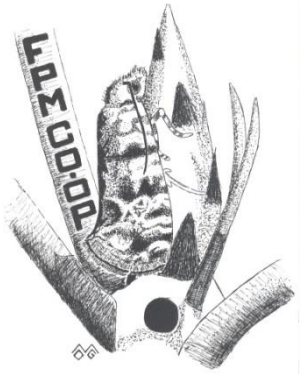
Forest Pest Management Cooperative



2013 Research Proposals

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2013 FPMC Members

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US Forest Service/FHP

March 2014

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SilvaShield™ Operational Treatment of Loblolly Pine Seedlings After Planting for Control of Pine Tip Moth

(Initiated in 2008)

Cooperators

Dr. Nate Royalty — Bayer Environmental Science, Research Triangle Park, NC

Objectives:

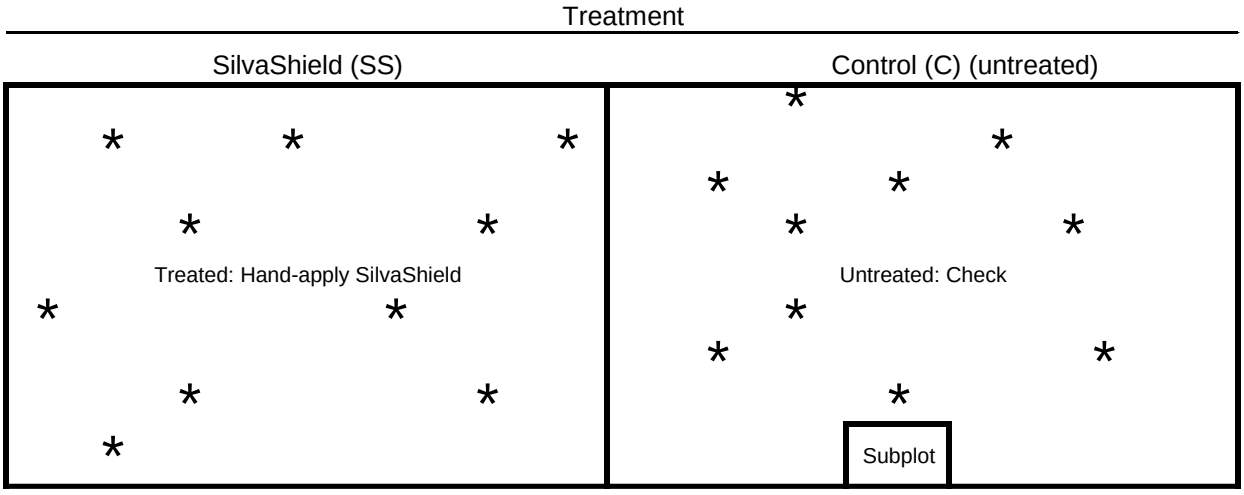
The objectives of this research proposal are to 1) determine the efficacy of SilvaShield™ tablets in reducing area-wide pine tip moth infestation levels on loblolly pine seedlings; 2) evaluate this product applied after planting to bedded or unwedded areas; and 3) determine the duration of protection provided by this insecticide application.

Justification: The Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock) (Lepidoptera: Tortricidae), is a serious pest in young pine plantations of the southeastern United States. Foliar applications of Pounce®, Warrior T®, dimethoate, and Mimic® have proven effective in reducing volume losses by this insect. However, there are several concerns about the use of insecticides in commercial forests, including cost effectiveness, public perceptions, and impact on nontarget organisms, including biological control agents. We propose to evaluate the efficacy and duration of SilvaShield™ (imidacloprid + fertilizer) tablets applied to the soil reducing volume losses caused by pine tip moth in first and second-year pine seedlings.

Research Approach:

A single family of loblolly pine containerized seedlings will be selected from the cooperator's nursery, Magnolia, AR. They are expected to be available for planting in November.

One recently-planted tract, and one one-year old tract, each 80 acres in size, will be selected in Texas or Louisiana based on uniformity of soil, drainage, topography and susceptibility to tip moth infestation (based on FPMC Tip Moth Hazard-Rating Model, Andy Burrow, Temple-Inland Forest Products.



Main treatment plots = 40 acres each; Internal treatment subplots = 0.5 acres each; ten 10-tree plots (*) evenly spaced within each main plot

Figure 1. Generalized Plot Design

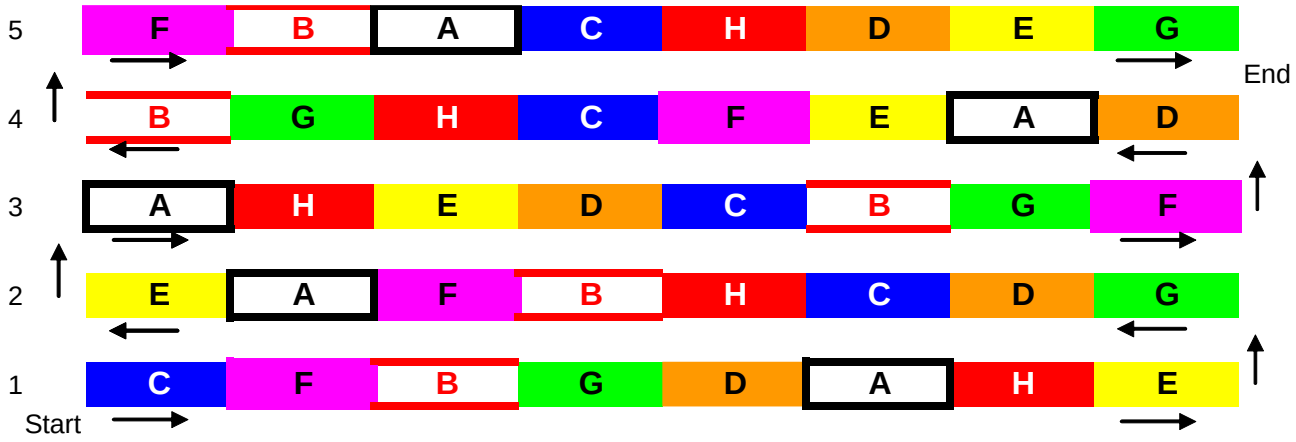
Treatments:

Main Plot (40 acres each)

1) SilvaShield™ (one tablet) applied after planting next to each seedling to a depth of 8 inches.
2) Check –seedlings planted by hand

Sub-plot (0.5 acres)

3) Check
4) SilvaShield™ (one tablet) applied after planting next to each seedling to a depth of 4 inches.
1) SilvaShield™ (two tablets) applied after planting next to each seedling to a depth of 4 inches.
2) SilvaShield™ (three tablets) applied after planting next to each seedling to a depth of 4 inches.
3) SilvaShield™ (one tablet) applied after planting next to each seedling to a depth of 8 inches.
4) SilvaShield™ (two tablets) applied after planting next to each seedling to a depth of 8 inches.
5) SilvaShield™ (three tablets) applied after planting next to each seedling to a depth of 8 inches.
6) SilvaShield™ (one tablet) applied at planting in plant hole with seedling (depth of ~8 inches).



A = White (Check)
B = Rd&Wht (1 tablet after planting 4"
C = Blue (2 tablet after planting 4")
D = Orange (3 tablets after planting 4")
E = Yellow (1 tablet after planting 8")
F= Pink (2 tablets after planting 8")
G = Green (3 tablets after planting 8")
H = Red (1 tablet in plant hole 8")

Figure 2. Randomized Block Design Layout for a Treatment Trial.

To evaluate the effects of treatment on large area tip moth damage levels a randomized complete block design, with sites as blocks, will be used. Each plantation will be hand or machine-planted. On one half of the plantation, the applicator will apply one SilvaShield™ tablet to each seedling after planting (Figure 1.). A lance will be used to create a 4 inch deep hole in the soil, angled toward the seedling. The tablet is then dropped into the hole and covered up. In the other half of the plantation, seedlings will be hand or machine planted at the same spacing.

Additionally, 0.75 acre subplot will be installed within check main treatment plot. Each treatment will be randomly assigned to ten trees on each of five rows (Fig 2).

Ten 10-tree plots will be spaced equally within each main plantation half (but outside the internal treatment plots) to evaluate tip moth damage levels in these area. A 50-tree plot will be positioned within each internal treatment subplot to evaluate tip moth damage levels in these areas. All stands will be treated with herbicide after planting to minimize herbaceous and/or woody competition.

Tip moth damage will be 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 will be calculated; and 3) separately, the terminal will be identified as infested or not. Observations also will be made as to the occurrence and extent of damage caused by other insects, i.e., coneworm, aphids, sawfly, etc. Each tree will be measured for diameter (at ground line) and height and ranked as to form in the fall (November) of the second year following planting. Form ranking of the seedling or tree will be categorized as follows: 0 = no forks; 1 = one fork; 2 = two to four forks; 3 = five or more forks. A fork is defined as a node with one or more laterals larger than one half the diameter of the main stem (Berisford and Kulman 1967).

Efficacy will be evaluated by comparing treatment differences for direct and indirect measures of insect-caused losses. Direct treatment effects include reduction in pine tip moth damage. Indirect treatment effects include increases in tree growth parameters (height, diameter and volume index). Data will be subjected to analyses of variance using Statview software (SAS Institute, Inc. 1999). Percentage and measurement data will be transformed by the arcsine % and log transformations, respectively, prior to analysis. Costs of treatment per acre also will be calculated.

If one or more treatments continue to be successful in reducing tip moth damage by > 75% in the 4th generation in 2009, the "best" treatment(s) will be followed into 2010 to continue evaluating duration of treatments.

Research Time Line:

CY2013

January – February 2013

- Begin trap monitoring of tip moth populations near each site

November - December 2013

- Measure diameter and height of seedlings.
- Conduct statistical analysis of data.
- Prepare and submit report to FPMC Executive Committee and Bayer Crop Science.

PTM™ and SilvaShield™ Comparison Trial

(Initiated in 2010)

Justification

Both fipronil (PTM™, BASF) and imidacloprid (SilvaShield™ Forestry Tablets, Bayer Environmental Science) have been proven effective in protecting pine seedlings against pine tip moth. A few cursory comparisons between these two products have been made in the past. We are interested in a more formal comparison in the Western Gulf region.

Objectives: 1) Evaluate the efficacy of PTM™ and SilvaShield™ Forestry tablets in reducing pine tip moth infestation levels on loblolly pine seedlings; 2) evaluate these products applied at different rates and timing to seedlings; and 3) determine the duration of treatment activity.

Cooperators

Mr. Ragan Bounds	Hancock Forest Management, Silsbee, TX
Mr. Tom Macom	Bayer Environmental Science, Research Triangle Park, NC
Mr. Bruce Monkey	Bayer Environmental Science, Waco, TX

Insecticides:

PTM™ (fipronil) –

SilvaShield™ Forestry tablet (Imidacloprid + fertilizer) – highly systemic neonictinoid with activity against Lepidoptera and fertilizer with NPK ratio of 12:9:4.

Research Approach:

A recently-harvested tract, 121 acres in size and owned by The Campbell Group, was selected NW of Jasper, TX (Jasper Co.).

Fifty seedlings for each treatment (A – O, see below) will be hand planted (standard spacing 8' X 8') on a first-year plantation site. The site has received an intensive site preparation and the soil was disked. A randomized complete block design will be used with beds or site areas serving as blocks, i.e., each treatment will be randomly selected for placement along a bed. Ten seedlings from each treatment will be planted on each of five beds. Treatments A, D, F, H, K & M will be applied as the seedling is planted. Just after seedling transplant, Treatments B, G, I, & N will be applied (pushed into the soil 4" deep and 2 cm from each assigned seedling [SS] or poured into one 4" deep probe hole near each seedling [PTM]. For treatments C, D, J & K, one tablet or solution will be applied to each seedling in Fall 2010. The remaining treatments (E,F,G, L, M & N) will be applied in early Spring 2011.

Treatments and Layout

Code	Treatment	Color
A	PTM in plant hole at planting (Dec. '09)	red
B	PTM post plant at 1 pt next to seedling (Dec. '09)	blue
C	PTM post plant at 2 pt next to seedling (Sep. '10)	orange
D	PTM at planting + PTM post plant (2 pts, Sep. '10)	pink/blue
E	PTM post plant at 2 pt next to seedling (Feb. '11)	white
F	PTM at planting + PTM post plant (2 pts, Feb. '11)	red/white
G	PTM post plant (1 pt, Dec. '09) + PTM post plant (2 pts, Feb. '11)	yellow/blue
H	SS in plant hole at planting (Dec. '09)	yellow
I	SS post plant next to seedling (Dec. '09)	green
J	SS post plant next to seedling (Sep. '10)	pink
K	SS at planting + SS post plant (Sep. '10)	blue/white
L	SS post plant next to seedling (Feb. '10)	green/orange
M	SS at planting + SS post plant (Feb. '11)	yellow/green
N	SS post plant (Dec. '09) + PTM post plant (Feb. '11)	blue/red
O	Check (lift and plant bare root seedlings)	green/white

Bed 1	Bed 2	Bed 3	Bed 4	Bed 5
J	G	L	I	K
E	H	E	O	E
F	J	C	H	I
L	E	H	G	O
A	C	J	E	H
N	B	M	M	A
K	L	B	B	F
O	F	F	K	M
B	M	A	A	N
D	I	K	C	C
G	A	D	N	G
C	N	I	F	J
I	D	G	L	D
M	K	O	D	B
H	O	N	J	L

Treatment description:

1) PTM solution (1.4ml product in 13.6 ml water) applied into plant hole at planting (Dec. ’09).
2) PTM solution (1.4ml product in 13.6 ml water) applied post plant at 1 point next to seedling (Dec. ’09).
3) PTM solution (0.7ml product in 14.3 ml water) applied post plant at 2 points next to seedling (Sept. ’10).
4) PTM solution (1.4ml product in 13.6 ml water) applied to plant hole at planting (Dec. ’09) and (0.7ml product in 14.3 ml water) applied post plant at 2 points next to seedling (Sept. ’10).
5) PTM solution (0.7ml product in 14.3 ml water) applied post plant at 2 points next to seedling (Feb. ’11).
6) PTM solution (1.4ml product in 13.6 ml water) applied to plant hole at planting (Dec. ’09) and (0.7ml product in 14.3 ml water) applied post plant at 2 points next to seedling (Feb. ’11).
7) PTM solution (1.4ml product in 13.6 ml water) applied post plant at 1 point next to seedling (Dec. ’09) and (0.7ml product in 14.3 ml water) applied post plant at 2 points next to seedling (Feb. ’11).
8) SilvaShield (SS) (1 tablet) applied into plant hole at planting (Dec. ’09).
9) SS (1 tablet) applied post plant next to seedling (Dec. ’09).

