Root Disease and Insect Infestations on Air-Pollution-Sensitive *Pinus strobus* and Studies of Pathogenicity of *Verticicladiella procera*

A. L. LACKNER, Graduate Research Assistant, and S. A. ALEXANDER, Associate Professor, Virginia Polytechnic Institute and State University, Blacksburg 24061

ABSTRACT

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Two lateral roots were excavated from each of 25 air-pollution-sensitive and 18 air-pollution-tolerant eastern white pines in the Blue Ridge Mountains in Virginia. *Verticicladiella procera, Heterobasidion annosum, V. serpens*, and a *Graphium* sp. were isolated from 24, 8, 8, and 4% of the sensitive trees, respectively. Two species of weevils, *Pissodes approximatus* and a *Hylobius* sp., were isolated from 20% of the sensitive trees. No pathogenic fungi or insects were isolated from the tolerant trees. The pathogenicity of three *V. procera* isolates was tested on eastern white pine and loblolly pine seedlings. Seedlings were inoculated using a root-dip method. Fifty-nine of 60 eastern white pines and 57 of 60 loblolly pines were dead 7 wk after inoculation. The total number of eastern white pines and loblolly pines killed was not significantly different ($P = 0.05$). One isolate killed significantly fewer loblolly pines than eastern white pines ($P = 0.05$).

The long-distance transport of ozone from the northern and northeastern parts of the United States into the Blue Ridge Mountains of Virginia has been suggested by Hayes and Skelly (5). In this area during the past 5 yr, ozone concentrations have been detected that exceed injury threshold levels for native forest vegetation, particularly eastern white pine (*Pinus strobus* L.) (13). Reduced radial growth, foliar injury, and death of eastern white pines have been attributed to these high ozone concentrations (14).

Effects of oxidant air pollution on forest tree species have been studied extensively in the San Bernadino Mountains in southern California (4,6). Ozone injury to the foliage of ponderosa (*P. ponderosa* Laws.) and Jeffrey (*P. jeffreyi* Gevr. and Balf.) pines increased susceptibility of the roots to infection and colonization by *Heterobasidion annosum* (Fr.) Bref. (syn. *Fomes annosus* (Fr.) Karst.). Ozone injury also appeared to predispose pine trees to infestations by bark beetles (16).

A survey was conducted to determine if such pollution-pest interactions exist among eastern white pines in the Blue Ridge Mountains in Virginia. During the survey, the fungus *Verticicladiella procera* Kendrick was frequently isolated from air-pollution sensitive trees. The pathogenicity of this fungus has recently been established on eastern white pine (8). Symptoms of *V. procera* infection include wilting of the needles, which then turn a uniform brown and remain attached to dead trees. Resin-soaking and black streaking are evident at the stem base and in the roots. In Virginia, *V. procera* has been isolated from dead and dying Austrian (*P. nigra*), Scotch (*P. sylvestris* L.), and eastern white pines in Christmas tree plantations and from loblolly pines in forest plantations (8). In addition to the survey, the pathogenicity of a *V. procera* isolate obtained during the survey was compared with two other isolates of the fungus on loblolly and eastern white pines.

Present address of first author: Department of Plant Pathology, North Carolina State University, Raleigh 27650.

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MATERIALS AND METHODS

Blue Ridge Mountain survey. Twenty-five air-pollution (primarily ozone)-sensitive eastern white pines scattered along a 105-km section of the Blue Ridge Parkway between Roanoke and Fancy Gap, VA were examined. The trees were selected on the basis of such pollutant symptoms as reduced terminal growth, thin crowns with only the current year's needles present, chlorotic or necrotic needles, and tip burn of needles. Eighteen eastern white pines showing no air-pollution injury served as controls. Each of these trees was located within 10 m of a separate sensitive tree.

