Distribution and Efficacy of Propagules of *Verticicladiella procera* in Soil

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ABSTRACT


The importance of soilborne propagules for spread of *Verticicladiella procera* root system in sandy and red soils was studied by examining propagule distribution in soil and their capability to cause disease. The distribution of soilborne propagules was closely associated with colonized roots of *Pinus strobus* and *P. sylvestris* Christmas trees. Propagule numbers were greatest in soil at the root collar (8-12 x 10^6 propagules per gram of soil) and decreased logarithmically toward the root tip (fewer than 40 propagules per gram at 30 cm). This distribution reflected the pattern of colonization in the root. No propagules were found near the roots of asymptomatic trees. Twelve seedlings were planted at 10, 25, and 40 cm from the root collar of each of four diseased trees. Colonization of seven seedings was related to proximity to the root collar rather than to presence of detectable levels of *V. procera* in soil. Colonized roots appeared to be the source of propagules in soil, and these propagules determined to be relatively unimportant for pathogen spread. The presence of conidiofores of *V. procera* in weevil galleries in roots of diseased trees, observation of weevil feeding signs on seedling stems, and the colonization of a potted seedling in the vicinity of diseased trees suggest that insects (Coleoptera) are potentially important vectors of *V. procera*.

*Verticicladiella procera* Kendrick is associated with Procera root disease (= white pine root decline) in the eastern United States (1,2,6,13). The fungus has been isolated most frequently from Christmas trees (3,13), but has also been isolated from pine plantations (2), natural stands (14), and seed orchards (23).

Symptoms of Procera root disease are a uniform chlorosis, wilting of the foliage, excessive resin exudation at the base of the stem accompanied by resin-soaked sapwood, and, at the base, black-staining beneath the bark at the base of infected trees (3). Significant economic losses have been observed in Christmas tree farms. Anderson and Alexander (3) observed annual mortality rates of 1-3% in Christmas tree plantations with a total stand mortality of 20%. Lackner and Alexander (13) isolated *V. procera* from trees in eight Virginia Christmas tree plantations and estimated a total loss of 750 marketable trees. Subsequently, disease incidence was monitored in two of these plantations and was found to increase over the 2 yr study period (15). The means of spread of *V. procera* between and within plantations was unknown.

Study of the fungus and the disease has provided knowledge of host range (1,4,10,25) and geographical distribution (1,9,10,12, 19,26). Disease occurrence has been associated with excessive soil moisture (18,19,22) and with insects (Scolytidae and Curculionidae) infestations (13,15,24,25). There is a lack of knowledge about the epidemiology of this disease.

Several lines of evidence indicate that infection of trees may occur through the roots by soilborne propagules. *V. procera* is most frequently recovered from the roots and root collar area (6,1,19), and the presence of *V. procera* in soil around these trees has been documented. Swai and Hindal (21) successfully isolated the fungus from 72 and 4% of soil samples of symptomatic and symptomless eastern white pine (*P. strobus* L.) Christmas trees. Lackner and Alexander (15) isolated *V. procera* from soil at the site of excavated, diseased Christmas trees but not from the base of asymptomatic trees. Of 25 seedlings planted at the site of excavated, diseased trees, 87% developed symptoms and *V. procera* was recovered from the root systems of asymptomatic trees. None of the seedlings planted in uninoculated soil became colonized with *V. procera*. The mechanism(s) of entry into the root system and the importance of soilborne inoculum in disease development are unknown. The role of soilborne inoculum in terms of location of the fungus in soil relative to diseased and healthy trees, quantity of propagules available for infection, number of propagules required for infection, and number of infections required for sufficient colonization to result in symptom development needs investigation. The association of root, stem, and soil-inhabiting insects with diseased trees has been documented (13,24,25).

The role of soilborne propagules of *V. procera* in disease development was studied by examining the density and distribution of propagules around symptomatic and asymptomatic trees and the ability of soilborne propagules to infect and colonize seedlings.

