TITLE: Evaluation of incidence and severity of Diplodia dieback of slash pine in peninsular Florida (pre-proposal).
LOCATION: Florida
DATE: September 30, 2013 (New Project)
DURATION: Year 1 of 2-year project **FUNDING SOURCE:** Base
PROJECT LEADER: Jason A. Smith, Associate Professor, School of Forest Resources and Conservation, University of Florida; 352-846-0843; jasons@ufl.edu
COOPERATORS: Jeffrey Eickwort and Chris Pearce, Florida Forest Service; Chris Bloodwell, Orlando-Orange County Expressway Authority
FHP SPONSOR/CONTACT: Dale Starkey, Pineville, LA; (318) 473-7293; dstarkey@fs.fed.us
PROJECT OBJECTIVES: The proposed research will focus on the following objectives: 1.) Determine extent (incidence and severity) of Diplodia dieback epidemic in peninsular Florida; 2.) Evaluate stand age, structure and historical weather effects on disease parameters.
JUSTIFICATION:

a. **Linkage to FHM program.** This project directly links to the FHM program by determining "extent, severity, and causes of undesirable changes in forest health". Although the preliminary mortality/damage data were not collected through the Detection Monitoring program, this project will provide data that will support both EM and DM goals.
b. **Significance/Impact of forest health issue.** Through initial surveys completed by the University of Florida, Florida Forest Service and Orlando-Orange County Expressway authority, a severe epidemic of Diplodia dieback is widespread and underway in slash pine stands in at least 9 counties (Brevard, Hernando, Lake, Nassau, Orange, Seminole, Pasco, Polk and Volusia) in peninsular Florida. Given the very high ecological and economic importance of slash pine in the southeast, attention should be given to this problem to determine the extent of the threat.
c. **Scientific basis.** This project will involve collaboration between a pathologist (Smith, PI), forest health specialists with experience in evaluation/monitoring and use of GIS-based techniques (Jeff Eickwort and Chris Pearce, Florida Forest Service) as well as those with expertise in forest management/silviculture (Chris Bloodwell).
d. **Cost/Economic efficiency.** This project has a modest budget ($52,465) given the scope of the work and will be highly leveraged, with funds coming from OOCEA and University of Florida ($52,654) as well as in-kind support from Florida Forest Service (Jeff Eickwort and Chris Pearce's time as well as use of their Digital Aerial Sketch Mapping (DASM) setup and Xplore GPS-linked tablet computer with Geolink and ArcGIS software for field data collection). In-kind support from OOCEA will include Chris Bloodwell's time and also assistance in access to field sites.
e. **Priority Issues.** This project directly supports the EM priority "Tree mortality – deviations from expected levels" and since this problem may be related to recent weather events, it also indirectly supports "Unusual weather events– deviations from normal".

DESCRIPTION:
a. Background: In the fall of 2012, dieback and mortality to slash pines planted as buffers along and near expressways in central Florida was observed. Symptoms included cankers, death of lower branches, resinosis (pitch exudation) and death of trees (Fig. 1). The Orlando-Orange County Expresswat Authority and Orange County Extension agent contacted the PI (Smith) about the cause of dieback and mortality, who requested samples be sent for analysis at the Forest Pathology Laboratory at the University of Florida. In November, samples were used to isolate potential pathogens and from that limited sampling, three fungal pathogens were isolated and identified based on sequencing the ITS-rDNA (15): *Diplodia pinea*, *Fusarium circinatum* and *Lasiodiplodia theobromae. Diplodia pinea* (syn. *Sphaeropsis sapinea*). In January 2013, additional sampling was completed from 30 trees at each of 3 different locations along SR 408, SR 417 and SR 429 in Orange and Seminole Counties. Laboratory analyses led to the recovery of the same pathogens identified in November, 2012, but two additional species, *Diplodia scrobiculata* and *Lasiodiplodia pseudotheobromae*, were found. Both of these pathogens are known to induce dieback symptoms in pines (7,9,13). Additional sampling in late 2012 and early 2013 from 5 sites in 4 counties revealed the consistent presence of *D. pinea* and *D. scrobiculata*. Symptomatic trees with significant damage to stands have been observed in 9 counties total (Fig. 2).

