Black Stain Root Disease in Douglas-Fir in Western Montana

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ABSTRACT

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Douglas-fir (*Pseudotsuga menziesii*) roots with black staining of the xylem were found in four root-disease centers in western Montana where previously only *Armillaria mellea* was thought to occur. Declining trees had mycelial fans of *A. mellea* at the root collar and often black stain in small, deeply positioned roots. Symptomless Douglas-fir adjacent to declining trees often had only the black stain. Laboratory and greenhouse studies indicated the stain was caused by *Verticicladiella wageneri*.

Observations in western Montana indicate that root disease of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is widespread with many stands severely damaged. For example, a 1979 survey of the Lolo National Forest indicated that about 7,700 ha (1.2%) of the commercial forest land was occupied by active root-disease mortality centers that could be detected from large-scale aerial photographs (8). This may be a very conservative estimate because a substantial, unmeasured amount of root disease apparent from the ground is undetected on the photos.

Many root pathogens occur on conifers in western Montana, including *Armillaria mellea* (Vahlex Fr.) Quel., *Heterobasidion annosum* (Fr.) Bref., *Phellinus weirii* (Murr.) Gilb., *Verticicladiella* sp. (possibly *V. wageneri* Kend.), and *Phaeolus schweinitzii* (Fr.) Pat. (2). *A. mellea*, however, is the root pathogen most commonly detected within mortality centers and is often the only pathogen located at the root crown of dead and dying trees.

This report summarizes observations at several locations in western Montana (1,7) indicating that black stain root disease caused by *V. wageneri* is present in some large mortality centers previously diagnosed as *A. mellea* infestations.

MATERIALS AND METHODS

Root examinations. Douglas-fir root systems were examined at four locations of active root disease in western Montana. Margins of active root-disease centers were ringed with dead and declining trees. Brush and coniferous seedlings, primarily Douglas-fir, had invaded the interiors. Trees within or on the margin of active root-disease centers were examined by cutting into the root crown and several major lateral roots and looking for stain or decay. Root systems were not completely dissected.

Site descriptions. *Saint Mary's Logging Unit, Flathead Indian Reservation.* The area contained a disease center of about 6 ha within an 80-yr-old Douglas-fir stand at about 1,000 m elevation. One dead tree and five declining trees with thin, chlorotic foliage and rounded tops were selected for root excavation. These trees were on the edge of the infection center. In addition, 15 nonsymptomatic trees outside the infection center but within 20 m of the margin were also selected. Selected trees were pushed with a D-6 Caterpillar tractor to expose roots, which were examined for decay, stain, and other indications of root disease. Several samples of stained roots were collected for microscopic examination and isolation.

Ducharme Creek, Flathead Indian Reservation. Twenty Douglas-fir trees within a 3-ha mortality center in an 80-yr-old mixed conifer stand were uprooted as described before and examined for symptoms and signs of root pathogens. Three of the trees were on the margin of the center.

McCormick Creek, Ninemile Ranger District, Lolo National Forest. Twenty windthrown Douglas-fir trees were examined in this area. Trees were located on the margins of several large disease centers in a 70-yr-old Douglas-fir stand.

Burnt Fork of Belmont Creek on land owned by Champion International Corporation. In a mortality center of about 20 ha in an 80- to 100-yr-old stand of Douglas-fir, several dozen trees with crown symptoms were examined for root pathogens at the root collar. The root of one symptomless tree was excavated to a depth of about 1 m below the soil line with hand tools.

Laboratory and greenhouse studies. Stained roots from each location were sectioned and examined microscopically (×100–400) for pigmented hyphae. Isolations from stained roots were attempted on water agar plates incubated at 20 C and at room temperature. Two species of *Verticicladiella* were consistently isolated from stained roots and used in pathogenicity tests. Two isolates identified as *V. wageneri* and an isolate of an undescribed species of *Verticicladiella* were grown in pure culture for 8 wk at 18 C on segments of ponderosa pine (*Pinus ponderosa* Laws.) twigs (about 1 cm diameter) that had been boiled for 2 hr in 10% malt extract and autoclaved (1 hr at 121 C). Ten wounded and 10 unwounded 2-yr-old bare-root Douglas-fir seedlings were inoculated with each isolate.

Wounds were made by cleaning the taproot surface with 95% ethanol, then excising a 1-cm-long section to a depth of about one-third the diameter of the taproot at about 3 cm below the ground line. A 1-cm segment of colonized pine twig was placed in contact with the fresh wound and secured with masking tape. Unwounded seedlings were inoculated by securing a segment 3 cm long to the taproot 5 cm below the ground line. Controls consisted of both wounded and unwounded seedlings inoculated with uncolonized twig segments. After inoculation, seedlings were potted in an unfertilized 1:1 mixture of peat and vermiculite, placed in a greenhouse, watered two or three times per week, and checked periodically for symptoms of root infections.

