Verticicladiella Root Disease of *Pinus strobus* in New Zealand

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ABSTRACT

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Verticicladiella sp. was isolated consistently from black stained sapwood of dead and dying *Pinus strobus* in two forests on the North Island of New Zealand. This is the first report of a species of *Verticicladiella* in New Zealand. The heaviest mortality associated with the fungus was along temporary access roads in the most poorly drained portion of one stand. Microscopic examination of infected sapwood showed hyphae in axial and ray tracheids and ray parenchyma. *Verticicladiella* sp. was reisolated consistently from inoculated damaged *P. strobus* roots but not from undamaged roots. Isolates in culture exhibited optimum growth near 20 C, more profuse growth at 25 C than 15 C, and no growth at 30 C. Sporulation occurred at 20 C and was most profuse at 25 C.

In 1974 mortality was noted in a 1952 planting of *Pinus strobus* L. at Gwavas State Forest (GSF) on the east coast of the North Island of New Zealand. Dying *P. strobus* had been reported since 1964 in a 1953 planting at Ngaumu State Forest (NSF) 140 km south of GSF. The dying trees were examined in detail to determine the cause of death.

MATERIALS AND METHODS

Field observations. In July 1976 dead and dying *P. strobus* in a 2-ha stand at GSF were examined. Dead and dying trees were concentrated along temporary access roads that had been used during a thinning of the lower hectare in 1970. Mortality was greatest along a road in the most poorly drained portion of the stand. In this latter 0.1-ha area more than 40% of the trees were dead or declining. The upper hectare of the stand had been precommercially thinned, and no roads were present; only a few scattered dead and dying trees were evident.

In February 1977, 69 dead trees and 30 trees in various stages of decline were present in the lower half of the stand. A year later 16 of the declining trees and seven with no previous crown symptoms were dead. The crowns of 20 more trees had symptoms of decline in February 1978.

Discs cut from bases of dead and dying trees exhibited a black stain across the outer rings of sapwood (Fig. 1). No basal resinosis was evident. Dead *P. strobus* at NSF exhibited similar symptoms of wood staining associated with crown decline.

Lateral roots were excavated on three dying and one dead *P. strobus* (all stained at the base) at GSF. On all trees the stain was most intense at the root collar and decreased distally along each root. Stain was not found further than 1.5 m from the root collar. Root contacts from adjacent trees were not observed in the region excavated. Adult *Pachycotes peregrinus* Chapuis, a native woodborer, were found in stained and unstained root wood of the four trees.

Isolation and culture of the fungus. The same fungus was isolated consistently on malt agar from typically black stained sapwood (Fig. 1) from GSF and NSF. Several isolations were also attempted from adult *P. peregrinus* that were boring within intensively stained wood. Some insects were flame-sterilized and plated whole. Others were sliced and isolations attempted from pieces of gut.

Three isolates of the fungus from different trees at GSF were cultured on 3% malt agar and incubated in the dark at 5, 10, 15, 20, 25, and 30 C. Colony diameters were recorded after 48 hr.

Inoculations. Blocks of sapwood, 10 × 2 × 2 cm, were cut from living *P. strobus* saplings, autoclaved in 1-L jars, and seeded with agar discs colonized by the fungus. These were incubated at approximately 20 C for 11 wk and used as inocula.

In February 1977, 11 pole-sized *P. strobus* in the healthy appearing portion of the GSF stand were selected for inoculations. Forty lateral roots 2–6 cm in diameter were exposed and treated at points 14 to 140 cm distal to the root collar. Twenty roots were wounded by removing an area of bark approximately 10 × 2 cm. Colonized inoculum blocks were tied firmly to the damaged region of 10 roots, and uncolonized sterile blocks were tied to the damaged region of 10 roots. The other 20 roots were left undamaged and treated similarly. After treatment all roots were reburied. Twelve of the treated roots were examined in August 1977 and 12 in February 1978. A total of 24 roots were examined; each of the following treatments was applied to six trees: 1) colonized inoculum blocks on injured roots, 2) colonized inoculum blocks on uninjured roots, 3) uncolonized sterile blocks on injured roots, and 4) uncolonized sterile blocks on uninjured roots. Results at 6 and 12 mo were combined because infection in the two time periods did not differ.

