TITLE: Evaluating distribution and structure of epidemic populations of *Sphaeropsis sapinea*

LOCATION: Michigan, Minnesota, and Wisconsin primarily, with less intensive sampling in other areas with history of epidemics (e.g., Kentucky, Nebraska, North Dakota, Ohio, Pennsylvania, and Vermont).

DURATION: Year 2 of 3-year project (first year funding received in Dec. 2002)
FUNDING SOURCE: Base

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COOPERATORS: USDA Forest Service: Joseph O'Brien and Linda Haugen, Forest Health Protection, NA, St. Paul (USFS cooperators will furnish field study locations, collect and provide sample materials for culture and characterization, and collaborate in preparation of USFS publications (FIDL or HOW TO) incorporating project results).
Michigan Dept. Natural Resources: Robert Heyd, Forest, Mineral and Fire Management Division
Minnesota Dept. Natural Resources: Jana Albers, Forest Health Unit, Division of Forestry
North Dakota Forest Service: Michael Kangas
Wisconsin Dept. Natural Resources: Jane Cummings-Carlson, Forest Health Management, Division of Forestry

PROJECT OBJECTIVES: The overall goal of the project is to evaluate distribution and structure of *S. sapinea* populations associated with epidemic disease. The projects three supporting objectives are to evaluate:

1) *S. sapinea* as a cause of red and jack pine damage and mortality in recently detected epidemics; isolates will be acquired and *S. sapinea* populations will be characterized;

2) structure of *S. sapinea* populations in nonepidemic situations, especially as it occurs beyond the range of historical reports of significant damage;

3) the potential for virulent populations to persist with asymptomatic hosts in federal, state, and private conifer nurseries.

JUSTIFICATION: Results should improve understanding by the USFS Forest Health Monitoring Program and Forest Health Protection staff of the causes of recent and severe damage including mortality. This severe damage appears to be increasing in frequency and distribution, occurring broadly across the northcentral region including both state and federal forest lands. Current damage increases costs and interferes with the ability to regenerate underrepresented native tree species, and throws suspicion on state and federal nursery stock as the potential source of distribution on an invasive, and possibly introduced pathogen. Investigator has record of productivity and is working from a base of knowledge in his own program and, thus, successful completion is highly probable. New knowledge should be utilized in the development

of biologically rational approaches for disease management. .

DESCRIPTION: . Background: Coniferous trees are important components of eastern woodlands, which provide economic value, aesthetic beauty, recreational opportunities, wildlife habitat, water protection, and biodiversity. Approximately 12 million acres of forests in the eastern US are categorized as the red/white/jack pine cover type (USFS 2001). Of these species, jack pine (*Pinus banksiana*) and red pine (*P. resinosa*) each was once more prevalent and/or dominant in portions of region than they are today (Radeloff et al. 1999). While natural regeneration of many forest tree species is abundant, successful regeneration of red pine is almost always accomplished through planting, and plantation establishment can represent the greatest monetary investment in forest management. In addition, occurrence of seedlings of these species and their recruitment to the 1-3 inch sapling stage is much less frequent in many areas. For example, numbers of jack pine saplings in Wisconsin decreased from 105 million in 1983 to 57 million in 1996 (WI DNR). While past harvesting and fire are cited as major factors contributing to the dominance of second- and third-growth hardwood forests (Mladenoff 1995), the influence of pathogens on regeneration of these important conifer species has been relatively unexplored.

Among the diseases that affect jack and red pines is Sphaeropsis shoot blight and canker, caused by *Sphaeropsis sapinea*. The disease occurs worldwide and has been associated with significant economic damage in exotic pine plantations in New Zealand, Australia, and South Africa. Although long recognized, the disease was not generally noted as cause of severe damage in the northcentral United States until the mid-1970's. Unprecedented epidemics were then reported affecting primarily red pines in nurseries and plantations, and also jack pines. Damages include not only typical shoot blight and canker, but excessive mortality (estimated as high as 95%) of recently planted seedlings or established saplings with which *S. sapinea* is frequently associated as a cause of collar rot (Stanosz and Cummings Carlson 1996). Such sudden outbreaks continue to occur, including in areas where severe damage and widespread mortality has not been previously observed, such as epidemics in the Northern Highland-American Legion State forest in northern Wisconsin (personal observation) and portions of northcentral Michigan (personal communication, Joseph O'Brien, USFS Northeastern Area, State and Private Forestry, Forest Health Protection; Bob Heyd, MI DNR).

