Root Disease Incidence in Eastern White Pine Plantations With and Without Symptoms of Ozone Injury in the Coweeta Basin of North Carolina

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ABSTRACT

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A survey was conducted in the Coweeta Basin, Macon County, North Carolina, to determine the incidence of root diseases and their relatedness to ozone symptomatology in two eastern white pine (*Pinus strobus*) plantations. *Heterobasidion annosum* was isolated from <1% of root segments sampled in a stand without symptoms of ozone-caused foliar injury. No root pathogens were found in a stand with symptoms of ozone-caused foliar injury. No relation was found between injury caused by ozone and the incidence of root diseases in these stands.

Eastern white pine (*Pinus strobus* L.) is susceptible to several root-inhabiting fungi, including *Heterobasidion annosum* (Fr.) Bref. (9), *Armillaria mellea* (Vahl ex Fr.) Kummer (9), and *Leptographium procerum* (Kendr.) Wingf. (15). Each of these fungi can kill roots, and *H. annosum* and *A. mellea* are known to cause decreases in white pine growth and stand productivity (9). In addition, each fungus is often more prevalent on trees under stress (9,11,15). There has been concern in recent years that increasing levels of air pollutants may predispose plants to be more susceptible to opportunistic pathogens such as those on roots of white pines (17,20). Ozone is a pollutant that has been implicated in declines of forest trees (10).

Ambient oxidant air pollution can affect annosus root rot etiology in field-grown Jeffrey (*Pinus jeffreyi* Grev. & Balf.) and ponderosa pine (*Pinus ponderosa* Douglas ex P. Laws. & C. Laws.) while having little effect on sporulation or spore germination of *H. annosum* (13). Oxidant injury to foliage of ponderosa and Jeffrey pines increased the susceptibility of roots to infection and colonization by *H. annosum* under field and fumigation chamber environments (12). The rate of surface area infection of freshly cut Jeffrey pine stumps and the rate of vertical colonization of freshly cut Jeffrey and ponderosa pine stumps by *H. annosum* increased with increased oxidant injury to tree crowns and was associated with decreased oleoresin exudation and colonization by other fungi (14). In the Blue Ridge Mountains of Virginia, *H. annosum* was isolated from 8% of ozone-sensitive white pines sampled but was not isolated from any ozone-tolerant trees (15).

In summer 1983, the canopy of eastern white pines on watershed 17 in the Coweeta Basin (35°3′ north latitude, 83°26′ west longitude), 95 km southwest of Asheville, North Carolina, showed symptoms of ozone injury. In 1984, white pines on watershed 1 showed no apparent foliar injury attributable to ozone, while 75% of the trees on watershed 17 across the basin were symptomatic for foliar injury caused by ozone. Symptoms were most noticeable in late summer 1984 when a Forest Service plant pathologist and pioneer in research of ozone injury to eastern white pine (4) inspected the pine canopies of watersheds 1 and 17. Based on the findings of the pathologist and the fact that symptoms occurred during peak periods of ambient ozone in the region, it was concluded that the damage to watershed 17 was caused by ozone (18). This study was designed to determine the extent, if any, to which root disease occurs in the white pine plantations on watersheds 1 and 17, and to determine if any disease found was related to the ozone symptoms on watershed 17.

MATERIALS AND METHODS

Root sampling and assay procedures. Roots were collected 17–20 September 1985 from 10 plots placed uniformly throughout each of the two white pine stands. Watershed 1 is 16.1 ha, faces south, and was planted to white pine in 1957; watershed 17 is 13.4 ha, faces north, and was planted to white pine in 1956. Plot centers were located in the middle of a quadrilateral formed by four dominant or codominant pines, each approximately 3.05 m from the plot center. This spacing was chosen to maximize the number of white pine roots sampled. The duff layer was removed from a square of forest floor 30.5 cm on a side beneath which a 30.5-cm-deep soil-root sample pit was excavated. All pine root segments 0.3 cm in diameter or larger were separated from the soil, placed in plastic bags, and put on ice in a cooler. Root segments were trans-

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ported to the Forest Pathology Laboratory, Virginia Polytechnic Institute and State University, where they were examined for signs and symptoms of disease. This procedure was adapted from a sampling protocol developed to assess annosus root rot in southern yellow pine stands (1).

Root segments were washed with tap water to remove soil and other debris from the rhizoderm. The diameter midway between the ends of the root segments and the length of segments were measured. Sections of rhizoderm were removed from segments with a knife so that the inner bark and xylem could be inspected. Symptoms of resin soaking and black stain and the presence of mycelia were recorded. The percentage of total segments with symptoms of disease per total segments collected was calculated. Segments with no live tissue were considered diseased and were included in the percentage of diseased segments.