10) SS (1 tablet) applied post plant next to seedling (Sept. ’10).
11) SS (1 tablet) applied into plant hole at planting (Dec. ’09) and SS (1 tablet) applied post plant next to seedling (Sept. ’10).
12) SS (1 tablet) applied post plant next to seedling (Feb. ’11).
13) SS (1 tablet) applied to plant hole at planting (Dec. ’09) and SS (1 tablet) applied post plant next to seedling (Feb. ’11).
14) SS (1 tablet) applied post plant next to seedling (Dec. ’09) and SS (1 tablet) applied post plant next to seedling (Feb. ’11).
15) Check –seedlings planted by hand without additional treatment.

Treatment Evaluation: Tip moth damage will be 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 will be calculated; and 3) separately, the terminal will be identified as infested or not. Observations also will be made as to the occurrence and extent of damage caused by other insects, i.e., aphids, weevils, coneworm, etc. Second-year trees will be measured for diameter and height (at 6”) in the fall (November) following planting. If warranted, third-year trees will be measured for height and diameter (at DBH) and ranked for form. Form ranking of the seedling or tree will be categorized as follows: 0 = no forks; 1 = one fork; 2 = two to four forks; 3 = five or more forks. A fork is defined as a node with one or more laterals larger than one half the diameter of the main stem (Berisford and Kulman 1967).

Tip Moth Damage Assessment or Tree Measurement Times for Jasper Co., TX site:
Generation 1: week of April 27
Generation 2: week of June 22
Generation 3: week of August 10
Generation 4: week of September 21
Generation 5: November 15 – December 31

Efficacy will be evaluated by comparing treatment differences for direct and indirect measures of insect-caused losses. Direct treatment effects include reduction in pine tip moth damage. Indirect treatment effects include increases in tree growth parameters (height, diameter and volume index). Data will be
subjected to analyses of variance using Statview software (SAS Institute, Inc. 1999). Percentage and measurement data will be transformed by the arcsine % and log transformations, respectively, prior to analysis.

Research Time Line:

CY 2013

January - February 2013

- Begin trap monitoring of tip moth populations near each site

November - December 2013

- Measure seedling and height of seedlings.
- Conduct statistical analysis of 2013 data.
- Prepare and submit report to BASF and Bayer, FPMC Executive Committee.

Reference:

Berisford, C.W., and H.M. Kulman. 1967. Infestation rate and damage by the Nantucket pine tip moth in six loblolly pine stand categories. For. Sci. 13: 428-438.

Fettig, C.J., J.T. Nowak, D.M. Grosman and C.W. Berisford. 2003. Nantucket pine tip moth phenology and timing of insecticide spray applications in the Western Gulf region. USDA Forest Service So. Res. Stat. Res. Pap. SRS-32. 13pp.

Evaluation of PTM™ Treatments for Containerized Pine Seedlings

(Initiated in 2010)

Justification

Several FPMC trials (2003 - 2005) showed that fipronil applied to bare root seedlings before or after planting was highly effective in reducing tip moth damage for 2+ years. Operationally, it would be desirable to apply chemical solutions to containerized seedlings because of these trees have higher value, it would be more economical to treat large numbers of seedlings in the nursery, and there may be less restriction on the amount of active ingredient that could be applied to each seedling.

A trial was initiated in 2006 to determine the efficacy of fipronil applied at different rates to containerized seedling. Seedlings were treated in July 2006 and outplanted in February 2007. Tip moth damage and tree growth were monitored through 2009. The results showed that again fipronil provided excellent protection against tip moth for 2+ years and improved tree volume growth by 21 to 63% compared to untreated checks.

Based on discussion at the PTM Strategy meeting on July 21, 2010, BASF is willing to support the development of a container plug injection system that would eliminate he Environmental Protection Agency (EPA) concerns about 1) movement of the active ingredient (AI, fipronil) out of containers during periodic watering in the nursery and 2) reduce exposure of handlers and planters to the AI when packaging and planting seedlings, respectively. It is of interest to evaluate the efficacy and duration of plug injection treatment of containerized seedlings.

Objectives: 1) Evaluate techniques for application of PTM™ (fipronil) to containerized seedling in the nursery; 2) evaluate efficacy of PTM™ (fipronil) applied to containerized and bareroot seedlings for reducing pine tip moth infestation levels; and 3) determine the duration of chemical activity.

Cooperators

George Lowerts, Keith Byrd	ArborGen LLC
Bill Stansfield, Rick Leeper	The Campbell Group
Jim Bean, Andy Goetz, Victor Canez	BASF
Nick Muir	Cellfor Inc.
Ragan Bounds	Hancock Forest Management
Wayne Bell, Mike Coyle, Chris Rosier	International Forestry Co
James West	North Carolina Forest Service
Alan Wilson, Greg Leach	Rayonier
Tony Fontenot, Wilson Edwards,	Weyerhaeuser Co.

Research Approach:

One family of loblolly pine containerized seedlings will be selected by (Cellfor).

Treatments**:**

1 = PTM™ High Concentration/Undiluted Plug Injection [5.6 ml PTM undilute/seedling (**110 tpa rate**)] - Injection into **container** seedling plug just prior to shipping.

2 = PTM™ High Concentration/Diluted Soil Injection [5.6 ml PTM in 9.4 ml water (15 ml total volume)/seedling] - Soil injection next to transplanted **container** plug just after planting.
3 = PTM™ High Concentration/Diluted Soil Injection [5.6 ml PTM in 9.4 ml water (15 ml total volume)/seedling] - Soil injection next to transplanted **bareroot** just after planting.
4 = PTM™ Mid Concentration/Undiluted Plug Injection [1.4 ml PTM undilute/seedling (**435 tpa rate**)] - Injection into **container** seedling plug just prior to shipping.
5 = PTM™ Mid Concentration/Diluted Plug Injection [1.4 ml PTM in 1.7 ml water (3ml total volume)/seedling] -Injection into **container** seedling plug just prior to shipping.
6 = PTM™ Mid Concentration/Diluted Soil Injection [1.4 ml PTM in 13.6 ml water (15 ml total volume)/seedling] - Soil injection next to transplanted **container plug** just after planting.
7 = PTM™ Mid Concentration/Diluted Soil Injection [1.4 ml PTM in 13.6 ml water (15 ml total volume)/seedling] - (**Standard 1**) Soil injection next to transplanted **bareroot** just after planting.
8 = PTM™ Low Concentration/Undiluted Plug Injection [1 ml PTM undilute/seedling (**600 tpa rate**)] - Injection into **container** seedling plug just prior to shipping.
9 = PTM™ Low Concentration/Diluted Plug Injection [1 ml PTM in 2 ml water (3ml total volume)/seedling] - Injection into **container** seedling plug just prior to shipping.
10 = PTM™ Low Concentration/Diluted Soil Injection [1 ml PTM in 14 ml water (15ml total volume)/seedling] - Soil injection next to transplanted **container plug** just after planting..
11 = PTM™ Low Concentration/Diluted Soil Injection [1 ml PTM in 14 ml water (15ml total volume)/seedling] - (**Standard 2**) Soil injection next to transplanted **bareroot** just after planting..
12 = Containerized Check (untreated)
13 = Bareroot Check (untreated)

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

Ten recently-harvested tracts will be selected in fall 2010 across the southeastern United States (TX, LA, AR, MS, GA, FL and NC) based on uniformity of soil, drainage and topography.

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

All stands will have been intensively site prepared, i.e., subsoil, bedding, and/or herbicide. A 1-acre (approximate) area within each site will be selected. A multiple Latin Square design will be established with single tree plots (1 tree X 13 treatments) serving as blocks, i.e., each treatment will be randomly selected for placement along a row (beds). Thirty-nine (39) blocks will be established on each site. Seedlings will be planted at 8 foot spacing along each row. Individual tree locations will be marked with different color pin flags prior to tree planting.

The plot corners should be marked with PVC pipe (1 at each end of the plot) and metal tags. It may be necessary to apply herbicide over the area in the spring to ensure that the seedlings remain exposed to tip moth attack throughout the year.

Damage and Tree Measurements

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

Efficacy will be evaluated by comparing treatment differences for direct and indirect measures of insect-caused losses. Direct treatment effects include reduction in pine tip moth damage. Indirect treatment effects include increases in tree growth parameters (height, diameter and volume index). Data will be subjected to analyses of variance (Table 3) using Statview software (SAS Institute, Inc. 1999). Percentage and measurement data will be transformed by the arcsine % and log transformations, respectively, prior to analysis. Costs of treatment per acre also will be calculated.

If one or more treatments continue to be successful in reducing tip moth damage by > 75% in the 4th generation in 2011, the "best" treatment(s) will be followed into 2012 to continue evaluating duration of treatments. In addition, the study may be expanded in 2012 to refine application rates and techniques for the promising treatment(s).

Treatments and Plot Design Example

Code	Treatment	Color	
A	High UD PTM container plug injection	red	R
B	High D PTM container soil injection	blue	B
C	High D PTM bareroot soil injection	orange	O
D	Med UD PTM container plug injection	pink/blue	P/B
E	Med D PTM container plug injection	white	W
F	Med D PTM container soil injection	red/white	R/W
G	Med D PTM bareroot soil injection (Standard 1)	yellow/blue	Y/B
H	Low UD PTM container plug injection	yellow	Y
I	Low D PTM container plug injection	green	G
J	Low D PTM container soil injection	pink	P
K	Low D PTM bareroot soil injection (Standard 2)	blue/white	B/W
L	Check (containerized)	green/orange	G/O
M	Check (bareroot))	blue/red	B/R

UD = undilute; D = dilute

	Block 1													Block 2						
Tree	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1	G	G	E	L	D	I	A	E	A	B	A	J	H	I	I	J	G	M	J	B
2	L	F	B	D	H	H	J	G	G	F	D	B	M	K	J	A	E	I	E	L
3	K	B	C	E	E	M	H	H	D	I	E	G	K	L	E	F	I	J	B	C
4	M	E	K	J	I	E	E	A	F	L	J	D	D	H	G	I	F	A	I	H
5	D	A	F	A	F	B	C	J	H	G	F	E	F	A	A	C	M	H	A	D
6	A	K	I	G	G	C	K	L	B	E	B	M	J	B	C	L	J	L	C	A
7	F	J	M	K	A	A	G	D	K	C	M	L	I	F	K	B	K	F	M	I
8	J	I	J	C	M	K	F	F	M	M	I	C	B	C	B	E	B	K	L	E
9	H	C	L	H	C	L	D	K	I	K	H	K	L	M	M	H	C	D	D	F
10	I	L	A	F	J	J	B	I	E	D	K	H	A	D	H	K	A	B	F	K
11	E	H	H	M	L	F	M	C	C	H	L	A	C	G	L	D	L	C	H	G
12	C	D	G	B	B	G	L	M	J	A	C	F	E	E	F	G	D	E	K	J
13	B	M	D	I	K	D	I	B	L	J	G	I	G	J	D	M	H	G	G	M

	Block 2						Block 3												
Tree	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
1	M	J	C	H	K	A	H	M	C	D	M	I	I	G	B	B	E	I	G
2	H	F	B	L	B	M	C	G	B	J	H	M	C	K	F	K	B	H	E
3	B	M	F	M	F	B	A	F	K	A	B	E	A	F	H	I	G	M	D
4	G	B	M	K	G	J	J	I	A	B	F	H	E	B	L	F	F	C	A
5	I	A	A	F	H	F	G	D	D	L	A	L	B	J	A	A	L	B	K
6	J	E	I	E	L	L	E	H	J	H	K	B	J	E	K	G	A	G	L
7	C	L	G	B	C	H	I	E	H	I	C	J	F	D	I	L	M	K	C
8	A	G	J	I	E	D	D	A	I	G	E	G	G	C	J	E	K	F	J
9	L	K	H	C	A	K	B	B	F	K	D	D	L	M	E	D	J	D	H
10	K	H	K	G	I	C	M	L	E	C	G	F	M	A	D	J	C	J	F
11	D	I	E	A	J	E	K	C	G	F	L	K	K	H	C	M	I	A	B
12	E	C	D	J	D	G	F	K	M	E	J	A	D	I	G	C	H	L	M
13	F	D	L	D	M	I	L	J	L	M	I	C	H	L	M	H	D	E	I

