Forest Pest Management Cooperative

2014 Research Proposals

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2014 FPMC Members

Forest Investment Associates
Hancock Forest Management, Inc
Plum Creek Timber Company, Inc
Rayonier
The Campbell Group
Weyerhaeuser Company
Anthony Forest Products
Arborgen, LLC
International Forestry Company
US Forest Service/FHP
Arborjet, Inc.

March 2014

Table of Contents

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).

<u>Treatments</u>**:**

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 2014

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 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)

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Justification

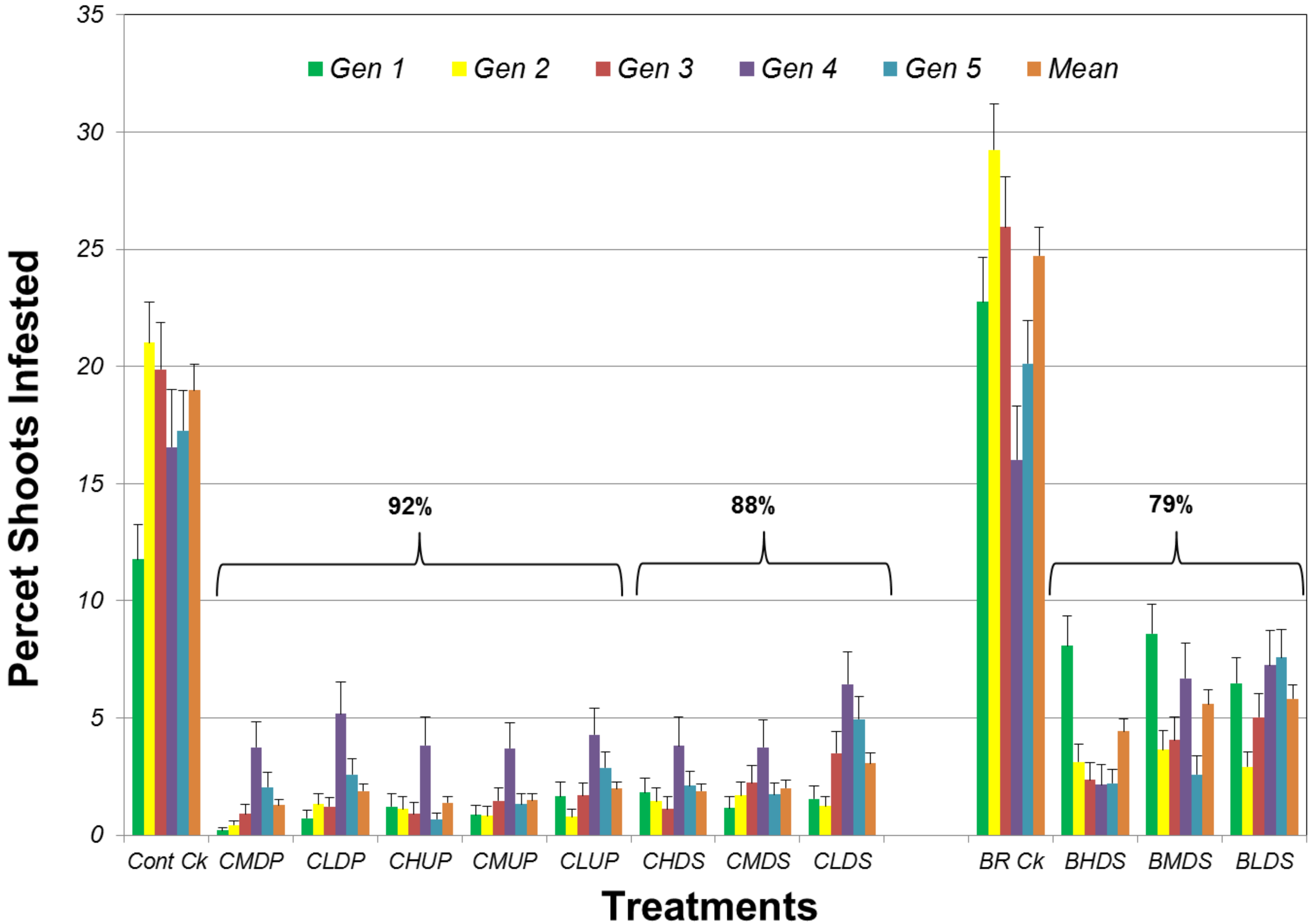
Several FPMC trials (2003 - 2005) showed that fipronil (PTM™) applied to bare root seedlings before or after planting was highly effective in reducing tip moth damage for 2+ years. Operationally, it would be desirable to apply chemical solutions to containerized seedlings because these trees have higher 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 5th 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).

<u>Treatments</u>**:**

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

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

Square 2

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

Square 3

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

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)

Don Grosman and Billi Kavanagh
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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	
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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

Square 2

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

Square 3

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

<u>January - February 2012</u>

- 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

<u>March - October, 2012</u>

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

<u>November - December 2012</u>

- 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

<u>January - February 2013</u>

- Begin trap monitoring of tip moth populations near each site

<u>March - October, 2013</u>

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

<u>November - December 2013</u>

- 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

<u>January - February 2014</u>

- Begin trap monitoring of tip moth populations near each site

<u>March - October, 2014</u>

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

<u>November - December 2014</u>

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

Don Grosman & Billi Kavanagh
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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

<u>November - December 2012</u>

- 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

<u>March - October, 2013</u>

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

<u>November - December 2013</u>

- 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.

<u>March - October, 2014</u>

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

<u>November - December 2014</u>

- 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.

OPTIMAL TIMING FOR A ONE-TIME SPRAY APPLICATION OF MIMIC^{TM} 2LV FOR CONTROL OF PINE TIP MOTH

Justification

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

Objectives

1. Determine which of four tip moth generations should be sprayed if only one spray treatment can be economically applied.

Cooperators: Plum Creek Timber Company, Weyerhaeuser, Valent BioSciences

Study site locations: Arkansas

Insecticides: Mimic^{TM} 2LV

Methods

In February 2014, three second-year plantations will be selected in Arkansas, 2 first-year plantations will be selected in LA, and 2 first-year plantations will be selected in NC. The plantations will be as variable as possible (wet, droughty, etc).

Experimental Design/ Statistical Analysis

A nested, randomized complete block design will be utilized. The first blocking factor will be site (plantation) and the second will be subplot, of which there will be five per site. There will be five treatments, which will be randomly assigned as a row of ten trees to the five subplots at each site (50 trees per treatment). The subplots will then contain 50 trees, for a total of 250 trees per site. Buffer rows will be placed between each treatment row (Figure 1).

