Occurrence of Verticicladiella wagenerii and Its Perfect State, Ceratocystis wageneri sp. nov., in Insect Galleries

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ABSTRACT

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Except for reports that *Verticicladiella wagenerii* can spread from tree to tree across root grafts and contacts, virtually no information is available on means of spread or inoculum production by the pathogen. Roots of 126 ponderosa pines located in infection foci were excavated and examined for evidence of insect activity and fungus sporulation. Conidiophores of *V. wagenerii* were found for the first time in one gallery each of a buprestid, a cerambycid, and an unidentified insect, in two galleries of *Dendroctonus valens*, and in 96 *Hylastes macer* galleries. The perfect state of *V. wagenerii*, described herein as *Ceratocystis wageneri* sp. nov., was found in 19 *H. macer* galleries. Evidence points to *H. macer* as a vector of the fungus.

Additional key words: black stain root disease.

Verticicladiella wagenerii Kendrick (Moniliales:Moniliaceae) causes a root disease of several western conifers, including ponderosa pine (*Pinus ponderosa* Laws.) in California. Diagnostic evidence of *V. wagenerii* infection is a dark brown to black stain in the sapwood of roots and lower stems. The dark-pigmented *V. wagenerii* hyphae grow almost exclusively through longitudinal tracheids, passing from cell to cell through bordered pit pairs and interfering with water uptake (11). Infected trees usually decline rapidly and die within a few years. Bark beetles often are involved in actually killing disease-weakened trees.

Verticicladiella wagenerii has been considered a relatively unimportant pathogen in commercial forest stands. However, within the past few years, it has been discovered in many western North American forests (12). Its potential to spread and to infect healthy stands is not known, but if it should be substantial, the pathogen could become a major problem.

Verticicladiella wagenerii spreads from tree to tree via root grafts and contacts (12). However, mechanisms of establishment of new infection centers have not been determined. It has been hypothesized (12) that insects, particularly members of the family Scolytidae, are involved in long distance spread of the fungus. The fungus has been isolated from *Pseudohylesinus grandis* Swaine in Douglas-fir (7), but no other evidence of any vector relationship has been reported.

Verticicladiella wagenerii was first isolated from stained ponderosa pine wood by C. T. Rumbold and was tentatively identified as *Leptographium lundbergii* Leg. and Melin (13). Kendrick (8) determined that the fungus produced sympodulae rather than annellophores as sporogenous cells, thus warranting its inclusion in the genus *Verticicladiella* Hughes (6). He proposed the specific name *wagenerii* in honor of W. W. Wagener, who conducted pioneering studies on the disease caused by the fungus.

In culture, the fungus produces a mass of septate, smooth-walled hyphae with stout conidiophores bearing ellipsoid, hyaline conidia on penicillately-branched heads. Conidia accumulate around the sporogenous apparatus in a mucilaginous mass. To date, there have been no reports of either sexual or asexual structures of *V. wagenerii* in nature.

Objectives of the present study were to determine whether reproductive structures of *V. wagenerii* are produced under field conditions and to determine if the character and location of these structures might suggest mechanisms of fungus dispersal.

MATERIALS AND METHODS

Investigations were made in 1974 and 1975 in the vicinity of Blodgett Research Forest at an elevation of 1400 m on the Georgetown Divide, El Dorado County, California. Approximately 700 *V. wagenerii* infection centers in ponderosa pine had been detected on 11,000 hectares in this area. The forest is generally characterized by the mixed conifer type composed of ponderosa pine, sugar pine, Douglas-fir, white fir, incense cedar, and California black oak. Most infection centers, however, occurred in relatively pure stands of ponderosa pine. Most ponderosa pines were 60-75 yr old and 25-80 cm in diameter 1.5 m above ground.

With the aid of the Growlersburg Conservation Crew, California Division of Forestry, root systems of all

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coniferous trees in and around entire infection centers or portions of centers were excavated by hand. Roots of 126 ponderosa pines in 19 sites were uncovered. Locations of all trees were mapped, and tree size, crown class, and general condition were recorded. Immediately after excavation, a search was made for injuries, insect galleries, vascular discolorations, and fungus fructifications on surfaces of stems and roots. The cambium and xylem of the lower stems of diseased trees plus 497 infected roots and 221 apparently healthy roots were further examined by removing the bark and dissecting the wood. The surrounding soil also was examined.