Inner bark and xylem cut from a single area of a symptomatic or asymptomatic root segment were placed in plastic petri dishes containing an agar medium selective for *H. annosum* (2). A second piece of tissue cut from the same area of the segment was placed in a dish containing an agar medium selective for *Leptographium* spp. (8). A third piece of tissue was placed on malt extract agar to assay fungi that were prevented from growing by the first two media. Feeder roots were removed from root segments, surface-sterilized with a 10% solution of 0.5% NaOCl, and placed on cornmeal agar amended with pimaricin, penicillin, and polymyxin B sulphate to allow selective isolation of *Phytophthora* spp. and *Pythium* spp. (19).

Media and root tissue in petri dishes were incubated in the dark at 23 C for 1–2 wk. Following incubation, fungi growing from root tissue or feeder roots were identified by spore and hyphal characteristics, conidiophore morphology, and architecture.

Onsite disease survey. Live roots in each quadrilateral plot were surveyed visually for signs and symptoms of diseases. The duff layer was removed from around the bases of three or four pines closest to plot centers, and tree bases and root collars were examined specifically for basidiocarps of *H. annosum* and for rhizomorphs and mycelial fans of *A. mellea*. Sections of bark were removed from root collars to examine the xylem for black stain. Xylem with this symptom was sampled, taken to the laboratory, and placed in a petri dish containing an agar medium selective for *Leptographium* spp.

Soil sampling and chemical analysis. Soil from the A and B horizons was collected from each soil root sample pit. If the A and B horizons were visually indistinguishable, the top 20 cm of soil was sampled as the A horizon. Standard soil chemical analyses included measuring soil solution pH using a pH electrode; measuring concentrations of K, Ca, and Mg by atomic absorption spectrometry; and measuring concentrations of P by flame photometry. Soil pH and concentrations of P, K, Ca, and Mg were compared between watersheds with the use of a one-way analysis of variance procedure.

Stand growth characterization. Because the steep topography of the watersheds prohibited more accurate measurements, the heights of two trees nearest the center of each plot on each watershed were estimated. Site indices for each plantation were estimated from site index tables for natural stands (6). Basal areas at each plot were measured by point-sampling techniques (3), with a 2.5-m^2 basal area factor optical prism. The diameter of the two trees used for height estimations was measured at breast height with a steel tape.

RESULTS

Isolations from root tissue. *H. annosum* was isolated from one resin-soaked and one asymptomatic root segment collected at one pit on watershed 1. The total number of root segments examined was 344 from watershed 1 and 330 from watershed 17. Mean root segment length and diameter were 20.0 cm (SD = 9.5 cm) and 0.28 cm (SD = 0.27 cm), respectively, at watershed 1, and 21.8 cm (SD = 11.5 cm) and 0.33 cm (SD = 0.39 cm), respectively, at watershed 17. Disease symptoms were observed on 12.5% of all root segments from watershed 1 and 7.3% from watershed 17. Fungi were isolated from 2.9% of symptomatic segments from watershed 1 and 1.8% from watershed 17.

Several root segments removed from pits on both watersheds had a thin weft of white or light buff-colored mycelia between the rhizoderm and xylem. This mycelial growth occurred on segments that were dead or had both live and dead tissue and may indicate the presence of a saprophyte or a facultative parasite such as *A. mellea*. Root segments collected at six pits on watershed 1 and five pits on watershed 17 had a mycelial weft or were resin-soaked. A species of *Leptographium* was isolated from root segments with resin-soaked or black-stained xylem, or both, collected at four pits on watershed 1 and three pits on watershed 17. *Leptographium* spp. were also isolated from three asymptomatic roots collected on watershed 1. No *Phytophthora* spp. were isolated from feeder roots collected on either watershed.

Onsite disease survey. No basidiocarps of *H. annosum* were found on trees near the soil root sample pit of watershed 1 where the fungus was isolated from root tissue. Black rhizomorphs were found in two pits and in the duff layer around the pits on both watersheds. Although basidiocarps were not found when the study was conducted, the presence of black rhizomorphs is unmistakable evidence of *A. mellea* within the soil. A diffuse black or dark blue stain occurred in root collar xylem of a dead tree at each of two pits on watershed 1. *Leptographium* spp. was isolated from xylem removed from these trees.

Soil chemistry. Concentrations of P, K, Ca, and Mg and soil solution pH of the A and B horizons did not differ between watersheds 1 and 17 ($P = 0.05$) (Table 1). Values for these parameters (except Ca) were also similar in the A and B horizons. Calcium in the A horizon was about twice that in the B horizon in each watershed.