Another 12 trees were damaged at the root collar and treated as described for inoculation of the roots. Six trees were inoculated with two colonized blocks each, and six were treated with two uncolonized sterile blocks each. Three trees treated with colonized blocks were examined in February 1978. The remaining trees were left undisturbed for future observations.

On the GSF study site, soil temperatures at a depth of 15 cm were recorded every 2 wk for 1 yr after inoculations.

Microscopic examinations. Thin sections of stained wood from naturally infected and inoculated roots were cut with a Reichert sledge microtome, mounted in lactophenol, and examined microscopically.

RESULTS

Identification of fungus. We identified the fungus as a species of *Verticicladiella*. This identification was confirmed by G. Samuels, Plant Diseases Division, DSIR, Auckland, New Zealand, and by D. Goheen, USDA Forest Service, Portland, OR. This is the first record of a *Verticicladiella* sp. in New Zealand and, to our knowledge, elsewhere in Australasia. The fungus was not isolated from *P. peregrinus*.

Growth in culture. One isolate of the *Verticicladiella* sp. grew noticeably slower than the other two tested (Table 1). In all isolates, sporulation was initiated at 20 C and profuse at 25 C. All isolates grew faster at 25 C than at 15 C, all had an optimum near 20 C, and all ceased growth at 30 C.

Inoculations. None of the inoculated trees exhibited crown symptoms. Black stain developed and *Verticicladiella* sp. was reisolated from all injured roots inoculated with colonized blocks. Stain was evident up to 16 cm proximally from the point of inoculation. Stain development was limited distal to the point of inoculation and tangentially outside the injured region. Radial development of

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stain into the root was generally minimal and, if present, was concentrated at the inoculation point. The fungus was reisolated readily from stained wood but not from wood in advance of the stain. Black stain developed in two uninjured roots inoculated with colonized blocks, and *Verticicladiella* sp. was reisolated from one of these. Its presence in this root, however, appeared unrelated to our inoculation. The other stained root had a small resinous spot at the inoculation point, suggesting unintentional injury during root exposure.

Black stain developed on two of the injured roots inoculated with uncolonized blocks, but *Verticicladiella* sp. was not isolated from either root. No stain developed and *Verticicladiella* was not reisolated from any of the uninjured roots inoculated with uncolonized blocks.

All trees inoculated at the base were alive after 12 mo. The three wounded trees examined were stained up the stem for a maximum extent of 81 cm. There was little radial or distal development of stain.

Soil temperatures fluctuated in gradual increments between 5.3 and 16 C during the study year.

Microscopic examinations. Microscopic examinations of thin sections from stained *P. strobus* wood showed hyphae concentrated in the axial tracheids but also frequently present in the rays (Fig. 2). Hyphae extended further in axial tracheids than in rays but were present in both ray tracheids and ray parenchyma. Individual hyphae extended through ray cells and continued into axial tracheids. Hyphae were occasionally present in tracheids a few millimeters in advance of macroscopically visible stain.

DISCUSSION

The results of our inoculation tests indicate that damaged roots and tree bases are the most probable infection courts and that roads made during the earlier thinning could have created these infection courts. Working with *P. ponderosa* Laws. and *Pseudotsuga menziesii* (Mirb.) Franco, Cobb and Platt (1) had much greater inoculation success with *V. wagnerii* Kendrick on damaged roots than on undamaged roots. Hansen (5) noted that *V. wagnerii* occurred more frequently along roadsides where *P. menziesii* were unnaturally exposed and therefore more likely to be damaged than in unexposed forest.

The occurrence of damage in the most poorly drained portion of the stand agrees with observations on *Verticicladiella* sp. in *P. strobus* in Yugoslavia (4) and *V. procera* on *P. strobus* in the eastern United States (8). High soil moisture also favored infections of *P. ponderosa* and *P. edulis* Engelm. by *V. wagnerii* (3,9).

Pathogenic *Verticicladiella* spp. generally grow faster in culture at 15 C than 25 C (D. J. Goheen, *personal communication*), contrary to results with our isolates. Wagner and Mielke (9) noted that an isolate of *Verticicladiella* sp. obtained by Bega had a growth optimum of 15 C. Although soil temperatures at our inoculation site were well below the optimum temperature for cultural growth of our isolates, disease did develop.

