Colonization Patterns of *Verticicladiella procera* in Scots and Eastern White Pine and Associated Resin-Soaking, Reduced Sapwood Moisture Content, and Reduced Needle Water Potential

W. Elliott Horner, S. A. Alexander, and Kathy J. Lewis

Graduate research assistant, associate professor, and graduate research assistant, respectively, Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute & State University, Blacksburg 24061. Present address of first author: Tulane Medical Center, 1500 Perdido St., New Orleans, LA 70112. Present address of third author: Ministry of Forests, Pest Management Division, Prince George, BC, Canada.

In partial fulfillment for the Ph.D. degree in plant pathology for the first author.
Research supported in part by McIntire-Stennis funds.
The authors wish to thank Jim Frith of Bentonville, VA, and Bill Larsen of Riner, VA, for their cooperation and for use of their plantations for this study. The statistical advice of R. L. Olson is gratefully acknowledged.
Contribution No. 556, Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University.
Accepted for publication 4 September 1986 (submitted for electronic processing).

ABSTRACT

Horner, W. E., Alexander, S. A., and Lewis, K. J. 1987. Colonization patterns of *Verticicladiella procera* in Scots and eastern white pine and associated resin-soaking, reduced sapwood moisture content, and reduced needle water potential. Phytopathology 77:557-560.

The colonization pattern of *Verticicladiella procera* in diseased pines was studied by plating tissue onto both general and selective media. A systematic sampling procedure was repeated on 10 *Pinus sylvestris* and 10 *P. strobus* Christmas trees (5–9 yr old) with symptoms of Procera root disease. Sampling points were at predetermined positions up the stem (four points on each of two or four sides), around the root collar (eight points), and along each root. Relative recovery frequencies of the fungus were, in decreasing order, root collars, roots, and stems. Root collar recoveries were distributed uniformly around the root collar. Recovery frequency along roots declined rapidly acropetally and fit a negative exponential distribution. Recovery of *V. procera* from bark was significantly more frequent than from wood. This recovery pattern suggests that colonization by *V. procera* involves initial establishment in bark at the root collar with subsequent acropetal development. The status of symptomatic trees was further characterized and shown to include needle water potentials lower than controls, low sapwood moisture content, and appreciable amounts of resin-soaked tissue in the stem cross-section.

Significant mortality of Christmas trees in Virginia has been associated in recent years with stem and root colonization by *Verticicladiella procera* Kendrick (12), referred to as Procera root disease. Lackner and Alexander (12,13) fulfilled Koch's postulates for *V. procera* on loblolly (*Pinus taeda* L.) and eastern white (*P. strobus* L.) pines, but the method of spread of *V. procera* among trees remains unclear. In early reports greater incidences were noted in low and wet sites (1,2,5,23). Lackner and Alexander (14), however, reported a scattered incidence of the disease within plantations and a rapid decline of propagule populations in the soil after removal of dead host tissue. Even so, seedlings planted in naturally infested soil became diseased, died, and yielded *V. procera* upon isolation (14). The apparent absence of insect activity on these seedlings led Lackner and Alexander (14) to conclude that infection probably occurred from soilborne inoculum. However, Lewis and Alexander (15) and Lewis et al (17) subsequently concluded that soilborne inoculum was probably not the major means by which this fungus spreads among trees. Germinability of propagules in artificially infested soil was found to decline rapidly both under controlled conditions and in the field. Propagule germinability decreased most sharply when soil dried beyond −1.5 mPk at moderate to high temperatures (20 C or above) under controlled conditions (15). Propagules in the soil were found only in the vicinity of colonized host tissue, with populations decreasing rapidly with distance from the host stem (17). In these studies, seedlings planted in naturally infested soil (24–170 colony-forming units per gram) did not become infected.

The present study was undertaken to describe the pattern of colonization of *V. procera* within naturally infected Christmas trees to determine the probable point of initial colonization. The sapwood moisture content, needle water potential, and extent of sapwood resin soaking were also measured to characterize these factors in colonized trees.

