USDA FOREST SERVICE- FHM- EVALUATION MONITORING PROPOSAL 2013

PROJECT TITLE: Monitoring the Health and Effects of a Scale/Pathogen Complex on Eastern White Pines in the Southern Appalachians

LOCATION: Southeastern U.S. **DATE OF SUBMISSION:** 9/30/2012

DURATION: 8/1/2013-3/31/2016 **FUNDING SOURCE:** EM Base

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PROJECT OBJECTIVES: The project goal is to determine the extent, severity, and nature of eastern white pine (*Pinus strobus*) health issues in the southeastern U.S. Since 2006, we have observed white pine trees (all diameter classes) with high densities of cankers on branches and main bole, branch flagging, and crown thinning in the southern Appalachians. Mortality of both young and mature white pine is occurring in multiple states including Georgia (GA), Virginia (VA), and West Virginia (WV). Associated with the cankers is a novel and previously unreported complex of a pine bast scale, *Matsucoccus macrocicatrices* (Hemiptera: Matsucoccidae) and multiple pathogens, including *Caliciopsis pinea, Diplodia spp., Fusarium spp.,* and *Phomopsis* spp. As linked to the project goal, our research objectives are to: 1) determine the range and severity of dieback, and fate of symptomatic white pine trees in the southern Appalachians; 2) assess if white pine health varies as based on site conditions such forest structure and composition, and various topographic features; and 3) determine the kinds of correlations between the scale insect, fungal pathogens, cankers, and health of eastern white pine.

JUSTIFICATION: a. Linkage to FHM Program- Our project is directly linked to the FHM program as we will assess and monitor the health of white pine trees in the southern Appalachians. Further, we will use FIA data to guide and establish plots for sampling in symptomatic areas. **b. Significance/ Impact of Forest Health Issue**- White pine is an ecologically and economically important tree canopy species in the southern Appalachians. Sampling will be conducted in 4-5 states on federal lands that are in the major range of white pine in southeastern U.S. **c. Scientific Basis/ Feasibility**- Our project is interdisciplinary and interagency, with diverse personnel including forest entomologists and pathologists, and a spatial scientist. **d. Cost/ Economic Efficiency-** We are requesting ~$30,000 per year to conduct this large-scale project with main requests for personnel salary and travel costs. **e. Priority Issues** - The priority area of our project is: "Tree mortality- deviations from expected levels".

DESCRIPTION: **a. Background:** Eastern white pine (*Pinus strobus*) is a widely distributed tree species in the Great Lakes region and in high-elevation Appalachian Mountains in eastern North America *(1)*. As an ecologically important species, white pine is associated with >23 forest-types and is a major component of five forest cover-types including a) white pine; b) red pine; c) white pine-red oak-red maple; d) white pine-hemlock; and e) white pine-chestnut oak forests *(1)*. Furthermore, seeds, bark, and foliage of white pine are used by a large number of wildlife species such as birds and mammals *(2)*. White pine is also economically important as shade trees, Christmas trees, timber, and is an ideal species for soil reclamation sites *(1)*.

Fig. 1. (a) Dying white pine tree; (b) dieback on a sapling; (c) dead seedling.

White pine is an especially critical associate of hemlock forests in the Appalachian Mountains *(1)*. Their role as canopy tree species has become even more important, as hemlock trees have been in a large-scale decline due to the exotic hemlock woolly adelgid (*Adelges tsugae*). Unfortunately, since 2006, there has been an increase in observations regarding white pine trees showing symptoms of dieback and death, especially in the mountains of GA, VA, and WV *(3)*. These symptoms include branch flagging, presence of cankers, high resin exudation associated with cankers, and mortality in almost all tree diameter classes (Fig. 1). As a result, we established 33 permanent plots in GA, WV, and VA in 2012. Preliminary results from an additional 25 temporary plots established in the Chattahoochee National Forest, GA revealed that 44% of the white pine trees were dead and another 30% of the trees showed >50% dieback (Fig. 2). In both GA and VA, the majority of mortality was observed on trees with <30 cm DBH (Fig. 2), but later visits indicated an increase in the number of both symptomatic and dead trees. Furthermore, dieback and mortality of white pines with similar symptoms has been documented during the last five years from Maine and New Hampshire *(4, 5)*.