Coincident with severe damage in Lake States has been recognition of variation within the pathogen, and confirmation of the biological significance of these differences. Palmer et al. (1987) recognized two morphological groups among isolates identified as *S. sapinea* from MI, MN, and WI. Two distinct groups, referred to as A and B, now have been confirmed on the basis of independent molecular genetic markers including isozymes, RAPDs, ITS sequences, and microsattelite fingerprints (Stanosz et al. 1999, Zhou and Stanosz 2001, Zhou et al. 2001). A group isolates appear to be highly genetically similar and isolates from the Lake States are not differentiable from those from other parts of the world. Inoculations trials reveal that these A group isolates are highly aggressive on red and jack pines (Blodgett and Stanosz 1997) and several other conifers (Blodgett and Stanosz 1999). In addition, it has been confirmed that virulent A group isolates to persistent on or in asymptomatic red pine nursery seedlings and later proliferate to kill seedlings under conditions inducing host stress (Stanosz et al. 1997, Stanosz et al. 2001). These discoveries raise significant questions for the USFS Forest Health Monitoring Program about the origins and current geographic range and invasiveness of each of these types, their association with recent epidemics, the potential distribution of an aggressive pathogen on nursery stock, and force the reevaluation of the strategies and measures currently

employed (but failing) to reduce its impact.

b. Methods: Objective 1: Based on information supplied by USFS Forest Health Protection and state forest health management personnel, sampling will be conducted by in areas of current or recent outbreaks. From 2001and new reports supplied by USFS personnel during this project, sites should include locations in northern Wisconsin, northcentral Michigan (lower peninsula), and Michigan's upper peninsula. Other potential sampling locations of current or historical damage include Ohio (personal communication Dan Houston, USFS, and Erico Bonello, OSU), southcentral Pennsylvania (personal observation), and the University of Nebraska Horning Farm (personal observation - site of previous USFS research by Glenn Peterson). Collection will employ hierarchical sampling with a defined number of isolates per fungal fruiting body, shoot, tree, pine species, and site. Determination of the *S. sapinea* group of each isolate and population structure within and between sites/regions will be determined by analysis of molecular markers as described in previous publications (Stanosz et al. 1999, Zhou and Stanosz 2001, Zhou et al. 2001).

Objective 2: Procedures for hierarchical sampling and analyses will be similar to those followed for objective 1, but isolates will be obtained in areas of known occurrence of the disease in the absence of current or historical reports of significant damage. USFS personnel and local cooperators (e.g. university disease diagnosticians, state FHM personnel) will be important sources of information used to locate appropriate sampling areas and will participate in the actual collection of samples. Group identity of isolates and structure of populations will be determined and compared with those from objective 1.

Objective 3: Both jack and red pine seedlings will be sampled to evaluate the frequency of persistence of *S. sapinea* on or in asymptomatic seedlings. Again, USFS personnel and local cooperators (e.g. university disease diagnosticians, state FHM personnel) will be important sources of information used to locate appropriate sampling areas and will participate in the actual collection of samples. Procedures involve surface disinfestation and incubation of shoot material as previously described for detection of virulent isolates of *S. sapinea* at two Wisconsin nurseries (Stanosz et al. 1997). Group identity of isolates and structure of populations will be determined and compared with those from objectives 1and 2.

c. Products: It is anticipated that two scientific papers will be published, two presentations will be made to state cooperators and USFS personnel, and that the USFS FIDL or HOW TO publications dealing with *Sphaeropsis sapinea* will be revised.

d. Schedule of Activities: Field sampling of epidemic areas for isolate collection will occur before the end of the 2004 growing season. Isolates will be characterized in the fall/winter of 2004.

e. Progress/Accomplishments: Due to requests by state cooperators, activity to date has focused on nursery sampling as described for objective 3, and provided confirmation of an important means of dissemination of the pathogen to field locations. Beds of red pine seedlings from seven nurseries (one USFS, one MI DNR, two MN DNR, and three WI DNR) were examined to determine the incidence of symptomatic seedlings, the presence of *S. sapinea* on symptomatic seedlings, the frequency with which *S. sapinea* was detected on the healthy appearing asymptomatic seedlings, and the group (A or B) of *S. sapinea* detected. Seedlings both in proximity to a potential inoculum source (if present) or distant from such a source (e.g.,

red pine windbreak) were sampled. **Approximately 5600 seedlings were examined visually and cultures obtained from 1400 seedlings.**

Detection of *S. sapinea* from asymptomatic seedlings varied among and within nurseries (Table 1). **The fungus was common from asymptomatic seedlings from Wilson, Griffith, Hayward, Badoura, and General Andrews nurseries.** The frequency of detection of *S. sapinea* from these asymptomatic seedlings was highly correlated with the incidence of symptomatic seedlings in the same rows. Thus, symptoms might be a good indicator of risk of dissemination of the pathogen with healthy-appearing stock from the same beds.