A preliminary report of portions of this work has been presented (8).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Ten trees each of eastern white and Scots (*P. sylvestris* L.) pine showing symptoms of Procera root disease were selected from commercial Christmas tree plantings where Procera root disease had been confirmed previously. Both plantings were established on former pastureland and managed for retail sales on a 'choose and cut' basis. Tree harvesting had occurred for at least the preceding 5 yr in both plantings. Scots pines were collected from Warren County and eastern white pines from Montgomery County, VA. After selection, trees were cut at about 0.5 m above the ground and the root system carefully excavated by hand with a mattock. Care was taken to keep root systems as intact as possible and to recover all woody roots, i.e., primary and low-order lateral roots (22), from within 0.5 m of the stem base and more distal portions if possible. Excavated root systems were returned to the laboratory for dissection and isolation. The 20 trees were collected between October 1984 and July 1985. The present study was designed to include trees at various stages of colonization, hence no attempt was made to examine effects of season on colonization.

Individual roots were cut from the root collar with pruning shears. Tissue platings were then made from tissue immediately adjacent to the root collar, 5 and 10 cm from the root collar, and every 10 cm thereafter to the root end. Isolations were generally made from eight equidistant positions around the root collar. One eastern white pine was sampled at four and one at six equidistant points around the root collar, because of small stem diameter. Two Scots pine were sampled at 12, one at 10, and one at seven points around the root collar. Two sides of the stem 180° apart were sampled at 5, 10, 20, and 30 cm above the root collar in the Scots pines. The last six eastern white pines collected were sampled along four sides of the stem, 90° apart at the same distances above the root collar. In total, 1,522 points were sampled from the 20 trees.

Tissue samples for isolation from predetermined positions on the collar, stem, or root were separated into a bark and a sapwood fraction. Each fraction type (bark or sapwood) was plated separately onto both acidified 2% malt extract agar and a medium selective for *V. procera* (18). The term bark, as used herein, includes both phloem and periderm, as defined by Sutton and Tinus (22). Surfaces were disinfested by swabbing lightly with 70% ethanol and flaming briefly. Tissue samples were then aseptically excised and transferred to agar. Plates were incubated at 20 C for at least 14 days. The presence of resin-soaked and/or black-stained tissue was noted at each sampling point, as well as any indications of insect activity.

Observations from each set of 10 trees were pooled within species. Results from stem, root collar, and roots were analyzed separately. The observed recovery pattern of each of the six categories (collar, stem, and roots of two species) was compared to a hypothetical uniform distribution by using a Kolgomorov-Smirnov goodness of fit test (19). The number of roots available for sampling varied with distance from the root collar. With increasing distance from the root collar there was an initial increase in the number of roots due to branching. Because the total length of some roots was in the range of 10–20 cm, sampling beyond 20 cm was from a decreasing number of roots. This caused a hypothetical uniform, or even, distribution to predict reduced frequencies at those distances, as seen in Figure 1.

Further analysis of the recovery patterns from stems consisted of regressing recoveries on position, then testing with Student's t test the hypothesis that the regression line slope was different from zero. Further analysis of the root recovery patterns of both species consisted of deriving negative exponential equations that fit the observed frequencies and comparing values predicted from this equation to the observed values, again with the Kolgomorov-Smirnov test. The number of isolation points that yielded the fungus from the bark only was compared with the number yielding the fungus from the wood only by a sign test (19).

The sapwood moisture content based on an oven-dry (105 C) weight basis (20) was determined for the basal stem section of 10 Scots and eight of the eastern white pine trees sampled. A visual estimate was made of the proportion of the stem circumference at groundline with resin-soaked sapwood for these same trees. Needle samples for water potential determinations were collected from 15 pairs of trees (eight eastern white and seven Scots). Each pair consisted of a sample tree (to be excavated as described above) and an asymptomatic neighbor tree of a similar age class. Branches with needles were collected with pruning shears and bagged separately in clean plastic bags with a moist paper towel. Bags were returned to the laboratory stored in ice chests. Kaufmann and Thor (10) demonstrated that minimal water potential changes occur in conifer tissue handled in this way. Needle water potentials were taken as replicate balancing pressure determinations from needle fascicles in a Scholander pressure bomb. Comparisons between mean water potentials for sample and neighbor trees were made with a paired Student's t test.