Tree	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	Y/B	Y/B	W	G/O	P/B	G	R	W	R	B	R	P	Y	G	G	P	Y/B	B/R	P	B
2	G/O	R/W	B	P/B	Y	Y	P	Y/B	Y/B	R/W	P/B	B	B/R	B/W	P	R	W	G	W	G/O
3	B/W	B	O	W	W	B/R	Y	Y	P/B	G	W	Y/B	B/W	G/O	W	R/W	G	P	B	O
4	B/R	W	B/W	P	G	W	W	R	R/W	G/O	P	P/B	P/B	Y	Y/B	G	R/W	R	G	Y
5	P/B	R	R/W	R	R/W	B	O	P	Y	Y/B	R/W	W	R/W	R	R	O	B/R	Y	R	P/B
6	R	B/W	G	Y/B	Y/B	O	B/W	G/O	B	W	B	B/R	P	B	O	G/O	P	G/O	O	R
7	R/W	P	B/R	B/W	R	R	Y/B	P/B	B/W	O	B/R	G/O	G	R/W	B/W	B	B/W	R/W	B/R	G
8	P	G	P	O	B/R	B/W	R/W	R/W	B/R	B/R	G	O	B	O	B	W	B	B/W	G/O	W
9	Y	O	G/O	Y	O	G/O	P/B	B/W	G	B/W	Y	B/W	G/O	B/R	B/R	Y	O	P/B	P/B	R/W
10	G	G/O	R	R/W	P	P	B	G	W	P/B	B/W	Y	R	P/B	Y	B/W	R	B	R/W	B/W
11	W	Y	Y	B/R	G/O	R/W	B/R	O	O	Y	G/O	R	O	Y/B	G/O	P/B	G/O	O	Y	Y/B
12	O	P/B	Y/B	B	B	Y/B	G/O	B/R	P	R	O	R/W	W	W	R/W	Y/B	P/B	W	B/W	P
13	B	B/R	P/B	G	B/W	P/B	G	B	G/O	P	Y/B	G	Y/B	P	P/B	B/R	Y	Y/B	Y/B	B/R

replicate

Tree	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1	B/R	P	O	Y	B/W	R	Y	B/R	O	P/B	B/R	G	G	Y/B	B	B	W	G	Y/B
2	Y	R/W	B	G/O	B	B/R	O	Y/B	B	P	Y	B/R	O	B/W	R/W	B/W	B	Y	W
3	B	B/R	R/W	B/R	R/W	B	R	R/W	B/W	R	B	W	R	R/W	Y	G	Y/B	B/R	P/B
4	Y/B	B	B/R	B/W	Y/B	P	P	G	R	B	R/W	Y	W	B	G/O	R/W	R/W	O	R
5	G	R	R	R/W	Y	R/W	Y/B	P/B	P/B	G/O	R	G/O	B	P	R	R	G/O	B	B/W
6	P	W	G	W	G/O	G/O	W	Y	P	Y	B/W	B	P	W	B/W	Y/B	R	Y/B	G/O
7	O	G/O	Y/B	B	O	Y	G	W	Y	G	O	P	R/W	P/B	G	G/O	B/R	B/W	O
8	R	Y/B	P	G	W	P/B	P/B	R	G	Y/B	W	Y/B	Y/B	O	P	W	B/W	R/W	P
9	G/O	B/W	Y	O	R	B/W	B	B	R/W	B/W	P/B	P/B	G/O	B/R	W	P/B	P	P/B	Y
10	B/W	Y	B/W	Y/B	G	O	B/R	G/O	W	O	Y/B	R/W	B/R	R	P/B	P	O	P	R/W
11	P/B	G	W	R	P	W	B/W	O	Y/B	R/W	G/O	B/W	B/W	Y	O	B/R	G	R	B
12	W	O	P/B	P	P/B	Y/B	R/W	B/W	B/R	W	P	R	P/B	G	Y/B	O	Y	G/O	B/R
13	R/W	P/B	G/O	P/B	B/R	G	G/O	P	G/O	B/R	G	O	Y	G/O	B/R	Y	P/B	W	G

Table 1. ANOVA Table and Expected Mean Squares for Fipronil Treatment Study

Source of Variation	df	Expected Mean Squares
Blocks (B)	r-1	
Treatments (T)	t-1	$\sigma^2_\varepsilon + rm\sigma^2_B$
BxT	(b-1) (t-1)	$\sigma^2_\varepsilon + m\sigma^2_{BT}$
Sampling error	rt (m-1)	σ^2_ε
Total	rtm-1	

Research Time Line:

CY 2010

June – September 2010

- Meeting with cooperators to discuss treatment options (June)
- Develop treatment techniques

November - December 2010

- Select research sites (November)
- Treat seedlings (December)
- Lift and plant all seedlings in plantation sites (December)
- Treat seedlings during and after planting with PTM via soil injection
- Begin trap monitoring of tip moth populations near each site

CY 2011

January - February 2011

- Continue trap monitoring of tip moth populations near each site

March - October, 2011

- Evaluate tip moth damage after 1st through 4th generations; photograph damage.

November - December 2011

- Evaluate tip moth damage after 5th generations; measure seedling and height of seedlings.
- Conduct statistical analysis of 2011 data.
- Prepare and submit report to FPMC Executive Committee, BASF.

CY 2012

January - February 2012

- Begin trap monitoring of tip moth populations near each site

March - October, 2012

- Evaluate tip moth damage after 1st through 4th generations; photograph damage.

November - December 2012

- Evaluate tip moth damage after 5th generations; measure seedling and height of seedlings.
- Conduct statistical analysis of 2012 data.
- Prepare and submit report to FPMC Executive Committee, BASF.

CY 2013

January - February 2013

- Begin trap monitoring of tip moth populations near each site

March - October, 2013

- Evaluate tip moth damage after 1st through 4th generations; photograph damage.

November - December 2013

- Evaluate tip moth damage after 5th generations; measure seedling and height of seedlings.
- Conduct statistical analysis of 2013 data.
- Prepare and submit report to FPMC Executive Committee, BASF.

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

(Initiated in 2012)

Justification

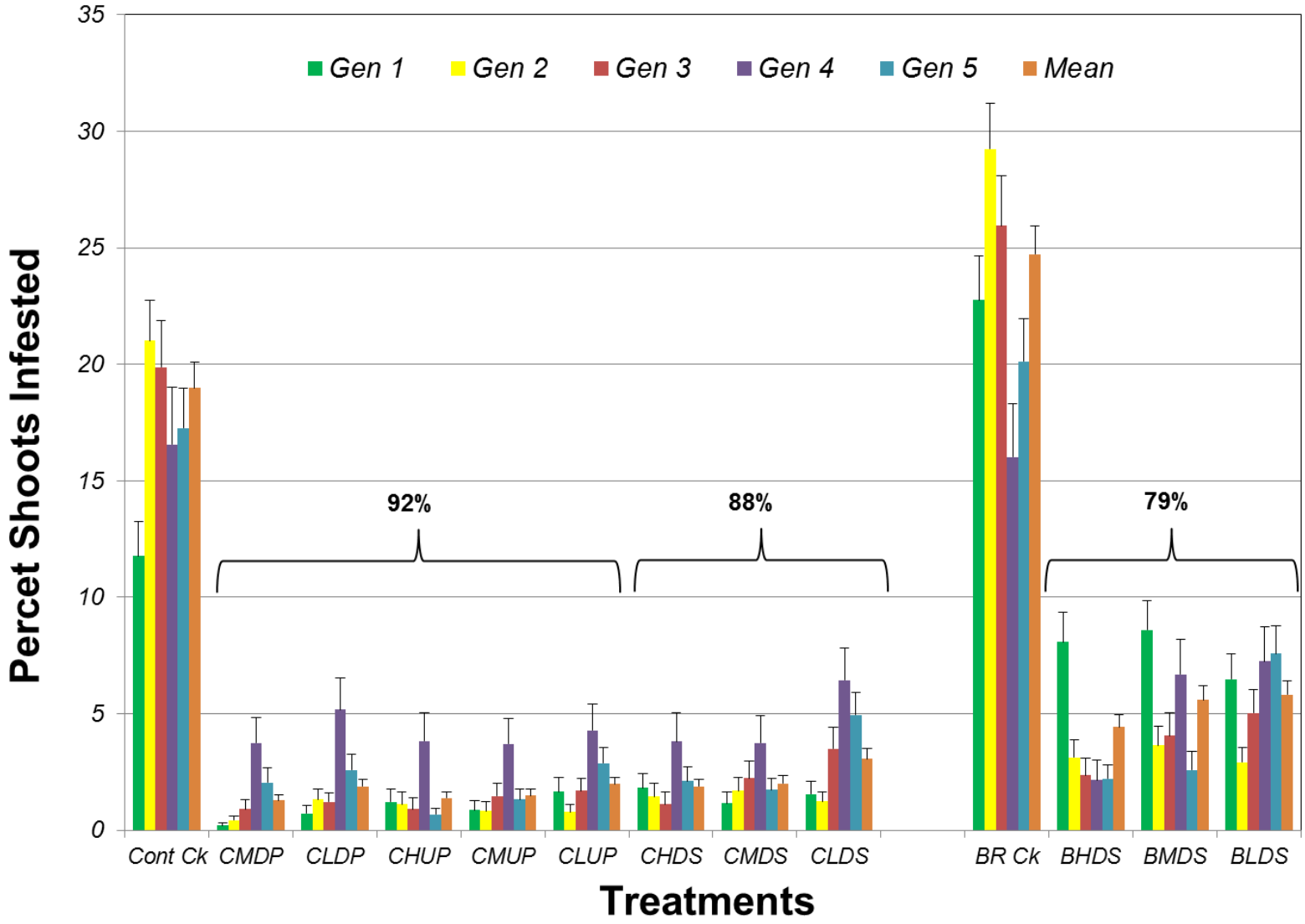
Several FPMC trials (2003 - 2005) showed that fipronil (PTM™) applied to bare root seedlings before or after planting was highly effective in reducing tip moth damage for 2+ years. Operationally, it would be desirable to apply chemical solutions to containerized seedlings because these trees have higher value, and it would be more economical to treat large numbers of seedlings in the nursery.

A trial was initiated in 2006 to determine the efficacy of fipronil applied at different rates to containerized seedling. Seedlings were treated in July 2006 and outplanted in February 2007. Tip moth damage and tree growth were monitored through 2009. The results showed that again fipronil provided excellent protection against tip moth for 2+ years and improved tree volume growth by 21 to 63% compared to untreated checks.

Based on discussion at the PTM Strategy meeting on July 21, 2010, BASF is willing to support the development of a container plug injection system that would eliminate the Environmental Protection Agency (EPA) concerns about 1) movement of the active ingredient (AI, fipronil) out of containers during periodic watering in the nursery and 2) reduce exposure of handlers and planters to the AI when packaging and planting seedlings, respectively. A containerized plug injection system is being developed by S&K Designs (Stewart Boots) to allow treatment of seedlings in the nursery. A prototype should be available for testing in December 2011.

In the meantime, it was of interest to evaluate the efficacy and duration of plug injection treatment (applied by hand) to containerized seedlings. A trial initiated in 2011 thus far (through the 5^{th} generation) shows that hand treatment of seedling plugs prior to planting provides somewhat better protection compared to container seedlings treated after planting and significantly better protection compared to bareroot seedlings treated after planting (Figure 1).

Pyraclostrobin (Insignia®SC) belongs to the strobilurin class of fungicides. In addition to excellent, broad-spectrum disease control, research has shown pyraclostrobin-based fungicides also provide additional plant health benefits. Pyraclostrobin-based fungicides control foliar fungal diseases by inhibiting respiration in the mitochondria of fungi. This inhibition prevents the breakdown of energy-rich carbon compounds the fungus needs to produce energy for growth. Pyraclostrobin-based fungicides also have activity on plant mitochondria and reduce respiration in the plant. Since the plant's primary source of energy comes from sunlight through photosynthesis, this decrease in respiration can have a positive effect on growth. Decrease in respiration allows the plant to keep more stored carbon compounds for growth and triggers a chain reaction of positive physiological changes in the plant. These positive physiological changes may include an increase in nitrate reductase activity, elevated levels of antioxidants and



C= Containerized; B= Bareroot; L= Low rate; M= Medium rate; H= High rate; D= Dilute; U= Undilute; P= Plug injection; S= Soil injection

Figure 1. Effect of PTM™ plug and soil injection dose on tip moth infestation of containerized or bareroot loblolly pine on ten sites across the southeastern United States, 2011.

defense signaling compounds, and a decrease in the stress hormone ethylene. The combination of disease control, stress reduction, and increased growth efficiency lead to the plant health benefits observed with the use of pyraclostrobin-based fungicides as described in this report (BASF Intrinsic™ report). It is of interest to evaluate the efficacy and duration of plug injection treatment of containerized seedlings with fipronil and pyraclostrobin alone or combined.

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

Cooperators

George Lowerts, Keith Byrd	ArborGen LLC
Jim Bean, Andy Goetz, Victor Canez	BASF
Bill Stansfield, Rick Leeper	The Campbell Group
Al Lyons, Ragan Bounds	Hancock Forest Management
Wayne Bell, Chris Rosier	International Forestry Co
Steve Meeks	Meeks' Farm and Nursery
James West, Bobby Smith	North Carolina Forest Service
Doug Sharp	Plum Creek Timber Co.
Alan Wilson, Becki Stratton	Rayonier
Billy Moore, Wilson Edwards Tony Fontenot	Weyerhaeuser Co.

Research Approach:

One family of loblolly pine containerized seedlings will be selected (from ArborGen, Cellfor or IFCo).

Treatments**:**

1 = Insignia®SC Mid Concentration/Undiluted Plug Injection [4.9 ml Insignia undilute/seedling (**435 tpa rate**)] - Injection into **container** seedling plug just prior to shipping.

2 = PTM™ Mid Concentration/Undiluted Plug Injection [1.4 ml PTM undilute/seedling (**435 tpa rate**)] - Injection into **container** seedling plug just prior to shipping.

3 = PTM™ + Insignia®SC Mid Concentration/Undiluted Plug Injection [1.4 ml PTM + 4.9 ml Insignia (6.3ml total volume)/seedling] -Injection into **container** seedling plug just prior to shipping.

4 = PTM™ Low Concentration/Undiluted Plug Injection [1 ml PTM undilute/seedling (**600 tpa rate**)] - Injection into **container** seedling plug just prior to shipping.

5 = PTM™ (Low) + Insignia®SC (Mid) Concentration/Diluted Plug Injection [1 ml PTM + 4.9 ml Insignia (5.9 ml total volume)/seedling] - Injection into **container** seedling plug just prior to shipping.

6 = Insignia®SC High Concentration/Diluted Soil Injection [13 ml Insignia in 17 ml water (30 ml total volume)/seedling] - Soil injection at two points next to transplanted **bareroot** just after planting.