Treatments:

1. Spray only for generation 1 (blue)
2. Spray only for generation 2 (yellow)
3. Spray only for generation 3 (red)
4. Spray only for generation 4 (orange)
5. Spray all generations (white)
6. Spray no generations (green) (Check)

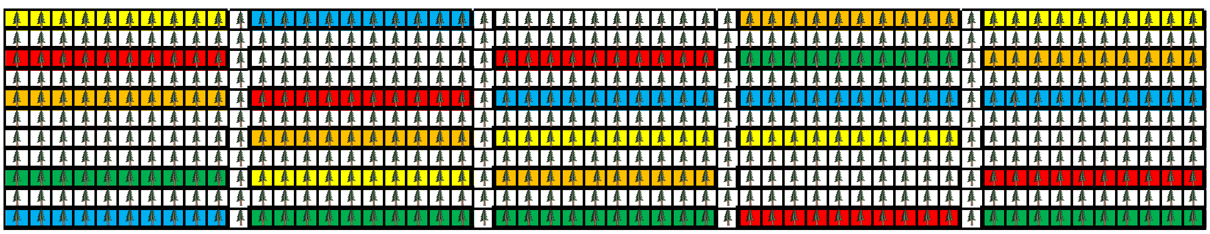

Figure 1. Example of nested block design at one site.

The timing of spray applications will be modeled after Fettig et al. (2003) and Fettig et al. (2000) (Table 1). Specific dates will depend upon the location of each site.

Table 1. General timing of spray applications

	Generation 1	Generation 2	Generation 3	Generation 4
Arkansas	April	June	July/August	September
North Carolina	April	June	July/August	n/a
Louisiana	March/April	May	July	August/September

Tip moth damage will be evaluated on all seedlings located in each subplot after each tip moth generation (3-4 weeks after peak moth flight) by 1). Identifying if the tree was infested or not, 2). If infested, the proportion of tips infested on the top whorl and terminal will be calculated, and

3). Separately, the terminal will be identified as infested or not. Additionally, a random sample of 10 trees per treatment at each site will be chosen to measure growth of the top terminal and 3 lateral shoots at the initiation of the study and at each generation. The height and diameter (at 15 cm or 6 in) of all trees will be measured at the initiation of the study and in the fall. All data will be analyzed using ANOVA with two blocking factors. If significant differences are found, Student's T will be used to determine where the differences lie. Experimentation may be repeated in 2015 if sufficient efficacy is found for a treatment other than the positive control (treatment 6).

Timeline

- February 2014: Choose sites, take initial height, diameter and shoot measurements
- March 2014: Begin spray treatments
- March 2014 through September 2014: Continue treatments, evaluate tip moth infestations, measure shoots
- November-December 2014: Measure height and diameter
- February 2015: Progress or Final Report

Literature Cited

Cade, S.C., Hedden, R.L., 1987. Growth impact of pine tip moth on loblolly pine plantations in the Ouachita mountains of Arkansas. Southern Journal of Applied Forestry 11**,** 128-133.

Fettig, C.J., Dalusky, M.J., Berisford, C.W., 2000. Nantucket pine tip moth phenology and timing of insecticide spray applications in seven southeastern states. Research Paper-Southern Research Station, USDA Forest Service.

Fettig, C.J., Nowak, J.T., Grosman, D.M., Berisford, C.W., 2003. Nantucket pine tip moth phenology and timing of insecticide spray applications in the Western Gulf region. USDA Forest Service, Southern Research Station Research Paper SRS-32.

Sun, J., Kulhavy, D., Roques, A., 2000. Effects of fertilizer and herbicide application on Nantucket pine tip moth infestation (Lep., Tortricidae). Journal of Applied Entomology 124**,** 191-195.

Budget:

Research period: February 2014-December 2014

Personnel	
Fischer (Coordinator, FPMC)	Contributed by TFS
Upton (Staff Forester II)	Contributed by TFS
Spivey Resource Specialist (10% of time)	$2,496.00
Benefits for Resource Specialist (30%)	$748.80
Jackson Seasonal Employee (10% of time)	$1,098.90
Benefits for Seasonal (8.6%)	$94.51
Materials and Supplies	
Misc. materials and supplies	$500.00
Vehicle Fuel (1180 miles @ $3.50 per gallon)	$266.45
Travel	
Total Meals ($40.25/day) Lodging ($83/ night) * 22 nights	$8,134.50
Other	
Maintenance	$600.00
Subtotal	$6,845.99
Indirect Costs (26%)	$1,779.96
Total	**$8,625.95**

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

(Initiated in 2013)

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Objectives: 1) Determine the efficacy of emamectin benzoate for protecting loblolly pine from PWN; and 2) efficacy of two emamectin benzoate concentrations.

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 and new 8% EB).
Rates: 1.25 or 2.5ml/inch DBH
Injection spacing: Root Flare Diameter/1.0 (~1 pt every 3"circ) spacing.
Season of Treatment: Fall 2013.
Duration: 8 month

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

Efficacy of Emamectin Benzoate

In fall 2013, 30 "healthy appearing" trees (25 cm (=10") DBH, ~25-YO) will be selected in an east Texas plantation. In mid-October ten (10) trees will be randomly assigned and treated with one of the treatments indicated below. The chemical will be allowed 8 months to circulate within each tree prior to felling. Immediately (within an hour of felling) 1.0 m bolts will be taken from the main stem of the lower crown (~6 m), and lower bole (0 m). The treatments include:

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

B = EB (8%) @ 1.25 ml @ **3"** spacing felled 8 month post injection (mid June)
C = Check (untreated) for each Treatment set above (10)

The 60 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 26-30 d after tree felling.

Monitoring Monochamus species and PWN occurrence in beetles
Modified funnel traps will be deployed (beginning in early 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 collection of wood samples, 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 live cerambycid larve present under bark.
2. Cerambycid activity, estimated by overlaying a 100 cm^2 grid over a portion of each bark strip and counting the number of squares overlapping area where cerambycid larvae have fed.
3. Number of oval cerambycid larvae entrance holes into sapwood.
4. Presence and percent area covered with blue stain.

Sampling logs for pinewood nematodes 28 days after felling
Each log is sampled at four locations: two points within each of the two bark plate areas. A wire brush is used to remove dirt and debris from the sample locations. 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 2013

October 2013

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

CY 2014

June - July 2014

- Cut trees and expose logs to cerambycids for 28 days (June)
- Collect tissue samples from trees and logs (July)
- Laboratory extraction and identification of nematode from plant tissue and adult *Monochamus* (July)
- Conduct statistical analysis of 2014 data (August).
- Prepare and submit preliminary report to participating members.