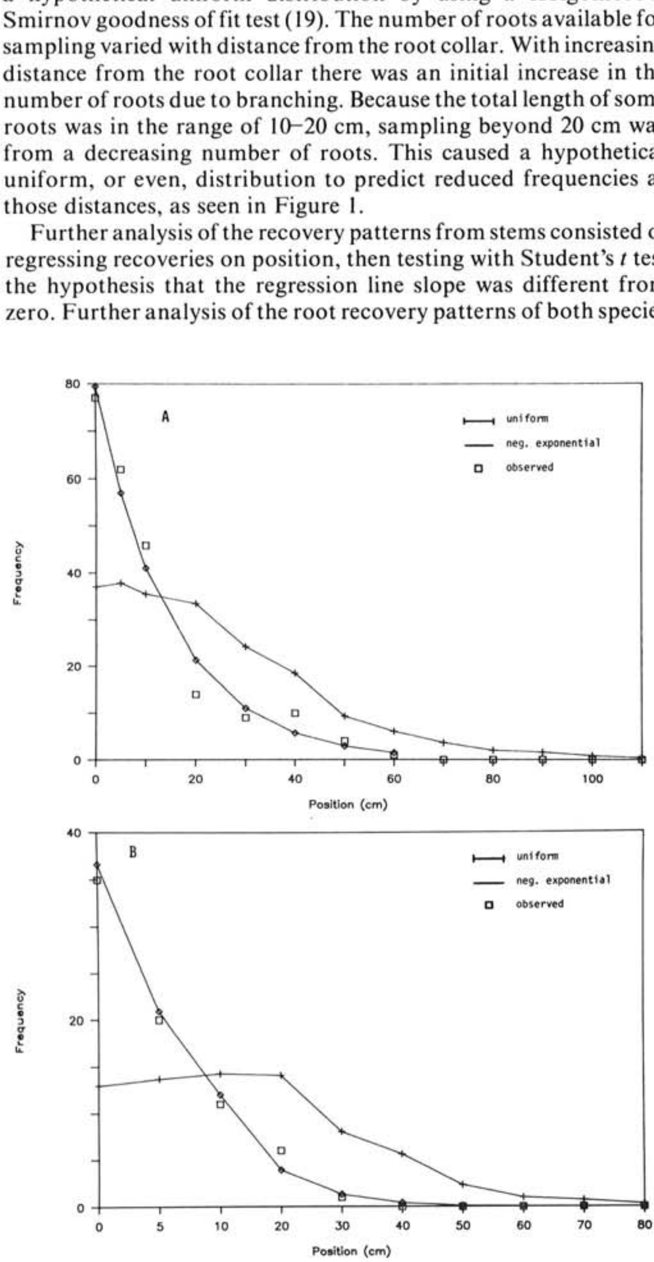

Fig. 1. Observed recovery patterns of *Verticicladiella procera* along roots of **A,** Scots and **B,** eastern white pines. Included in each graph are the hypothetical uniform distribution and negative exponential distribution to which the observed distribution was compared. Note difference in frequency scale. Position refers to centimeter from root collar.

RESULTS

The recovery pattern of *V. procera* was similar on Scots and eastern white pine. In both cases, relative recovery frequencies of *V. procera* in decreasing order were collars, roots, and stems. Recovery frequencies were higher overall in Scots pine than in eastern white pine.

The fungus was recovered from 97% of the points sampled from Scots pine root collars. A range of 85–100% of the sample points from individual trees yielded *V. procera*. Comparatively, 42% of all points sampled from eastern white pine root collars yielded the fungus, with a range of 33–44% of the sample points from individual trees positive for *V. procera*. In both cases, the goodness of fit test did not reject ($P = 0.05$) an assumption of uniform distribution around the root collar ($D = 0.02$ for both species, where D is the Kolgomorov-Smirnov test statistic; $n = 31$, eastern white; $n = 86$, Scots).