7 = Insignia®SC Mid Concentration/Diluted Soil Injection [4.9 ml Insignia in 25.1 ml water (30 ml total volume)/seedling] - Soil injection at two points next to transplanted **bareroot** just after planting.
8 = PTM™ Mid Concentration/Diluted Soil Injection [1.4 ml PTM in 28.6 ml water (30 ml total volume)/seedling] - Soil injection at two points next to transplanted **bareroot** just after planting.
9 = PTM™ + Insignia®SC Mid Concentration/Diluted Soil Injection [1.4 ml PTM + 4.9 ml Insignia in 23.7 ml water (30 ml total volume)/seedling] - Soil injection at two points next to transplanted **bareroot** just after planting.
10 = PTM™ Low Concentration/Diluted Soil Injection [1 ml PTM in 29 ml water (30 ml total volume)/seedling] - Soil injection next to transplanted **bareroot** just after planting.
11 = PTM™ (Low) + Insignia®SC (Mid) Concentration/Diluted Soil Injection [1 ml PTM + 4.9 ml Insignia in 25.5 ml water (30 ml total volume)/seedling] - Soil injection next to transplanted **bareroot** just after planting.
12 = Containerized Check (untreated)
13 = Bareroot Check (untreated)

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

Five (5) recently-harvested tracts will be selected in fall 2011 across the southeastern United States (most likely in TX, AR, AL, GA, and NC) based on uniformity of soil, drainage and topography.

Potential Cooperators
TX – Rayonier (Leach), Hancock (Bounds), Stansfield (Campbell Group)
AR or LA – ArborGen (Byrd), Weyerhaeuser (Edwards), Plum Creek (Fristoe)
AL – Weyerhaeuser (Birks)
FL or GA – Rayonier (Wilson, Stratton)
NC – Weyerhaeuser (Edwards), NCDENR (Smith)

All stands will have been intensively site prepared, i.e., subsoil, bedding, and/or herbicide. A 1-acre (approximate) area within each site will be selected. A triple Latin square design will be established with single tree plots (13 rows X 13 treatments) serving as blocks, i.e., each treatment will be randomly selected for placement along each row (bed). Thirty-nine (39) rows will be established on each site. Seedlings will be planted at 8 foot spacing along each row. Individual tree locations will be marked with different color pin flags prior to tree planting.

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

Damage and Tree Measurements

Tip moth damage will be 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 will be calculated; and 3) separately, the terminal will be identified as infested or not. Observations also will be made as to the occurrence and extent of damage caused by other insects, i.e., coneworm, aphids, sawfly, etc. Measurements of tree health will be collected periodically and/or at the end of each growing season. Tree health measurements include tree height and diameter; crown diameter, density and color (vigor); number and length of shoots in the top whorl, and tree survival. All study trees will be measured for height & diameter at ground line) at the beginning of the study (when seedlings are planted). Measurements also will be taken when tree growth has stopped in mid- to late November for at least the first 2 years of the study. Tree form will be evaluated at end of year 3. Form ranking of the seedling or tree will be categorized as follows: 0 = no forks; 1 = one fork; 2 = two to four forks; 3 = five or more forks. A fork is defined as a node with one or more laterals larger than one half the diameter of the main stem (Berisford and Kulman 1967).

Efficacy will be evaluated by comparing treatment differences for direct and indirect measures of insect-caused losses. Direct treatment effects include reduction in pine tip moth damage. Indirect treatment effects include increases in tree growth (height, diameter and volume index; shoot length) and health (crown density and color; nuber of shoots and tree survival) parameters. Data will be subjected to analyses of variance (Table 1) using Statview software (SAS Institute, Inc. 1999). Percentage and measurement data will be transformed by the arcsine % and log transformations, respectively, prior to analysis. Costs of treatment per acre also will be calculated.

If one or more treatments continue to be successful in reducing tip moth damage by > 75% in the 4th generation in 2012, the "best" treatment(s) will be followed into 2012 to continue evaluating duration of treatments. In addition, the study may be expanded in 2013 to refine application rates and techniques for the promising treatment(s).

Table 1. ANOVA Table and Expected Mean Squares for Fipronil Treatment Study

Source of Variation	df	Expected Mean Squares
Site (S)	s-1	
Blocks (B)	r-1	
Treatments (T)	t-1	$\sigma^2_\varepsilon + rm\sigma^2_B$
SxB	(s-1) (b-1)	$\sigma^2_\varepsilon + m\sigma^2_{SB}$
BxT	(b-1) (t-1)	$\sigma^2_\varepsilon + m\sigma^2_{BT}$
SxBxT	(s-1) (b-1) (t-1)	$\sigma^2_\varepsilon + m\sigma^2_{SBT}$
Sampling error	srt (m-1)	σ^2_ε
Total	srtm-1	

Square	1														2														3													
row/column	1	2	3	4	5	6	7	8	9	10	11	12	13		1	2	3	4	5	6	7	8	9	10	11	12	13		1	2	3	4	5	6	7	8	9	10	11	12	13
1	L	H	M	D	E	K	G	B	C	F	I	J	A		C	A	M	H	J	E	K	F	B	L	G	I	D		I	M	G	H	F	D	J	L	B	E	C	K	A
2	I	E	J	A	B	H	D	L	M	C	F	G	K		H	F	E	M	B	J	C	K	G	D	L	A	I		G	K	E	F	D	B	H	J	M	C	A	I	L
3	G	C	H	L	M	F	B	J	K	A	D	E	I		I	G	F	A	C	K	D	L	H	E	M	B	J		C	G	A	B	M	K	D	F	I	L	J	E	H
4	M	I	A	E	F	L	H	C	D	G	J	K	B		A	L	K	F	H	C	I	D	M	J	E	G	B		H	L	F	G	E	C	I	K	A	D	B	J	M
5	J	F	K	B	C	I	E	M	A	D	G	H	L		G	E	D	L	A	I	B	J	F	C	K	M	H		M	D	K	L	J	H	A	C	F	I	G	B	E
6	C	L	D	H	I	B	K	F	G	J	M	A	E		J	H	G	B	D	L	E	M	I	F	A	C	K		B	F	M	A	L	J	C	E	H	K	I	D	G
7	B	K	C	G	H	A	J	E	F	I	L	M	D		B	M	L	G	I	D	J	E	A	K	F	H	C		E	I	C	D	B	M	F	H	K	A	L	G	J
8	D	M	E	I	J	C	L	G	H	K	A	B	F		M	K	J	E	G	B	H	C	L	I	D	F	A		K	B	I	J	H	F	L	A	D	G	E	M	C
9	A	J	B	F	G	M	I	D	E	H	K	L	C		K	I	H	C	E	M	F	A	J	G	B	D	L		F	J	D	E	C	A	G	I	L	B	M	H	K
10	E	A	F	J	K	D	M	H	I	L	B	C	G		E	C	B	J	L	G	M	H	D	A	I	K	F		D	H	B	C	A	L	E	G	J	M	K	F	I
11	K	G	L	C	D	J	F	A	B	E	H	I	M		F	D	C	K	M	H	A	I	E	B	J	L	G		A	E	L	M	K	I	B	D	G	J	H	C	F
12	F	B	G	K	L	E	A	I	J	M	C	D	H		L	J	I	D	F	A	G	B	K	H	C	E	M		J	A	H	I	G	E	K	M	C	F	D	L	B
13	H	D	I	M	A	G	C	K	L	B	E	F	J		D	B	A	I	K	F	L	G	C	M	H	J	E		L	C	J	K	I	G	M	B	E	H	F	A	D

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/blue
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

Research Time Line:

CY 2012

January - February 2012

- Select research sites (January)
- Treat seedlings (January)
- Lift and plant all seedlings in plantation sites (January)
- Treat seedlings during and after planting with PTM via soil injection
- Begin trap monitoring of tip moth populations near each site

March - October, 2012

- Evaluate tip moth damage after 1st through 4th generations; photograph damage.
- Continue trap monitoring of tip moth populations near each site

November - December 2012

- Evaluate tip moth damage after 5th generations; measure seedling and height of seedlings.
- Conduct statistical analysis of 2012 data.
- Prepare and submit report to FPMC Executive Committee, BASF.

CY 2013

January - February 2013

- Begin trap monitoring of tip moth populations near each site

March - October, 2013

- Evaluate tip moth damage after 1st through 4th generations; photograph damage.

November - December 2013

- Evaluate tip moth damage after 5th generations; measure seedling and height of seedlings.
- Conduct statistical analysis of 2013 data.
- Prepare and submit report to FPMC Executive Committee, BASF.

CY 2014 (if warranted based on CY 2013 results)

January - February 2014

- Begin trap monitoring of tip moth populations near each site

March - October, 2014

- Evaluate tip moth damage after 1st through 4th generations; photograph damage.

November - December 2014

- Evaluate tip moth damage after 5th generations; measure seedling and height of seedlings.
- Conduct statistical analysis of 2014 data.
- Prepare and submit report to FPMC Executive Committee, BASF.
- Present results at annual Entomological Society of America meeting.

Evaluation of PTM™ and Insignia®SC Rates for Bareroot Pine Seedlings

(Initiated in 2012)

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Justification

Several FPMC trials (2003 - 2005) showed that fipronil (PTM™) applied to bare root seedlings before or after planting was highly effective in reducing tip moth damage for 2+ years.

Pyraclostrobin (Insignia®SC) belongs to the strobilurin class of fungicides. In addition to excellent, broad-spectrum disease control, research has shown pyraclostrobin-based fungicides also provide additional plant health benefits. Pyraclostrobin-based fungicides control foliar fungal diseases by inhibiting respiration in the mitochondria of fungi. This inhibition prevents the breakdown of energy-rich carbon compounds the fungus needs to produce energy for growth. Pyraclostrobin-based fungicides also have activity on plant mitochondria and reduce respiration in the plant. Since the plant's primary source of energy comes from sunlight through photosynthesis, this decrease in respiration can have a positive effect on growth. Decrease in respiration allows the plant to keep more stored carbon compounds for growth and triggers a chain reaction of positive physiological changes in the plant. These positive physiological changes may include a defense signaling compounds, and a decrease in the stress hormone ethylene. The combination of disease control, stress reduction, and increased growth efficiency lead to the plant health benefits observed with the use of pyraclostrobin-based fungicides as described in this report (BASF Intrinsic™ report). It is of interest to evaluate the efficacy and duration of soil injection treatment of bareroot seedlings with fipronil and pyraclostrobin alone or combined.

Objectives: 1) Evaluate efficacy of PTM™ (fipronil) and Insignia®SC (pyraclostrobin) alone or combined applied to bareroot seedlings at different rates for reducing pine tip moth infestation levels and improving seedling health; and 3) determine the duration of chemical activity.

Cooperators

Greg Leach	Rayonier
Jim Bean, Andy Goetz, Victor Canez	BASF

Research Approach:

One family of loblolly pine bareroot seedlings will be selected (from ArborGen, Cellfor or IFCo).

Treatments**:**

1 = PTM™ High Concentration/Diluted Soil Injection [5.6 ml PTM (**110 tpa rate**) in 24.4 ml water (30 ml total volume)/seedling] - Soil injection at two points next to transplanted **bareroot** just after planting.

2 = PTM™ Mid Concentration/Diluted Soil Injection [1.4 ml PTM (**435 tpa rate**) in 28.6 ml water (30 ml total volume)/seedling] - Soil injection at two points next to transplanted **bareroot** just after planting.
3 = PTM™ Low Concentration/Diluted Soil Injection [1.0 ml PTM (**600 tpa rate**) in 29.0 ml water (30 ml total volume)/seedling] - Soil injection at two points next to transplanted **bareroot** just after planting.
4 = Insignia®SC High Concentration/Undiluted Soil Injection [51.6 ml 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 [13.1 ml Insignia (**435 tpa rate**) in 11.9 ml water (30 ml total volume)/seedling] - Soil injection at two points next to transplanted **bareroot** just after planting.
6 = Insignia®SC Low Concentration/Diluted Soil Injection [9.5 ml Insignia (**600 tpa rate**) in 20.5 ml water (30 ml total volume)/seedling] - Soil injection at two points next to transplanted **bareroot** just after planting.
7 = PTM™ + Insignia®SC High Concentration/Undiluted Soil Injection [5.6 ml PTM + 51.6 ml Insignia (57.2 ml total volume)/seedling] - Soil injection at four points next to transplanted **bareroot** just after planting.
8 = PTM™ + Insignia®SC Mid Concentration/Diluted Soil Injection [1.4 ml PTM + 13.1 ml Insignia in 15.5 ml water (30 ml total volume)/seedling] - Soil injection at two points next to transplanted **bareroot** just after planting.
9 = PTM™ + Insignia®SC Low Concentration/Diluted Soil Injection [1.0 ml PTM + 9.5 ml Insignia in 19.5 ml water (30 ml total volume)/seedling] - Soil injection at two points next to transplanted **bareroot** just after planting.
10 = Bareroot Check (untreated)

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

One (1) recently hand planted tracts will be selected in January 2012 in Texas based on uniformity of soil, drainage and topography.

Potential Cooperators
TX – Rayonier (Leach)

All stands will have been intensively site prepared, i.e., subsoil, bedding, and/or herbicide. A half-acre (approximate) area will be selected. A triple Latin square design will be established with single tree plots (10 rows X 10 treatments) serving as blocks, i.e., each treatment will be randomly selected for placement along each row (bed). Thirty (30) rows will be established on each site. Seedlings will be planted at 6 foot spacing along each row. Individual tree locations will be marked with different color pin flags prior to tree planting.

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

Damage and Tree Measurements

Tip moth damage will be 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 will be calculated; and 3) separately, the terminal will be identified as infested or not. Observations also will be made as to the occurrence and extent of damage caused by other insects, i.e., coneworm, aphids, sawfly, etc. Measurements of tree health will be collected periodically and/or at the end of each growing season. Tree health measurements include tree height and diameter; crown diameter, density and color (vigor); number and length of shoots in the top whorl, and tree survival. All study trees will be measured for height & diameter at ground line) at the beginning of the study (when seedlings are planted). Measurements also will be taken when tree growth has stopped in mid- to late November for at least the first 2 years of the study. Tree form will be evaluated at end of year 3. Form ranking of the seedling or tree will be categorized as follows: 0 = no forks; 1 = one fork; 2 = two to four forks; 3 = five or more forks. A fork is defined as a node with one or more laterals larger than one half the diameter of the main stem (Berisford and Kulman 1967).

Efficacy will be evaluated by comparing treatment differences for direct and indirect measures of insect-caused losses. Direct treatment effects include reduction in pine tip moth damage. Indirect treatment effects include increases in tree growth (height, diameter and volume index; shoot length) and health (crown density and color; nuber of shoots and tree survival) parameters. Data will be subjected to analyses of variance (Table 1) using Statview software (SAS Institute, Inc. 1999). Percentage and measurement data will be transformed by the arcsine % and log transformations, respectively, prior to analysis. Costs of treatment per acre also will be calculated.