Two lateral roots were severed 1 m from the trunk of the sensitive and control trees and brought to the laboratory. Root chips about 1 cm^3 were removed and placed onto *V. procera*-selective medium (VPSM) (10), ortho-phenylphenol medium selective for *H. annosum* (1), 2% malt extract-agar medium (MEA), and acidified potato-dextrose agar medium. After incubating for 2 wk at 20 C, the various fungi growing from the chips were identified. The excavated roots were also examined for insect activity. Damage was noted and insects were removed and identified.

After *V. procera* was isolated from the roots of one of the sensitive trees, soil samples were collected around excavated roots of the 18 subsequently sampled trees. About 300 g of soil was collected per sample. Samples were prepared using the agar plate method (2) and plated onto *V. procera* soil isolation medium (17).

Pathogenicity study. Three *V. procera* isolates were obtained from the following hosts: 1) a 6-yr-old eastern white pine in a Christmas tree plantation in Montgomery Co., VA (isolate 1); 2) a 28-yr-old eastern white pine located along the Blue Ridge Parkway in Carroll Co., VA (isolate 2); and 3) a 6-yr-old Scotch pine in a Christmas tree plantation in Fairfax Co., VA (isolate 3). Chips were removed from the edge of resin-soaked or stained root and stem tissue, placed onto *V. procera*-selective medium, and incubated at 20 C. The isolates were then transferred to 9-cm-diameter petri plates containing 2% malt-extract agar, four plates per isolate, and incubated at 20 C for 14 days.

Conidial suspensions were prepared by pouring sterile distilled water (SDW) over each plate and swirling gently. These suspensions were further diluted with SDW until the concentration of the conidia, determined with a hemacytometer, was about 1×10^6 conidia per milliliter for each isolate.

Root systems of 15-mo-old loblolly and eastern white pine seedlings grown from seed in a greenhouse were rinsed

Table 1. Pathogens isolated from roots of 25 air-pollution-sensitive and 18 air-pollution-tolerant eastern white pines located along the Blue Ridge Parkway in Virginia

Pathogens isolated	Trees infected/ infested: Sensitive (%)	Tolerant (%)
Fungi		
Verticicladiella procera	24	0
Heterobasidion annosum	8	0
V. serpens	8	0
Graphium sp.	4	0
Insects		
Pissodes approximatus	20	0
Hylobius sp.	20	0

free of soil with tap water. Roots of 20 seedlings of each of the two host species were dipped in the spore suspension of one isolate. This procedure was repeated for the other two isolates. Ten seedlings of each host species were dipped in SDW as a control. Immediately after treatment, all seedlings were planted individually in 11-cm pots containing steam-sterilized Spasoff's potting mixture (Weblite, vermiculite, peat, 2:2:1, v/v/v) and placed in a greenhouse.

As seedlings died, isolations were made from root and stem tissue onto VPSM and 2% MEA. The study was concluded after 7 wk, at which time isolations were made from all remaining seedlings.

RESULTS

The most commonly isolated pathogenic fungus from ozone-sensitive eastern white pine was *V. procera*, which was isolated from 24% of the sensitive trees (Table 1). *Heterobasidion annosum* was isolated from two of the sensitive trees. The infected roots showed symptoms typically induced by these fungi: resin-soaking and black staining with *V. procera* and a white stringy rot with *H. annosum*. Two blue-stain fungi, *V. serpens* (Goid.) Kendrick and a *Graphium* sp., were isolated from dark-stained tissue of 12% of the sensitive trees. No pathogenic fungi were isolated from the control trees.

Two species of weevils, *Pissodes approximatus* Hopk. and *Hylobius* sp., were found in the roots of five of the sensitive trees (Table 2). Galleries, pupal chambers and adult insects were observed. *V. procera* was isolated from the same roots in which these insects were found. No insects were found in the control trees.

Three of the 18 soil samples collected around the roots of sensitive trees yielded *V. procera* (Table 2). The three soil samples were collected from the immediate vicinity of roots also infected with the fungus.