The proximal parts of roots also yielded the fungus in high frequencies. A rapid decline in recovery frequency occurred acropetally along roots in both pine species (Fig. 1). In eastern white pine, 35% of the roots yielded the fungus from the sample point adjacent to the root collar. Recovery declined to 0% of points sampled at or beyond 40 cm from the root collar. Scots pine roots showed a similar pattern, declining from 84% of sample points adjacent to the root collar to 0% at or beyond 70 cm from the root collar. Goodness of fit tests rejected ($P = 0.01$) values expected from a uniform distribution for Scots ($D = 0.3$, $n = 223$) and eastern white pine ($D = 0.38$, $n = 73$). When expected values derived from negative exponential distributions were compared with the observed freqencies from roots of both species, goodness of fit tests did not reject ($P = 0.05$) the fit ($D = 0.02$).

Recoveries along stems declined acropetally, although not as rapidly as in roots. *V. procera* was recovered from 45, 35, 35, and 25% of stem points sampled in Scots pine at positions 5, 10, 20, and 30 cm respectively, above the root collar. White pine stems yielded the fungus from 9, 6, 3, and 0% of the points sampled from these positions. This acropetal decline of recovery frequency was not significantly different ($P = 0.05$) from a uniform distribution by goodness of fit testing for Scots ($D = 0.07$, $n = 28$) or eastern white pine ($D = 0.32$, $n = 6$). Subsequent regression of frequency on stem position did, however, yield slopes significantly different from zero ($P = 0.05$) in both species.

The frequency of 'bark only' recoveries was greater than 'sapwood only' recoveries in both species for each category sampled, except for the eastern white pine stems (Table 1). These differences were significant in Scots pine roots and collar ($P = 0.01$) and in eastern white pine roots ($P = 0.05$). These trends were accentuated when categories were pooled.

Means and standard deviation (and ranges) of percent sapwood moisture content for 10 Scots and eight eastern white pine in this study were 86 ± 6% (34–123%) and 50 ± 5% (32–93%), respectively. The needle water potentials of Scots pine symptomatic sample and asymptomatic neighbor trees averaged −2.7 and −1.5 mPk, respectively. Sample and neighbor eastern white pine needle water potentials averaged −3.2 and −1.3 mPk, respectively. A paired Student's *t* test showed the mean difference was significant for both Scots ($P = 0.05$) and eastern white pine ($P = 0.01$). The estimated amount of stem cross-sectional area at groundline that was resin-soaked and/or black-stained averaged 73 ± 5% (30–100%) and 53 ± 6% (5–95%) for eastern white and Scots pine, respectively.

DISCUSSION

In both Scots and eastern white pine the greatest recovery frequency of *V. procera* was from the root collars. Recovery frequency declined acropetally both up the stem and out the roots in both species. Regression analysis indicated an acropetal decline in frequency along the stem in both species, although this decline was not significantly different from a uniform distribution by goodness of fit testing. This lack of significance is probably related to low values overall for stem frequencies. The acropetal decline along roots of both species did not fit a uniform distribution but was described adequately by a negative exponential function. These data strongly suggest that the fungus occurs more frequently in proximal than in distal portions of roots. This agrees with data from stem and root collar isolations indicating that in both species, the root collar is the most frequently colonized part of the tree and that stem and root parts adjacent to the root collar are more frequently colonized than those away from the root collar. This pattern strongly suggests that initial colonization develops in the vicinity of the root collar and moves into root and stem tissues from the root collar.

Root tip infection, followed by basipetal root colonization, could also lead to root collar colonization. Subsequent acropetal colonization of other roots could then yield a pattern similar to that observed in this study. If this were true, the presence of some roots (at least one per tree) colonized at the distal end but not at the root collar would be expected. When colonization data were examined by individual root, however, only rare instances of this were seen. Three of 20 trees examined had a single root colonized at portions distal from but not proximal to the root collar. These three roots are less than 2% of the total 219 roots in the study. We feel that this is not sufficient to support root tip colonization preceding root collar colonization.

