

# Root Disease, Associated with *Verticicladiella alacris*, of Pines in South Africa

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## ABSTRACT

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A disease of *Pinus pinaster* and *P. radiata* associated with root infection by *Verticicladiella alacris* was common at roadsides, and wounding appeared to be necessary for infection. All infected trees were colonized by the European bark beetle, *Orthotomicus erosus*, and *V. alacris* was isolated from *Hylastes* spp. in diseased roots.

*Verticicladiella wagnerii* Kendrick, *V. procera* Kendrick, and other *Verticicladiella* spp. are known root pathogens in other parts of the world (6,15,17,18). Infected trees are attractive to bark beetles (Coleoptera: Scolytidae) (1,9,11-13), which have been recorded as possible vectors of *V. wagnerii* (5). During a preliminary survey of diseases of exotic forest trees in South Africa, *Verticicladiella alacris* Wingfield & Marasas (19) was found associated with the roots of diseased *Pinus pinaster* Ait. and *P. radiata* D. Don. This paper describes the disease and its association with bark beetles.

## MATERIALS AND METHODS

Diseased *P. pinaster* and *P. radiata* trees were examined in 13 centers in the following areas of the Western Cape Province: Cape Peninsula (Tokai State Forest), Franschhoek (La Motte State Forest), and Grabouw (Grabouw and Lebanon State Forests).

Root systems were excavated and sampled. A sledge microtome was used to section infected roots, and isolations were made on half-strength malt extract agar (MEA) (10 g of Difco Malt extract and 15 g of Difco Bacto Agar per liter). Cultures were routinely maintained at 24 C.

One lateral root on each of 10 10-yr-old *P. pinaster* trees was inoculated by removing a 9-mm cambial disk with a cork borer and replacing it with a disk from a 2-wk-old culture of *V. alacris* on MEA. Inoculation points were covered with moist paper towels and plastic film, and the soil was replaced. Two-month-old *P. pinaster* and *P. radiata* seedlings (20 of each species) were inoculated by tying to their taproots, 20.0 × 3.0 mm wooden dowels, boiled in potato dextrose broth (200 g of potatoes and 15 g of dextrose per liter), and were colonized for 2 mo by *V. alacris*. Seedlings were repotted and kept in a glasshouse

maintained between 15 and 25 C. Equal numbers of control inoculations were made.

Naturally infected trees in all areas were examined for bark beetles. Beetles found in the roots and root crowns of trees in an infection center in the Grabouw State Forest were surface sterilized in a commercial sodium hypochlorite solution (1% available chlorine) with Tween 80 added, rinsed in sterile distilled water, and squashed onto MEA containing 4% vancomycin.

## RESULTS

**Symptoms.** Infection centers in 10- to 15-yr-old plantings of *P. pinaster* and *P. radiata* ranged from a few trees to 2 ha in size. Many occurred at roadsides (Fig. 1). Trees showed reduced terminal growth and wilting, with chlorosis and death of the needles, which were retained after death. According to foresters in the area, trees took 2-3 yr to die, and during this period an abnormal number of cones was produced (Fig. 1). Infected roots and root crowns showed heavy impregnation of resin and dark blue discoloration with staining in areas parallel to the annual rings (Fig. 2). Staining was never seen above the root crown area. Symptoms on both *Pinus* spp. were similar, except that *P. radiata* roots stained darker and exuded more resin. Microtome sections through infected wood showed dark hyphae of *V. alacris* in the tracheids.

Natural regeneration (*P. pinaster*) replaced dead trees in infection centers, but many of the young trees were also diseased. In these trees healthy roots in contact with diseased roots developed lesions at the point of contact, and resin exuded from the bark of the roots (Fig. 3), which also stained dark blue (Fig. 4).

**Inoculation tests.** All roots inoculated after wounding exuded resin, and dark lesions similar to those observed in naturally infected trees developed in the sapwood. Six months after inoculation, lesions had extended an average of 10.0 cm on either side of the inoculation points. The pathogen was reisolated from

all lesions. Control inoculations with uninoculated MEA disks showed no disease symptoms. Seedlings inoculated with dowels were unaffected 8 mo after inoculation.