All isolates obtained from asymptomatic seedlings from Wilson, Griffith, Hayward, and Badoura nurseries were characterized as belonging to the aggressive A group. This is consistent with the relatively high incidences of shoot blight and mortality in these nurseries. In contrast, seven of the ten isolates from asymptomatic seedlings from the General Andrews nursery, where incidence of symptoms was much lower, were of the less aggressive B group. It is possible that the lower incidence of disease at the General Andrews nursery reflects the relative dominance of the less aggressive form of the fungus at this location. Detection of some A group strains indicates the potential for additional damage and measures to prevent further establishment and spread of this aggressive group at that location may be justified.

Symptoms were rare and *S. sapinea* was not associated with symptomatic seedlings collected from the Wyman and Toumey nurseries. Similarly, *S. sapinea* was never detected from asymptomatic seedlings from these nurseries. These nurseries lack red pine windbreaks, and thus do not provide abundant primary inoculum from them to initiate disease. Other management practices might also limit disease there. Finally, it is possible that Wyman and Toumey nurseries are located in geographic areas in which *S. sapinea*, especially the aggressive A group, is absent or relatively rare.

Table 1. Occurrence of symptomatic seedlings[1] and frequency of detection of *S. sapinea* from asymptomatic seedlings in selected red pine beds seven Lake States nurseries.

Nursery	Inoculum[2] pressure	Seedlings symptomatic (%) (dead, or live but blighted) mean, range[3]	Asymptomatic seedlings but positive for *S. sapinea* (%) mean, range[4]
1 Wilson (*S. sapinea* detected on 10/10 symptomatic seedlings)			
(Boscobel, WI)	Low	8,0-14	8, 0-28
	High	43,25-59	63, 35-75
2 Griffith *(S. sapinea* detected on 9/10 symptomatic seedlings)			
(WI Rapids,WI)	Low	1,0-4	3, 0-10
	High	21,14-34	35, 20-70
4 Hayward (*S. sapinea* detected on 9/10 symptomatic seedlings)			
(Hayward, WI)	Low	12, 5-16	24, 15-40
	High	26, 20-31	30, 20-35
7 Badoura (*S. sapinea* detected on 10/10 symptomatic seedlings)			
(Badoura, MN)	Low	4, 1-7	19, 5-50
	High	58, 41-72	88, 75-95
5 General Andrews (*S. sapinea* detected on 10/10 symptomatic seedlings)			
(Willow River, MN	NA	4, 2-6	26, 5-40
	NA	4, 1-6	20, 10-35
3 Wyman (*S. sapinea* not detected on symptomatic seedlings)			
(Manistique, MI)	NA	3, 0-7	0, 0-0
	NA	1, 0-2	0, 0-0
6 Toumey (*S. sapinea* not detected on symptomatic seedlings)			
(Watersmeet, MI)	NA	1, 0-4	0, 0-0
	NA	0, 0-1	0, 0-0

[1]Seedlings had completed two seasons of growth, except at Toumey where seedlings in one of the two areas had completed a third season of growth (spring-sown 3 years earlier).

[2] Except at Wyman, Andrews, and Toumey, two areas within each nursery were selected to differ in proximity to a primary inoculum source (red pine windbreak) and preliminary visual estimate of incidence of symptoms. At these three nurseries, neither such a difference in proximity to a primary in inoculum source nor incidence of symptoms were apparent.

[3] Mean and range (to nearest %) for five sampled beds; approx. 1-m-long portion of an interior row inspected in each.

[4] Mean and range (to nearest %) for five groups of 20 asymptomatic seedlings each from an inspected 1-m-long portion of an interior row.

COSTS:				
	Item	**Requested FHM EM Funding**	**Other-Source Funding**	**Other Source**
YEAR (2 of 3)				
Administration	Salary[1]	$22,600		
	Fringe[2]	7,458		
	Travel	4,500		
	Overhead[3]		$18,226	UW-Madison
Procurements	Contracting			
	Equipment			
	Supplies[4]	5,500		
Totals		$40,058	$18,226	UW-Madison

[1] Salary for research specialist for 6 months at 100% appointment
[2] Fringe benefit rate is 33% of salary
[3] Indirect cost rate is 45.5% of the total of requested funds
[4] Supplies include sampling bags, laboratory culture media, petri dishes, reagents, computer paper, toner, etc.