Occurrence and Pathogenicity of *Verticicladiella procera* in Christmas Tree Plantations in Virginia

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ABSTRACT

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Verticicladiella procera was identified as the cause of severe losses in some Christmas tree plantations in Virginia. Losses for 1980 totaled about 700 marketable trees in eight plantations. *V. procera* was isolated from *Pinus strobus*, *P. sylvestris*, and *P. nigra*. Pathogenicity was determined by inoculating 2-yr-old *P. strobus* seedlings with *V. procera*. Inoculation was accomplished by dipping root systems in a spore suspension or by inserting small blocks of white pine stem wood colonized with *V. procera* into a slit wound in the taproot. Inoculated seedlings began dying in 2 wk and continued to die over a 10-wk period. *V. procera* was isolated from 50% of the seedlings inoculated by root-dip and from 25% of the seedlings inoculated by colonized blocks. *V. procera* was isolated only from dead, inoculated seedlings.

Recently, an increasing number of eastern white pines (*Pinus strobus* L.) have died in Christmas tree plantations in Virginia. Mortality in some plantations has reached epidemic levels. Affected trees exhibited needle wilt, a uniform browning of the needles, and resin-soaking with black streaks at the base of the stem. These symptoms are characteristic of white pine root decline, which is usually associated with *Verticicladiella procera* Kendrick (1).

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V. procera has been isolated from several *Pinus* spp. in the eastern United States, New Zealand, and Yugoslavia (4,5,7–11). Dochinger (2) observed similar symptoms on eastern white pines and reported that a *Leptographium* spp., later identified as *V. procera*, was responsible for inciting these symptoms. The role of *V. procera* as the primary pathogen inciting white pine root decline, however, has not been completely accepted by many plant pathologists because the reports are inconclusive. The objectives of this study were to report on the occurrence of this disease in Virginia and to determine the pathogenicity of *V. procera* on *P. strobus*.

MATERIALS AND METHODS

Field observations and isolations. Christmas tree plantations with dead or dying trees were identified and located, symptoms noted, and tree samples removed to the laboratory. Isolations were made from the edge of resin-soaked or stained tissues and cultured on potato-dextrose agar and *V. procera* selective medium (6).

Pathogenicity tests. The *V. procera* isolate used for inoculations was isolated from a *P. strobus* root system on *V. procera* selective medium. The fungus was cultured in 500-ml bottles containing 80 ml of oatmeal agar (3) and incubated at 20 C for 14 days. Spore suspensions were prepared by pouring sterile distilled water into the bottles and gently scraping the agar surface with a glass rod. This suspension was diluted with sterile distilled water to yield 6×10^6 spores per milliliter. Spore viability in the suspension was tested by spreading 1 ml of suspension onto each of 15 petri plates of malt extract-agar medium.

Root systems of 32 *P. strobus* seedlings were rinsed free of soil with tap water. Roots of 16 seedlings were further rinsed for 2 min with 1% sodium hypochlorite, and roots of the other 16 were further rinsed for 2 min with sterile distilled water. Roots of 10 seedlings from each pretreatment were soaked in spore suspensions for 15 min. The other six seedlings from each pretreatment were soaked for 15 min in sterile distilled water as a control. Immediately after treatment, seedlings were planted individually in 11-cm pots containing a pasteurized potting

Table 1. Losses due to *Verticicladiella procera* in Virginia Christmas tree plantations in 1980

County	Tree species infected	Number of trees killed
Albemarle	*Pinus sylvestris*	60
Fairfax	*P. sylvestris*, *P. strobus*	70
Goochland	*P. nigra*, *P. sylvestris*	75
Madison	*P. strobus*	40
Montgomery	*P. strobus*	200
Nelson	*P. strobus*	2
Wythe	*P. strobus*	150
Warren	*P. sylvestris*	100

Table 2. Mortality of *Pinus strobus* seedlings 10 wk after dipping roots in a spore suspension of *Verticicladiella procera*

Treatment	Seedlings (no.)		Seedlings dead (%)		Reisolation (%)[b]
	NaClO[a] rinse	H_2O rinse	NaClO rinse	H_2O rinse	
Inoculated	10	10	50	50	100
Control	6	6	0	0	0

[a] Sodium hypochlorite.
[b] From dead seedlings only.

