Incidence and Development of *Verticicladiella procera* in Virginia Christmas Tree Plantations

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ABSTRACT

Lackner, A. L., and Alexander, S. A. 1984. Incidence and development of *Verticicladiella procera* in Virginia Christmas tree plantations. Plant Disease 68:210-212.

The rates of incidence (r) of *Verticicladiella procera* in two Christmas tree plantations in Virginia were 0.17 and 0.049 from February to April 1980. During the summer, fall, and winter, r values decreased to near zero. By spring of 1981, r values were only 0.008 and 0.02 for the two plantations. Populations of *V. procera* in the soil were found to decline from August to October. The fungus was present in low numbers in the soil from October 1980 to June 1981. Many eastern white pines infected with *V. procera* were also infested with bark beetles. The fungus was isolated from 7% of bark beetles collected from six trees. Fifty diseased eastern white pines were cut in October 1980 and the stumps were excavated in April 1981. *V. procera* was isolated from 45% of the stumps, indicating that the fungus can overwinter in infected tree stumps. Mortality during this study averaged 26% in the two plantations.

Incidences of *Verticicladiella procera* Kendrick have been reported in New York (8,14), Pennsylvania (17), Virginia (10), New Zealand (13), and Yugoslavia (6). Bertagnole and Partridge (2) isolated *V. procera* from bark beetles and insect galleries in several species of pine in the western United States. Kendrick (9) reported isolation of the fungus from pupal chambers of *Pissodes pini* L. in pine in Sweden. Other than these examples, ecological and epidemiological information concerning this pathogen is scarce. Consequently, studies were conducted to monitor development of *V. procera* in Christmas tree plantations, quantify populations in the soil, study persistence of the fungus in plantations, and investigate insect associations.

Accepted for publication 7 September 1983.

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MATERIALS AND METHODS

Plantation 1 was a 22-ha Christmas tree planting in Montgomery County, VA. Most of the plantation had been planted to eastern white (*Pinus strobus* L.) and Scots pines (*P. sylvestris* L.), with a few hectares planted to several spruce, fir, and other pine species. Plantation 2 was a 30-ha eastern white pine plantation in Wythe County, VA.

Epidemiology study. Distribution of diseased trees in the two plantations was observed from February 1980 to June 1981. In plantation 1, an 8-ha area containing 545 4- to 8-yr-old eastern white pines was observed and the distribution of healthy and diseased trees was mapped at 2-mo intervals. An 18-ha area in plantation 2 containing 1,166 4- to 8-yr-old eastern white pines was observed and mapped as in plantation 1.

Populations of *V. procera* in the soil were also determined at 2-mo intervals. Six plots were established in each of the two plantations. Each plot (1 m^2) was established where a single diseased tree ranging from 5 to 7 yr old had been cut and the root system excavated in August 1980. In addition, five plots, each

containing one symptomless tree of the same age class, were established in each plantation. A 300-g soil sample was collected to a depth of 20 cm at each site. Samples were prepared using the agar-plate method of Clark (3) and plated onto *V. procera* soil isolation medium (VPSIM) (16). Plates were incubated at 20 C for 10 days. The pH of all soil samples was measured using equal amounts of soil and distilled water (v/v).

Eight trees in each plantation were observed for insects in the spring of 1981. Diseased trees ranging from 5 to 7 yr old were excavated from each plantation and brought to the laboratory. Bark was removed from the stem and roots and insects were collected, rinsed in sterile distilled water, and crushed onto plates of *V. procera*-selective medium (VPSM) (11).

Seedling study. Eastern white pine seedlings were planted in naturally infested soil to determine if they could become infected under natural conditions. Six 1-m^2 plots were established in each of the two plantations. Five plots in each plantation were located at sites where trees infected with *V. procera* had been excavated in August 1980. The remaining plot in each plantation was located between healthy trees, at least 30 m from any diseased tree, where isolations from the soil did not yield the fungus.

Sixty-five 1-yr-old eastern white pines obtained from the Virginia Division of Forestry were planted in individual pots, placed in a greenhouse, and observed for disease symptoms. Root and stem tissue of five of the seedlings were plated onto VPSM and 2% malt-extract agar (MEA) to determine if *V. procera* or any other pathogenic fungi were present. In September 1980, five healthy seedlings were planted at the corners of a 1-m square and one was planted in the center of the square. Plots were observed at monthly intervals for 8 mo. When seedlings died, isolations were made on VPSM and MEA. At the end of the experiment in June 1981, all remaining seedlings were removed, observed for insect activity, and isolations made from each.

