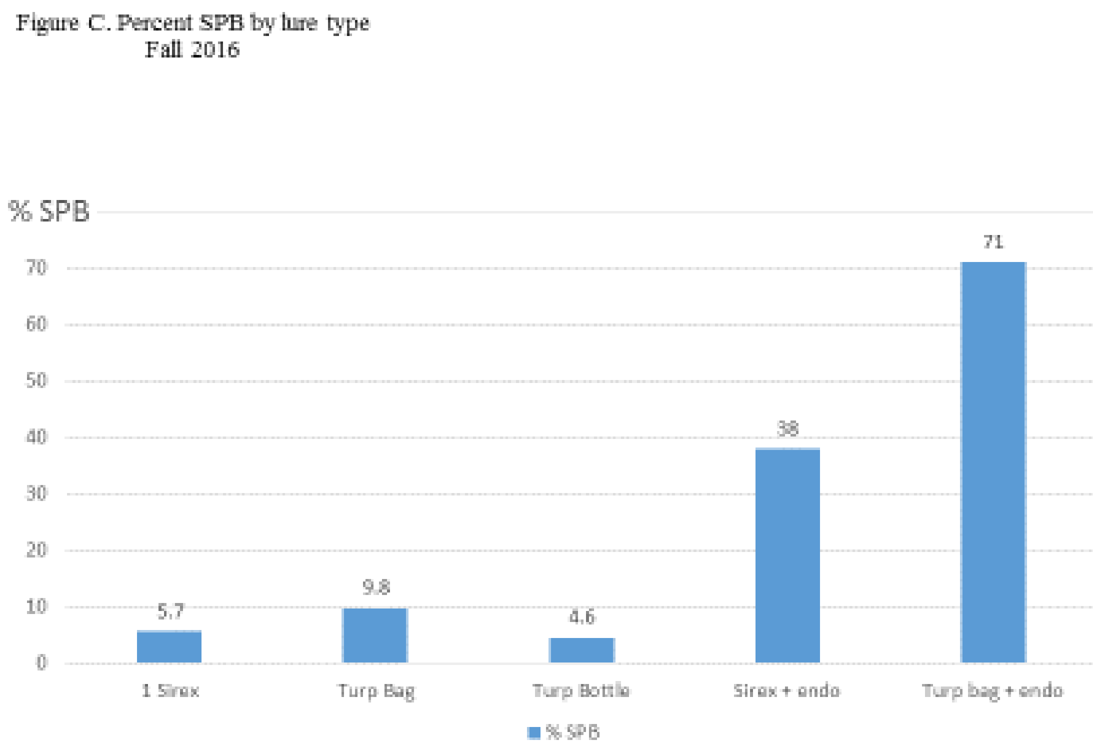


Figure 1: Results of fall bioassays to test the attractiveness of different pheromone lures for southern pine beetle and clerids; **D:** Mean numbers of SPB (*Dendroctonus frontalis*) and clerids (*Thanasimus dubius*) caught by lure type for all 8 locations combined; **E:** Percent of total catch of SPB by trap location and lure type; **F:** Percent SPB (SPB/SPB + clerids) by lure type for all 8 locations combined. October – December 2016.

These results provide a basis for making changes in the SPB annual prediction survey. Starting in 2018, recommendations are to 1) bait survey traps exclusively with a combination of frontalin + Sirex lure + *endo*-brevicomin, the latter displaced 4 m from the trap. Initiate spring surveys when redbuds begin to bloom (usually in mid-February) and monitor the traps for 4 consecutive weeks, collecting insects at the end of each week. Use the modified prediction chart (Fig. 1 H) to predict SPB infestation trends and levels. For those cooperators wishing to continue using the previously standard lure of frontalin + Sirex lure, results should be interpreted using modified prediction chart shown in Figure I. The combination of frontalin, Caribbean turpentine and *endo*-brevicomin, even though it attracted the most SPB, is not recommended as a survey lure because the quality and composition of pine turpentine varies from batch to batch. Also, this lure combination may attract more SPB than cooperators are willing to count. The increased attractiveness of this lure combination in comparison to the Sirex lure with *endo*-brevicomin suggests that there is an additional attractive component in turpentine or a component inhibitory to SPB in the Sirex lure.