References:

Craighead. F.C. 1950. Insect enemies of eastern forests. Misc. Publ. 657. Washington, DC: US Department of Agriculture. 679 pp.

Cran, M. and J. Hanson. 2004. How to identify and manage pine wilt disease and treat wood products infested with pinewood nematode. NA-FR-01-04.

Dwindell, L.D. 1997. The pinewood nematode: regulation and mitigation. Ann. Rev. of Phytopathology. 35: 153-166.

Dwinel,l L.D. and W.R. Nickle. 2004. An overview of the pine wood nematode ban in North America. USDA www.forestpests.org

EPPO. *Bursaphelenchus xylophilus*. 1990. http://www.eppo.org/QUARANTINE/nematodes/Bursaphelenchus_xylophilus/BURSXY_ds.pdf. 12 pp.

Glass, B. 2012. The Campbell Group LLC, Timber Trends, Dec, '11/Jan. '12. https://www.campbellgroup.com/_assets/downloads/public/publicationdoc/Dec_10_TT.pdf. 12 pp.

Linit, M. 1988. Nematode-vector relationships in the pine wilt disease system. J. Nematol. 20: 227-235.

Linit, M.J., E. Kondo, and M.T. Smith. 1983. Insects associated with the pinwood nematode, *Bursaphelenchus xylophilus* (Nematode: Aphelenchoididae(, in Missouri. Environ. Entomol. 12: 467-470.

Miller, D.R., C. Asaro, C.M. Crowe, and D.A. Duerr. 2011. Bark beetle pheromones and pine volatiles: Attractant kairomone lure blend for longhorn beetle (Cerambycidae) in pine stands of the southeastern United States. J. Econ. Entomol. 104: 1245-1257.

Mamiya, Y. and T. Kiyohara. 1972. Description of *Bursaphelenchus lignicolus* n. sp. (Nematoda: Aphelenchoididae) from pine wood and histopathology of nematode-infested trees. *Nematologica* 18: 120-124.

Mamiya, Y. 1983. Pathology of pine wilt disease caused by *Bursaphelenchus xylophilus*. *Annual Review of Phytopathology* 21: 201-220.

Takai, K., T. Soejima, T. Suzuki, and K. Kawazu. 2000. Emamectin benzoate as a candidate for a trunk-injection agent against the pine wood nematode, *Bursaphelenchus xylophilus.* Pest Manag. Sci. 56: 937-941.

Takai, K., T. Soejima, T. Suzuki, and K. Kawazu. 2001. Development of a water-soluble preparation of emamectin benzoate and its preventative effect against the wilting of pot-grown pine trees inoculated with the pinewood nematode, *Bursaphelenchus xylophilus.* Pest Manag. Sci. 57: 463-466.

Takai, K., T. Suzuki, and K. Kawazu. 2003a. Development and preventative effect against pine wilt disease of a novel liquid formulation of emamectin benzoate. Pest Manag. Sci. 59: 365-370.

Takai, K., T. Suzuki, and K. Kawazu. 2003b. Distribution and persistence of emamectin benzoate at efÞcacious concentrations in pine tissues after injection of a liquid formulation. Pest Manag. Sci. 60: 42-48.

Webb, J.L. 1909. The southern pine sawyer. Bull. 58. Washington, DC: US Department of Agriculture Bureau of Entomology: 41-56.

Budget:

Research period: October 2012 – August 2014

Personnel	
Grosman / future FPMC Coordinator	Contributed
Student Worker (65%)	Contributed
Student Worker (35%)	$ 3,499.65
Benefits for Seasonal Technician (8.45%)	$ 295.72
	$ 3,795.37
Materials and Supplies	
4 liters of TREE-äge (emamectin benzoate) and Arborplugs	Contributed
20 kairomone blend baits ($17/bait)	$ 340.00
PPE equipment (chaps, hard hat, gloves, eye protection)	$ 100.00
Lab equipment (funnels, pans, slides, chemicals)	$ 150.00
	$ 590.00
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)	$ 500.00
Subtotal	$ 4,885.37
Indirect Costs (26%)	$ 1,270.19
TOTAL REQUESTED	**$ 6,155.57**

Incorporating Emamectin Benzoate into Control Strategies for Southern Pine Beetle

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Forest Pest Management Cooperative
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Lufkin, TX 75904

David L. Cox — Syngenta Crop Protection LLC
14446 Huntington Rd.
Madera, CA 93636

Justification: The southern pine beetle (SPB) (Coleoptera: Curculionidae, Scolytinae) is considered the most destructive insect pest of southern pine forests. Since 1997, no SPB infestations have been detected in Western Gulf states (TX, AR, LA & OK) and very few SPB have been caught in pheromone traps in East Texas since 2001 (11 SPB). Pheromone traps deployed during the spring have proven effective for predicting SPB population increases since 1988 across the South (Billings and Upton 2010). SPB populations in 2012 and 2013 were at unprecedented low population levels throughout the South and Northeast, with the exception of southern New Jersey, the Hommochitto National Forest and surrounding private lands in Mississippi, and local areas in Alabama and Virginia. The SPB Prevention Program, sponsored by US Forest Service/Forest Health Protection, has cost shared the thinning of high hazard pine stands as an SPB prevention measure since 2003; some 100,000 acres have been treated as of November 20, 2013 on small private landholdings in Texas (Billings 2009 and personal communication,); over a million acres have been treated throughout the South under this program. Although prevention efforts are important, they are not being applied to all land ownerships, suggesting that it is just a matter of time before SPB outbreaks reoccur in Texas and other southern states. A method for effectively dealing with SPB outbreaks in early stages of development is needed. Much is known about SPB biology and seasonal habits (see Coulson and Klepzig 2011). Most new SPB infestations are initiated following long-distance dispersal in the spring (March-May) and to a lesser extent in the fall (October-December). A new systemic insecticide (emamectin benzoate) has been developed by the Texas Forest Service Forest Pest Management Cooperative and is sold by Syngenta under the trade name Tree-äge™. This insecticide is effective against SPB (Grosman et al 2009, 2010) and has been registered and is now available for pine bark beetle control in forest situations. This is the only insecticide registered for control of SPB in forests. Allee effects (positive density dependence) have been shown to play an important role in the establishment and spread of invasive species. A certain population density is essential before an invasive species can become established and spread in a new environment (and because of Allee effects, many new introductions of invasive plants and animals fail to succeed). Increased interest in recent years is being focused on the potential to exploit Allee effects as a means to manage invasions of exotic species (Tobin et al. 2011). We propose to exploit this same phenomenon for control of SPB when populations begin to return, treating this native bark beetle as if it were an invasive species.