Goheen and Cobb (2) suggested that species of wood-boring insects are vectors of *V. wagnerii*. Our efforts to isolate the fungus from adult *P. peregrinus* boring in infected tissues were unsuccessful. Although our attempts and methods of isolation were limited, *P. peregrinus* appeared to be a secondary invader of trees killed or weakened through attack by *Verticicladiella* sp. Similar observations were made on insect invasion of *P. strobus* attacked by *Verticicladiella* spp. in Pennsylvania (8) and Yugoslavia (4).

Our observation of hyphae in axial tracheids, ray tracheids, and ray parenchyma (Fig. 2) contrasts with other microscopic observations of *Verticicladiella* sp. in infected wood. Wagner and Mielke (9) noted the absence of hyphae in medullary rays of *P. ponderosa*. Landis and Helburg (6) found hyphae exclusively within the tracheids of the axial system of *P. edulis*. Halambek (4) reported hyphae only in tracheids of *P. strobus*. Smith (7) found hyphae only in mature xylem tracheids of *P. ponderosa* and *P. edulis*; in *P. ponderosa*, hyphae were present in both axial and ray tracheids but only in axial tracheids of *P. edulis*. Hyphae moved between tracheids via bordered pit pairs. All of these observations, except those of Halambek (4), involved *V. wagnerii*.

Cultural characteristics, the pattern of staining in diseased trees, and general disease symptomatology suggest that this

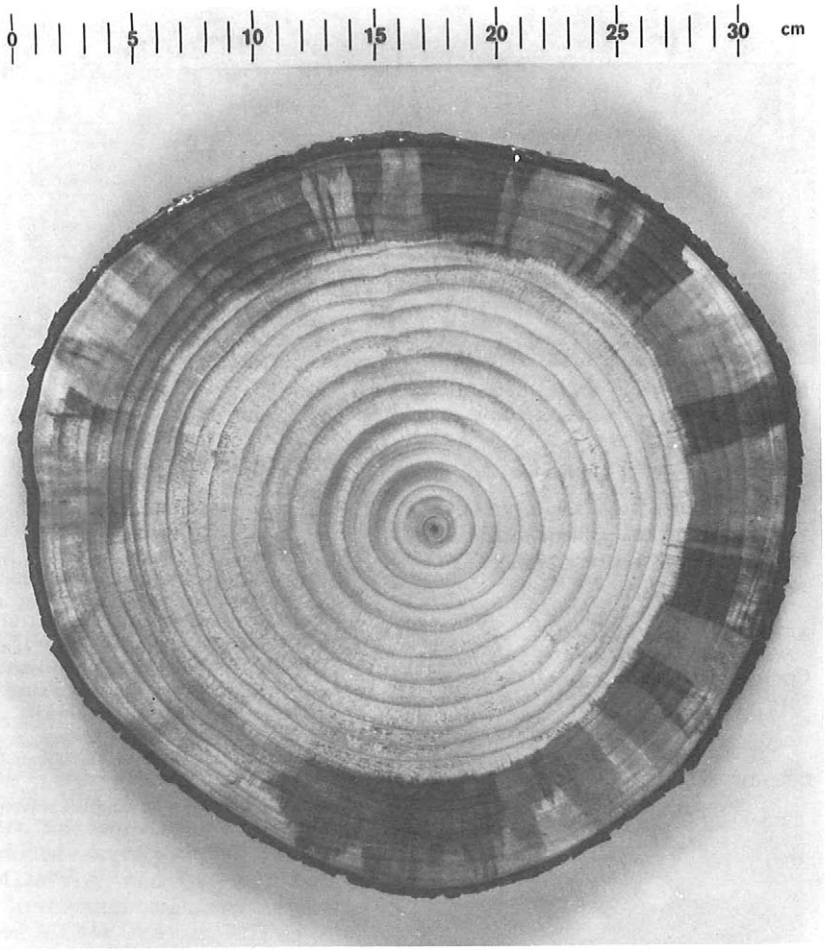

Fig. 1. Cross section from the base of a dead *Pinus strobus* shows the pattern of stain in the outer rings of sapwood. *Verticicladiella* sp. was readily isolated from such stained wood.

Table 1. Growth in culture of three isolates of *Verticicladiella* sp. incubated 48 hr in the dark

Isolate	Colony diameter (mm) at temperature (°C)[z]					
	5	10	15	20	25	30
A	10 a	22 b	26 b	51 e	48 e	0 f
B	9 a	15 c	23 b	33 d	25 b	0 f
C	9 a	22 b	32 d	52 e	48 e	0 f

[z]Mean of five observations; values followed by different letters differ significantly ($P = \leq 0.01$).