If one or more treatments continue to be successful in reducing tip moth damage by > 75% in the 4th generation in 2012, the "best" treatment(s) will be followed into 2012 to continue evaluating duration of treatments. In addition, the study may be expanded in 2013 to refine application rates and techniques for the promising treatment(s).

Table 1. ANOVA Table and Expected Mean Squares for Fipronil Treatment Study

Source of Variation	df	Expected Mean Squares
Site (S)	s-1	
Blocks (B)	r-1	
Treatments (T)	t-1	$\sigma^2_\varepsilon + rm\sigma^2_B$
SxB	(s-1) (b-1)	$\sigma^2_\varepsilon + m\sigma^2_{SB}$
BxT	(b-1) (t-1)	$\sigma^2_\varepsilon + m\sigma^2_{BT}$
SxBxT	(s-1) (b-1) (t-1)	$\sigma^2_\varepsilon + m\sigma^2_{SBT}$
Sampling error	srt (m-1)	σ^2_ε
Total	srtm-1	

Square	1									
row/column	1	2	3	4	5	6	7	8	9	10
1	C	J	H	A	D	F	B	G	I	E
2	I	F	J	G	H	B	E	D	A	C
3	F	G	A	E	I	D	H	C	B	J
4	B	D	G	C	A	H	J	I	E	F
5	H	I	C	F	E	A	G	B	J	D
6	D	C	E	J	B	I	A	F	H	G
7	J	A	I	B	C	G	D	E	F	H
8	E	H	D	I	G	J	F	A	C	B
9	G	E	B	H	F	C	I	J	D	A
10	A	B	F	D	J	E	C	H	G	I

	2									
	1	2	3	4	5	6	7	8	9	10
	E	H	I	F	C	B	J	D	A	G
	D	E	J	I	A	G	B	C	H	F
	J	I	H	A	G	D	E	B	F	C
	B	J	E	H	F	C	D	G	I	A
	G	B	D	E	I	A	C	F	J	H
	I	F	A	C	B	E	H	J	G	D
	H	A	F	G	D	J	I	E	C	B
	F	G	C	D	J	H	A	I	B	E
	C	D	B	J	H	F	G	A	E	I
	A	C	G	B	E	I	F	H	D	J

	3									
	1	2	3	4	5	6	7	8	9	10
	I	B	J	G	E	H	C	D	F	A
	C	F	D	J	B	G	E	A	I	H
	A	J	C	I	G	F	H	E	D	B
	E	I	A	D	F	J	B	H	C	G
	B	C	H	A	I	D	F	G	E	J
	F	E	G	H	C	A	I	J	B	D
	J	H	F	B	A	E	D	I	G	C
	G	A	B	E	D	C	J	F	H	I
	D	G	I	F	H	B	A	C	J	E
	H	D	E	C	J	I	G	B	A	F

Treatments and Plot Design Example

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

UD = undilute; D = dilute

Research Time Line:

CY 2012

January - February 2012

- Select research site (January)
- Treat seedlings after planting with PTM and Insignia via soil injection
- Begin trap monitoring of tip moth populations near each site

March - October, 2012

- Evaluate tip moth damage after 1st through 4th generations; photograph damage.
- Continue trap monitoring of tip moth populations near each site

November - December 2012

- Evaluate tip moth damage after 5th generations; measure seedling and height of seedlings.
- Conduct statistical analysis of 2012 data.
- Prepare and submit report to FPMC Executive Committee, BASF.

CY 2013

January - February 2013

- Begin trap monitoring of tip moth populations near each site

March - October, 2013

- Evaluate tip moth damage after 1st through 4th generations; photograph damage.

November - December 2013

- Evaluate tip moth damage after 5th generations; measure seedling and height of seedlings.
- Conduct statistical analysis of 2013 data.
- Prepare and submit report to FPMC Executive Committee, BASF.

CY 2014 (if warranted based on CY 2013 results)

January - February 2014

- Begin trap monitoring of tip moth populations near each site

March - October, 2014

- Evaluate tip moth damage after 1st through 4th generations; photograph damage.

November - December 2014

- Evaluate tip moth damage after 5th generations; measure seedling and height of seedlings.
- Conduct statistical analysis of 2014 data.
- Prepare and submit report to FPMC Executive Committee, BASF.
- Present results at annual Entomological Society of America meeting.

Machine Planter Evaluation in a Flex Stand Situation

Initiated in 2012

Cooperators:

Anthony, Fontenot	Weyerhaeuser, Natchitoches, LA
Chris Dowden	Forestry LLC, Coushatta, LA

Objectives: 1) Evaluate the efficacy of PTM applied to genetically improved trees located every fourth tree along a row with trees of standard root stock and 2) determine the duration of PTM activity.

Description: In 2012, Weyerhaeuser provided the Forest Pest Management Cooperative with 2 sites in Louisiana to test the efficacy of PTM when used in a "Flex Stand" setting. In the case, the Flex Stand consists of 75% trees of standard rootstock (biomass trees) and 25% improved genetic stock (crop trees). Trees were planted by machine. Generally, trees were planted at the rate of three biomass trees followed by one crop tree. All crop trees were treated at the 435 TPA rate or 1.4ml PTM/tree. This was done by the person feeding the coulter wheel. Once the crop tree was in the furrow, the operator pushed a button to dispense PTM into the furrow before it was closed.

Design: Two recently planted sites were selected in Natchitoches and Creston, Louisiana. At each site, 10 subplots were randomly selected. Each subplot consists of 10 crop trees and 10 biomass trees selected along a single row.

Damage and tree measurements: Study trees will be evaluated for damage from pine tip moth after each generation of tip moth has occurred. Height and ground line diameter measurements will be taken immediately after plot establishment and again at the end of the year.

Duration: Monitoring for damage and volume will continue until significant differences can no longer be seen between the crop trees and the biomass trees.

Evaluation Effects of Cold Storage Time on Efficacy of Fipronil Injection Treatments on Containerized Loblolly Pine Seedlings

Don Grosman & Billi Kavanagh
Forest Pest Management Cooperative
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Cooperators

Wayne Bell	International Forest Company
Jim Bean	BASF, Research Triangle Park, NC

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

Justification

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

Research Approach:

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

Treatments**:**

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

Note: If possible, Trt **A** seedlings (150 for each site; 300 total) should be treated first (Nov. 12) and Trt **A** & **E** seedlings placed in cold storage; Trt **B** seedlings would be treated on Nov. 26 and Trt **B** & **F** seedlings placed in cold storage; Trt **C** seedlings would be treated on Dec. 3 and Trts **C** & **G** seedlings placed in cold storage; and Trt **D** seedlings would be treated on Dec. 10 and Trt **A, B, C, E, F,** and **G** seedlings would be taken out of cold storage. All

seedlings, including checks (**D** & **H**), would be planted on Dec. 10 or 11. The TX seedlings would be shipped immediately.

Square 1

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

Square 2

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

Square 3

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

Square 4

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

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

Containerized seedlings will be individually treated at the IFCo nursery prior to planting using the plug injection system developed by Stewart Boots, S&K Designs. The seedlings will be treated with PTM™ at 1.4 ml per seedling (435 tpa) based on the restricted rate of 59 g AI/acre/year (PTM™).

Two recently harvested tracts will be selected; one in east Texas and one near Moultrie, GA. A 1 acre (approximate) area within each site will be selected. A quadruple Latin square design will be established with single tree plots (8 rows X 8 treatments) serving as blocks, i.e., each treatment will be randomly selected for placement along each row (bed). Thirty-two (32) rows will be established on each site. Seedlings will be planted at 8 foot spacing along each row. Individual tree locations will be marked with different color pin flags prior to tree planting.

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

Damage and Tree Measurements

Tip moth damage will be evaluated by determining percent of trees infested, percent of infested shoots in top whorl and percent terminals infested about 4 weeks after peak moth flight of each generation for at least the first 2 years. Observe and record presence and extent of damage caused by other insects, i.e., weevils, coneworm, webworm, aphids, etc. All study trees will be measured (height & diameter @ 6 inches) at the beginning of the study (just after seedlings are planted). Measurements also will be taken when tree growth has stopped in mid- to late November for at least the first 2 years of the study. Tree form will be evaluated at end of year 3. Form ranking of the seedling or tree will be categorized as follows: 0 = no forks; 1 = one fork; 2 = two to four forks; 3 = five or more forks. A fork is defined as a node with one or more laterals larger than one half the diameter of the main stem (Berisford and Kulman 1967). Data will be analyzed by GLM and the Tukey's Compromise test using Statview or SAS statistical programs.

Research Time Line:

CY 2012

November - December 2012

- Select research sites
- Treat containerized seedlings with fipronil via plug injection system at 4wk, 2 wk, 1 wk and on day of planting. Place selected seedlings in cold storage for designated periods.
- Begin trap monitoring of tip moth populations near each site

March - October, 2013

- Evaluate seedlings for survival and tip moth damage after 1st through 4th generations; photograph damage.

November - December 2013

- Evaluate tip moth damage after 5th generation; measure seedling diameter and height.

- Conduct statistical analysis of 2013 data.
- Prepare and submit report to FPMC Executive Committee and BASF.

March - October, 2014

- Evaluate seedlings for survival and tip moth damage after 1st through 4th generations; photograph damage.

November - December 2014

- Evaluate tip moth damage after 5th generation; measure seedling diameter and height.
- Conduct statistical analysis of 2013 data.
- Prepare and submit report to FPMC Executive Committee and BASF.

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

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Abstract: The black turpentine beetle (BTB, *Dendroctonus terebrans*) can cause mortality of pines in the Southeastern US. Historically, control is obtained with periodic bole sprays of insecticides. Recently, an injected systemic insecticide, emamectin benzoate (TREE-age™; Arborjet Inc., Woburn, MA) has shown to be effective against certain bark beetles. In this study, the effectiveness of different rates of TREE-age™ will be evaluated for protecting individual loblolly pine from black turpentine beetle.

Objectives:

1) Evaluate the efficacy of systemic injections of TREE-age™ (emamectin benzoate) for protection of pine against black turpentine beetle (BTB); and
2) Determine the effect of injection height on success of BTB attacks.

Background/Justification Statement:

The black turpentine beetle (BTB, *Dendroctonus terebrans*) may attack all pines native to the South. It is most serious in pine naval stores and pines stressed by serious drought, flooding, storms, wildfires, and cutting operations. Use of mechanized harvesting equipment, which damages residual trees, compacts the soil and injures the roots, has increased damage by black turpentine beetle (Merkel 1981, Staeben et al. 1910).

The black turpentine beetle, a close cousin of the southern pine beetle (*Dendroctonus frontalis*), is found from New Hampshire south to Florida and west to east Texas. The adult BTB is dark brown to black in color and 3/8 inch in length. The posterior end is rounded (this contrasts with the concave posteriors of the *Ips* engraver beetles). Full grown larvae are white with a reddish brown head and about 1/3 inch long. Pupae are about ¼ inch in length and yellowish white.

Black turpentine beetles attack fresh stumps and the lower trunk of living pine trees. Initial attacks are generally within 2 feet of the ground. Attacks are identified by white to reddish-brown pitch tubes about the size of a half dollar. The pitch tubes are located in bark crevices on

the lower tree bole, usually below a height of 10 feet. Infested pines are often attacked by other bark beetles (i.e., southern pine beetle and Ips engraver beetles).

Adult beetles bore into the cambium and construct galleries which usually extend downward. Eggs are laid in clusters and hatch in 10 to 14 days. Larvae feed side by side, excavating a large continuous area. The life cycle takes from 2 ½ to 4 months, depending on the season. There are two to four generations per year.

Natural enemies and good tree vigor generally keep black turpentine beetle populations at low levels. Historically, prevention treatments have consisted of bole spraying the base to the about 10 feet with approved insecticide. Currently-registered chemicals include bifenthrin or chlorpyrifos. Recently, a systemic insecticide, emamectin benzoate (TREE-age™), has been shown to be effective in protecting southern pines against southern pine beetle and *Ips* engraver beetles. It is of interest to determine if TREE-age™ is equally effective against BTB and if injection height will affect treatment efficacy.

Completion of proposed objectives will:

1) Document the efficacy of the recommended rate of the TREE-age™ formulation of emamectin benzoate for protecting individual loblolly pine from attack by black turpentine beetle.

2) Determine the effect of injection height on TREE-age™ efficacy against black turpentine beetle.

Research approach:

Locations, Treatments, and Environmental Conditions

This study will be conducted near or within the Fairchild State Forest, Rusk, TX (about 31°78 N, 95°36 W, elev. 451ft). Forty (40) loblolly pine, >13 " DBH, will be randomly selected for insecticide treatment. An additional ten trees will serve as untreated checks.

There will be five treatments: TREE-age (5.0 ml / inch DBH) treatment applied at ground level (treatment **1**); TREE-age (2.5 ml / inch DBH) applied at ground level (treatment **2**); TREE-age (2.5 ml / inch DBH) applied at 36 inches above ground (treatment **3**); Scimitar (lambda-cyhalothrin, Syngenta) spray applied from ground to 10 feet (treatment **4**); and untreated tree (treatment **5**).

Each treatment will be applied to 10 randomly-assigned trees. Test trees will be located in spaced >160 m apart, 20 to 76 cm dbh, and within 100 m of access roads to facilitate the treatment. Each systemic insecticide treatment (treatments 1, 2, & 3) will be injected at the labeled rate after dilution in 1 part water with the Arborjet Tree IV™ microinfusion system (Arborjet, Inc. Woburn, MA) into evenly spaced points (number is calculated by DBH/2). Injections will occur in September 2012. In October 2012 (30 days post-injection), the bole of treatment 4 trees (up to 10 ft) will be sprayed to runoff using a backpack sprayer.

In October 2012 (30 days post treatment), each tree will be baited with fontalin and endo-brevicomin lures and turpentine (in amber bottle and wick).

Two multiple funnel traps will be deployed in the area and each baited with frontalin, endo-brevicomin and turpentine (Payne et al. 1987). Traps will be checked every two weeks during the course of the study. Captured BTB will be sexed and counted.