The results of the sign tests (Table 1) indicate that *V. procera* occurs more frequently in the bark than in the wood. We therefore infer that initial colonization is in the bark or at least adjacent to bark tissues.

Recent data implicate weevils (Coleoptera) as vectors of *V. procera* in eastern white pine Christmas tree plantings in Virginia (1n). Pertinent features of the ecology of these weevils (*Hylobius pales* (Hbst.) and *Pissodes* sp., probably *P. approximatus* (Buch.)) are that they typically infest the root collars and roots of trees and that they feed in the phloem and oviposit in the cambium (3). The colonization patterns seen in this study implicate an infection mechanism operating in the bark at the root collar. The suggestion that weevils are vectors of *V. procera* in eastern white pine (16) implies that initial colonization by *V. procera*, at least in that species, would be at the root collar, which agrees with the findings of this study.

Horner and Alexander (7) presented evidence demonstrating that resin-soaked or black-stained sapwood was much less permeable to water flow than was clear sapwood. All basal stem sections examined in the present study were resin-soaked. Although the amount varied widely, average values for the cross-sectional areas that were resin-soaked were 53 and 73% for Scots and eastern white pine, respectively. This suggests that symptomatic trees are functioning with less than half of their cross-sectional area at the stem base. This is probably a conservative estimate because some degree of resin-soaking likely occurs and probably decreases permeability at levels too low to detect visually.

TABLE 1. Ratios of isolations from bark only and wood only from individual sampling points by species and tree part

Tree part	E. White	Scots	Combined
Stem	2:3 (TF)[a,b]	8:3 (NS)	10:6 (NS)
Collar	5:3 (NS)	12:0 (**)	17:3 (**)
Roots	29:12 (*)	56:28 (**)	85:40 (**)
Total	36:18 (*)	76:31 (**)	112:49 (**)

[a] The first and second values of the ratio are, respectively, the number of sampling points that yielded *Verticicladiella procera* from the bark only and from the wood only.

[b] Indicators of significance levels are: TF, too few observations to test; NS, not significant; *, significantly different at $P = 0.05$; **, significantly different at $P = 0.01$.

Even though statistical testing is precluded by the absence of appropriate controls, a comparison of published values with sapwood moisture content values observed in the present study is informative. Mean sapwood percent moisture content of eastern white pine samples in this study was 50%. Reported values for the percent moisture content of eastern white pine sapwood are 120 (6) and 175% (4). The Scots pine (a diploxylon species) in this study had a mean basal sapwood moisture content of 86%. Koch (11) presents a range of values for diploxylon pine species (southern yellow pines) of 115–135% for trees less than 25 yr old in the southeastern United States. Scots pine in Siberia has sapwood moisture content values of 102–164% (9). Trees in the present study were less than 12 yr old and therefore contained mostly juvenile wood (21), which has a higher percent moisture content than mature wood (11, p. 269). Values expected from the present study would therefore be as high, or higher, than the cited values, which are for stems containing mature wood. Contrary to expectation, however, values from this study vary from the low end of to well below the cited range of sapwood moisture content.

Evidence gathered during the course of this study indicates that colonization by *V. procera* in pine Christmas trees in Virginia originates in the vicinity of the root collar, most likely in the bark. This conclusion is particularly interesting in regard to recent evidence that certain weevils common in Christmas tree plantings in Virginia may serve as vectors of *V. procera* (16).

Additional evidence gathered in this study suggests that Procera root disease is a xylem dysfunction leading to crown desiccation. The significantly reduced needle water potentials of symptomatic trees indicate the presence of abnormal plant water relations in trees with symptoms of Procera root disease. Lower than expected sapwood moisture contents support this contention. Resin-soaked sapwood, which is known to have a drastically reduced permeability to water (7) is prevalent at the root collar in trees with Procera root disease, which could explain the aforementioned effects on plant water relations.