Diplodia spp. can be opportunistic (secondary) on stressed hosts (1,4,5,8,15,18) or may be primary pathogens if the strains have become more virulent (2,12) or a particularly susceptible seed

source was used for planting (14). In preliminary artificial inoculation experiments, all were pathogenic on potted slash pine with some variation in virulence (aggressiveness) observed between the pathogens. Stress to pine trees can be caused by numerous factors including drought (1,4,8,10,11), too much competition and over fertilization. It was discovered during the preliminary investigation that many of the trees were over-fertilized during the first few years following planting. Nitrogen increases susceptibility to *D. pinea* infection (3).

Diplodia pinea affects 150 species of pines, firs, spruce, beech, larch, cypress, and other conifers (13). The only host so far reported in Florida is slash pine, *Pinus elliottii*, on which the disease is described as "dieback" and "blue stain." However, this outbreak is unusual and reported mortality from this disease in Florida has been extremely limited. In Georgia, loblolly (*P. taeda*), shortleaf (*P. echinata*), and Virginia pine (*P. virginiana*) seedlings have been found susceptible (7). The seed source of the trees has also been called into question. Orlando is near the southern limits for the natural range of slash pine (*Pinus elliottii* var. *elliottii*), but at the northern limits for south Florida slash pine (*Pinus elliottii* var. *densa*). Inherently, the source of the trees can make them more or less prone to stress and subsequent disease outbreaks (14). If slash pines from a nursery in Georgia are planted in Orlando, they may become stressed and perform poorly. Unfortunately, we do not currently know source origins of the planted pines. In preliminary inoculation experiments conducted on both slash pine (*Pinus elliottii var. elliottii*) and south Florida slash pine (*Pinus elliottii var. densa*), *D. pinea* caused death within 5 weeks. It appeared as though *D. pinea* is quite virulent as the trees were not stressed and being grown under optimum controlled conditions.

Dieback due to *D. pinea* infection has been regularly noted after hailstorms, wounding from pruning and insect damage (12,16). Slash pines (*P. elliottii*) attacked by the deodar weevil (*Pissodes nemorensis*) are susceptible; drought or nutrient stress also creates opportunities for entry (12). The fungus may directly penetrate unwounded epidermal tissue of new shoots, or enter through needle stomata (12). Its rapid appearance after wounding may be due to its pre-existing residency within the plant as an endophyte (5). When environmental conditions become unfavorable, *D. pinea* turns pathogenic and moves into internal tissues of the tree (5, 16). Mature cones act as a reservoir for fungal inoculum (12, 13) and latent infections are well known for all of these pathogens (13). Given the extremes of drought and above average rainfall that has occurred during 2010-2013 in the region, it is possible that this outbreak is related to specific weather events.

b. Methods: <u>1.) Determine extent (incidence and severity) of Diplodia dieback epidemic in peninsular Florida</u>: We will begin by surveying the nine counties identified previously for stands of symptomatic trees. In year 2, we will expand to include counties not previously identified as infested. FFS county foresters will be trained on what to look for and will carry out county-wide surveys for suspect stands. Samples will be collected and sent back to the UF Forest Pathology Lab for confirmation of pathogen presence. The area and distribution of each "epidemic center" will be recorded using a GPS Digital Aerial Sketch Mapper. Each center will also be photographed every 3 months and assigned a severity rating (0-5 scale). We will also set up nine linear, 1/5th acre monitoring plots in slash pine stands displaying 3 stages of disease development: 1. No disease symptoms observed; 2. Early stages (less than 10% of trees with symptoms and symptomatic trees displaying less than 20% of crown with symptoms) and 3. Advanced stages of disease development (> 50% of trees showing > 50% of crown symptoms). The plots will be mapped, marked (with non-permanent metal tags fixed to corner trees) and GPS located. Photography will be used to document symptom progression at monthly intervals for 12 months. The data will be fitted to epidemiological models to evaluate the disease progression over time. Area under the disease progress curves (AUDPC) will be determined for each stand type and mortality rates will be calculated and analyzed. The data obtained from this objective will provide a basis for management decisions within a context of the timing of the epidemic.