Precipitation and temperature data will be obtained from the nearest weather station during the course of this study from 1 September 2012 to October 2014.

Experimental Design – Treatment Efficacy

The number, height of attack, and success of BTB attacks will be evaluated monthly. The success can be determined by the size and composition of the pitch tubes exuding from each BTB attack site. Large pitch tubes containing frass (phloem tissue and beetle waste) and brood emergence indicate success of females alone or with males in colonizing the host. Small, crystalized pitch tubes with little or no frass and no brood emergence indicates failure to successfully colonize host (or attacks by *Ips*).

At the termination of the experiment in June 2013 (about 9 months after treatment), final crown ratings will be made. An analysis of variance will be used to test for differences among injection treatments.

Research timetable:

Research Activity	Date
1. Study plan	Completed
2. Lufkin Parks contacted, liaison	Completed
3. Field site selection	Completed
4. Trees selected, tagged and treatments assigned	September 2012
5. Treatments 1, 3, 5 & 7 applied	September 2012
6. Trees baited and traps deployed	October 2012
6. Post-treatment assessment of efficacy	Nov and Dec 2012
7. Post-treatment assessment of efficacy	Jan - June 2013
8. Data summary and analyses (Grosman and new FPMC Coordinator)	Fall 2013
9. Final report, peer-reviewed publication submitted (coauthored by Grosman and new FPMC Coordinator)	Fall 2013

Literature Cited

Merkel, E.P. 1981. Control of the Black turpentine beetle. Georgia Forest Research Paper 15. Georgia Forestry Commission. 7 p.

Payne, T.L., R.F. Billings, J.D. Delorme, N.A. Andryszak, J. Bartels, W. Franke, and J.P. Vite. 1987. Kairomonal-pheromonal system of the black turpentine beetle, *Dendroctonus terebrans* (Ol.). J. Appl. Entomol. 103: 15-22.

Staeben, J.C., S. Clarke, and K.J.K. Ganghi. 2010. Black turpentine beetle. Forest Insect and Disease Leaflet 12. U.S. Dep of Agriculture Forest Sevice. 8 p.

Budget:

BTB:- CY 2012-2013

Personnel	
Grosman	Contributed
Seasonal Technician (30%)	$ 2,997
Benefits for Seasonal Technician (8.45%)	$ 253.25
Materials and Supplies	
50 frontalin and endo-brevicomin lures (Synergy Chemical Co.)	$ 200
Miscellaneous materials and supplies	$ 200
Travel	
Vehicle fuels and maintenance	$ 318
Indirect Costs (26%)	$ 1,031.75
TOTAL REQUESTED	**$ 5,000**

New Systemic Insecticide to Protect High-Value Pines on Bastrop State Park

Ronald F. Billings
Texas A&M Forest Service

Justification:
In 2011, the Bastrop wildfire scorched over 32,000 acres including some 6,000 acres of Bastrop State Park. The small portion of the pines that survived this destructive fire is now being threatened by pine bark beetles, also known as engraver beetles (*Ips* spp.). Of particular concern to park managers are large diameter pines that provide shade in campground areas of the park. A new systemic insecticide known as emamectin benzoate (EB) may provide protection from bark beetle attack.

Objective:

- Evaluate the efficacy of Tree-äge for protection of loblolly pine against *Ips* engraver beetles.

Methods:
One hundred loblolly pine were selected at the Bastrop State Park, Bastrop, TX. An additional 63 trees were selected at the Lost Pines Scout Reservation near Bastrop. Seventy one and 43 trees, respectively, were injected with TREE-äge™ at 5 ml/ inch DBH when < 20 inch DBH or 10 ml/ inch DBH when > 20 inch DBH in October 2012. Twenty -thirty trees were included as untreated controls. All trees will be evaluated in October 2013 for survival.

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

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

Objectives:

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

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

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

Background/Justification Statement:

Thousand cankers disease (TCD) is an insect-disease complex recognized in 2008 consisting of the walnut twig beetle (WTB, *Pityophthorus juglandis*) and the associated fungus (*Geosmithia morbidia*) that it carries to walnut trees, primarily black walnut (*Juglans nigra*) (Tisserat et al. 2009, Utley et al. 2009, Kolarik et al. 2010, Seybold et al. 2010). Beetles tunnel through the bark of limbs and stems and introduce the fungus. The fungus grows, producing cankers, or areas of infected phloem tissue. As thousands of small cankers grow together to girdle branches, tree health declines and the tree eventually dies. Thousand cankers disease has caused widespread death of walnuts in western states (AZ, CA, CO, ID, NM, NV, OR, UT, and WA) over the past decade. In 2010, TCD was found in Tennessee (currently in Anderson, Blount, Knox, London, Sevier, and Union counties), within the native range of black walnut. More recently (July and August 2011), TCD was discovered in Virginia (Chesterfield and Henrico Cos.) and Pennsylvania (Bucks Co.), respectively (Mielke et al. 2011).

Currently, there is no known means of reliably controlling this disease. Standard pesticide treatments (drenching trunk/branch sprays with permethrin or bifenthrin) to control the bark beetle vector have been tested (Cranshaw and Tisserat 2010). However, infected black walnut trees continue to decline and die even after repeated insecticide spray applications. Similarly, soil-applied systemic neonicotinoid insecticides (e.g., imidacloprid, dinotefuran, clothianidin) are largely ineffective. Trunk injections of glycosides (e.g., emamectin benzoate, abamectin) have not been tested. However, emamectin benzoate has been used successfully against pine wood nematode, *Buraphelenchus xylophilus* (Takai et al. 2001), pine bark beetles (southern pine beetle, western pine beetle, *Ips* engraver beetle) (Grosman and Upton 2006, Grosman et al. 2009, 2010), and wood borers (emerald ash borer, soapberry borer, eucalyptus longhorn borer) (McCullough et al. 2011, Grosman, Cox unpublished data). In the cases of the emerald ash borer and soapberry borer, treatments made after decline symptoms are also effective (Smitley et al. 2010, Billings et al. 2011). This chemical may also be effective against defoliators and boring insects that attack walnut, as related to pest claims on the TREE-äge™ label, including: walnut caterpillar, *Datuna integerrima,* codling moth, *Laspeyresia pomonella*, walnut weevils, *Conotrachelus retentus (Say) and C. jugandis*, flat-headed apple tree borer, *Chrysobothris femorata (Oliv.)*, walnut husk fly, *Rhagoletis completa Cresson*, walnut leaf gall mite, *Aceria erinoea*, and root lesion nematode, *Pratylenchus vulnus (Williams 1990)*.

Most bark beetles have complex associations with fungi, including non-staining *Ceratocystiopsis* spp. (carried in the mycangium) and staining *Ophiostoma* and *Ceratocystis* spp. (carried on external body surfaces) (Paine et al. 1997). As beetles bore into the phloem, spores are inoculated and serve to help beetle colonization by interfering with host tree defenses. The fungi alone may disrupt water transport and cause tree death (Nelson and Beal 1929). In their study of pine bark beetles, Grosman et al. (2009) suggested that blue stain fungal infection was the

primary cause of tree mortality as attacking beetles must contact and consume tree phloem prior to mortality occurring from emamectin benzoate injection (i.e., with bole sprays, beetles contact insecticides prior to entering the bark and therefore blue stain inoculation is rare). Accordingly, combining TREE-age™ with a fungicide, such as propiconazole (Alamo™) may hold promise for single tree protection.

In this study, we propose to evaluate the effectiveness of recommended rates of emamectin benzoate (TREE-äge™; Syngenta) alone and combined with the fungicide propiconazole (Alamo®; Syngenta) for reducing the attack success of WTB (and other insect pests) on individual black walnut trees and the progression of fungi introduced during initial phases of tree colonization. We will also determine the distribution and concentration of emamectin benzoate in walnut tissue. If funds are provided, we will also determine propiconazole distribution and concentration in selected plant tissue.

This study will address FS-PIAP National Priorities for systemic forest use insecticides, specifically those requesting additional studies on the "physical transport and disposition of priority systemic insecticides (emamectin benzoate) and fungicides (propiconazole) with application via trunk injection into trees of interest . . . (as well as) … investigate pest control efficacy . . . " The assembled team has extensive field and laboratory experience conducting studies of this nature (*see* Qualifications).

Completion of proposed objectives will:

1) Document the efficacy of the recommended rate of the TREE-age™ formulation of emamectin benzoate for protecting individual black walnut from decline and/or mortality attributed to walnut twig beetle and other insect pests.

2) Document the efficacy of the recommended rate of TREE-age™ + the ALAMO® formulation of propiconazole for protecting individual black walnut from decline and/or mortality attributed to WTB attack and associated fungal infection.

3) Determine the efficacy of TREE-age™ and TREE-age™ + ALAMO® as therapeutic treatments after WTB attack and associated fungal infection.

4) Provide data on the distribution and concentration of TREE-age™ and ALAMO® in black walnut phloem following injection.

Research approach:

Locations, Treatments, and Environmental Conditions
This study will be conducted at two primary locations: TCD-confirmed location(s) within or around Knox Co., TN (about 35°59 N, 83°55 W, elev. 955 ft) and uninfested locations in Rusk Co., TX (about 31°44 N, 95°12 W, elev. 397 ft). There will be as many as seven treatments: emamectin benzoate (TREE-äge™) alone injected into TCD symptomatic (treatment **1**) and non-symptomatic (treatment **2**) trees; propiconazole (Alamo®) alone injected into TCD symptomatic (treatment **3**) and non-symptomatic (treatment **4**) trees; TREE-äge™ + Alamo® injected into TCD

symptomatic (treatment **5**) and non-symptomatic (treatment **6**) tree; and an untreated control (treatments **7**).

Each treatment will be applied to 10 randomly-assigned trees (*N* = 40-70 per site). Test trees will be located in areas with abundant insect activity, spaced >10 m apart, 13 to 38 cm dbh, and within 100 m of access roads to facilitate the treatment. Each insecticide, fungicide or insecticide + fungicide treatment (treatments 1-6) will be injected with the Arborjet Tree IV™ or QUIK-jet™ microinfusion system (Arborjet, Inc. Woburn, MA) into 4-8 evenly spaced points 0.3 m above the ground. Injections will occur in March or April (i.e., about 1 month prior to initiation of WTB adult flight and tunneling). All experimental trees (treated and untreated) in TN will be baited with WTB pheromones (provided by Steve Seybold) beginning in June, 2012 and throughout the growing season. All surviving treated trees in treatments 1-6, and the untreated control trees (treatment 7) will be baited for the same length of time in June, 2013. WTB populations will be monitored throughout the season at the TN location with 3-5 baited 4-unit Lindgren funnel traps placed at 10 feet on steel conduit poles. Trap catches will be recovered every two weeks throughout the season.

In April, 2012 (at the time of treatment) and then every other month (June, August & October), the stem and crown of each tree will be ranked as to the extent of insect damage. In addition, three small branchs (12" length) will be collected from the low, mid and upper crown of each study tree. The branches will be evaluated for the presence of and ranked on the level of WTB (TN) and other insect damage (TX and TN).

Two HOBO data loggers (Onset Computer Corp., Bourne, MA) will be placed in the study area for accumulation of temperature data. These data will later be used to describe the general temperature regime (i.e., maximum, minimum, mean) during the course of this study from 1 April through 30 October 2012 and 2013. Precipitation will be obtained from the nearest weather station for the same periods of time.

Experimental Design – Treatment Efficacy

A photograph of the crown of each study tree in TN will be taken at the time of treatment. Trees will be evaluated for crown condition every other month for 18 months. The date of appearance of TCD symptoms will be recorded. Each walnut crown will be given a rating of 0 (healthy), 1 (wilt symptoms comprising < 20% of the crown), 2 (wilt symptoms comprising 20-80% of the crown), 3 (wilt symptoms comprising >80% of the crown) (Mayfield et al. 2008), or 4 (dead tree). At each rating period, trees with a crown rating of 2 will have wood samples taken from the stem and branches to determine the presence of WTB galleries and *G. morbidia*.

At the termination of the experiment in November 2013 (about 18 months after treatment), final crown ratings will be made. An analysis of variance will be used to test for differences among injection treatments. A χ^2 (Chi-square) test for homogeneity will be used to test the null hypothesis that the percentage of trees with a crown rating of 2 did not differ between the insecticide-, fungicide- or combination-treated trees and the untreated control group (Mayfield et al. 2008). The null hypothesis will be rejected if more than 20% of the treated trees reached a

crown rating of 2. The test will be invalidated if fewer than 60% of the control trees reach a crown rating of 2.

Experimental Design – Residue Analyses

Xylem and phloem samples will be collected at the TX site in June 2012 and June 2013 (treatments 2, 4, 6 & 7). Nut samples will be collected in June and September 2012 and 2013 (treatments 2, 4, 6 & 7). If sufficient concentrations exist in phloem collected in September 2013, we may continue sampling in 2014 if additional funding can be obtained.

Propiconazole residues will be extracted with ethylacetate, cleaned up by Gel Permeation Chromatography and analyzed by gas chromatography (GLC) utilizing a N-P detector. Positive pesticide residues will be confirmed by GC-Mass Spectroscopy. The GC columns to be utilized are SPB-5 and SPB-35 megabore capillary columns. The column oven will be temperature programmed from 135-275 °C at 5 degrees/min. A fortified sample and reagent blank will be included with each set of analyses. In the past, the average propiconazole residue recovery has been 72.4% and the method is well recognized. Emamectin benzoate residues will also analyzed, but the exact methodology that will be used has not yet been determined [i.e., we are currently reviewing the efficiency and effectiveness of recently developed methods employed by Syngenta Corp. (unpublished).

This study involves the use of pesticides, but the findings are not intended to be submitted to the U.S. Environmental Protection Agency in support of a research or marketing permit. This research is therefore not covered by the Federal Insecticide, Fungicide, and Rodenticide Act Good Laboratory Practices regulations.