MATERIALS AND METHODS

Density and distribution of soilborne propagules in a large plot. Plots were established in an eastern white pine Christmas tree plantation in Montgomery County, VA, where trees on one hillside of the plantation were affected by the disease. The four 8 x 6-m plots were situated at the top, midcline (two plots), and bottom of the hill. Symptomatic trees surrounded by asymptomatic trees served as plot centers. Ten soil sampling locations per plot were located on a 2 x 2-m grid. These locations were at least 50 cm from the base of the center symptomatic tree. A soil auger (1-L capacity) was used to remove a sample from each location each month (starting May 1984) for 3 mo. A subsample (approximately 60 g) was removed after thoroughly mixing the soil in a plastic bag. To verify the presence of *V. procera*, the symptomatic tree in each plot...
was sampled by removing wood chips from the root collar and plating them on cycloheximide-amended, 1.5% malt extract agar (AMA) (17).

Propagule density and distribution around individual trees. Ten symptomatic trees were sampled from each of two plantations on sites typical for Christmas tree plantations in Virginia. Plantation A was planted with Scots pine (*Pinus sylvestris* L.) (Warren County, VA) and Plantation B with eastern white pine (Montgomery County, VA). Samples were collected over a 8-month period excluding the winter months. This was done because of a report that the fungus population declines during the winter months (15). Two roots (I and II) approximately 180° apart were selected and carefully excavated by brushing soil away from the top of the root to minimize soil disturbance. Small soil samples (approximately 30 g) were removed aseptically from precise locations along the two roots (Fig. 1). The samples were placed in plastic bags labeled by tree, root, and sample location. Samples a, b, c, and f were taken at the root at 0, 10, 20, and 30 cm, respectively, from the root collar. Sample pairs d, g, and e, h were taken at 5 and 10 cm, respectively, perpendicular to the root surface adjacent to positions c and f. All samples were from the same depth as the root. After dilution plating of the samples, the mean number of germinating propagules at each position was determined for each root and averaged for the two tree species separately.

Dilution plating of soil samples followed the method of Clark (5). Diluted suspensions were plated on a medium selected for *V. procer*a (VPIM) (21) and incubated at room temperature (18–22°C) for 14 days. Colonies were counted and numbers of propagules per gram of soil were corrected for oven-dry (105°C for 24 hr) soil weight.

Association of soilborne propagules with colonization patterns in roots. The lower bole and remaining root system were excavated, carefully examined for signs of insect activity, and removed to the laboratory. A drawing was made of the stump and the major roots were labelled with particular attention paid to roots I and II (Fig. 1). Tissue samples were taken along each major root at 0 (point of attachment to the root collar), 10, 20, and 30 cm from the root collar. The samples taken from roots I and II corresponded with the soil samples from locations a, b, c, and f (Fig. 1). The tissue samples were removed aseptically with a cork borer (0.8 cm diameter) and cut in half. The bark and wood were separated and plated individually. One half was plated onto 2% malt extract agar (MEA) and the other half onto AMA. After incubation at 20°C for 14 days, tissue scores were recorded for the presence or absence of *V. procer*a. The positions from which *V. procer*a was recovered from tissue and/or soil isolations were marked on the drawing. Colonization patterns of *V. procer*a in wood and the corresponding distribution of propagules in the soil adjacent to the wood tissue were noted from the drawing. Each root tissue and soil isolation pair was placed in a category according to the following criteria: category 0 = no recovery from tissue and/or soil tissue; category 1 = recovery only from soil sample; category 2 = recovery only from root tissue; category 3 = recovery from both soil and root tissue. The frequencies of occurrence for each category for the four positions along the roots were determined and plotted over sampling position for the two tree species separately.