To ascertain why the Caribean turpentine + *endo*-brevicomin was so much more attractive than the Sirex lure + *endo*-brevicomin when combined with frontalin, Dr. Brian Sullivan (USFS, Southern Research Station) conducted a chemical analysis of vapors emitted after 3 weeks' exposure of the two host lures. Results (Table 1) showed significant differences in the proportion of terpenes and other components.

Table 1: Chemical composition of volatiles from SPB lures (Sullivan 2017, unpublished data)

Lure	*Alpha*-pinene	*Beta*-pinene	3-carene	Eucalyptol
Sirex	68%	29%	0%	0.28%
Turpentine sleeve	90%	2.2%	3.3%	0%

Vapors from Caribbean turpentine had a chemical composition that varied markedly from those of the Sirex lure. The turpentine contained a much higher component of *alpha*-pinene (synergist of frontalin) and a lower component of *beta*-pinene. Also, the turpentine contained signiificant amounts of the terpene 3 carene which may have added to its attractiveness to SPB. The Sirex lure contained no 3-carene. In turn, the Sirex lure contained eucalyptol, a volatile from eucalyptis trees, which may be repellent to SPB. This assumption requires further testing. The turpentine was free of eucalyptol.

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SPB PREDICTION CHARTS

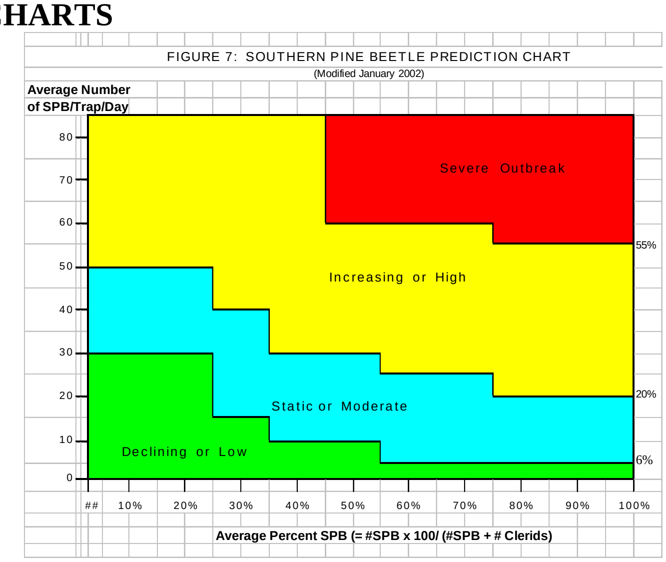

Figure 2: Original SPB prediction chart used up to 2017

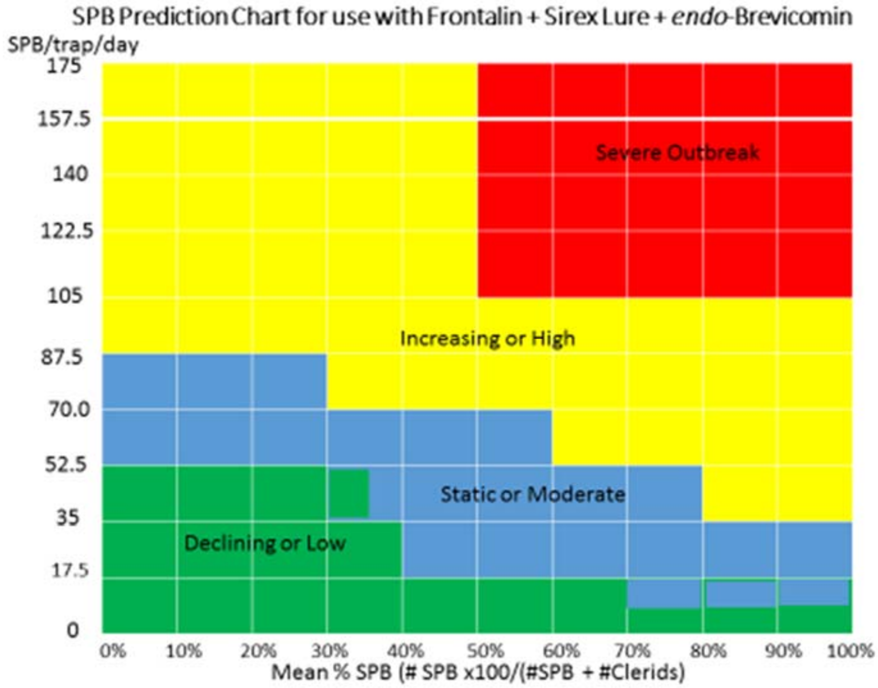

Figure 3: Modified SPB prediction chart to use when traps are baited with frontalin, Sirex lure and endo-brevicomin.