Stand characteristics. Average basal areas for pine on the watersheds were nearly identical, whereas the average diameter at breast height, estimated heights, and site indices were slightly

Table 1. Mean soil chemical parameters of the A and B soil horizons on watersheds 1 and 17 in the Coweeta Basin, Macon County, North Carolina

	Watershed 1		Watershed 17	
Parameter[a]	Horizon A	Horizon B	Horizon A	Horizon B
pH	5.24[b]	5.32	5.22	5.10
P	2.90	2.40	2.60	2.20
K	27.90	30.10	27.70	28.40
Ca	231.60	105.60	169.20	88.80
Mg	32.10	35.80	30.40	24.50

[a] Concentrations of P, K, Ca, and Mg are in ppm.
[b] All values are the mean of 10 samples from each soil horizon collected at each watershed. A and B horizon soil parameters did not differ significantly between watersheds at $P = 0.05$.

Table 2. Characteristics of stands of eastern white pine on watersheds 1 and 17 of the Coweeta Basin, Macon County, North Carolina

Parameter[a]	Watershed 1	Watershed 17
Basal area	56.25	56.50
DBH	23.65	21.44
Estimated height	17.60	16.23
Site index	85	80

[a] Basal area (m^2/ha), DBH (diameter at breast height) (cm), and estimated height (m) are averages of two dominant or codominant trees measured at 10 plots on each watershed.

higher for watershed 1 than for watershed 17 (Table 2).

DISCUSSION

A low level of root diseases and little foliar injury attributable to ozone in watershed 1 and no root diseases and high foliar injury attributable to ozone in watershed 17 indicates no relation between injury caused by ozone and the incidence of root diseases in these stands. This report documents *H. annosum* in the roots of pines on watershed 1 at the time of the study. Because the incidence of *H. annosum* was low or nonexistent in the stands, annosus root rot is not likely to limit stand productivity. However, trees in either watershed may become more susceptible to root diseases, especially under continued stress from ozone or other abiotic or biotic stresses.

It remains unclear why most trees in watershed 17 were damaged by ozone when most trees in watershed 1 were uninjured. Perhaps a factor peculiar to the two plantations is responsible for the differing symptoms. For example, the origin of the trees planted in the watersheds 1 yr apart is unknown and may differ to the extent that white pines on watershed 17 are more inherently sensitive to damage by ozone. Soil chemistry and stand characteristics of both watersheds were similar, so they were unlikely to have contributed to any differences in root disease levels at the time of the study. However, the presence of root-inhabiting fungi in the pines on both watersheds could be important to root disease etiology in the future.

Rhizomorphs of *A. mellea* were found in the soil and duff layer of the forest floor on watersheds 1 and 17. This fungus possesses the unique ability among fungi to degrade xylem like a saprophyte and to parasitize the cambium in living trees (17). *A. mellea* can become a pathogen when cutover areas are replanted and the fungus, using dead stumps as a food source, attacks young trees (17). The fungus can destroy white pine seedling and sapling reproduction up to 9 m or more from colonized hardwood stumps (9). Because *A. mellea* preferentially attacks stressed trees (11), it can be a contributing factor in many tree declines (17). The association of *A. mellea* with roots of declining and dead red spruce (*Picea rubens* Sarg.) in New England and New York indicated that the fungus may be involved in, but is not the primary cause of, red spruce decline in the Northeast (5). The white pines of watersheds 1 and 17 could be parasitized by *A. mellea* if sufficiently stressed by some biotic or abiotic factor. It would be interesting to determine at that point if the trees on watershed 17, with a history of sensitivity to ozone, had more root disease.

The species of *Leptographium* recovered from black-stained roots and root collars on watersheds 1 and 17 was not *L. procerum*, the causal agent of black-stain root disease common on ornamental and naturally occurring eastern white pines. Gross cultural characteristics and conidiophore morphology of the isolates appear to be near those of *Leptographium serpens* (Goid.) Siem., yet morphologically and electrophoretically distinct from *L. serpens* (T. C. Harrington, *personal communication*). *L. serpens* was reported to have caused a "killing root disease" associated with stained wood in Italian stone pine (*Pinus pinea* L.) in Italy (7,16). The report of Lorenzini and Gambogi (16) is now believed to be false; *H. annosum* was identified as the causal agent of the disease (21). This species of *Leptographium* is not known to be pathogenic and thus is of little consequence to the health of the pines on watersheds 1 and 17.

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