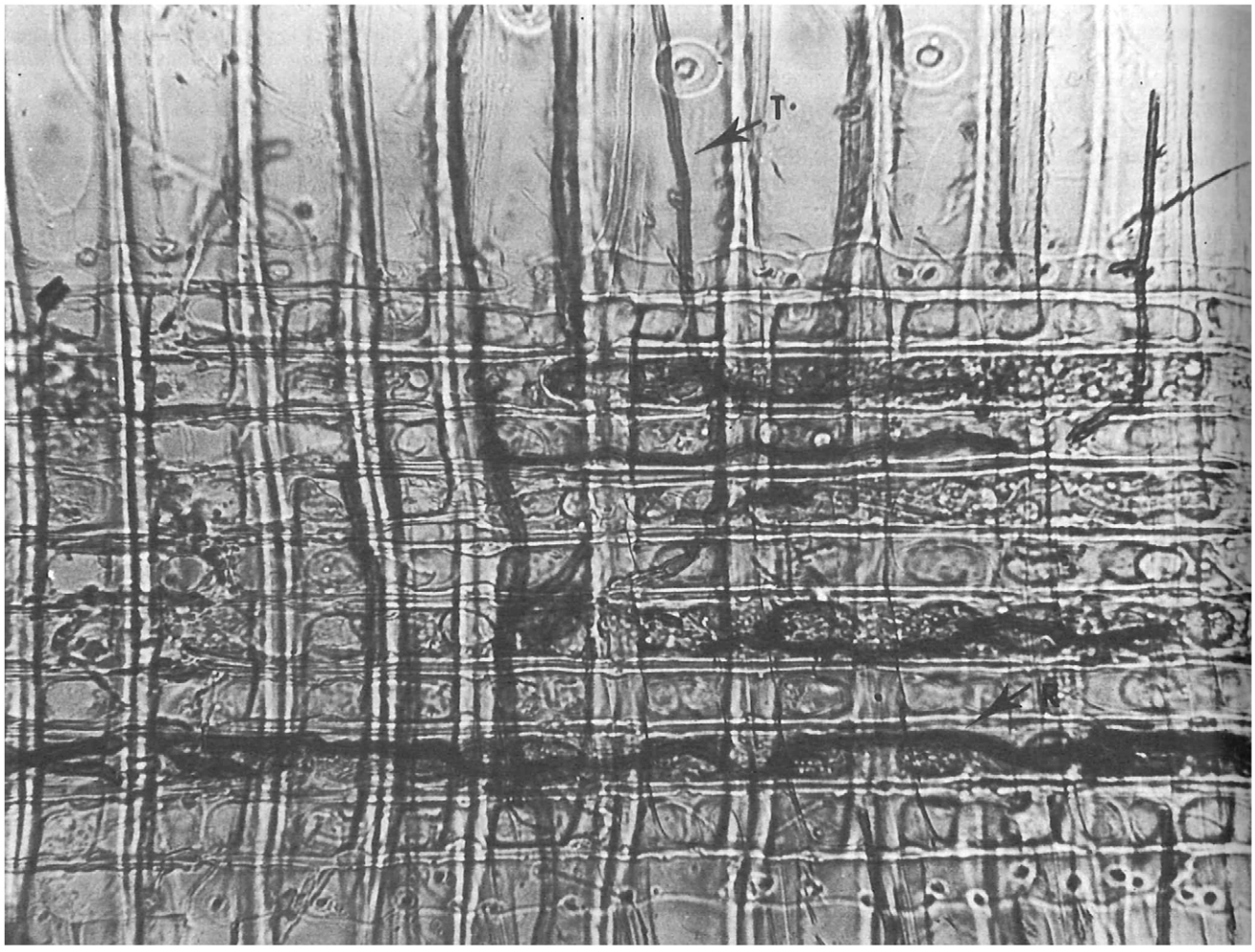

Fig. 2. Radial section of *Pinus strobus* sapwood infected with *Verticicladiella* sp. Hyphae were present in axial tracheids (**T**), ray tracheids, and ray parenchyma (**R**). This section is from an inoculated root; patterns of hyphal development were similar in naturally infected roots. (Approximately ×440)

fungus differs from the aggressive *V. wagnerii* and may be similar to *V. procera*, a known cause of dieback in *P. strobus* (8).

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