Infection and colonization of seedlings by soilborne propagules. An 8 × 10-m plot was established in Plantation B in an area encompassing several symptomatic trees and the sites of excavated trees from which *V. procer*a had been isolated. Twenty 2-year-old eastern white pine seedlings were planted at 2 × 2-m intervals. A control of one seedling in a pot with a soil mix (2:1:1; vermiculite : peat mix : perlite) was placed next to each of the planted seedlings. In addition, before selecting seedlings for outplanting, five seedlings from the same source were randomly selected for root isolations. A soil sample (approximately 60 g) was removed from the location of each planted seedling and dilution-plated onto VPIM. The plot was established in August 1984 and maintained for 10 mo. When a planted or potted seedling died, it was replaced with a healthy seedling. Dead seedlings were labelled by position in the grid and taken back to the laboratory. Isolations were made from the taproot and one lateral root of each seedling as described above. Three segments were removed from each of two lateral roots and the taproot. The three segments were from positions distal, middle, and proximal relative to the root collar. Each segment was cut in half, one half plated on MEA and the other on AMA. The agar plates were marked on the bottom such that the three segments from one root could be placed at predetermined positions on a single plate. Colony formation at particular segment indicated the location of the fungus in the sampled roots. After incubation at 20°C for 14 days, the plates were examined for colonies of *V. procer*a. This procedure was to ensure no pretreatment colonization of the outplanted seedlings by *V. procer*a. At the termination of the experiment (June 1985), all of the seedlings were lifted and labelled according to their position in the grid. Inspection of potted seedlings verified that no roots were growing into the soil through holes in the bottom of the pot. Crowns and stems were examined for discoloration, length of new growth, presence of resin-soaked areas, or lesions. Roots were examined for presence of new roots and health of cortex. Seedlings were checked for evidence of insect activity (puncture holes or feeding marks). Isolations were then made from the roots and root collar as previously described. The number of seedlings showing symptoms of *Procer*a root disease and/or in which *V. procer*a was recovered was recorded.

Seedlings also were planted directly adjacent to individual symptomatic trees to study the effects of soilborne propagule density and spatial distribution on infection and colonization of seedlings. Three seedlings in each of four perpendicular directions were planted at 10, 25, and 40 cm from the root collar of five symptomatic trees ( termed “center trees”) and one asymptomatic tree.

Fig. 1. Soil sampling scheme for eight positions at each of two roots (I, II) of 10 symptomatic eastern white pine and 10 symptomatic Scots pine Christmas trees.
RESULTS

Density and distribution of soilborne propagules in a large plot. *V. prosera* was not recovered from any of the soil samples from the four plots. However, *V. prosera* was recovered from the four symptomatic plot center trees.

**Propagule density and distribution around individual trees.** Mean propagule densities at positions a, b, c, and f (those immediately adjacent to the root surface) for both species are plotted in Figure 2. The data were fit to a negative exponential curve, which describes a very high number of propagules at position a, decreasing logarithmically towards the root tip. With both species, the slope of the regression line of the transformed data was significantly different from zero (*P* = 0.05), indicating a significant difference in propagule density between sample locations. The equations were for Scots pine, \( Y = 51.021 e^{-0.77x} \), and for eastern white pine, \( Y = 158.419 e^{-0.205x} \), where \( Y \) = number of propagules per gram of soil at \( x \) cm from the root collar.

Analysis of variance on the four sets of lateral samples (positions c, d, e and f, g, h) showed that the number of propagules recovered from the root surface and at 5 and 10 cm from the root surface were not significantly different for either species.

Association of soilborne propagules with colonization patterns in roots. The spatial pattern of propagules in the soil was similar for both Scots and eastern white pine. However, the numbers of propagules recovered were different. Recovery of *V. prosera* from only one component of the tissue-soil sample pair was consistent from the root collar toward the root tip (Fig. 3). Category 0 occurred more frequently with the eastern white pine samples than with the Scots pine samples. Likewise, category 3 occurred much more frequently with Scots pine than with eastern white pine. The incidence of recovery of *V. prosera* from soil was greatest with the Scots pine. However, when propagules were recovered from soil samples from eastern white pines, the numbers were generally higher than those from Scots pines.