Preliminary examination of cankers yielded a number of pathogens including *Fusarium chlamydosporium*, *F. acuminatum*, *Diplodia scrobiculata*, *Pestalotiopsis* spp., *Phomopsis* spp., and *Caliciopsis pinea*. The majority of the previous focus has been on *C. pinea (4, 5)*, which was found on 86% of branches. Embedded within these various cankers were also immature pine bast scales (genus *Matsucoccus*) which were later identified via DNA analysis by Dr. Penny Gullan (Australia) as *Matsucoccus macrocicatrices*. Our discovery of *M. macrocicatrices* is novel because: 1) this species called the "Canadian pine scale" has never been reported or collected from the southeastern U.S. (we also reviewed holdings of >15 museums). It is known only from Massachusetts, New Brunswick, New Hampshire, Nova Scotia, Ontario, and Quebec; 2) it has never been reported with trees that are showing signs of dieback or mortality; and 3) it has never been found associated with pathogens, and only with *Septobasidium pinicola*, an epiphytic, non-pathogenic and mutualistic fungus *(6, 7)*. Since the immature stages of *M. macrocicatrices* are either deeply embedded in the cankers or present on top of the bark with clear necrotic tissue under their feeding area (Fig. 3), we hypothesize that *M. macrocicatrices* may be creating wounds for pathogenic fungi such as *C. pinea* to infest. Relative contribution of the scale insect and fungi to canker formation are currently unknown.

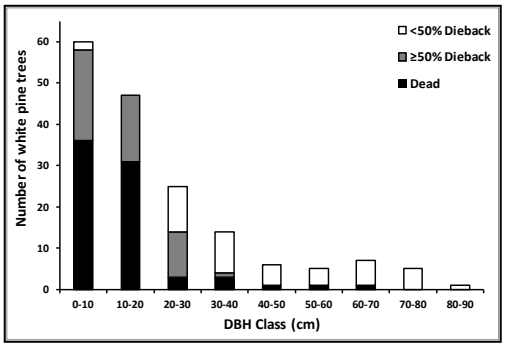

Fig. 2. Condition of white pine trees in GA.

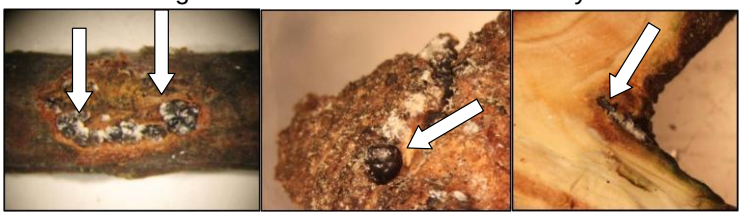

Fig. 3. Arrows indicate immature cyst stages of *Matsucoccus macrocicatrices* embedded in cankers on white pines.

Clearly, we are dealing with a previously undocumented insect and fungal complex on white pine trees in the southern Appalachians. At present, there are many unanswered questions about this phenomenon such as: 1) what is the full status of white pine health in the southern Appalachians; 2) are these health issues prevalent in certain regions and localities as based on site conditions; 3) why are this insect and pathogens now associated with an increase in white pine mortality, especially in natural forest stands; 4) is the Canadian pine scale a recent introduction to the southern Appalachians or just recently discovered; and 5) what is the nature of correlation between the incidence of insect and pathogens on cankers? As a part of an interdisciplinary and multiagency team, we propose to monitor and survey white pine trees in the southern Appalachians, and to better understand the impacts of the insect and pathogen complex on an ecologically important tree species.