The Forest Pest Management Cooperative initiated a trial in 2012 to evaluate the ability of emamectin benzoate- treated trap trees to manage southern pine beetle populations at low levels in AL and VA. The first year results indicate that baited EB-treated trees can absorb SPB in areas with low population levels (<2.0 SPB/trap/day). It is unknown whether emamectin benzoate-treated trees would continue to show efficacy against SPB past the first year of treatment.

Objectives:

1. Evaluate the efficacy of trunk injections of emamectin benzoate for protection of southern yellow pines against SPB
2. Determine the duration of efficacy of emamectin benzoate for protection of southern yellow pines against SPB (in the second year following injection).
3. Develop and evaluate a new management strategy to monitor and respond to SPB populations to maintain them below the Allee threshold required for re-establishment and spread, using current knowledge of SPB seasonal behavior, available methods of SPB monitoring, and new technology for suppression.

Cooperators:

Ms. Cindy Ragland	Oakmulgee R.D, Talladega N.F., Brent, AL
Dr. Christopher Asaro	VA Dept. of Forestry, Charlottesville, VA
Dr. Steve Clarke	USDA Forest Service – FHP R8, Lufkin, Texas
Dr. Don Grosman	Arborjet, Inc., Woburn, MA

Study Sites: The study is to continue to be conducted in the Talladega National Forest, Oakmulgee Ranger District in Bibbs and Perry Co., Alabama and in the Prince Edward and Appomattox-Birmingham State Forests, Virginia with SPB attacking loblolly pine, *Pinus taeda*. Forest tracts (18-22) where loblolly pine predominate, of similar age (>30 years old) and density (>90 basal area), were selected on the State and National Forests.

Insecticides:

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

Treatments (2013):

1. Baited (frontalin + Sirex lure + endo-brevicomin (EB)), emamectin benzoate-treated trees surrounded by 2-6 unbaited, emamectin benzoate-treated (5ml/ inch DBH) trees (within 15 ft of baited trap tree).
2. Baited (frontalin + Sirex lure + endo-brevicomin (EB)), emamectin benzoate-treated trees surrounded by 2-6 unbaited, untreated trees (within 15 ft of baited trap tree).
3. Baited (frontalin + Sirex lure + endo-brevicomin (EB)), emamectin benzoate-treated tree isolated from other pine (no pine within 25ft) located within a pine/ hardwood stand with a closed canopy (10- 30 BA).
4. Control: Isolated trees (no pine within 25ft) located in pine/hardwood, closed-canopy stands. Trees will be baited (frontalin + Sirex lure + endo-brevicomin (EB)), but not treated.

Treatment Methods and Evaluation:

In 2013, three Lindgren funnel trap baited with frontalin + turpentine + endo-brevicomin (displaced by 4 m) bait were deployed in the area 300 m away from plots, to monitor local beetle populations.

The sites chosen in AL and VA forests were selected based on low trap catch levels (<2 SPB/trap/day). Within each forest, loblolly stands with higher BA (>90 sq. ft./acre) were selected. Within each stand (within 150 ft of an access road to facilitate treatment), a center tree was selected and for treatments 2 and 3, all trees within 15 ft of the center tree were flagged and tagged. One of four treatments was randomly assigned to each tract. Note: Where possible, poor quality (form, health, etc.) trees were selected as trap trees.

TREE-äge™ was injected at 5 ml per inch DBH in trees < 12" and 10 ml per inch DBH in trees $\geq$ 12". The Tree IV™ microinfusion system (Arborjet, Inc. Woburn, MA) was used to inject TREE-äge™ into 4 (trees <12" DBH) -8 (trees $\geq$12" DBH) points 0.3 m above the ground. The injected trees were allowed 4 weeks to translocate chemicals prior to being challenged by the application of synthetic pheromone baits. All center trees were baited with species-specific lures (Synergy Semiochemical, Delta, BC) for three 5 week periods in 2012.

In 2014, these sites/ treatments will be revisited. Three Lindgren funnel traps baited with frontalin + turpentine + endo-brevicomin (displaced by 4 m) will again be deployed approximately 300 m away from plots, to monitor local beetle populations. The control plots (Treatment 4) will need to be re-established due to tree mortality the previous year. All center trees will be baited with species-specific lures (Synergy Semiochemical, Delta, BC) for three 5 week periods in 2014. The treatments will be evaluated as described below to determine if emamectin benzoate- treated trees continue to show efficacy against the SPB as trap trees in the second year of treatment.

Treatment evaluation:

- Monitor attack level (occurrence of pitch tubes) of SPB and health on study (baited, injected or untreated) trees at intervals of 5 12 and 19 weeks after the installation of baits.
- At the end of the field season (October in VA; November in AL), each study tree (trap tree and treated and untreated within 30 ft of trap tree; N = 18-30 per block), will be nondestructively sampled. Using a head lamp and hand lens, the number of SPB successful attacks (i.e., oxidized phloem material present in pitch tubes or points of attack containing phloem boring dust and/or dry frass) and unsuccessful attacks (i.e., pitch tubes without oxidized phloem material) in 20 X 25 cm (500 cm2) sample windows at approximately 1.5, 4.0 and 6.5 m in height at northern and southern aspects will be recorded.
- All dead study trees will be felled. Bark plates (10 X 10 cm = 100 cm2) will be collected at approximately 1.5, 4.0 and 6.5 m height at northern and southern aspects. SPB gallery length and density of emergence holes will be measured.
- Compare the number of new SPB infestations that become established in treated and untreated areas with similar host/climatic conditions.

Project Timetable:

CY 2014:

1) Bait trees (AL: April, VA: May)
2) Post-bait evaluations at five and twelve and nineteen weeks after baiting (May, late June, September)
3) End of field season evaluation (VA: October, AL: November)
4) Data summary and analyses (December)

CY 2015:

1) Progress report (January)

Literature Cited:

Billings, R.F. 2009. Delivering science-based information to forest landowners: The southern pine beetle prevention project in east Texas", pp. 159 – 166. *In*: Proceedings, Southern Region Conference on Technology Transfer and Extension, August 2-4, 2006, S. Ashton, W.G. Hubbard, and H.M. Rauscher, eds. USDA Forest Service, Southern Research Station General Technical Repot SRS-116. Hot Springs, AR.

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

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

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

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

Smith, Richard H. Trapping western pine beetle with baited toxic trees. Res. Note PSW- 382, Berkeley,CA: Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture; 1986. 9 P.