**Infection of seedlings by soilborne propagules.** The field study involving planted and potted seedlings was established to compare incidence of infection with recovery of *V. prosera* from soil and to use eastern white pine seedlings as bait for propagules not detected in soil isolations. None of the 20 soil samples from the sites of planted seedlings in the 8-x-10-m plot yielded *V. prosera*. All of the
planted seedlings removed at experiment termination had healthy, green tops and new, white root tips. During the course of the experiment a few seedlings died and were replaced by healthy seedlings, but *V. procera* was not recovered from the dead seedlings. None of the isolations from the 20 planted seedlings yielded colonies of *V. procera*. Soilborne propagules were not detected by either the selective medium or the seedling bait.

A third purpose of the field study was to subject potted seedlings to infection by means other than soilborne propagules. One of the 20 potted seedlings was found to be colonized by *V. procera*. The fungus was recovered from the proximal portion of a lateral root.

However, no symptoms were observed in this seedling or any of the other potted seedlings.

Of the five symptomatic center trees around which seedlings were planted, four were colonized by *V. procera* (Table 1). The fungus was recovered from root collar samples and samples 15 cm from the root collar. *V. procera* was not recovered from cork borer plugs of bark and wood tissue removed from the root collar of tree G, the symptomless tree. Conidiophores and spore masses were observed in insect galleries in roots of two center trees (Fig. 4). *V. procera* was recovered from at least one of the 12 seedlings planted around each of the four colonized center trees (Table 1). A total of seven seedlings was infected. Three had been planted at 10 cm from the root collar and four had been planted at 25 cm from the root collar. Recoveries from the tap and lateral roots of these seedlings were equal. The fungus was not detected in any of the seedlings planted around tree E (no *V. procera* recovered) and the symptomless tree G.

*V. procera* was infrequently recovered from the soil samples taken at each of the 12 planting sites (Table 1). Recovery of *V. procera* from seedlings did not correspond to the locations from which *V. procera* was recovered from the soil. Colonization of seedlings was associated with proximity to the colonized root collar of the center tree and with the presence of *V. procera* in the soil. There was no difference in root condition between colonized seedlings and seedlings from which *V. procera* was not recovered. *V. procera* was recovered from one seedling with obvious signs of weevil feeding on the lower stem.

**DISCUSSION**

*V. procera* was found in the soil in association with diseased trees. Soil samples taken in the general area of, but not directly adjacent to, diseased trees did not yield the fungus. *V. procera* was not recovered from soil sampled around asymptomatic trees. These results concurred with those of Lackner and Alexander (15) who recovered *V. procera* from soil at symptomatic trees only. However, Swai and Hindal (21) recovered *V. procera* from soil at 4% of the asymptomatic trees sampled. There was no mention of tissue sampling from the symptomatic and asymptomatic trees. Therefore, some of the asymptomatic trees may have been colonized by *V. procera* but not showing symptoms, or propagules may have been brought to the site by other means such as insects vectors. *V. procera* was recovered from a much higher percentage of soil samples from symptomatic trees by Swai and Hindal (21). Colonized roots and root collar of diseased trees may be a source of propagules in the soil.

Sampling of soil immediately surrounding diseased trees revealed a definite pattern of propagule distribution that reflected the presence of *V. procera* in the adjacent root and root collar. Colonization was greatest at the root collar and decreased up the stem and down toward the root tips indicating that colonization originated at the root collar (11). Large numbers of propagules in

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**TABLE 1. Recovery of *Verticillium lobata* from six center trees and 12 seedlings planted around each center tree**

<table>
<thead>
<tr>
<th>Tree</th>
<th>Roots colonized (no.)</th>
<th>Ratio root collar isolations</th>
<th>Root condition</th>
<th>Position of seedlings with <em>V. lobata</em></th>
<th>Position of soil samples with <em>V. lobata</em></th>
<th>Propagules per gram of soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>2/3</td>
<td>RS, IBS</td>
<td>N-25</td>
<td>E-10</td>
<td>24.5</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>0/3</td>
<td>RS, IBS, <em>V. lobata</em> in insect gallery of one root</td>
<td>E-25</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>1/3</td>
<td>RS, IBS, some RS and BS</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>3/3</td>
<td>roots not excavated</td>
<td>N-10</td>
<td>W-10</td>
<td>88.8</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
<td>0/3</td>
<td>healthy RS, insect feeding sites</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
<td>0/3</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*Root collar isolations: successful recoveries/ attempts.
*RS = resin-soaked tissue, BS = black-stained tissue.
*Letters refer to compass direction from root collar of the center tree. Values represent distance (cm) along the compass direction.
soil corresponded with frequent recovery of *V. procera* from the adjacent tissue sample, another line of evidence suggesting that soilborne propagules originate from colonized root systems.