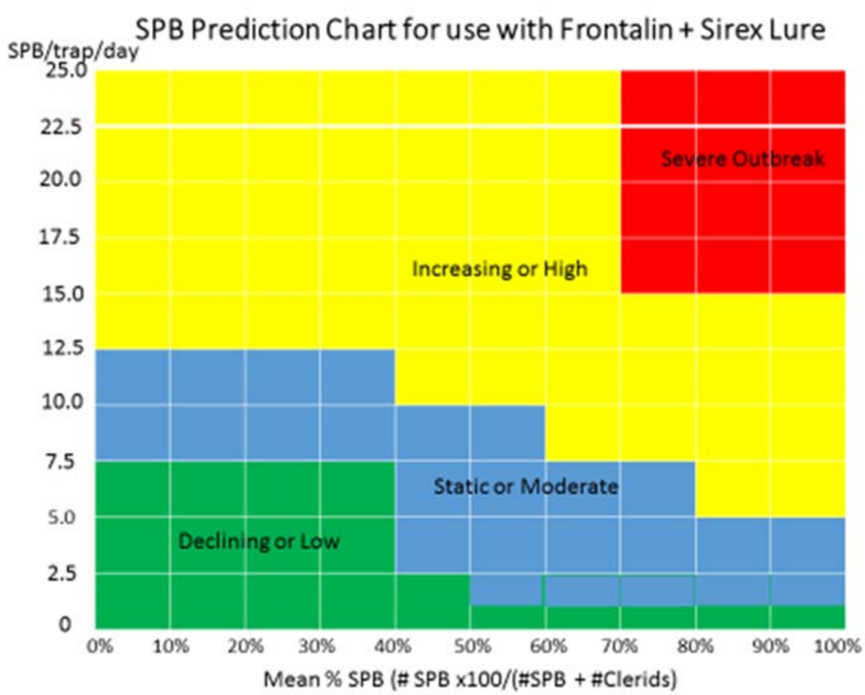

Figure 4: Modified SPB Prediction chart to use when frontalin + Sirex lure are used to bait traps.

Evaluation of Botanigard™ (a. i. *Beauveria bassiana*) for Longevity and Control of Southern Pine Beetle

Initiated in 2016

Cooperators: Brian Strom and Rabiu Olatinwo, US Forest Service, Southern Research Station

Funding: FPMC

Objectives:

The objectives of this study are to:

1. Evaluate the duration of Botanigard™ 22WP on loblolly pine logs under various environmental conditions in east Texas.
2. Conduct a preliminary assessment of the efficacy of Botanigard 22WP for control of southern pine beetle populations using standing loblolly pine trap trees in Mississippi.

Methods:

Objective 1: This study will be conducted on state and private forestlands in East Texas. In May (spring-summer conditions), and November (fall-winter conditions), the following trial will be established. Six log sections, each 4-feet in length, will be cut from two 8-inch loblolly pine trees and treated with Botanigard. Two log sections will be placed horizontally under each of the following conditions: full sun, partial shade, and full shade in a typical pine forest. The treated logs will be sampled at intervals of 4, 8, 12 and 16 weeks following treatment by removing 100 square cm samples of bark from the upper and lower surfaces of each log. Samples will be sent to Rabiu Olatinwo (Southern Research Station) to sample for *Beauvaria bassiana* presence and activity.