Tobin, P.C., L. Berec, and A.M. Leibhold. 2011. Exploiting Allee effects for managing biological invasions. Ecology Letters 14: 615-624.

Budget:

Research period: April 2014-January 2015

<u>Personnel</u>	
Coordinator, FPMC	Contributed
Staff Forester II	Contributed
Resource Specialist (12% of time)	$2,995.00
Benefits for Resource Specialist (30%)	$898.56
<u>Materials and Supplies</u>	
SPB combo lures + EB (4 sites x 4 treatments x 6 replicates x baited 3 times minus 100 contributed @ $19.22/ea)	$3,613.36
Misc. materials and supplies	$500.00
Vehicle Fuel (50% of 15,730 miles @ $3.50 per gallon)	$1,911.00
<u>Travel</u>	
Meals ($40.25/day) Lodging ($83/ night) * 29 days	$5,423.00
<u>Other</u>	
Maintenance	$600.00
<u>Subtotal</u>	$15,941.12
<u>Indirect Costs</u> (26%)	$4,144.69
Total	**$20,085.81**

Incorporating Emamectin Benzoate into a Control Strategy for Southern Pine Beetle

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Justification:
The Forest Pest Management Cooperative initiated a trial in 2012 in AL and VA to evaluate the ability of emamectin benzoate-treated pines to serve as trap trees for maintaining southern pine beetle populations at low levels. It was found that the southern pine beetle was more likely to attack **untreated** trees surrounding a central-baited, treated tree compared to **treated** trees surrounding a central-baited, treated tree. This may suggest that the southern pine beetle can detect emamectin benzoate within the tree and therefore has a preference for attacking nearby untreated trees rather than the baited, treated tree in the center of the plot. For this reason, it is of interest to assess the efficacy of emamectin benzoate for protection of southern yellow pines against SPB by applying injection and baiting treatments at different timings. Perhaps if a tree is baited at the time it is injected, quickly thereafter, or baited before injection, beetles would not detect the chemical, as the emamectin benzoate has not had opportunity to move upward from the basal injection points. The bait may attract beetles that then attack the tree and produce brood, but the brood would not be expected to live, as the chemical should move into the upper region of the bole and kill the brood before emergence can occur.

Objectives:

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

Cooperators:
Jim Meeker, USDA Forest Service, Forest Health Protection, Pineville, LA
Roger Menard, USDA Forest Service, Forest Health Protection, Pineville, LA
Cynthia Ragland, USDA Forest Service, Oakmulgee Ranger District, Brent, AL
Steve Clarke, USDA Forest Service – FHP R8, Lufkin, Texas
Don Grosman, Arborjet Inc., Woburn, MA

Study Site:
Talladega National Forest, AL

Systemic Insecticide:

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

Treatment Methods and Evaluation:

Sites chosen for this study will be selected based on low-moderate trap catch levels in early spring. In 2013, an average of 17.6 SPB/day were caught during spring trapping on the Oakmulgee NF. The ratio of SPB to clerids during this time was 1:2. Trap catches throughout the rest of the season (June – October) averaged at 43.9 SPB/day with a 1:1 ratio of SPB to clerids. Given that no expanding infestations were detected on the Forest at these population levels in the previous year, it should be safe to use these numbers as a baseline for site selection in 2014. Two weeks of spring trap catch will be analyzed prior to tree baiting to ensure SPB population levels are not significantly higher than in 2013.

Three Lindgren funnel traps baited with frontalin + turpentine + endo-brevicomin (displaced by 4 m) bait will be deployed in the area 300 m away from plots to monitor local beetle populations. Loblolly pine chosen for experimentation will be located in closed-canopy, pine-hardwood stands. The trees will be isolated (no pine within 25ft). There will be four treatments (listed below) with four to six replicates of each treatment. All trees will be baited with species-specific lures (frontalin, Sirex lure, and endo-brevicomin) for three 5 week periods in 2014. Baiting of all treatments will be synchronous. TREE-äge™ will be injected into the lower trunk of trees at 5 ml per inch DBH in trees < 12” and 10 ml per inch DBH in trees ≥ 12”. The Tree IV™ microinfusion system (Arborjet, Inc. Woburn, MA) will be used to inject TREE-äge™ into 4 (trees <12” DBH) -8 (trees ≥12” DBH) points 0.3 m above the ground. Where possible, poor quality (form, health, etc.) trees will be selected as trap trees. Treatments will be applied in spring 2014 first. If spring treatments 1, 2 and/or 3 show efficacy, the treatments will be replicated in the fall to test for seasonal effects of treatment efficacy between spring and fall dispersal periods.

Treatments:

1. Inject trees 4 weeks prior to baiting with 5 mL per inch DBH rate
2. Inject trees 4 weeks prior to baiting with 2.5 mL per inch DBH rate
3. Inject trees 2 weeks prior to baiting with 5 mL per inch DBH rate
4. Inject with 5 mL per inch DBH rate and bait experimental trees on same day
5. Control: Bait, but do not inject

Treatment evaluation:

- Monitor SPB and BTB attack level (occurrence of pitch tubes) and health of study (baited, injected or untreated) trees at one to two week intervals following installation of baits and at five (5) week intervals throughout the summer until final evaluations.
- At the end of the field season (September), each study tree will be nondestructively sampled. Using head lamps and hand lens, the number of SPB and BTB successful attacks (i.e., oxidized phloem material present in pitch tubes or points of attack

containing phloem boring dust and/or dry frass) and unsuccessful attacks (i.e., pitch tubes without oxidized phloem material) in 20 X 25 cm (500 cm^2) sample windows at approximately 1.5, 4.0 and 6.5 m in height at northern and southern aspects will be counted.

- All dead study trees will be felled at the end of the field season. Bark plates (10 X 10 cm = 100 cm^2) will be collected at approximately 1.5, 4.0 and 6.5 m height at northern and southern aspects. SPB gallery length and density of emergence holes will be measured.

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

Project Timetable:
CY 2014:

5) Begin spring treatments: injections (mid-March)
6) Bait spring treatments (early April)
7) Post-bait evaluations of spring treatments (see timeline, May-Oct.)
8) Begin fall treatments: injections and baiting (October)
9) Finish baiting fall treatments (late October)
10) End of field season sampling for spring treatments (November)

CY 2015:

11) Post-bait evaluations of fall treatments (once a month, Nov.-Feb.)
12) End of field season sampling for fall treatments (March)
13) Data summary and analyses (April)
14) Final report (May)

Timeline*

Treatment	Week 0	Week 2	Week 4	Week 10	Week 16	Week 24
1	Inject		Bait	Bait	Bait	Sample
2	Inject		Bait	Bait	Bait	Sample
3		Inject	Bait	Bait	Bait	Sample
4			Inject and Bait	Bait	Bait	Sample
5			Bait	Bait	Bait	Sample

- USFS or National Forest staff will monitor spots between TFS visits and TFS will return if control (tree felling) is warranted.