All samples with apparent fruiting bodies were examined microscopically. Single spores or, if spores could not be obtained, hyphal fragments, from *V. wagenerii*-like conidiophores and possible perfect-state fruiting structures were cultured on 10% potato-dextrose agar and incubated at temperatures of 15-18 C. The structure of conidiophores grown in culture was compared with Kendrick's description of *V. wagenerii* and other *Verticicladiella* species (8). Ponderosa pine seedlings were inoculated with confirmed isolates of *V. wagenerii* in the greenhouse by taping a dowel colonized by the fungus to the taproot 3 to 6 cm below ground level. Development of typical symptoms by inoculated seedlings and reisolation of *V. wagenerii* constituted final proof of identity.

RESULTS

Conidiophores were found in insect galleries in 29 roots on 18 trees. All of the roots exhibited *V. wagenerii* stain. In two trees that had only one root stained, the stain was limited to a few cm above and below insect galleries. In five cases, roots in which conidiophores were found showed evidence of past injury, and insect galleries were adjacent to the wounds. Most galleries and conidiophores were found in small roots (2-5 cm in diameter) on relatively small trees (40 cm or less in diameter 1.5 m above ground) in the intermediate to suppressed crown classes.

Of the 18 trees in which conidiophores were discovered, four had been infested and killed recently by *Dendroctonus brevicomis* Lec. and 14 were still alive. All but three of the latter trees exhibited crown thinning and needle chlorosis.

Conidiophores were located in one unidentified gallery, one buprestid (probably *Buprestis arrulenta* Linn.) gallery, one cerambycid (*Spondylis upiformis* Mannerheim) gallery, two *D. valens* Lec. galleries, and 96 *Hylastes macer* Lec. galleries. Conidiophores were found in fewer than 1% of the buprestid, cerambycid, and *D. valens* galleries examined but were in 24% of the *H. macer* galleries. Most of the insect galleries were occupied by larvae, pupae, or adult insects. Galleries containing conidiophores were found in roots at depths of 10-100 cm below the soil surface. Most *H. macer* galleries which contained conidiophores were located on undersides of roots.

Most galleries with conidiophores were found in April, May, and June. However, a few *H. macer* galleries containing fresh-looking conidiophores were observed in October and November. Some dried structures that may have been conidiophores were found during the summer months, but these could not be cultured or definitely identified. Soil around conidiophore-containing roots was usually moist.

Perithecia were found in insect galleries in seven roots on five of the 18 trees in which conidiophores of *V. wagenerii* also were found (Fig. 1). The five trees were alive, and three of them did not exhibit crown symptoms. Perithecia were found on roots at the same depths in the soil and at the same times of year as were conidiophores.

The perithecia, similar to but larger than those of *Ceratocystis penicillata* (Grosm) C. Moreau (8), are those of an undescribed species. The description of the new species is as follows: *Ceratocystis wageneri* sp. nov. (Fig. 1). Perithecia in porticis de *Hylastes macer* Lec. in radicis de *Pini ponderosa* Laws. cum primus contagione a *Verticicladiella wagenerii* Kendrick; perithecia fere inundati partin; basi nigris, globosis, 74-343 μm (medium = 145 μm) diam., leves, interdum leviter asparati praesipue cum perithecia senescet. Rostra 345-786 μm (medium = 566 μm) longa, gracila, nigra qui muntantes ad fulvos in apicis, basi 21-43 μm (medium = 31 μm) diam. et apicis 14-20 μm (medium = 16 μm) diam., apicia interdum leviter inflata; non ostiolar hyphae visum. Asci non visum. Ascosporae subcurvate, hyaline, 7.0-7.2 μm (medium = 7.1 μm) $\times$ 2.0 $\times$ 2.1 μm (medium = 2.0 μm).