<u>b. Methods</u>: *<u>Field Studies</u>:* We have already established 33 permanent plots in GA, VA, and WV that are being monitored every six months. Using guidance from FIA data, field scouting, and incoming reports of white pine health issues, we will establish 10 sites and 30 plots on federal lands in each state within the major range of white pine including GA, VA, and WV, and North Carolina and Tennessee. Data from field scouting may assist in finding closest FIA plots with symptomatic white pines. These 150 permanent plots will assist in determining temporal changes in the health of white pines. Plots will be circular and 10 m in radius. Three plots will be placed along a transect at each site; transects will be separated by >500 m.

Within each plot, we will assess the following stand characteristics: 1) forest composition such as overstory and understory plant species; 2) forest structure e.g., vertical and horizontal heterogeneity (basal area, tree density, percentage of plants in different vertical layers, coarse-woody debris, etc.); 3) soil characteristics e.g., physical and chemical attributes; and 4) topographic features e.g., elevation, slope and aspect.

We will also assess the following white pine tree characteristics in each plot: 1) DBH and height; 2) crown condition and class. A scale from 1-5 may be developed for white pine trees where 1 will be a healthy tree with full crown, 2-4 as gradual thinning of the crown, and 5 as the tree being dead. About 1-2 mature pine trees may be cut next to the plots to assess the number of branch cankers; 3) five saplings will be randomly selected per plot on which we will catalog the proportion of necrotic branches; 4) within each site, but not within the plots, five white pine seedlings will be collected to quantify canker and scale densities. This will also allow us to look for potential relationships between the cankers, scale, and leaf area. Data collected will include the number of each life-stage of the scale, the number and sizes of cankers, and the presence/absence of *C. pinea* fruiting bodies.

<u>Laboratory Studies</u>: A subset of cankers without *C. pinea* fruiting bodies will be collected from all diameter classes of white pine and plated on three different media in an attempt to isolate and identify any pathogens. Subsequent pathogenicity tests will be conducted with isolates of unknown pathogenicity on white pine saplings. Any pathogenicity testing will be performed within the county and state where the potential pathogen was found. Two isolates per species will be tested in 4 plots (809 m^2 or 16 m radius) on 5 white pine saplings. Branches at breast

height will be wounded (2-4 mm) and inoculated with mycelial plugs or water agar, then sealed with parafilm. After one year, resulting cankers will be measured and placed on media for reisolation of the inoculant.
Statistical Analyses: We will use analysis of variance tests (ANOVA) to determine differences in the number of healthy, symptomatic, and dead white pines across DBH class in different states, and use repeated measures ANOVA for detecting changes in pine health over time *(8)*. Correlation analyses will be used to assess relationships between the health of white pines and various stand characteristics with the prevalence of cankers, scale insect, and fungi *(8)*.
c. Products: Overall, we are attempting to assess the status of white pine health in the Appalachian Mountains, and better understand the associated fungal pathogen and scale insect complex to assist with management decisions related to this tree species. Our target audience will include managers and scientists working on white pine health issues both locally and regionally. Results will be presented at national and international meetings, and 2-3 peer-reviewed and extension papers will be published from the project. We expect results from this study will be used to guide management issues related to white pines in the Appalachian Mountains.
d. Schedule of Activities: August 2013 to July 2014- Assess FIA data; select plots in five states; finalize project design; establish plots; collect stand-level data and samples in these states. August 2014 to July 2015- continue collecting plot and stand-level data; analyze collected data; make presentations at meetings. August 2015 to March 2016- submit final report and manuscripts for publication; present results and papers to forest managers and post results online.
e. Progress/Accomplishments: None as this is a new proposal.
f. Relevant Citations: ***(1)*** Wendel and Smith. 1990. Silvics of North America. ***(2)*** Rogers and Lindquist. 1992. The White Pine Symposium. ***(3)*** Asaro, C. 2011. Forest Health Review, May 2011, Virginia Department of Forestry. ***(4)*** Lombard. 2003. Caliciopsis Canker (pine canker): *Caliciopsis pinea*. ***(5)*** Maine Forest Service. 2008. Forest and shade tree insect and disease conditions for Maine: a summary of the 2007 situation. ***(6)*** Booth, J.M., and Gullan, P.J. 2006. Proceedings of the Entomological Society of Washington 108: 749-760. ***(7)*** Watson, W.Y., Underwood, G.R., and Reid, J. 1960. The Canadian Entomologist XCII: 662-667. ***(8)*** Zarr, J.H. 1996. Biostatistical Analysis.
COSTS:

	Item	**Requested FHP EM Funding**	**Other-Source Funding**	**Source**
Year 8/1/2013 – 7/31/2014				
Administration	Salary- Research Coordinator	4,828		**NONE**
	Fringe benefits	1,299		
	Salary- M.S. Student	16,500		
	Fringe benefits	825		
	Overhead- 26%	8,048		
	Travel	5,000		
Procurements	Contracting			
	Equipment			
	Supplies	500		
	Vehicle Expense	2,000		
Year Totals		**39,000**		
	Item	**Requested FHP EM Funding**	**Other-Source Funding**	**Source**
Year 8/1/2014 – 7/31/2015				
Administration	Salary- Research Coordinator	4,925		**NONE**
	Fringe benefits	1,325		
	Salary- M.S. Student	16,830		
	Fringe benefits	842		
	Overhead- 26%	8,048		

	Travel	4,500		
Procurements	Contracting			
	Equipment			
	Supplies	530		
	Vehicle Expense	2,000		
Year Totals		**$39,000**		
	Item	Requested FHP EM Funding	**Other-Source Funding**	**Source**
Year 8/1/2015 – 3/31/2016				
Administration	Salary- Research Coordinator	5,023		**NONE**
	Fringe benefits	1,351		
	Salary- M.S. Student	4,292		
	Fringe benefits	215		
	Salary - PI - Kamal Gandhi			
	Fringe benefits			
	Overhead- 26%	3,349		
	Travel	1,500		
Procurements	Contracting			
	Equipment			
	Supplies			
	Vehicle Expense	500		
Year Totals		**$16,230**		
Project Totals		**$94,230**		

JUSTIFICATION: SALARIES & FRINGE BENEFITS: A Project Coordinator will assist with all the laboratory and field aspects of this project ($4,828 for the first year with an estimated 2% increase starting January 2014; ~13.65% of their time or ~1.6 months in the first year). Fringe benefits are estimated at 26.9% for the Project Coordinator. A M.S student will conduct research with salary of $16,500 for the first year with 2% increase each year. Fringe benefits are estimated at 5% for the students.
TRAVEL: Travel funds are requested to visit field sites to collect data in several states in the Southeast. Travel will be for at least 25 days at ~$40/day per diem each for the field personnel ($2,000) and hotel expenses at ~$60/night/each for two rooms ($3,000). In the second year, travel will be for at least 20 days at ~$40/day per diem each for the field personnel ($1,600) and hotel expenses at ~$60/night for two rooms ($2,400). Another $500 will be used to travel to conferences. In the third year, travel will be to present papers at conferences in the last year for $1,500. Total for travel is: $5,000 + $4,500 + $1,500 = $11,000.
SUPPLIES/OPERATING EXPENSE: Supplies for the project include (but are not limited to) GPS unit, flagging, DBH tape, loggers' tape, tree tags, rite-in-rain paper, field-vests, ethanol, vials, slides and chemicals for fungal isolations, soil analyses, etc. Vehicle expenses in a Warnell vehicle to travel to field sites for at least 25 days at a daily rate of $20 ($500), and ~ 2,587 miles at 0.58/mile (~$1,500). Vehicle expenses total $2,000 in the first and second year and $500 in the third year.
INDIRECT COSTS: 26% for off-campus research. COST-SHARE: NONE needed/provided as this project will take place on Federal lands.