Budget:

Research period: April 2014-January 2015

Personnel	
Fischer (Coordinator, FPMC)	Contributed by TFS
Upton (Staff Forester II)	Contributed by TFS
Spivey Resource Specialist (5% of time)	$1,248.00
Benefits for Resource Specialist (30%)	$374.40
Materials and Supplies	
SPB combo lures + EB ($19.22/ea)	$1,383.84
Misc. materials and supplies	$250.00
Vehicle Fuel (4,000 miles @ $3.50 per gallon)	$875.00
Travel	
Total Meals ($40.25/day) Lodging ($83/night) x 6 nights	$1,848.00
Other	
Maintenance & repairs	$500.00
Subtotal	$6,479.99
Indirect Costs (26%)	$1,684.80
Total	**$8,164.79**

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

Justification

The black turpentine beetle (BTB, *Dendroctonus terebrans*), 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.

The black turpentine beetle 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).

Emamectin benzoate (TREE-age™) was shown to be effective in protecting loblolly pine from black turpentine beetle for one year. It is of interest to determine if the efficacy of TREE-age™ against BTB continues into the second year following treatment.

Objectives:

1) Determine the duration of efficacy of systemic injections of TREE-age™ (emamectin benzoate) for protection of pine against black turpentine beetle (BTB)

Research approach:

Locations, Treatments, and Environmental Conditions
This study will be conducted within the Fairchild State Forest, Rusk, TX (about 31°78 N, 95°36 W, elev. 451ft). Forty loblolly pine, >13 "DBH that were previously treated with emamectin benzoate and ten untreated control trees will be included in this study.

The treatments that had been applied the year before included: 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 was applied to 10 randomly-assigned trees. Trees are spaced >160 m apart and within 100 m of access roads. Each systemic insecticide treatment (treatments 1, 2, & 3) was injected at the labeled rate after dilution in 1 part water with the Arborjet Tree IV™ microinfusion system (Arborjet, Inc. Woburn, MA) into evenly spaced points (number is calculated by DBH/2). Injections occurred in September 2012. In October 2012 (30 days post-injection), the bole of treatment 4 trees (up to 10 ft) was sprayed to runoff using a backpack sprayer.

Treatment Efficacy

Trees will be baited three times, beginning in April. 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 October 2014, 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. Trees baited	April 2014
2. Re-bait	June 2014
3. Re-bait	August 2014
4. Final Assessment	October 2014
5. Data summary and analyses	Fall 2014
6. Final report	Fall 2014

Literature Cited

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

Staeben, J.C., S. Clarke, and K.J.K. Ganghi. 2010. Black turpentine beetle. Forest Insect and

Budget

Personnel	
Fischer (Coordinator, FPMC)	Contributed
Spivey (Research Specialist)	Contributed
Seasonal Technician (16% of time)	$1,758.24
Benefits for Seasonal Technician (8.6%)	$151.21
Materials and Supplies	
Frontalin lures (50% of 150 @ $14.00/ea)	$1,050
Misc. materials and supplies	$50.00
Vehicle Fuel (50% of 1800 miles @ $3.50 per gallon)	$50.00
Other	
Maintenance	$60.00
Subtotal	$3,119.45
Indirect Costs (26%)	$811.06
Total	**$3,930.51**

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 branches (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. Post-treatment assessment of efficacy — Spring 2014
16. Data summary and analyses — Fall 2014
17. Final report, peer-reviewed publication submitted — Fall 2014

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)

Literature Cited

Billings, R.F., D.M. Grosman and H.A. Pase III. 2011. Soapberry Borer, *Agrilus prionurus* (Coleoptera: Buprestidae): An Exotic Pest Threatens Western Soapberry in Texas. Southeastern Naturalist. In Review.

Cranshaw, W., and N. Tisserat. 2010. Questions and answers about thousand canker disease of walnut. http://www.coopext.colostate.edu/pf/pdfdocs/thousand_canker_questions_answers.pdf.

Grosman, D.M., and W.W. Upton. 2006. Efficacy of systemic insecticides for protection of loblolly pine against southern pine engraver beetles (Coleoptera: Curculionidae: Scolytinae) and wood borers (Coleoptera: Cerambycidae). Journal of Economic Entomology 99: 94-101.

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

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

Kolarik, M., E. Freeland, C. Utley, and N. Tisserat. 2010. *Geosmithia morbida* sp. nov. a new phytopathogenic species living in symbiosis with the walnut twig beetle (*Pityophthous jugandis*) on *Juglans* in the USA. Online. Mycologia doi:10.3852/10-124.

Mayfield III, A.E., E.L. Benard, J.A. Smith, S.C. Bernick, J.M. Eickwort, and T.J. Dreaden. 2008. Effect of propiconazole on laurel wilt disease development in redbay trees and on the pathogen in vitro. Arboriculture & Urban Forestry. 34: 317-324.

Mccullough, D., T. Poland,A. C. Anulewicz, P. Lewis, and D. Cappaert 2011. Evaluation of *Agrilus planipennis* (Coleoptera: Buprestidae) control provided by emamectin benzoate and two neonicotinoid insecticides one and two seasons after treatment. J. Econ. Entomol. 104: 1599-1612.

Mielke, M., N. Schneeberger, N. Dart, and B. Moltzan. 2011. Report on site visits in Virginia and Pennsylvania to examine the site, host and pest characteristics associated with thousand canker disease (TCD) infections in eastern black walnut.

Nelson, R.M, and J.A. Beal. 1929. Experiments with blue-stain fungi in southern pines. Phytopathology 19: 1101-1106.

Paine, T.D., K.F. Raffa, and T.C. Harrington. 1997. Interactions among scolytid bark beetles, their associated fungi and live host conifers. Annual Review of Entomology 42: 179-206.

Seybold, S.J., D. Haugen, J. O'Brien, and A.D. Graves. Thousand cankers disease. Pest Alert, NA-PR-02-10. May 2010. Newton Square, PA: U.S. Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry. 2 p.

Seybold, S.J., Graves, A.D., and Coleman, T.W. 2010. Walnut twig beetle: Update on the biology and chemical ecology of a vector of an invasive fatal disease of walnut in the western U.S., pp. 55—57, *in* K. A. McManus and K. W. Gottschalk (eds.). Proceedings, 21st U.S. Department of Agriculture Interagency Research Forum on Invasive Species 2010, January 12-15, 2010; Gen. Tech. Rep. NRS-P-75. 156 pp., Newtown Square, Pennsylvania: USDA, Forest Service, Northern Research Station, December 2010.