Cultare juvenili hyalinis cum non aerii mucelibus qui mutant ad virides de olearum ad spadicis et dinique spadicis obscuris ad negris post 10-14 dies. Hyphae ramosa, muri densi, 3-12 μm (medium = 4 μm) diam. Multi erecta conidiophora de *Verticicladiella wagenerii* Kendrick apparent post 10-14 dies; stipes spadicis obscuris, muri densi, 8-12 (medium = 10 septatae, 540-914 μm (medium = 714 μm) longi et 10-12 μm (medium = 11 μm) diam.; caputis ramosis usque ad 60 μm transverisis fulvis in basi mutantes hyalinis in apicis. Sympodulosporae hyalinae, obovoideis, 6.5-7.0 μm (medium = 6.7 μm) $\times$ 1.8-2.0 μm (medium = 1.9 μm). Perithecia non formabant in culturae.

Perithecia in galleries of *Hylastes macer* Lec. in roots of *Pinus ponderosa* Laws. with early infection by *Verticicladiella wagenerii* Kendrick; perithecia almost always partially submerged; bases black, globose, 72-343 μm (mean = 145 μm) in diameter, smooth or occasionally slightly roughened, especially as they become older. Necks 345-786 μm (mean = 566 μ) long, slender, black becoming light brown at the extreme apex, bases 21-43 μm (mean = 31 μm) in diameter, and tips 14-20 μm (mean = 16 μm) in diameter, tips sometimes slightly flared; no ostiolar hyphae observed. Asci not seen. Ascospores subcurvate (or bean-shaped), extruded from the ostiole in a sticky, gelatinous droplet.

Cultures hyaline at first with no aerial mycelium, becoming olive green to brownish and finally dark brown to black after 10 to 14 days; linear growth rate on potato dextrose agar moderate to fast, 25-45 mm (mean = 31 mm) in 10 days at 18 C, little or no growth at 24 C; odor slightly citrus-like. Hyphae hyaline at first, becoming brownish-green, branched, thick-walled, septate, 3 to 12 μm (mean = 4 μm) in diameter. Numerous erect conidiophores of *Verticicladiella wagenerii* appear after 10-14 days; stipe dark brown, thick-walled, 8-12 (mean = 10) septate, 540-914 μm (mean = 714 μm) long and 10-12

μm (mean = 11 μm) in diameter; ramified heads up to 60 μm across made up of repeatedly branching metulae, light brown at the bases becoming hyaline at the tips. Sympodioconidia hyaline, obovate, 6.5-7.0 μm (mean = 6.7 μm) × 1.8-2.0 μm (mean = 1.9 μm). Perithecia not formed in culture.

Holotype; collected from roots of *Pinus ponderosa,* Butcher's Corral, El Dorado Co., California; deposited in the herbarium of the University of California at Berkeley-Accession No. UCB 1445965.

DISCUSSION

The discovery of both the imperfect and perfect states of *Verticicladiella wageneri* in the galleries of root-inhabiting insects has provided concrete evidence supporting the hypothesis that insects are involved in overland or long distance spread of the pathogen. Both imperfect and perfect fructifications bore spores in slime droplets and occurred in galleries while larvae and adult insects were present.

Other *Verticicladiella* and *Ceratocystis* species are transmitted by beetles in the family Scolytidae (1, 5, 8, 9, 10). Conidiophores of *V. wageneri* were found in galleries of a cerambycid and a buprestid as well as in those of two scolytids, *Dendroctonus valens* and *Hylastes macer*. However, the conidiophores were relatively common only in galleries of the scolytid, *H. macer*. In addition, perithecia of the fungus were found only in