Objective 2:. There will be two treatments conducted on the Bienville National Forest in Mississippi; a Botanigard treatment and a control. The treatments will consist of two loblolly pine trees each applied during the late spring of 2016..

Pines to be treated will be sprayed from the ground with 4 liters of Botanigard formulation to contain a nominal $8X10^{7}$ conidia/ mL. The Botanigard mix will include:

- 20 liters clean water
- 450g of *Beauveria bassiana* (Bb) wettable powder formulation
- 10ml Silwet L-77 Ag (.05% final concentration)
- 2ml of biologically benign Sigma life sciences Antifoam O-30 at a concentration of 1% previously mixed into cold water.

The spore formula will be mixed/shaken vigorously in 20 L plastic carboys 1-2 hours before use and mixed repeatedly thereafter.

Botanigard will be applied using a Solo hand pump backpack with an adjustable spray tip. Spray using this backpack has been found to reach approximately 8 m (Products).

At the time of application, trees will show no evidence of southern pine beetle attack. Trees will be sprayed vertically with the narrowest pattern in short controlled bursts onto each aspect of tree as high as possible until wet and just starting to drip, but not running. The spray will then be adjusted to a narrow cone for the middle range (10'-20'), moving to every face of tree until wet. Finally, standing farther from the tree and the nozzle will be adjusted to the width of the bottom until wet and dripping slightly, but not washing/running off of tree. Each tree will be checked for dry areas and spot sprayed if necessary.

Treated and control trees will be baited with species specific pheromone attractants (frontalin, Sirex lure, and *endo*-brevicomin) immediately after application to attract beetles.

Treatment evaluation

Each study tree will be nondestructively sampled every four weeks through the end of November following application of Botanigard. Following successful colonization and progeny emergence, all study trees will be felled. Bark plates 20 X 25 cm (500 cm^2) will be collected at approximately 1.5, 4 and 7 m in height at northern and southern aspects. Southern pine beetle gallery length and density of emergence holes will be measured.

The average number of SPB attacks, the density of emergence holes, and lengths of galleries per 500 cm^2 will be compared between treated and check trees. The number of Bb-infected SPB adults or immature stages and/or predators will be counted and recorded from each bark sample.

Similarly, 100 cm bark samples will be collected at heights of 2, 5 and 8 m from the northern and southern aspects of each treated tree and sent to the Southern Research Station to evaluate presence and level of *Beauvaria bassiana* activity. The treated logs were sampled at intervals of 4, 8, 12 and 16 weeks following treatment by removing 100 square cm samples of bark from the upper and lower surfaces of each log. Samples were sent to Dr. Rabiu Olatinwo (USDA Forest Service, Southern Research Station) to sample for *Beauvaria bassiana* presence and activity.

Results (Table 1) suggest that the fungal spores don't survive for long periods of time, particularly when exposed to full sunlight and Texas summer heat. No viable spores were found on treated bark after just 4 weeks of exposure to full sunlight on the top of treated logs. When exposed in partial sunlight, 50% of the sampling points had viable spores on the top of logs after 4 weeks, but this percentage dropped to 0 by week 16. On the bottom side of the same logs, 62% of the sampling points had viable spores after 4 weeks, which declined to 25% after 16 weeks.

For logs maintained in full shade, viable spores were detected on 100% of the points sampled on the top of logs after 4 weeks, but none were found at week 16. On the bottom side of shaded logs, viable spores were detected on 100% of the sampling points after 8 weeks, but this level of viability dropped to 50% by week 16. When data for all sampling sites were combined, the average percentage of points with viable fungal spores declined from 67% after four weeks to just 14% after 16 weeks. Whether the viability of *Beauvaria* spores in BotaniGard applications is sufficient to have an effect on southern pine beetle during its 4-5 week life cycle within host trees was the objective of a field test applied to standing trees colonized by SPB.

Table 1: Percent of four sampling points per 100 cm2 with viable spores of *Baeuvaria bassiana* under different environmental conditions in East Texas (June-October 2015).