**Association with bark beetles.** The aboveground parts of all trees infected with *V. alacris* were colonized by the European bark beetle *Orthotomicus erosus* (Woll.). *O. erosus* and *Hylurgus ligniperdus* (F.) were present in some of the root crowns, and *Hylastes angustatus* Herbst. and *H. linearis* Erichson were in the roots well below soil level. Isolations from a mixed sample of the two *Hylastes* spp. yielded cultures of *V. alacris*, but the pathogen was not isolated from other bark beetle species.

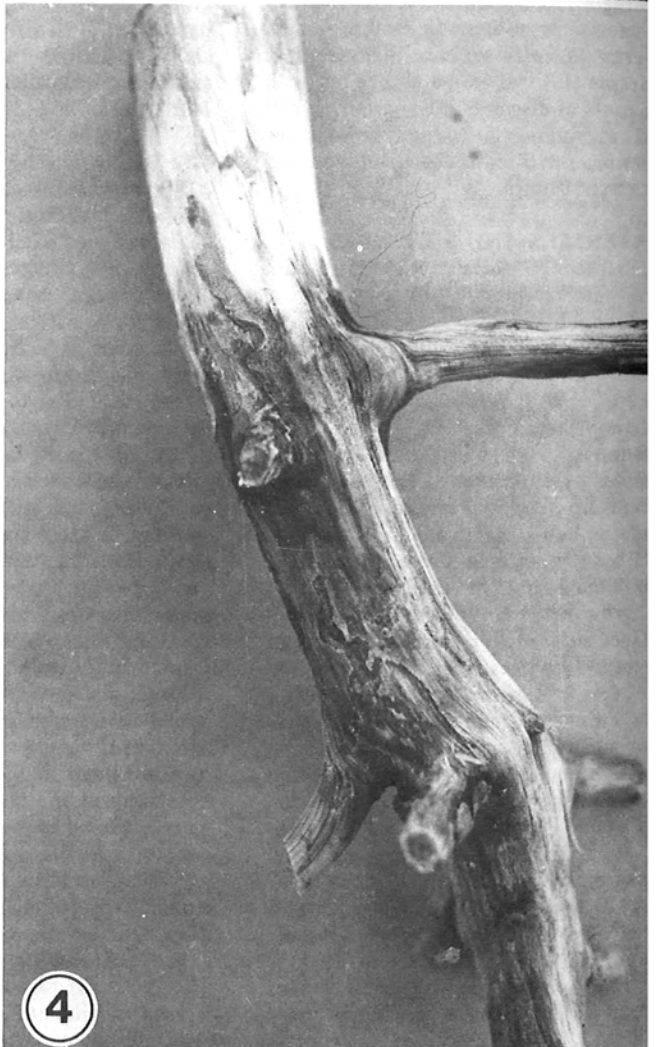
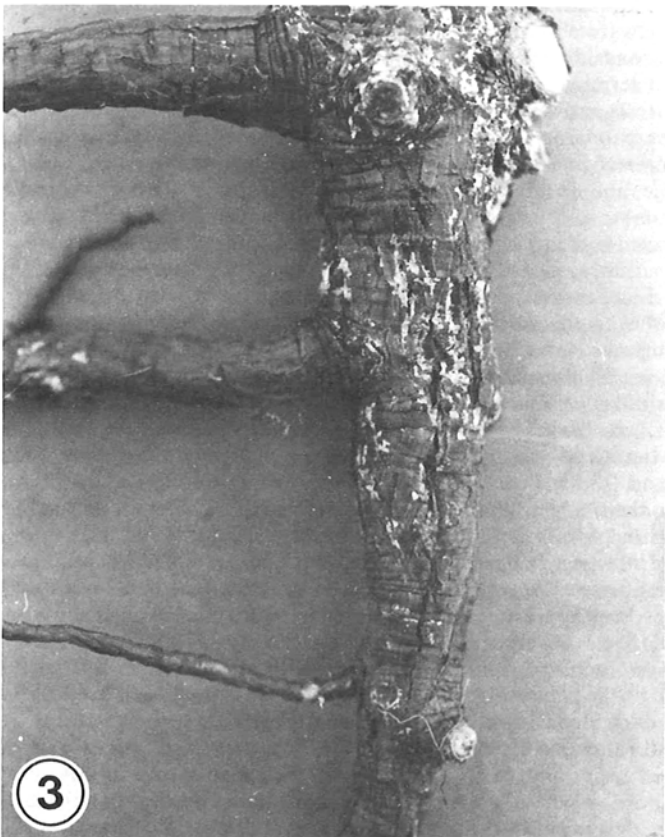
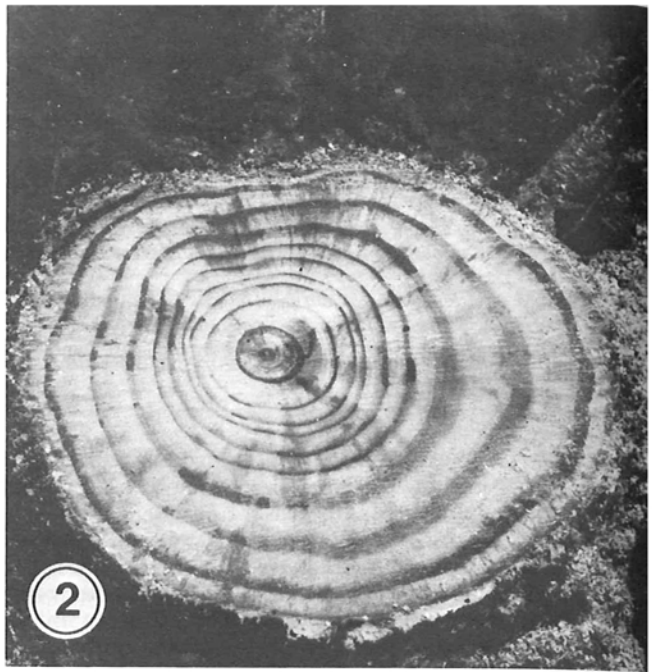
## DISCUSSION