Table 3. Mortality after 12 wk of *Pinus strobus* seedlings inoculated with wood blocks colonized by *Verticicladiella procera* and inserted into a slit wound in the taproot

Treatment	Seedlings (no.)	Seedlings dead		Reisolation (%)[a]
		No.	%	
Inoculated	8	2	25	100
Control	6	0	0	0

[a] From dead seedlings only.

mixture (2 parts weblite, 2 parts vermiculite, 1 part peat) and placed in a greenhouse.

Eight *P. strobus* seedlings were also inoculated by inserting wooden blocks colonized with *V. procera* into a 2-cm-long incision made in the taproot just below the soil line. Inoculum blocks were 0.1-cm^3 pieces of healthy white pine stem wood. Blocks were autoclaved for 30 min and incubated in a culture of the fungus for 4 wk. One block was inserted into a slit wound in the taproot of each seedling. Sterile blocks were inserted into slit wounds in six seedlings as controls. Seedlings were further treated as previously described.

As seedlings died, isolations were made from symptomatic root and stem tissue and placed on *V. procera* selective medium and malt extract-agar medium. After 10 wk, roots and stems of all seedlings were examined for symptoms of disease development, and isolations were made from each. *V. procera* was identified by its conidial stage on agar plates.

RESULTS AND DISCUSSION

Field observations and isolations. Eight affected plantations were identified and their losses estimated for 1980 (Table 1). Affected species in these plantations were eastern white pine, Scotch pine (*P. sylvestris* L.), and Austrian pine (*P. nigra* Arnold). Losses for 1980 totaled about 700 trees, with losses over the past 3 yr estimated to be more than 2,000 trees killed. These losses are important because the affected trees were 6–10 yr old and 1–2 m in height and, therefore, ready to be marketed at prices from $5 to $15 each.

A significant number of trees observed in the field began to exhibit needle discoloration and wilting in late February and early March, and they were dead by mid-April. Other trees exhibited no budbreak in mid-April and then developed similar symptoms and were dead by middle to late May. Infected trees that did break bud in the spring died at various times throughout the summer and into the early fall. Few trees died during the winter.

Resin-soaking and black staining were observed primarily at the base of the stem and extending upward to 45 cm. In most of the trees, infection apparently was initiated at the base of the stem and root collar zone and developed both up the stem and into the roots. Wilting was an obvious symptom in eastern white pine but not in Austrian and Scotch pines, because of their short and stiff needles. Needle coloration went from light green to reddish brown over a period of 4–8 wk. Bark beetles were observed in symptomatic as well as asymptomatic trees. Two species of weevils, *Hylobius pales* Herbest. and *Pissodes approximatus* Hopk., were observed only in the stem base of dead trees.

The fungus most frequently isolated from affected trees was *V. procera*. The fungus was recovered from 60% of the symptomatic trees. *V. procera* was isolated from eastern white pine and from two previously unreported hosts, *P. nigra* and *P. sylvestris*.

Pathogenicity tests. Inoculated seedlings began dying in 2 wk and continued to die throughout the 10-wk period. Typical symptoms of *V. procera* infection developed only on dying seedlings. Needles began to droop, turned rusty brown, but remained attached. Root systems became reduced and had black discoloration that extended into the stem.

Fifty percent of the seedlings inoculated by root-dipping died in both pretreatments (Table 2). In the germination tests, 75% of the spores germinated; therefore, the viable spore concentration in the suspensions was 4.5×10^6 spores per milliliter. *V. procera* was isolated from root and stem tissue of all dead seedlings, but not from any live inoculated or control seedlings.

In the block inoculation treatment, 25% of the *P. strobus* died (Table 3). In these seedlings, black streaking extended both above and below the inoculation points. *V. procera* was isolated from root tissue up to 10 cm below and 23 cm above the root collar. Root vascular systems were entirely black, but discoloration did not extend more than 3 cm up the stem from the inoculation point. The fungus grew several centimeters in advance of staining in both the stem and root, but advanced more rapidly up the stem than down into the roots. As observed with some *Verticicladiella* spp. (8,10) but not with all (7), the fungus appeared confined to the xylem.

White pine root decline has suddenly become an important factor in the culture of Christmas trees in Virginia. The disease has reached epidemic levels in some plantations with significant losses. Although the common name of the disease implies a root decline, in Virginia the disease is expressed most often as a sudden wilt. This would suggest that a more proper name for the disease would be white pine wilt. Completion of Koch's postulates confirmed that this isolate of *V. procera* was pathogenic on *P. strobus* seedlings and provided evidence that *V. procera* incites white pine wilt in Virginia, as inoculated seedlings that died exhibited symptoms identical to those observed on diseased trees in the field.

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