Smitley, D.R., J.J. Doccola, D.L. Cox. 2010. Multiple-year protection of ash trees from emerald ash borer with a single trunk injection of emamectin benzoate, and single-year protection with an imidacloprid basal drench. Arbor. & Urban For. 2010. 36: 206–211.

Takai K., T. Soejima, T. Suzuki, and K. Kawazu. 2001. Development of a water-soluble preparation of emamectin benzoate and its preventative effect against the wilting of pot-grown pine trees inoculated with the pine wood nematode, *Bursaphelenchus xylophilus. Pest Manag Sci* 57:463–466.

Tisserat, N.**,** W. Cranshaw, D. Leatherman, C. Utley, and K. Alexander. 2009. Black walnut mortality in Colorado caused by the walnut twig beetle and thousand cankers disease. Online. Plant Health Progress doi:10.1094/PHP-2009-0811-01-RS.

Utley, C., W. Cranshaw, S. Seybold, A. Graves, C. Leslie, W. Jacobi, and N. Tisserat. 2009. Susceptibility of *Juglans* and *Carya* species to *Geosmithia*; cause of thousand canker disease. [Abstract] Phytopathology. Supplement. 99: S133.

Williams, R.D. Black Walnut. In R.M. Burns and B.H. Honkala (eds). Silvics of North America: 2. Hardwoods. U.S.D.A. Forest Service, Washington, D.C. 877 p.

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

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

Research Approach:

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

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

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

1) Treatment 1: Experimental bait will be spread uniformly over CNA at 10.0 g/m^2.

2) Treatment 2: Amdro Ant Block® (AI = hydramethylnon; bait standard) bait will be spread uniformly over CNA at label rate.
3) Treatment 3: Control - Blank Bait will be spread uniformly over CNA at 10.0 g/m^2.

It is of interest to determine the rate at which leaf-cutting ants retrieve the applied bait formulation. To do this, five petri dishes containing four bait particles (= $10g/m^2$) will be distributed evenly within the CNA just after each colony is treated. The dishes will be checked at 3 hour intervals during the first 24 - 36 hours after treatment. At each interval, the number of particles removed will be recorded. In addition, observations will be made to determine if animals (birds), other than leaf-cutting ants, are feeding on the applied bait.

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

Research Time Table:

Spring 2014

- Acquire insecticide formulation from Syngenta
- Select 3 leaf-cutting ant colonies for preference trial
- Conduct preference trials
- Select 30+ candidates for efficacy trial from mapped leaf-cutting ant colonies and randomly assign treatments
- Evaluate ant activity on day of treatment
- Treat colonies with assigned treatment
- Revisit treated and check nests at 2, 4, 8 & 16 weeks after treatment date to evaluate ant activity
- Conduct statistical analysis of data and submit seasonal report to Syngenta

Note: If spring treatments are effective, an additional efficacy trial could be established during the fall 2014/2015

Literature Cited:

Cameron, R.S. 1989. Control of the Texas leaf-cutting ant, *Atta texana* (Hymenoptera: Formicidae) with thermal fog application of resmethrin. p. 236-244. *In*: Alfaro, R.I., and Glover, S. eds. Insects Affecting Reforestation: Biology and Damage. Proc. IUFRO Conference, XVIII International Congress of Entomol. Vancouver, B.C. July 3-9, 1988. Forestry Canada. Pacific Forestry Centre, Victoria, British Columbia, Canada. 256 pp.

Cameron, R.S. 1990. Potential baits for control of the Texas leaf-cutting ant, *Atta texana* (Hymenoptera: Formicidae). *In*: Vander Meer, R.K., Jaffe, K., and Cedeno, A. eds. Applied Myrmecology: A World Perspective.

Budget: Below is the requested funding for Spring 2014

Personnel	
Fischer	Contributed
Resource Specialist (10% of time)	$ 2,496.00
Benefits (30%)	$ 748.80
Materials and Supplies	
Flagging, pin flags, spreaders, gloves, Petri dishes	$ 200.00
Travel	
Vehicle fuels and maintenance (1,500 miles at $3.50/gallon)	$ 825.00
Subtotal	$ 4,269.80
Indirect Cost (26%)	$ 1,110.15
TOTAL REQUESTED	***$ 5,379.95***

Evaluation of Trunk Injections of PGRs for Phytotoxicity and Reduction of Fruit Production on Sweetgum

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Don Grosman	Arborjet Inc. 99 Blueberry Hill Road, Woburn, MA 01801 dgrosman@arborjet.com	339-227-7538 (c)

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

Plant growth regulators (PGRs) have been evaluated and used extensively to manage the vegetative growth of trees, shrubs, and grass along utility rights of way, and residential landscapes, etc. Commercial orchardists regularly use PGRs to thin fruit crops (Byers et al. 1983, Elfving and Cline 1993). Some of these materials are also registered for use on ornamental trees and shrubs to eliminate or reduce fruit production. Dikegulac-sodium (Atrimmec™, PBI/Gordon and Pinscher™, ArborSystem) spray applications were shown to reduce sweetgum ball production by 57% (Banko & Stefani 1995) and also labeled for suppression of flowers and fruit on ornamental olive, glossy privet, and multiflora rose. Ethephon (Florel™, Monterey) is labeled for home garden tomato ripening, mistletoe shoot removal, and undesirable fruit elimination on a number of ornamental shrubs and trees (including sweetgum and olive). Indol-3-butyric acid (Snipper, Tree Tech) is registered for use on several ornamental shrubs and tree species (including sweetgum and olive) for undesirable fruit elimination. Mefluidide (Embark™, PBI/Gordon) is labeled for suppression of flowers and fruit on ornamental olive. Methyl chlorflurenol (Maintain CF 125™) is labeled for use to eliminate fruit on olive.

Timing of chemical spray applications is often critical to obtain optimal fruit reduction. However, injection of chemical treatments may reduce the importance of timing. Pinscher and Snipper are injectable formulations, while the others are labeled for use as foliar sprays. Although Florel™, Embark™, Atrimmec™, and Maintain CF 125™ are registered for use as foliar sprays, we would like to attempt injecting them to see if they have systemic activity. Two trials will be conducted to evaluate the phytotoxic effects and efficacy of injectable formulations of dikegulac sodium, indole-3-buttyric acid, ethephon, medfluidide, and methyl chlorflurenol for elimination of sweetgum fruit in landscape situations.