Symptoms associated with *V. alacris* were generally similar to those of other *Verticicladiella* diseases (6,15,17,18). However, trees infected with *V. wagnerii* (the best documented *Verticicladiella* root pathogen) usually lose their needles before dying (4), whereas those infected with *V. alacris* retain their needles; and stained sapwood was never found above the root crown area in trees infected with *V. alacris*, whereas trees infected with *V. wagnerii* show extensive discoloration of aboveground parts (4).

*V. wagnerii* has a low optimum growth temperature in a narrow growth temperature range, and infection is limited by high soil temperatures (16,18). *V. alacris* has a rapid growth rate over a wide temperature range, with an optimum at 25 C (19). The pathogen is favored by the Mediterranean climate of the Western Cape with its cool, wet winters and hot, dry summers.

*V. wagnerii* infects unwounded roots (2,8), but *V. alacris* and *Verticicladiella* sp. on *P. strobus* L. in New Zealand (15) appear incapable of colonizing uninjured roots. The *V. alacris* infection centers at roadsides suggests that these trees were stressed by disturbance of the soil or damage to the roots (7). Stressed trees (7) and trees with other *Verticicladiella* root diseases (5,9,11) attract bark beetles that carry *Verticicladiella* spp. (3,5,10,14).

Further studies are necessary to establish the relationship between *V. alacris* and bark beetles (particularly *Hylastes* spp.) as possible vectors of this root pathogen and to establish the role of bark beetles such as *O. erosus*, which attack aboveground parts of the trees, in the total disease syndrome.



**Figs. 1-4.** *Pinus pinaster* and *P. radiata* infected with *Verticillium alacris*: (1) Infection center at a roadside *P. radiata* planting showing excessive production of cones on diseased trees. (2) Staining pattern of wood in the root crown area of an infected *P. pinaster* tree. (3) Resin exudation from the bark of an infected root of a *P. pinaster* sapling. (4) Stained root of an infected *P. pinaster* sapling.

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