LITERATURE CITED

1. Alexander, S. A. 1980. White Pine Root Decline. VPI & SU Pest Management Guide 503. 4 pp.
2. Anderson, R. L., and Alexander, S. A. 1979. How to identify and control eastern white pine root decline. U.S. For. Serv. Southeast. For. Bull. SA-FR/P6 4 pp.
3. Baker, W. L. 1972. Eastern Forest Insects. U.S. For. Ser. Misc. Publ. 1175. 560 pp.
4. Cech, M. Y., and Pfaff, F. 1977. Kiln Operator's Manual for Eastern Canada. Report OPX192E, Eastern Forest Products Laboratory, Ottawa. 189 pp.
5. Dochinger, L. S., 1967. Leptographium root decline of eastern white pine. (Abstr.) Phytopathology 57:809.
6. Harrar, E. S. 1957. Hough's Encyclopedia of American Woods. Vol. I. Robert Speller and Sons, New York. 204 pp.
7. Horner, W. E., and Alexander, S. A. 1985. Permeability of asymptomatic, resin-soaked, and *Verticicladiella procera*-black-stained pine sapwood. (Abstr.) Phytopathology 75:1368.
8. Horner, W. E., Lewis, K. J., and Alexander, S. A. 1985. Colonization patterns of *Verticicladiella procera* in Scots and eastern white pine.

(Abstr.) Phytopathology 75:625.
9. Isaeva, L. N. 1970. Moisture distribution in growing trunks of the main varieties of east Siberian trees. Pages 1-11 in: Properties and Preservation of Wood and New Materials from Wood. V. A. Bazhenov, ed. Indian National Scientific Documentation Centre, New Delhi. 157 pp.
10. Kaufmann, M. R., and Thor, G. L. 1982. Measurement of water stress in subalpine trees: Effects of temporary tissue storage methods and needle age. Can. J. For. Res. 12:969-972.
11. Koch, P. 1972. Utilization of the Southern Pines. Vol. I. The Raw Material. USDA Agric. Handb. 420. 734 pp.
12. Lackner, A. L., and Alexander, S. A. 1982. Occurrence and pathogenicity of *Verticicladiella procera* in Christmas tree plantations in Virginia. Plant Dis. 66:211-212.
13. Lackner, A. L., and Alexander, S. A. 1983. Root disease and insect infestations on air-pollution-sensitive *Pinus strobus* and studies of pathogenicity of *Verticicladiella procera*. Plant Dis. 67:679-681.
14. Lackner, A. L., and Alexander, S. A. 1984. Incidence and development of *Verticicladiella procera* in Virginia Christmas tree plantations. Plant Dis. 68:210-212.
15. Lewis, K. J., and Alexander, S. A. 1985. Germinability over time of *Verticicladiella procera* propagules in artificially infested soil and their inability to cause disease. (Abstr.) Phytopathology 75:1337.
16. Lewis, K. J., and Alexander, S. A. 1986. Insects associated with the transmission of *Verticicladiella procera*. Can. J. For. Res. 16:1330-1333.
17. Lewis, K. J., Alexander, S. A., and Horner, W. E. 1987. The distribution of natural populations of *Verticicladiella procera* in soil and their efficacy in causing disease. Phytopathology 77:000-000.
18. McCall, K. A., and Merrill, W. 1980. Selective Medium for *Verticicladiella procera*. Plant Dis. 64:277-278.
19. Ostle, B., and Mensing, R. W. 1975. Statistics in Research. Iowa State University Press, Ames. 596 pp.
20. Panshin, A. A., and de Zeeuw, C. 1970. Textbook of Wood Technology, 3rd ed. McGraw-Hill, New York. 705 pp.
21. Senft, J. F., Bendtsen, B. A., and Galligan, W. L. 1985. Weak wood. J. For. 83:476-483.
22. Sutton, R. F., and Tinus, R. W. 1983. Root and root system terminology. For. Sci. Monogr. 24. 137 pp.
23. Towers, B. 1977. The occurrence of *Verticicladiella procera* in Pennsylvania: 1976. Plant Dis. Rep. 61:477.