Pathogenicity studies. No significant difference in the pathogenicity of isolates on eastern white pine was found (Table 3). On loblolly pine, there was a significant difference ($P = 0.05$) among isolates, with isolate 3 being less pathogenic than the other isolates. The total number of eastern white pines and loblolly pines killed was not significantly different.

Dying seedlings showed typical symptoms of *V. procera* infection and the fungus was isolated from each. The fungus was also isolated from two of three symptomless loblolly pines and one eastern white pine.

DISCUSSION

Results of this study indicate that eastern white pines expressing oxidant air-pollution injury are more susceptible to root disease and insect infestations. Air-pollution injury has been shown to decrease the amount and rate of oleoresin flow and reduce sapwood and phloem moisture content in several tree species (3). These conditions are associated with increased susceptibility of trees to infection by *H. annosum*, blue-stain fungi, and other pathogenic fungi (9). These conditions have also been shown to facilitate invasions by insects (12). Such pollutant-pest interactions appear to be operating among eastern white pines in the Blue Ridge Mountains in much the same manner as they do in the San Bernadino Mountains in California. Results of this study suggest that the injurious effects of air pollution are not adequately assessed by observations of foliar injury alone but should include evaluation of root disease and insect infestations.

Isolation of *V. procera* from eastern white pines in this study verifies the presence of the fungus in natural stands in Virginia. The fungus was found in the soil but only around some of the infected roots. Failure to recover the fungus from soil around the roots of all infected trees may have been due to very low populations in the soil. If more soil had been collected per sample, it might have increased the recovery rate of the fungus. Weevils were found in five of six trees infected with *V. procera*. Isolation of this fungus from bark beetles and their galleries (8) and from pupal chambers of *Pissodes pini* (L.) (7) indicates the possibility that the fungus may be introduced into the roots by insects.

Inoculation experiments conducted by Smith (15) showed that two strains of *V. wagenerii* were not host specific. In this experiment, infection of loblolly and eastern white pines with isolates obtained from Scotch and eastern white pines indicate that these isolates of *V. procera* similarly are not host specific. Except for isolate 3, all isolates were equally pathogenic on loblolly pine. Such variations between virulence of the pathogen and resistance of the host has been demonstrated with *Verticillium* spp., another wilt disease fungus (11). Further study is needed to determine if either or both of these mechanisms do occur among *V. procera* isolates and their hosts.

Table 2. Root and soil isolations made from the nine of 25 air-pollution-sensitive eastern white pines found infected/infested along the Blue Ridge Parkway, VA

Tree no.	Fungi	*V. procera* soil samples[a]	Weevils[b]	Condition of roots
1	*Verticicladiella procera* *Graphium* sp.	+	+	Resin-soaked, dark stain
2	*V. procera*	−	−	Resin-soaked
5	*V. procera*	−	+	Resin-soaked, dark stain
6	*V. procera*	+	+	Resin-soaked, dark stain
10	*Heterobasidion annosum*	−	−	White, stringy rot
13	*V. serpens*	−	−	Dark stain
14	*V. serpens*	−	−	Dark stain
16	*V. procera*	−	+	Resin-soaked, dark stain
20	*V. procera, H. annosum*	+	+	Resin-soaked, dark stain

[a]+ = *V. procera* isolated from soil samples; − = *V. procera* not isolated.
[b]+ = Weevils found in roots; − = weevils not found.

Table 3. Results of inoculation of eastern white pine and loblolly pine seedlings with three isolates of *Verticicladiella procera* after 7 wk[a]

Host	Isolate	Inoculated (no.)	Symptomatic or dead (no.)	Reisolation[b] (%)
Eastern white	1	20	20	100
	2	20	20	100
	3	20	19	100
Loblolly	1	20	20	100
	2	20	20	100
	3	20	17[c]	100

[a]Ten loblolly and 10 eastern white pines dipped in sterile distilled water as controls remained asymptomatic and no *V. procera* was isolated.
[b]Percentage of symptomatic or dead seedlings from which *V. procera* was isolated.
[c]Significantly different based on the chi-square test ($P = 0.05$).

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