Week	4	8	12	16
Full sun/top	0%	0%	0%	0%
Full sun/bottom	37%	75%	12%	25%
Partial shade/top	50%	37%	37%	0%
Partial shade/bottom	62%	100%	12%	25%
Full shade/Top	100%	37%	50%	0%
Full shade/Bottom	87%	100%	50%	50%

A preliminary evaluation of the effectiveness of BotaniGard™ for control of southern pine beetle was conducted on the Oakmulgee National Forest in Alabama in June, 2015. Two pines were treated with BotaniGard 22 WP from ground level to a height of 12 feet using a backpack sprayer. On the same day, the treated trees were baited with SPB lures (frontalin and alpha-pinene) to induce attacks. The trees were monitored until the crowns began to fade, indicating successful SPB. Colonization. Examination of bark samples taken at heights of 4 and 10 feet revealed no apparent treatment effect (Table 2). Beetles had attacked the baited trees at typical densities and SPB brood developed and emerged at densities comparable to baited trees without BotaniGard™ application.

Table 2: Summary of 2015 BotaniGard SPB Trap-tree Trial

Height	Treatment	SPB Attacks #	SPB Emerg. holes #	Cerambycid egg niches #	Mean/100 cm2 SPB egg galleries (cm)
1.5 m	BotaniGard	3.2	30	0.5	114
	Check	3.5	17	0.2	75
4 m	BotaniGard	2.7	47.2	1.2	76
	Check	2.3	26.5	1	100
6.5 m	BotaniGard	2.5	48.5	1.7	51
	Check	2	22.5	1.2	75
15 m	BotaniGard	0.7	18.7	0.7	75
	Check	2.2	20.5	1.7	17
Mean	BotaniGard	2.3	36.1	1.02	79.0
	Check	2.5	21.6	1.02	66.7

EVALUATION OF MACRO- AND MICRO-INJECTION SYSTEMS FOR APPLICATION OF PROPICONIZOLE IN LIVE OAK TO PREVENT OAK WILT

Initiated: 2016

Sponsor: USDA Forest Service, Forest Health Protection (Pesticide Impact Assessment Project)

Grant: $58,000 for 3 years (shared between FPMC and TAMU Department of Plant Pathology and Microbiology)

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Abstract:

This project will compare the effectiveness of macro- (high volume, low concentration) versus micro-injection (low volume, high concentration) systems for treating live oak trees with propiconazole for prevention of oak wilt, caused by the vascular fungus *Ceratocystis fagacearum*. The field trials will be conducted in central Texas on the leading edge of expanding oak wilt centers.

Objectives:

1) Evaluate effectiveness of macro-infusion compared to one micro-infusion (the Arborjet's Tree I.V.) system for injecting propiconazole (Alamo® or Propizol™) into live oak for prevention of oak wilt.
2) Evaluate the standard macro-infusion system versus one micro-infusion system (Tree I.V.) for speed and distribution of propiconazole movement within live oaks by monitoring uptake and movement of the fungicide in study trees at periodic intervals following injection.

Background/Justification Statement: Several cultural control techniques (minimize fungal inoculum, timing of branch pruning, painting wounds and pruning cuts on oaks, prompt removal of infected red oaks, and root disruption/trenching around expanding infection centers, among others) are available for management of oak wilt, caused by the plant pathogen, *Ceratocystis fagacearum* (Billings, 2001, Koch et al. 2010). However, these techniques are often impractical for treatment of high value individual trees or small groups at risk to infection. Currently, the most widely used treatment recommended for protecting high-value oaks 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 1995). Until recently, applications of propiconazole have been made almost exclusively through the use of macro-injection systems to deliver 20ml Alamo® diluted in 1 liter water per inch 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 in red oaks and longer in live oaks (Blaedow et al. 2010), but is labor intensive to perform. It often involves exposing root flares with an air spade or other tool. Arborists are interested to know if propiconazole can be applied at more concentrated levels to the lower trunk of live oak trees using available micro-injection/infusion systems and whether these applications are effective in preventing/reducing fungal infection and spread within the host. An initial comparison of various micro-infusion systems revealed that the Arborjet Tree I.V. system outperformed several other commercially available systems for injecting propiconazole into live oak (Grosman et al. 2015). Propiconazole is one of the fungicides undergoing Forest Service Health and Ecological Risk Assessment and is being reviewed by U. S. EPA for reregistration. Propiconazole is the fungicide most effective in preventing oak wilt and few other fungicide alternatives exist for this specific purpose. A new formulation of propiconazole, sold under the trade name Propizol by Arborjet, Inc., also will be tested. Propizol contains the same concentration of propiconazole as Alamo (14.3%), but has a different carrier.