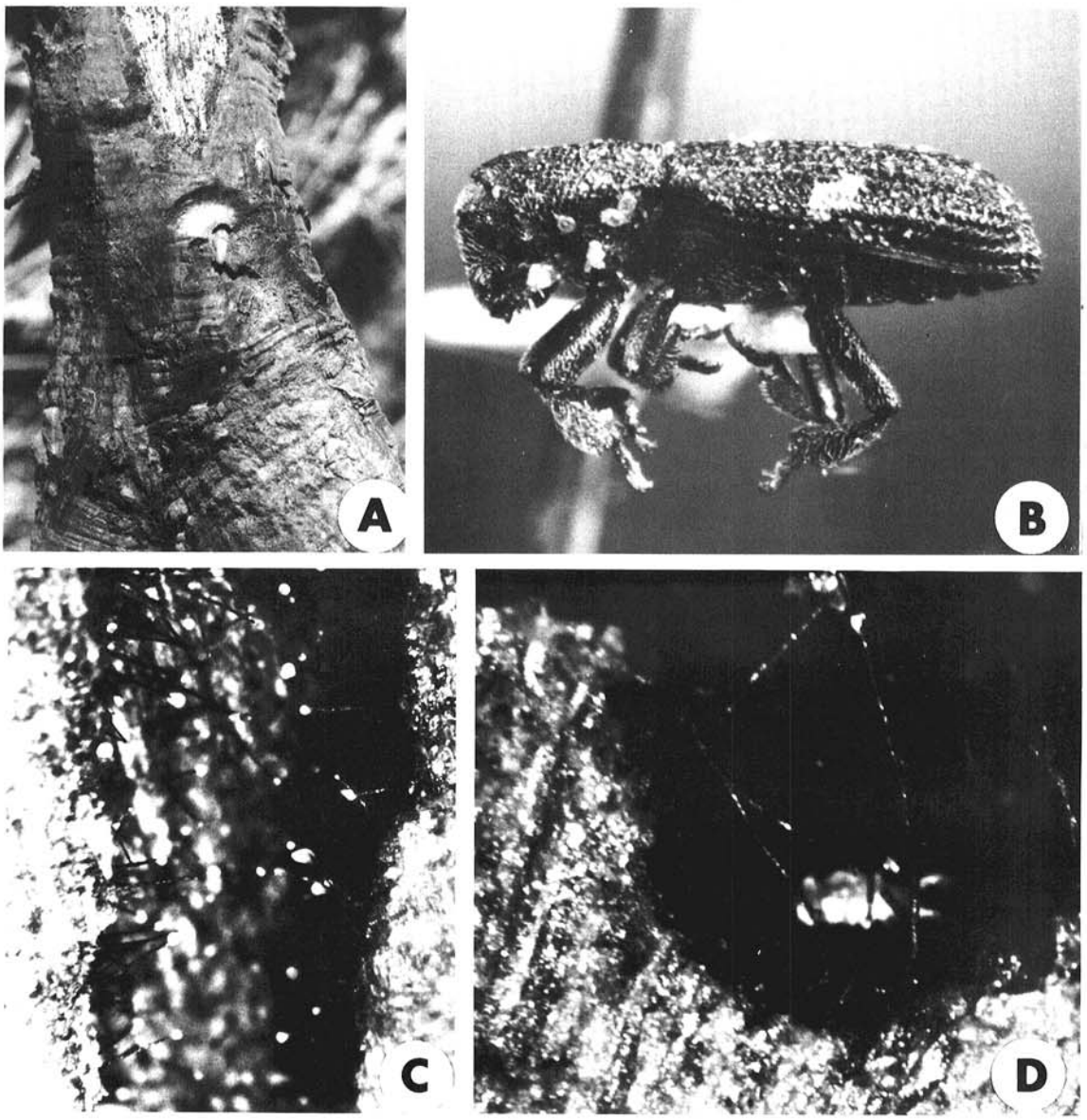

Fig. 1-(A to D). **A)** *Hylastes macer* gallery and larva on ponderosa pine root (×1). **B)** *Hylastes macer* adult (×20). **C)** *Ceratocystis wageneri* perithecia in *Hylastes macer* gallery (×18). **D)** *Ceratocystis wageneri* perithecium in *Hylastes macer* gallery (×32).

galleries of that beetle. A close association between xylem staining and insect galleries, especially those of *H. macer,* far down some of the roots is further evidence that an insect-vector relationship exists.

The biology of *H. macer* has not been studied thoroughly (2, 3, 4), but the insect is known to infest several pine species. As an adult, it often attacks the roots of weakened trees, appears to be attracted to wounds and is more active when the soil is moist. That the fruiting structures of *V. wagenerii* were found mostly on small roots of small, intermediate or suppressed trees, some of which had wounded roots, would further implicate *H. macer* or insects of similar habit. In addition, the apparent time of fruiting when the soil is moist would coincide with the period when the insect appears to be most active. Although there is not enough evidence to rule out other insects as potential vectors of *V. wagenerii, H. macer* does appear at this time to be the most likely candidate.

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