<u>2.) Evaluate stand age, structure and previous weather effects on disease parameters</u>: For this objective we will expand our studies aimed at understanding the epidemic and its causes. Detailed stratified sampling will be carried out with approximately 900 trees (300 asymptomatic, 300 lightly symptomatic and 300 in advanced symptomatic stage) growing in infested OOCEA slash pine buffers on 6 sites (50 trees per category/per site). Sites will be chosen based on age (< 5 years old; 6-15 years old and > 20 years old) and density (plantings vary from ~ 100 to 500 trees per acre). Climate data from 2010-2014 will be collected from local weather data collection sites. We will evaluate, using statistical models, how these factors influence disease development over time.

c. Products: Overall, this project will provide a resource for forest managers in the region as little is currently known about the problem. We anticipate 1-2 peer-reviewed journal publications and a final report in 2014. In addition, the work will be presented at local and regional as well as 2 national meetings (including FHM). The distribution maps and information from Obj. 2 will be used by agencies and landowners and used for future modeling of disease epidemics in the region.

d. Schedule of Activities:

	Task Timeline			
Task	Winter 2014	Spring 2014	Summer 2014	Fall 2014
Begin county wide survey	X			
Set up plots for Obj.1 and 2	X			
Evaluate data	X	X	X	
Collect samples for Obj. 1	X			
Carry out lab analysis for Obj. 1		X		
Write up final report and BMP Manual			X	X

e. **Progress/Accomplishments:** N/A

f. **Relevant Citations:**

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7. House, E.S. 2007. *Diplodia pinea, Diplodia scrobiculata,* and *Lasiodiplodia theobromae* in South Georgia pine plantations and their respective roles in causing tip dieback in Southern pine species. M.S. thesis: Univ. of Georgia, Athens, GA.
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13. Sinclair, W.A. and H. H. Lyon. 2005. *Diseases of Trees and Shrubs* (2d ed.). Cornell University Press: Ithaca, NY, p. 130.
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15. Smith, D. R., and Stanosz, G. R. 2006. A species-specific PCR assay for the detection of *Diplodia pinea* and *D. scrobiculata* in dead red and jack pines with collar rot symptoms. Plant Dis. 90:307-313.
16. Smith, H., Wingfield M. J., and Coutinho , T. A. 2002. The role of latent *Sphaeropsis sapinea* infections in post-hail associated dieback of *Pinus patula*. For. Ecol. Manage. 164:177- 184.

COSTS:

	Item	Requested FHM EM Funding	Other-Source Funding	Source
YEAR 1				
Administration	Salary	Technician (8% time + fringe) = $3,200 M.S. student (100% time + tuition + fringe) = $24,900 Smith salary (10%+ fringe) = $11,372 _____	Matching funds for salary+fringe for PI (10% time) = $17,211; Partial salary for UF field/laboratory technician + fringe = $14,350 (25% time + fringe)	University of Florida and OOCEA
	Overhead	25% of direct UF IDC = $13,068	Unrecovered IDC (standard UF IDC is 51% of direct on federal grants) (26%) = $10,913	UF
	Travel	Travel to field sites (8-10 visits @ $250/site) and 2 national meetings, including FHM National Meeting = $2,500	$4,440	OOCEA
Procurements	Contracting			
	Equipment			
	Supplies		$5,740 (field and lab supplies)	OOCEA
Total Direct		$52,465	$52,654	