Expected Accomplishments:

1. A side-by side comparison of two injection systems (macro- versus micro-) will demonstrate the advantages and disadvantages of each system for delivery of the fungicide propiconazole.
2. The field comparison will determine which system provides better distribution of propiconazole within the tree and corresponding prevention of oak wilt infection in live oaks challenged by oak wilt.

Research Approach:

One microinjection system and one macro-injection system will be evaluated:

<u>Tree IV</u> System (Arborjet, Inc.; contact: Joe Doccola) – low volume (20 ml fungicide/injection point); moderate pressure (60 psi) (Fig. 1A).

<u>Macro Injection</u> System (Standard) (Rainbow Treecare Scientific Advancements; contact: Shawn Bernick) - high volume (1 liter water and 20ml fungicide/inch diameter); low pressure (20 - 30 psi) (Fig. 1 B)

A portion of the treated trees will be injected with Alamo® and a similar number will be injected with Propizol™, using both injection systems to determine if treatment effectiveness varies due to formulations produced by the two manufacturers (Syngenta and Arborjet).

<u>Treatment Methods and Evaluation</u>**:**

A Master's student was trained to inject trees with the macro-infusion system, including use of an air spade to expose root systems prior to injection. The micro-injections were applied by Texas A&M Forest Service staff forester experienced in this process (Figure 1A). Foliage samples were removed from macro- and micro-injected trees 1 day, 1 week, and 1 month after infection to assay them for the presence of the fungicide. A bioassay was used to estimate the relative levels of the funigcide in the leaves by extracting the tissues with a mix of organic solvents and processing the extract on thin layer chromatography plates. The dried plates were oversprayed with a suspension of a dark-spored fungus, a *Cladosporium* spp. Inhibition of fungal growth will appear on the plates, providing evidence for the presence of fungicide in the original foliar tissues. A minimum of 10 samples to a maximum of 30 samples will be collected from the crowns, depending on the diameters of the trees. Unfortunately, the Master's student involved with early phases of this project has decided to drop out of the program to seek other opportunities. A replacement has been found and will be trained to fulfill project objectives.

The study will be conducted in central Texas within untreated, expanding oak wilt centers on privately-owned property within the range of live oak and oak wilt in central Texas (specific locations to be determined). Non-symptomatic test trees (ca. 120), ranging from 15 to 46 cm (6 – 18 in) dbh (diameter at breast height), will be selected in proximity with trees showing oak wilt symptoms (veinal necrosis). In July and August, 2016, a minimum of forty (40) trees per delivery system will be injected with Alamo® (Syngenta) or Propizol™ (Arborjet) at the label rate (20 ml/inch tree dbh) using the two systems described above. Forty (40) trees (5 trees per study site) will serve as untreated controls. The application procedure used to inject the propiconazole formulation will be based on the recommendations of each system manufacturer. The injected trees will be selected according to proximity to symptomatic trees naturally infected with *Ceratocystis fagacearum*. All of the injected or check trees will be located adjacent to the infected trees, at a distance of at least 50 - 75 ft but exhibiting no symptoms of oak wilt infection. The treatment will therefore be tested under conditions of natural infection with the pathogen.

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Trees will be evaluated for oak wilt symptoms after one, six, twelve and eighteen months. Each oak crown will be 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 sampled from the stem and branches to determine the presence of *Ceratocystis fagacearum*.

Activities Completed

The numbers of injected trees (n=38) and uninjected control trees (n=31) located in 8 study plots for each treatment type can be seen in Table 1 below. Table 1 also contains the dates of injection. All plots have been surveyed for spread of the pathogen into treated trees, but no injected trees or uninjected control trees have developed symptoms of infection during the reporting period.