Stump isolations. Diseased 5- to 7-yr-old trees were cut down in both plantations in October 1980. Twenty-five stumps from diseased trees in each plantation were flagged. In April 1981, the stumps were excavated and examined for root grafts or contacts. Root and stem chips were removed and placed on VPSM and MEA. Five stumps from healthy trees in each plantation were also excavated and isolations made from each.

RESULTS

Epidemiology study. Progress of the *V. procera* epidemic is illustrated in Figure 1. Sporocarps of the fungus were not observed in the field during the 16-mo study period. Consequently, we assumed there was no exponential increase of the inoculum during the epidemic and the disease was treated as a "simple interest disease" (18).

In plantation 1, symptoms of *V. procera* infection were first observed among a few eastern white pines in 1976 and spread over a 2-ha area of the plantation in 3 yr. Most losses in this section occurred in 1977 and 1978, when about 150 trees died. The area used in this study was about 50 m from the initially infected area. In 1980, the disease extended over a 4-ha area and killed about 180 eastern white pines. The disease was not nearly as severe in 1981. The *r* value for spring of 1980 was 0.17 compared with 0.008 for spring of 1981. During the 16-mo study, mortality was 34%.

The disease developed in a similar manner in plantation 2. Six-year-old eastern white pines were first observed dying in 1974. Diseased trees were initially concentrated in a 0.4-ha area, but in 6 yr, the disease had spread throughout the 30-ha plantation. The disease was most serious in 1980, with losses totaling nearly 150 trees. As with plantation 1, the disease was not as severe in 1981. The *r* values for spring of 1980 and 1981 were 0.049 and 0.02, respectively. Mortality in plantation 2 was 17% for the study period.

Detectable *V. procera* propagules in the soil decreased after infected host material was removed in June and were not detected by December (Fig. 2). The fungus appeared to overwinter successfully at only one of the 10 infected sites. Soil samples collected at the base of healthy trees did not yield any *V. procera*. The pH of the soil samples ranged from 5.1 to 5.6, with no significant difference between infested and uninfested soil.

All 16 diseased trees examined for insects were heavily infested with bark beetles, particularly species of *Pityokteines* Fuchs, *Pityogenes* Bedel, and *Pityophthorus* Eichh. Bark beetle galleries were found mainly in the lower half of the stem, associated with black-stained wood. Several trees were also infested with the weevil *Pissodes approximatus* Hopk. Pupal chambers of the weevil were concentrated around the stem base and were also associated with black-stained wood. A total of 320 adult bark beetles and 10 adult weevils were plated. Twenty-two bark beetles collected from six trees yielded *V. procera*. The fungus was not isolated from any of the weevils.

Seedling study. Twelve of 25 seedlings in plantation 1 and 11 of 25 seedlings in plantation 2 had died by the end of the experiment (Table 1). *V. procera* was

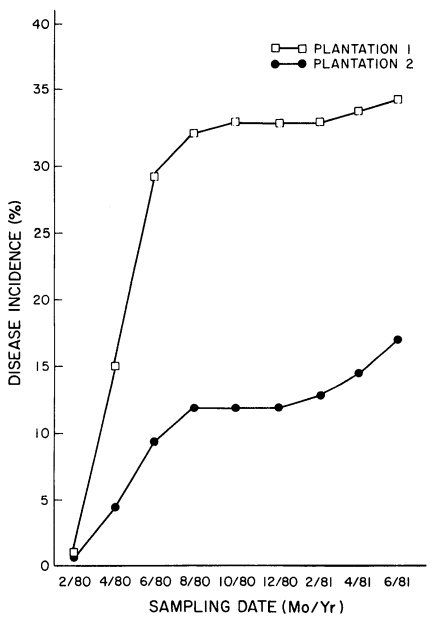

Fig. 1. Progress of *Verticicladiella procera* epidemics in two Christmas tree plantations from February 1980 to June 1981.