Objectives: 1) Evaluate phytotoxic effects of trunk injections of dikegulac sodium, indole-3-buttyric acid, ethephon, medfluidide, and methyl chlorflurenol on sweetgum; 2) evaluate the efficacy of these five chemicals for elimination of fruit production on sweetgum; and 3) determine the longevity of treatments.

Cooperators:

Mr. Joseph Doccola — Arborjet, Inc., Woburn, MA

Private landowners

Anticipated Products: This project will provide an alternative treatment (to spray applications) for reducing/eliminating nuisance fruit on certain ornamental trees, including sweetgum and olive.

Methodology:

Trial 1 (Evaluation of phytotoxic effects): This study will likely be conducted on private or forest industry lands in East Texas. Individual 2-inch (diameter at ground level) sweetgum trees (66) will be selected. One of eleven treatments will be randomly assigned to each of six trees. Note: Where possible, healthy (unstressed by drought, insect, or disease, etc.) trees will be selected as study trees.

The treatments are:

1) Atrimmec (18.5% dikegulac sodium) at 2.0 ml per inch diameter injected at 1 point;

2) Atrimmec (18.5% dikegulac sodium) at 6.0 ml per inch diameter injected at 3 points;

3) Snipper (4% indole-3-buttyric acid) at 2.0 ml per inch diameter injected at 1 point,

4) Snipper (4% indole-3-buttyric acid) at 6.0 ml per inch diameter injected at 3 points,

5) Florel (3.9% ethephon) at 2.0 ml per inch diameter injected at 1 point,

6) Florel (3.9% ethephon) at 6.0 ml per inch diameter injected at 3 points,

7) Embark 2-S (3.2% medfluidide) at 5.0 ml per inch diameter injected at 1 point,

8) Embark 2-S (3.2% medfluidide) at 15.0 ml per inch diamete injected at 3 points,

9) Maintain CF125 (12.5% methyl chlorflurenol) at 5.0 ml per inch diameter injected at 1 point,

10) Maintain CF125 (12.5% methyl chlorflurenol) at 15.0 ml per inch diameter injected at 3 points,

11) Control – water at 15 ml per inch diameter injected at 3 points.

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

Trees will be evaluated visually for phytotoxic symptoms (yellowing or browning of leaves, excessive sap flow around injection points or pruning cuts) in March, 2014 (~120 DAT). The trees will be cut at ground level and a 12-inch bolt (containing the injection points) retained for evaluation. The bolts will be sent to Arborjet and examined for presence and length of phytotoxic lesions in the sapwood.Trial 2 (Efficacy for fruit reduction): This study likely will be conducted on private or forest industry lands in East Texas. Individual (36-60) sweetgum trees, 8-10" DBH, will be selected. One of six treatments will be randomly assigned to each of 6-10 trees. Note: Where possible, healthy (unstressed by drought, insect, or disease, etc.) fruit-producing trees will be selected as study trees.

The treatments are:

1) dikegulac sodium (18.5% Atrimmec);

2) indole-3-buttyric acid (4%, Snipper),

3) ethephon (3.9%, Florel),

4) medfluidide (3.2% Embark),

5) methyl chlorflurenol (12.5%, Maintain),

6) untreated control

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

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

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

Project Timetable:

CY 2013:

15) Identify study area and select trees for Trial 1 (October)

16) Implementation (injection) of treatments for Trial 1 (October)

CY 2014:

17) Post-treatment evaluations and harvest trees for Trial 1(March)

18) Identify study area and select trees for Trial 2 (March)

19) Implementation (injection) of treatments for Trial 2 (March)

20) Post-treatment evaluations for Trial 2 (September)

21) Reapply (injection) treatments for Trial 2 (September)

22) Data summary and analyses (November)

23) Progress report (December)

CY 2015:

2) Post-treatment evaluation for Trial 2(March)

3) Data summary and analyses (April)

4) Progress report (May)

References

Banko, T.J. and M. Stefani. 1995. *Growth regulators for management of fruit production on American sweetgum. J. Aboriculture.* 21: 88-89.

Byers, R.E., J.A. Barden, and D.H. Carbaugh. 1990. *Thinning of spur 'Delicious' apples by shade, terbacil, carbaryl, andethephon.* J. Amer. Soc. Hort. Sci. 115:9-13.

Dirr, M.A. 1983. (3rd ed.) Manual of Woody Landscape Plants: Their Identification, Ornamental Characteristics, Culture, Propagation and Uses. Stipes Publishing Company, Champaign, IL 826 pp.

Elfving, D.C.and R.A. Cline. 1993. *Benzyladenine and other chemicals for thinning 'Empire' apple trees.* J. Amer. Soc. Hort. Sci. 118:593-598.

Budget:

PGR:- CY 2013-2014

Personnel

Fischer	Contributed by FPMC
Seasonal Technician (30%)	$ 2,997.00
Benefits for Seasonal Technician (8.45%)	$ 253.25

Materials and Supplies

Five PGR products	Contributed by Arborjet
Injection equipment and Arborplugs	Contributed by Arborjet
Miscellaneous materials and supplies	$ 318.00

Travel

Vehicle fuels and maintenance (50% of 1,600 miles @ $0.50 / mile)	$ 400.00
Indirect Costs (26%)	$ 1,031.75
TOTAL REQUESTED	**$ 5,000.00**

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 EcoMite Plus, 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:

2) Evaluate the potential efficacy of tree injection of TREE-age™ (emamectin benzoate), and spray applications of EcoMite Plus, 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 conifer mites (Cordell et al. 1989). Pine, hemlock, spruce, juniper, fir, and white-cedar are often heavily attacked.

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

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

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

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 mid-February 2014 of the general health of five-year-old loblolly pines in a polymix trial containing several families. Each pine will be evaluated for tip moth damage and presence of conifer mites. Twenty (20) trees will be randomly selected for treatment. An additional ten trees will serve as untreated checks.

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

Each treatment will be applied to 10 randomly-assigned trees. Test trees will be located in areas with abundant conifer mite and tip moth 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 mid-February 2014. Arborjet spray treatment (2) will be applied in late February and again two weeks later.

In February, 2014 (at the time of initial spray treatment) and then 7, 14, 21, 28, 42, 56 days (for Trt 1 & 2) and 4, 8 and 12 months (for Trt 1 only) 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	February 2014

5. Treatments 1- 9 applied — February 2014
6. Post-treatment assessment of efficacy — Mar 2014-Feb 2015
8. Data summary and analyses — March 2015
9. Final report, peer-reviewed publication submitted — May 2015

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 2014-2015

Personnel	
Fischer	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 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**