Research timetable:

Research Activity	Date
1. Study plan	Completed
2. Forest/District contacted, liaison	Completed
3. Field site selection	Completed
4. Trees selected, tagged and treatments assigned	March-April 2012
5. Treatments 1 - 6 applied; monitoring traps installed	April 2012
6. Trees baited	May 2012
7. Xylem, phloem & nut samples collected (treatments 2, 4, 6 & 7)	June 2012
8. Nut sampled (treatments 2, 4, 6 & 7)	September 2012
9. Post-treatment assessment of efficacy	Jun, Aug & Oct 2012
10. Presentation at Bark Beetle Technical Working Group	October 2012
11. Trees baited (all) and xylem, phloem and nut samples collected (treatments 2, 4, 6 & 7)	May 2013
12. Post-treatment assessment of efficacy	Jun, Aug & Oct 2013
13. Presentation at Southern Forest Insect Work Conference	July 2013
14. Nut samples collected (treatments 2, 4, 6 & 7)	September 2013

15. Data summary and analyses — Fall 2013
16. Final report, peer-reviewed publication submitted — Fall 2013
17. Presentation at Society of American Foresters — November 2013

Technology transfer plan:

The proposed research team includes members of Forest Health Protection (S&PF), Pacific Southwest Research Station, Texas Forest Service and Syngenta Crop Science. Research findings will be delivered in a timely manner in both verbal and written formats. Technology transfer will be sustained through training sessions, consultations with other FHP and state-level entomologists, presentations at local, regional and national meetings, and subsequent publications. In addition, significant conduits for technology transfer activities already exist based on previous requests for the information that this study will provide.

Planned deliverables during and within one year of completion of this study include:

- Bark Beetle Technical Working Group presentation (oral)
- Society of American Foresters presentation (poster)
- East Texas Forest Entomology Seminar presentation (oral)
- Southern Forest Insect Work Conference presentation (oral)
- Southern Journal of Applied Forestry research (research paper)

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

(Initiated in 2012)

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Justification

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

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

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

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

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

Objectives: 1) Determine the efficacy of emamectin benzoate for protecting loblolly pine from PWN; 2) efficacy of chemical treatments at different rates, 3) effects of injection spacing on treatment efficacy, 4) duration of treatment efficacy.

Potential Cooperators

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

Research Approach:

Parameters:

Tree Species: loblolly pine
Chemical: emamectin benzoate (EB, TREE-age™ w 4% EB).
Rates: 0.75, 1.25, 2.5ml and 5.0 ml/inch DBH
Injection spacing: DBH/1.25 (~1 pt every 4" circ) and DBH/2.5 (~1 pt every 8"circ), DBH/5 (~1 pt every 16" circ) spacing.
Season of Treatment: Fall 2012 and Spring 2013.
Duration: 1, 2, 3, 12, 24, 36, and 48 months

During the initial season (fall), one site will be selected in east Texas, within 40 miles of Lufkin/Nacogdoches. Additional sites may be added across the South in later seasons if there is interest.

Trial 1: Testing chemical rate and injection point spacing

In November 2012, 60 "healthy appearing" trees (23-25 cm (=9-10") DBH, ~20-YO) will be selected in an east Texas plantation. Six (6) trees will be randomly assigned and treated with one of the treatments indicated below. The chemical will be allowed 3 months (March) to circulate within each tree prior to felling. Immediately (within an hour of felling), wood samples and 1.0 m bolts will be taken from the main stem of the lower crown (~6 m), mid bole (3 m), and lower bole (0 m). The treatments include:

A = EB @ 0.75 ml/inch @ 4" spacing
B = EB @ 0.75 ml/inch @ 8" spacing
C = EB @ 0.75 ml/inch @ 16" spacing
D = EB @ 1.25 ml/inch @ 4" spacing
E = EB @ 1.25 ml/inch @ 8" spacing
F = EB @ 1.25 ml/inch @ 16" spacing
G = EB @ 2.50 ml/inch @ 4" spacing
H = EB @ 2.50 ml/inch @ 8" spacing
I = EB @ 5.00 ml/inch @ 4" spacing
J = Check (untreated)

The 180 bolt sections will be placed about 1 m apart on discarded, dry pine bolts to maximize surface area available for colonization as well as to discourage predation by ground and litter-inhabiting organisms. A bait blend (ethanol, (-) a-pinene, ipsenol, ipsdienol, and monochamol) will deployed in the harvest area to attract cerambycid beetles. All logs will be sampled for PWN 28d after tree felling.

Trial 2:Testing Treatment Duration

In Spring 2013, 96 "healthy appearing" trees (23-25 cm (=9-10") DBH, ~20-YO) will be selected in an east Texas plantation. In April, six (6) trees will be randomly assigned and treated with one of the treatments indicated below. The chemical will be allowed 2 weeks to48 months to circulate within each tree prior to felling. Immediately (within an hour of felling), wood samples and 1.0 m bolts will be taken from the main stem of the lower crown (~6 m), mid bole (3 m), and lower bole (0 m). The treatments include:

A = EB @ best rate and spacing (trial 1) felled **2 week** post injection
B = EB @ best rate and spacing (trial 1) felled **1** month post injection
C = EB @ best rate and spacing (trial 1) felled **2** mo post injection
D = EB @ best rate and spacing (trial 1) felled **3** mo post injection
E = EB @ best rate and spacing (trial 1) felled **12** mo post injection
F = EB @ best rate and spacing (trial 1) felled **24** mo post injection
G= EB @ best rate and spacing (trial 1) felled **36** mo post injection
H = EB @ best rate and spacing (trial 1) felled **48** mo post injection
I = Check (untreated) for each Treatment set above

The 36 bolt sections (for each treatment set) will be placed about 1 m apart on discarded, dry pine bolts to maximize surface area available for colonization as well as to discourage predation by ground and litter-inhabiting organisms. A bait blend (ethanol, (-) a-pinene, ipsenol, ipsdienol, and monochamol) will deployed in the harvest area to attract cerambycid beetles. All logs will be sampled for PWN 28d after tree felling.

Monitoring *Monachamus* species and PWN occurrence in beetles.

Modified funnel traps will be deployed (beginning in ealy March) at 2-3 nearby harvest sites. Traps will be baited with kairomone blend (ethanol, (-)alpha-pinene, ipsenol, ipsdienol, & monochamol) placed inside the funnels and using a wet cup (Miller et al. 2011, Dave Wakarchuk, personal communication). Traps will be monitored year around at two week intervals. Collected cerambycids will be identified to species. *Monochamus* specimens will be dissected to determine presence/absence of PWN (Linit 1988, Linit et al. 1983).

Inspecting logs for wood borer and bark beetle colonization

At 28 days after felling borders of two 10 X 50 cm strips (total = 1000 cm^2) will be marked on the bark surface and the number of cerambycid egg niches and bark beetle attacks counted within each strip.

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

1. Number of unsuccessful *Ips* attacks - penetration to phloem, but no egg galleries.
2. Number of successful *Ips* attacks - construction of nuptial chamber and at least one egg gallery extending from it.
3. Number and lengths of *Ips* egg galleries with brood galleries radiating from them.
4. Cerambycid activity, estimated by overlaying a 100 cm^2 grid over a portion of each bark strip and counting the number of squares overlapping area where cerambycid larvae have fed.
5. Number of oval cerambycid larvae entrance holes into sapwood.
6. Presence and percent area covered with blue stain.

Sampling logs for pinewood nematodes 28 days after felling
Each log is sampled at five locations: at two points approximately one-third distance from the ends and 3 times at the end of the log, 1.5 cm below the cambium, in a triangular pattern (holes may overlap on small logs). A wire brush is used to remove dirt and debris from the sample locations. At log ends, the first 5 cm from the sample locations should be discarded due to contaminates. Place a clean container beneath the work site to catch shavings throughout the process. Using a 5.4 cm (2 1/8 in) drill bit, slowly drill to the center of the log, reversing and removing the bit from the hole every 3.81 – 5.08 cm (1.5 – 2.0 inches) to collect the shavings. For large diameter trees a utensil will be required to remove the final shavings.

Pool into a bucket all of the material drilled (except the external discard, as recommended on the protocol) from a given log, mix it well, placed in a sealable plastic bag and keep at room temperature. In the lab, half of the material is used for nematode extraction (the remaining half will serve as a backup, in case there is a need to repeat the test).

Extraction of nematodes from wood shavings:
The following extraction method using a pie-pan is commonly used by nematologists to extract PWN. This method is only good for extracting live, motile nematodes.

- Each sample is assigned a Lab ID number.
- Make a single layer of wood shavings inside plastic or wire baskets lined with double-folded large Kimwipes™. Make sure the wood shavings are completely wrapped in the Kimwipes. Place the baskets into plastic containers. Add water to the containers until the wood shavings are completely submerged. Incubate for 24 hours at room temperature to allow nematodes to move out.
- After incubation, the supernatant water is decanted from the containers, after gently removing the wood-containing baskets.
- The nematode suspension in the container is left to settle for about 10 minutes at a slant, approximately 45 degrees. Decant supernatant water again.
- Approximately 100 ml of the nematode solution is decanted into beakers and allowed to settle for 60 minutes.
- Supernatant water is then collected to approximately 20ml.

- Pour the sample into a counting dish. Identify and count nematodes under inverted microscope. Use publications by Mamiya & Kiyohara, 1972 and Mamiya, 1984 as references for identification.
- Save the samples in water and 4% Formalin accordingly for further test and future reference.
- Left over wood with paper is heat-treated in a dry heat oven for 2 hours at 250°F and disposed in a receptacle for biodegradable items.
- Observe for female, male, and dauer larvae of *Bursaphelenchus xylophilus* and any suspects with a stylet. Prepare permanent slides following the procedure described below for fixing and mounting specimens and take digital photos of any positively identified specimens.

Identification of nematodes:
Nematodes extracted from the wood samples will be identified based on morphological characteristics. In cases where morphological diagnosis is not conclusive (e.g., for juveniles only, insufficient specimens) an identification as *B. xylophilus* cannot be ruled out.

The nematodes will be identified and counted under the microscope. Live nematodes will be heat killed gently for about 5 seconds on a hot-plate and placed in temporary water mounts for all measurements and microphotographs to assure quality and accuracy. For suspect specimens, nematodes will be heat killed and fixed in 4% formalin for long term preservation. The nematodes will be processed with glycerin by a modification of a glycerin-ethanol series of Seinhorst's rapid method (1959) and permanently mounted on 25 × 75-mm microscope slides. Specimens will be examined with a compound microscope with interference contrast at up to 1,000× magnification.

Data Analysis: The number of cerambycid egg niches, bark beetle attacks, nematodes present per log treatment, position on tree, and interval after felling and debarking, will be used to measure the degree of risk of PWN export. Risk of export will be then analyzed statistically using Statview software (SAS Institute, Inc. 1999) to contrast and determine the difference between treatments at each observation. Percentage and measurement data will be transformed by the arcsine % and log transformations, respectively, prior to analysis.

Project Support: This trial is supported in part by FPMC funds. Additional funds will be requested from participating members.

Research Time Line:

CY 2012

December 2012

- Select stand and study trees for Trial 1
- Inject trees with EB at different rates and injection spacing

CY 2013

January - April 2013

- Install and monitor traps (January - April)
- Cut trees and expose logs to cerambycids for 28 days (March)

- Collect tissue samples from trees and logs (April)
- Laboratory extraction and identification of nematode from plant tissue and adult *Monochamus* (April)
- Select stand and study trees for Trial 2 (April)
- Inject trees with EB at best rate and spacing (April)

May - August 2013

- Continue to monitor traps (May - August)
- Cut trees after 1, 2 & 3 months post treatment and expose each log set to cerambycids for 28 days (May August)
- Collect tissue samples from trees and logs (June - August)
- Laboratory extraction and identification of nematode from plant tissue and adult *Monochamus* (June - August)

September - December 2013

- Continue to monitor traps (September - December)
- Conduct statistical analysis of 2013 data (Sept).
- Prepare and submit preliminary report to participating members.
- Present results at ETFES.

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Budget:

Research period: November 2012 – August 2013

Personnel	
Grosman / future FPMC Coordinator	Contributed
Student Worker (50%)	Contributed
Student Worker (50%)	$ 4,995.00
Benefits for Seasonal Technician (8.45%)	$ 422.08
	$ 5,417.08
Materials and Supplies	
4 liters of TREE-age (emamectin benzoate) and Arborplugs	Contributed
35 kairomone blend baits ($17/bait)	$ 595.00
PPE equipment (chaps, hard hat, gloves, eye protection)	$ 300.00
Lab equipment (funnels, pans, slides, chemicals)	$ 300.00
	$ 1,195.00
Travel	
Vehicle fuels and maintenance (50% of 4,000 miles @ $0.50/mile)	Contributed
Vehicle fuels and maintenance (50% of 4,000 miles @ $0.50/mile)	$ 1,000.00
Subtotal	$ 7,612.08
Indirect Costs (26%)	$ 1,522.42
TOTAL REQUESTED	**$ 9,134.50**

Evaluation of Miticides for Control of Conifer Mites on Loblolly Pine

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Abstract: Stressed loblolly pine trees (due to drought) tend to be more susceptible to attack by secondary pests including conifer mites. Recently, new insecticide/miticides (emamectin benzoate and Experimental Products DYM1, DYM2, DYM3, and NSM, Arborjet Inc.) have shown some promise for control of mites. In this study, the effectiveness of these chemicals will be evaluated for protecting trees from secondary conifer mites.

Objectives:

1. Evaluate the potential efficacy of tree injection of TREE-age™ (emamectin benzoate), and spray applications of DYM1, DYM2, DYM3, NSM, for control of secondary conifer mites.

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

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

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

Several miticides (insecticidal/miticidal oils and soaps, Dicofol™, Kelthane™, Avid™,

Floramite™, Hexagon™, Sanmite™, and Forbid™) are available for control, but resistance can develop if the applicator relies too heavily on one product (Shetlar 2011). Recently, Arborjet has developed several formulations of botanical miticides.

Research approach:

Locations, Treatments, and Environmental Conditions

This study will be conducted at The Campbell Group's Seed Orchard, Jasper, TX (about 30°57 N, 94°09 W, elev. 105 ft). An initial survey will be conducted in early September 2012 of the general health of four-year-old loblolly pines in a polymix trial containing several families. Each pine will be evaluated for tip moth damage and presence of conifer mites. Fifty (50) trees will be randomly selected for treatment. An additional ten trees will serve as untreated checks.