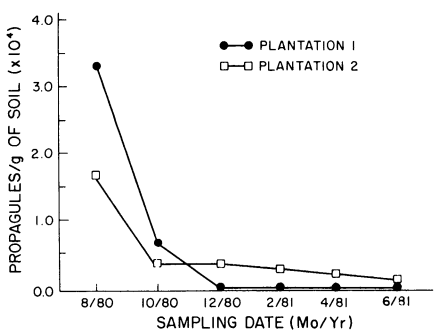

Fig. 2. Populations of *Verticicladiella procera* in soil collected in two Christmas tree plantations. Points represent the average of six samples for each plantation.

Table 1. Results of isolations made from 50 eastern white pine seedlings planted in soil naturally infested with *Verticicladiella procera* and 10 seedlings planted in uninfested soil

Location	Total no. planted	Symptomatic[a] No.	Symptomatic[a] Isolations (%)	Asymptomatic No.	Asymptomatic Isolations[b] (%)
		Infested soil			
Plantation 1	25	12	83	13	0
Plantation 2	25	11	91	14	14.3
		Uninfested soil			
Plantation 1	5	1	0	4	0
Plantation 2	5	0	0	5	4

[a] Seedling symptomatic of *V. procera* infection.
[b] Isolation of *V. procera*.

isolated from 83 and 91% of the dead seedlings, respectively. Two asymptomatic seedlings yielded the fungus. Three seedlings planted in infested soil and one control seedling died but none showed symptoms of *V. procera* colonization and the fungus was not isolated. There was no insect or other damage observed on any of the seedlings.

Stump isolations. All stumps of diseased trees had extensive black staining in the roots and stems. *V. procera* was isolated from 40 and 50% of the diseased stumps excavated from plantations 1 and 2, respectively. The fungus was not isolated from any healthy stumps. There were no root contacts or grafts observed between trees in the two plantations.

DISCUSSION

Several species of *Verticicladiella* have been associated with bark beetles and their galleries (1,2,4,9). Barras and Perry (1) found germinating spores and hyphal fragments of *Verticicladiella* spp. in the mycangium of *Dendroctonus adjunctus* Blandford. Both conidia of *V. wagenerii* Kendrick and perithecia of its perfect stage, *Ceratocystis wagenerii* Goheen & Cobb, were found in galleries of beetles by Goheen (4). Isolations of *V. procera* from bark beetles and their galleries by Bertagnole and Partridge (2) and in this study indicate a possible insect-vector relationship with this fungus as well.

V. wagenerii has been shown to spread from tree to tree through root grafts and major root contacts (5,19). In this study, there were no root contacts or grafts observed between any trees in either plantation. Newly infected trees were observed as far as 50 m from other diseased trees and completely surrounded by healthy trees. This scattered distribution of infected trees may be explained at least in part by insect transmission. Bark beetles were observed on both diseased and symptomless trees.

The *r* values decreased rapidly in the summer. This decrease may be attributed to the decrease in the number of susceptible hosts or to the behavior of the bark beetles implicated as vectors. Rudinsky (12) suggested that host selection by bark beetles depends on the attractiveness of the first attacking beetles. After these pioneer beetles become established, more beetles are attracted until there is no additional space for invasion. The numerous trees killed in the spring may have provided adequate feeding and breeding material for the bark beetle populations present in the two plantations.

V. procera was isolated successfully from soil around roots of diseased trees. Isolation of the fungus after removal of diseased material indicates the capacity of the organism to survive in the soil. Studies have shown that *V. wagenerii* can exist in the soil, at least in part, as mycelium and possibly as spores (7). Closer examination is needed to conclusively determine the form(s) in which this fungus exists in soil. The fungus had successfully overwintered in diseased stumps, surviving for at least 6 mo. Houston (8) found that *V. procera* remained viable in dead bark tissue of eastern white pines for at least 2 yr. The fungus appears to survive for limited periods of time in the soil and to survive extended periods in dead host tissue.

At least one seedling planted in naturally infested soil at each of the 12 sites died and *V. procera* was isolated from them. Absence of the fungus in the seedlings before planting and absence of insect activity indicates that *V. procera* can infect seedlings through root systems in the field. Seedlings have been inoculated with this pathogen through roots in the laboratory (10). Several investigators have hypothesized that roots can serve as an infection court for other *Verticicladiella* spp. (4,15,19).

In this study, seedlings were planted in the fall and the majority died in the spring. They could have been infected at any time between September and the end of the experiment in June. Because populations of *V. procera* in the soil were observed to decline so rapidly through the winter, whether or not seedlings planted in the spring would also be infected needs investigation.

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