RESULTS

Root excavations. Mycelial fans of *A. mellea* were detected at the root collars of standing dead and declining Douglas-fir in each survey area. The root collars of trees without crown symptoms did not reveal symptoms or signs of any pathogens although roots often had black stain within the xylem. Black stain was not found in sapwood at the root crown of any trees. Most stain was restricted to deeply positioned sinker roots less than 5 cm in diameter 0.5–2 m below the ground line. Stain occurred parallel to the annual rings when viewed in cross section, a pattern characteristic of *V. wageneri* (11). Dead and declining regeneration were

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usually infected only with *A. mellea*.

At the St. Mary's site, four of five uprooted declining trees had fans of *A. mellea* in their roots and root collars; however, the roots of two of these trees also had black stain in the xylem. Black stain was also found in the roots of one declining tree that lacked signs of *A. mellea* and in the one uprooted dead tree. Of the 15 symptomless trees outside the disease center, six had black-stained roots; none showed indications of *A. mellea*.

At Ducharme Creek, all declining trees examined were infected with *A. mellea* but not with black stain. Black stain was found in the roots of one of the three trees examined from the margin of the center. This tree lacked crown symptoms and was not infected with *A. mellea*.

At McCormick Creek, all windthrown Douglas-fir trees with crown symptoms had fans of *A. mellea* at the root collar and in many roots. Black stain was present in eight of 20 declining or symptomless windthrown trees near the margins of the centers. Neither *A. mellea* nor black stain was detected in the roots of windthrown trees farther than 40 m from a root-disease center. Extensive brown cubical decay typical of that caused by *Phaeolus schweinitzii* was also present in the roots of several trees.

Declining trees at Burnt Fork had *A. mellea* colonizing their root collars. Black stain was found in the excavated root of the one symptomless tree. Most trees also had decay typical of *P. schweinitzii*.

Laboratory and greenhouse studies. Stained roots contained pigmented hyphae restricted to the xylem tracheids, a common indication of black stain root disease (10). Stained hyphae were not present in ray parenchyma. Two fungal species were isolated from stained roots collected at St. Mary's. One grew very slowly or not at all at room temperature and fit Kendrick's (9) description of *V. wageneri*. The second species, which grew faster than *V. wageneri* at 25 C and differed in conidiophore morphology (9), did not fit the descriptions of *Verticicladiella* and may represent an undescribed species. Stain fungi isolated from the other sites corresponded to descriptions of *V. wageneri*.

The two isolates of *V. wageneri* tested for pathogenicity killed five and seven of the wound-inoculated seedlings and five and three of the unwounded seedlings. Mortality was first evident after 4 mo and continued for 7 mo after inoculation. All killed seedlings had extensive black staining of the xylem (average length 16 cm). Two unwounded seedlings had black staining but were still alive 9 mo after inoculation. Microscopic examination of stained xylem showed hyphae restricted to the tracheids. Reisolation from stained xylem yielded *V. wageneri*.

All seedlings inoculated with the unidentified *Verticicladiella* sp. survived. Some wound-inoculated seedlings showed limited (less than 2 cm) gray to black discoloration near the wound. The unidentified fungus was not reisolated from these seedlings. Seedlings inoculated without wounding as well as unwounded control seedlings showed no evidence of infection.

DISCUSSION

Black stain root disease of Douglas-fir has been reported in California (3), Oregon (4–6), and Washington (4,5). We verified *V. wageneri* associated with black stain root disease at one site in western Montana and found staining typical of the disease at several other locations. Two isolates of *V. wageneri* from stained roots killed inoculated seedlings and produced symptoms identical to those described for black stain root disease. An unidentified *Verticicladiella* sp. was also associated with stained roots, but this fungus was not pathogenic in our inoculations. We have isolated this species from *Hylastes nigrinus* (Mannerheim), a root-feeding bark beetle commonly found in stumps and diseased roots of Douglas-fir. This fungus may be secondary to *V. wageneri*, ie, introduced by *H. nigrinus* into roots previously colonized by *V. wageneri*.

Etiology of root disease in western Montana appears to be complex. Examination of the root collar and major roots at several sites revealed only *A. mellea*. Our observations after root excavation indicate that *A. mellea* may sometimes be secondary to *V. wageneri*, as was reported by Goheen and Hansen (5). We suspect that *V. wageneri* infects trees first because the fungus was present on many roots of trees immediately outside of the visible advancing disease-center margin on trees that were not yet infected by *A. mellea*. Some large trees, however, were invaded and killed by *A. mellea* in apparent absence of *V. wageneri* and only *A. mellea* was found on dead and dying regeneration. Perhaps, *A. mellea* initially colonized trees weakened by *V. wageneri* and used that food base to attack and kill more vigorous trees and regeneration. Because black stain root disease was rarely detected at the root collar, the disease may be more common than previously thought.

Presence of a third root pathogen, *P. schweinitzii*, at two of the evaluated sites raises further questions regarding root-disease etiology. Dubreuil and Martin (*personal communication*) found *A. mellea* to be secondary to *P. schweinitzii* in Idaho. This may also occur in western Montana.

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