There will be six treatments: TREE-age (emamectin benzoate) tree injection (treatment **1**); Arborjet product DYM1 spray (treatment **2**); Arborjet product DYM2 spray (treatment **3**), Arborjet product DYM3 spray (treatment **4**), Arborjet product NSM spray (treatment **5**),and untreated control (treatment **6**).

Each treatment will be applied to 10 randomly-assigned trees. Test trees will be located in areas with abundant TM activity, and spaced >4 m apart. The injection treatment (treatment 3) will be injected at the labeled rate (2.5 ml TREE-age per inch ground line diameter) after dilution in 1 part water with the Arborjet Tree IV™ microinfusion system (Arborjet, Inc. Woburn, MA) into a three points (use #3 Arborplugs) at staggered heights up to 6 inches above the ground. Injections will occur in early September 2012. Arborjet spray treatments (6, 7, 8, & 9) will be applied in mid-September 2012.

In September, 2012 (at the time of initial spray treatment) and then 7, 14, 21, and 28 days after treatment application, two lower branches will be shaken over a white sheet of paper. The conifer mites found on the paper will be counted and identified.

Precipitation and temperature data will be obtained from the nearest weather station during the course of this study from 1 September to 1 December 2012.

Research timetable:

Research Activity	Date
1. Study plan	Completed
2. Campbell Group contacted, liaison	Completed
3. Field site selection	Completed
4. Trees selected, tagged and treatments assigned	September 2012
5. Treatments 1- 9 applied	September 2012
6. Post-treatment assessment of efficacy	Oct - Nov 2012
8. Data summary and analyses	Dec 2012
9. Final report, peer-reviewed publication submitted	Mar 2013

Literature cited:

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

Budget:

Conifer Mite: - CY 2012-2013

Personnel	
Grosman	Contributed
Research Specialist (7.5%)	$ 2,448.00
Benefits (30%)	$ 734.40
	$ 3,182.40
Materials and Supplies	
Miscellaneous materials and supplies	$ 267.60
Travel	
Vehicle fuels and maintenance	$ 518.25
Subtotal	$ 3,968.25
Indirect Costs (26%)	$ 1,031.75
TOTAL REQUESTED	**$ 5,000.00**

Evaluation of Microinjection Systems for Application of Propiconazole in Live Oak

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Justification: Several cultural control techniques (minimize fungal inoculum, timing of branch pruning, prompt removal of infected red oaks, and root disruption/trenching, among others) are available for management of oak wilt, caused by the plant pathogen, *Ceratocystis fagacearum* (T.W. Brentz) (Koch et al. 2010). However, these techniques are often impractical for treatment of high value individual trees or small groups at risk to infection. Currently, the only effective treatment available 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). Applications of propiconazole have been made almost exclusively through the use of macroinjection 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 (Blaedow et al. 2010), but is very labor intensive to perform. Arborists are interested to know if propiconazole can be applied at more concentrated levels to live oak using available microinjection/infusion systems and whether these applications effective in preventing/reducing fungal infection spread within the host.

Objectives:

1) Evaluate ability of various delivery systems to inject propiconazole formulation based on time to prepare/load, install and treat each tree and safety.
2) Evaluate speed and distribution of propiconazole movement based on protection 4 weeks after injection, and then every 8 weeks for 18 months.

Methodology:

Five (5) microinjection systems and one (1) macroinjection system will be evaluated:

Mauget System (Mauget; contact: Marianne Waindle) low volume (10 ml/inj pt); low pressure (5 psi)

Pine Infuser System (Rainbow Treecare Scientific Advancements; contact: Shawn Bernick); moderate volume (10 - 30 ml/inj pt); moderate pressure (40 psi)

Portle System (ArborSystems; contact: Chip Doolittle) – moderate volume (1-10 ml/inj pt); moderate pressure determined by applicator (50+ psi)

Tree IV System (Arborjet, Inc.; contact: Joe Doccola) – high volume (40+ ml/inj pt); moderate pressure (60 psi)

Chemjet System (Scenic Hills Nursery; contact: Jim Rediker) – moderate volume (20 ml/inj pt); low pressure (23 - 37 psi)

Macro Injection System (Standard) (Rainbow Treecare Scientific Advancements; contact: Shawn Bernick) - high volume (30 ml/inj pt); low pressure (20 psi)

Information about the systems will be requested from each manufacturer/distributor. In particular, information will be requested on:

1) system cost
2) need for peripheral parts (plugs, needles)
3) system capacity (volume of product)
4) recommended procedures for installation and injection of trees
5) Is system reusable?
6) Does chemical product need to be prepackaged or mixed?

Each system will be ranked on the following criteria with potential points in parentheses:

1) System cost (10 pts)
2) Need for peripheral parts (plugs, needles, battery chargers) (5 pts)
3) System capacity (volume of product) (3 pts)
4) Is system disposable or reusable? (2 pts)
5) Does chemical come prepackaged; can you inject product undiluted or is it necessary to dilute with water? (5 pts)
6) Time and ease to fill system with chemical product (5 pts)
7) Time and ease to install system on tree (5 pts)
8) Number of injection points required per tree (5 pts)
9) Can the system be left alone on tree or does the applicator need to manually operate the system continuously? (5 pts)
10) Time and ease to inject X amount of product. (10 pts)
11) Cumulative time applicator spends at each tree. (10 pts)
12) Potential for chemical exposure. (10 pts)
13) Time and ease to clean system. (10 pts)
14) Weather restrictions (moisture, temperature) (5 pts)
15) Effectiveness of treatment 1 month after treatment (10 pts)
16) Effectiveness of treatment at 6, 12 and 18 months (10 pts each period)

*Treatment Methods and Evaluation**:***

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

system manufacturer. The injected trees were allowed 6 weeks to translocate chemicals prior to being challenged with fungal inoculations.

Inoculations were/will be performed using standard procedures (Camilli et al. 2009, Peacock and Fulbright 2009) on three of the four groups of trees at each site. Two *Ceratocystis fagacearum* isolates were cultured from samples recovered from infected live oak and Spanish oak in spring 2011 from an active oak wilt center in Central Texas. The pathogen cultures were serially "plated" on petri plates containing Potato Dextrose Agar. Following 2 weeks of growth, the plates were flooded with 20 ml of sterile distilled water. The surfaces of the plates were scraped with a glass rod, resulting in a suspension of conidia. The conidia were harvested by pouring the water from the plates, combining the aliquots, and quantifying the total suspension with a hemacytometer. The suspension was adjusted to a level of 1×10^6 spores/ml with appropriate dilutions to make a quantity of the inoculum sufficient for the inoculations. On July 28, 2011, two inoculation points (North and South sides) were selected on each roots 23 cm below injection points. At each point, a 14mm-wide wood chisel was used to cut through the bark into the xylem tissue (~ 2 cm deep). A dropper was used to apply 1 ml of conidia suspension into each wound site. Due to extreme drought conditions, it may be necessary to reinoculate trees in November, 2011.

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

A photograph of the crown of each study tree will be taken at the time of fungal inoculation. Trees will be evaluated for crown condition every week. The date of oak wilt symptoms appearance will be recorded and then evaluations will switch to once every 4 weeks thereafter for 80 weeks (18 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 felled and wood samples taken from the stem and branches to determine the presence of *Ceratocystis fagacearum*.

At the termination of the experiment in November 2012 (about 18 months after pathogen inoculation), final crown ratings will be made. An analysis of variance will be used to test for differences among injection systems. A X^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.

Once the trial is complete, infected trees and any new oak wilt centers will be destroyed to prevent further spread into other areas.

Research Time Line**:**

CY 2013

April - December, 2013

- Monitor for tree decline (April - October)
- Sample infected trees to confirm presence of *Ceratocystis fagacearum*.
- Conduct statistical analyses of data (November)
- Prepare and submit report to FPMC Executive Committee, Syngenta and System manufacturers (December).
- Destroy all infested trees and treat new oak wilt centers created by this study.

References:

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Camilli, K., D.N. Appel, and W.T. Watson. 2009. Studies on pruning cuts and wound dressings for oak wilt control, pp. 115-128. *In* R.F. Billings and D. N. Appel (eds.). Proceedings National Oak Wilt Symposium, June 4-7, 2007, Austin, TX. Texas Forest Service Publ. 166.

Koch, K.A., G.L. Quiram, and R.C. Venette. 2010. A review of oak wilt management: A summary of treatment options. Urban Forestry & Urban Greening. 9: 1-8.

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Peacock, K.L. and D.W. Fulbright. 2009. Effective longevity of propiconazole following injection into Quercus rubra, pp. 175-184. *In* R.F. Billings and D. N. Appel (eds.). Proceedings National Oak Wilt Symposium, June 4-7, 2007, Austin, TX. Texas Forest Service Publ. 166.

Cooperators:

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Rainbow Treecare Scientific Advancements; President: Shawn Bernick; Ph: (952) 252-0547; Fax: 952) 252-4720; Email: sbernick@treecarescience.com; Website: http://www.treecarescience.com/; Address: 11571 K-TEL DR., Minnetonka, MN 55343

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

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Abstract: Hypoxylon canker (HC) has caused considerable mortality of oaks in Texas in association with severe drought in 2011 and into 2012. There was no known control or treatment for HC other than maintaining tree vigor. Recently, an injected systemic fungicide, containing salts of phosphorous acid (PHOSPHPO-jet™; Arborjet Inc., Woburn, MA) has shown some promise for improving the health of HC-infected oaks. In this study, the effectiveness of recommended rates of PHOSPHO-jet™ will be evaluated for protecting or improving the health of individual red oak trees infected with hypoxylon canker.

Objectives:

3) Evaluate the potential efficacy of systemic injections of PHOSHO-jet (salts of phosporous acid) as a therapeutic treatment of oaks against hypoxylon canker; and
4) Determine the duration of treatment efficacy.

Background/Justification Statement:

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

Hypoxylon canker activity usually increases during and shortly after prolonged droughts. When drought stresses trees, the fungus is able to take advantage of these weakened trees. The moisture content of living wood in live, healthy trees is typically 120% - 160%. It is difficult for HC to develop in wood that has a normal moisture content. However, any of the factors listed

above could weaken or stress trees causing the moisture content of the wood to reach levels low enough for the hypoxylon fungus to develop. When this happens, the fungus becomes active in the tree and invades and decays the sapwood causing the tree to die. Once hypoxylon actively infects a tree, the tree will likely die.

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

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

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

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

Completion of proposed objectives will:

1) Document the efficacy of the recommended rate of the PHOSPHO-jet™ formulation of salts of phosphorous acid for protecting individual red oak from decline and/or mortality attributed to hypoxylon canker.

2) Determine the efficacy of PHOSPHO-jet™ as a therapeutic treatment after hypoxylon canker infection.

Research approach:

Locations, Treatments, and Environmental Conditions

This study will be conducted near or within Kit McConnico Park, Lufkin, TX (about 31°22 N, 94°41 W, elev. 249 ft). A survey will be conducted in August 2012 of the general health of red oaks along the Kit McConnico Hiking and Biking Trial (5.1 miles in length). Each oak will be assigned to one of four health categories: **Healthy**; "healthy", full crown with no apparent

evidence of HC infection; **Light**: some evidence HC infection and < 20% of crown showing dieback; **Moderate**: evidence HC infection and 20-80% of crown showing dieback; **Severe**: obvious HC infection and > 80% of crown showing dieback. Ten (10) red oaks from each of the healthy, light and moderate health categories will be randomly selected for PHOSPHO-jet treatment. An additional ten trees from each category will serve as untreated checks.

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

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

In September, 2012 (at the time of treatment) and then the folowing spring (April), summer (July) and fall (October) 2013 and 2014, the stem and crown of each tree will be ranked as to health and the extent of fungal infection. In addition, where possible, small branchs (12" length) will be collected from the low, mid and upper crown of each study tree. The branches will be evaluated for the presence of HC.

Precipitation and temperature data will be obtained from the nearest weather station during the course of this study from 1 September 2012 to October 2014.

Experimental Design – Treatment Efficacy

A photograph of the crown of each study tree in TX will be taken at the time of treatment and again in April, July, and October of 2013 and 2014. Trees will be evaluated for crown condition every three months for 24 months. Each oak crown will be given a rating of 0 (healthy), 1 (HC symptom comprising < 20% of the crown), 2 (HC symptoms comprising 20-80% of the crown), 3 (HC symptoms comprising >80% of the crown) (Mayfield et al. 2008), or 4 (dead tree). At each rating period, trees with a crown rating of 2 will have wood samples taken from the stem and branches to determine the presence of HC fungi.

At the termination of the experiment in November 2014 (about 24 months after treatment), final crown ratings will be made. An analysis of variance will be used to test for differences among injection treatments. A χ^2 (Chi-square) test for homogeneity will be used to test the null hypothesis that the crown rating of treated trees of a particular health category did not differ from untreated control tree in the same health category (Mayfield et al. 2008).

Research timetable:

Research Activity	Date
1. Study plan	Completed
2. Lufkin Parks contacted, liaison	Completed
3. Field site selection	Completed
4. Trees selected, tagged and treatments assigned	September 2012
5. Treatments 1, 3, 5 & 7 applied	September 2012
6. Post-treatment assessment of efficacy	Apr, Jul & Oct 2013
7. Post-treatment assessment of efficacy	Apr, Jul & Oct 2014
8. Data summary and analyses (Grosman and new Coordinator)	Nov 2014
9. Final report, peer-reviewed publication submitted (co-authored by Grosman and new Coordinator)	Dec 2014

Literature Cited

H.A. Pase III. 2012. Hypoxylon Canker. http://txforestservice.tamu.edu/main/article.aspx?id=1262.

Budget:

Hypoxylon Canker:- CY 2012-2013

Personnel	
Grosman	Contributed
Seasonal Technician (30%)	$ 2,997
Benefits for Seasonal Technician (8.45%)	$ 253.25
Materials and Supplies	
Miscellaneous materials and supplies	$ 400
Travel	
Vehicle fuels and maintenance	$ 318
Indirect Costs (26%)	$ 1,031.75
TOTAL REQUESTED	**$ 5,000**