Table 1. Plots, dates, and numbers of trees treated with the two injection systems and the uninjected control trees (near Pipe Creek, TX).

Plot No.	Date	Micro Injections (no. trees)	Macro Injections (no. trees)	Uninjected Controls (no. trees)
1	6/16/2016	2	2	4
2	6/23/2016	3	2	4
3	6/24/2016, 7/7/2016	2	2	2
4	7/7/2016	3	3	4
5	7/7/2016,7/8/2016	3	3	6
6	7/8/2016	2	2	2
7	7/8/2016	1	0	5
8	9/23/2016	4	4	4

At the termination of the experiment in June 2018, final crown ratings will be made. An analysis of variance will be used to test for differences among injection systems. 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 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. The test will be invalidated if fewer than 60% of the control trees reach a crown rating of 2.

Research Timetable:

<u>October - December, 2016</u>

- Monitor for tree decline (October)
- Sample infected trees to confirm presence of *Ceratocystis fagacearum*.
- Conduct statistical analyses of data (Novembe

CY 2017

<u>April - December, 2017</u>

- Monitor for tree decline (April - October)
- Sample infected trees to confirm presence of *Ceratocystis fagacearum*.
    - If sufficient nubers of trees are not infected by natural spread of the oak wilt fungus, inoculate the study trees to ensure they are challenged by the oak wilt fungus (June, 2017)
- Prepare and submit progress report to US Forest Service (October).

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<u>April – September, 2018</u>

- Conduct final evaluation of treated and check trees (June)
- Sample infected trees to confirm presence of *Ceratocystis fagacearum*.
- Conduct statistical analyses of data (July)
- Prepare and submit final report to U. S. Forest Service (September)
- Present final results at annual International Society of Arboriculture meeting (September).

In summary, the project is progressing on schedule. In case an insufficient number of injected and check trees are challenged by natural spread of the fungus as of June, 2017, treatment trees will be artificially inoculated with spores of *Ceratacystis fagaceaurm* to complete the objectives of this project.

A B

Figure 1: Injecting live oaks with the Tree I.V. microinjection system (A) and the standard macroinjection system (B) to compare ease of application and efficacy for preventing oak wilt infection. Pipe Creek, Texas. November 2016.

Final Words from Coordinator Ron Billings

Following 21 years of successful operation, the Forest Pest Management Cooperative has been disbanded. The Forest Pest Management Cooperative was truly a team effort and I take this opportunity to recognize the many employees and others who have made the FPMC a success over the years. Particular appreciation is due to our two previous coordinators, Dr. Don Grosman (1996-2012) and Dr. Melissa Fisher (2013-2014). Without their leadership, dedication and vision, the FPMC would not have been so successful in addressing forest pest problems and meeting the needs of members. Of course, they were ably assisted by an experienced field staff over the years which included William Upton, Jeff Anderson, Frank McCook, Jason Helvey, Billi Kavanagh, Allen Smith, Amanda Zumwalt, Larry Spivey, and numerous seasonal workers. I thank Allen Smith for devoting part of his busy schedule to overseeing research activities since February, 2015, and for providing statistical analyses of research data. Several TFS Forest Health personnel helped out as needed with FPMC field and laboratory studies. These included Joe Pase, Michael Murphrey, and Aleksandar Dozic. Outstanding office support in Lufkin has been provided since 1996 by a succession of staff assistants: Martha Johnson, Cathy Wallace, Harold Read and Patricia Faries. I also appreciate the administrative support provided at TFS headquarters in College Station by Directors Bruce Miles, Jim Hull, and Tom Boggus, Forest Resource Development Department Heads Ed Barron and Bill Oates, and budget gurus Carrie Chesbro, Sharon Klinker and Travis Zamzow.

Finally, the FPMC would not have functioned or persisted for more than two decades without the support of recent and past members. The many forest industries and organizations which elected to be members over the years provided not only financial support but also frequent professional guidance for research projects. I have enjoyed serving as Administrative Coordinator (1996-2014) and Coordinator (2015-2017) and thank all of the above for their contributions to the FPMC.