The patterns of propagule distribution were similar in soil around Scots and eastern white pine, but there were differences in the density of propagules. How much of this difference may be attributed to host species is unknown. In the two plantations studied, the incidence of *V. procera* in soil at the root collar and along the roots was greatest for the Scots pine plantation, but the total number of propagules recovered was greatest in the eastern white pine plantation. In a few cities that play a role in propagation distribution, the difference in habitats of insects associated with the two tree species may account for the difference in propagule numbers. For example, two weevils associated with white and Scots pine are *Hyllobius pales* (Hbst.) and *H. radicus* (Buch.), respectively. Both weevils oviposit in the inner bark of the root collar area of stumps and trees which is also the area most frequently colonized by *V. procera* (11). Larvae of *H. radicus* may move from the inner bark several centimeters into the surrounding soil, whereas larvae of *H. pales* remain in the inner bark (7,8,20). This movement into soil by contaminated larvae could account for more frequent isolation of *V. procera* from soil around diseased Scots pine trees compared with eastern white pine.

Both planted and potted seedlings in the 8 x 10-m field plot should be susceptible to incoculation by a fungus-bearing insect. Only planted seedlings are susceptible to infection from propagules in soil, because none of the potted seedling roots were in contact with soil propagules in soil where weevils were not detected on the selective medium or with the planted seedlings. This, together with the lack of recovery of *V. procera* from soil sampled systematically in plots centered on diseased trees, supports the conclusion that *V. procera* is not generally distributed in soil, even in the vicinity of diseased trees. If undetected soilborne propagules were present, they did not cause sufficient infection or colonization to result in symptom expression and colonization was not extensive enough to be detected by root isolation. The infection of a potted seedling but not of planted seedlings indicates that infection can occur by means other than propagules in soil. The most likely alternative is an insect vector(s). Two explanations are proposed for the colonization of 14.5% of these seedlings and of those planted at the site of an excavated, diseased tree in the 1984 study by Lackner and Alexander. One is that infection occurred through small wounds in the roots with propagules in soil as the source of inoculum. The second is that insects carrying propagules of *V. procera* that enter the fungus to the seedling while feeding on the roots or root collar. The low incidence of recovery of *V. procera* from soil sampled at the sites where planted seedlings became colonized, and the lack of infection and colonization of seedlings planted in infested soil supports the latter possibility. Studies with artificially infested soil (16) suggest that infection and colonization requires very high numbers of propagules, which are not found in nature except for soil closely associated with colonized roots. In the study by Lackner and Alexander (15), 46% of the seedlings planted at the sites of excavated diseased trees became colonized by *V. procera*, a higher rate of colonization than the 14.5% observed in the present study. Lackner and Alexander (15), using the same dilution plating technique, determined the density of propagules in soil where the seedlings were planted to be between 3 and 300 x 10^3 propagules per gram of soil. This number is much greater than the number of propagules recovered from the sites of planted seedlings in the present study. Small pieces of colonized wood tissue left from the tree excavations may account for high soilborne propagule numbers observed by Lackner and Alexander (15) and also may have provided inoculum for infection. It appears doubtful that infection of the seedlings in the study by Lackner and Alexander and in this study in particular, are due solely to propagules in the soil.

This study indicates that the role of soilborne propagules in disease spread is unimportant except where new hosts are directly adjacent to a concentrated source of inoculum. Furthermore, an alternative method of infection, most likely insect transmission, was suggested based on the observed insect activity. This would be of major importance when developing control measures.

**LITERATURE CITED**