Pine Mortality in Hawaii Associated with *Botryosphaeria dothidea*

CHARLES S. HODGES, Chief Plant Pathologist, Institute of Pacific Islands Forestry, Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Honolulu, HI 96813

ABSTRACT

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A rapid wilt and dying of *Pinus taeda* and *P. elliottii* var. *elliottii* in Hawaii was found to be caused by *Botryosphaeria dothidea*. Infection took place through the roots. This is believed to be the first report of this infection pathway for *B. dothidea*. Tree mortality was apparently enhanced by a severe drought and overstocking.

In 1960–1965, about 300 ha of slash (*Pinus elliottii* Engelm. var. *elliottii*) and loblolly (*P. taeda* L.) pines were planted on the tops of several ridges on the northwestern portion of the Hawaiian island of Kauai. Trees in these plantations grew well until about 1976, when a few dying trees were observed. By 1977, extensive mortality was occurring and studies were initiated to determine the cause of the problem. This paper describes the results of those studies.

SYMPTOMS

Mortality occurred in groups of two or three to as many as 100 or more trees. The first noticeable symptom was slight yellowing of the upper portion of the crown, followed by yellowing of the entire crown. The crown then rapidly changed to brick-red and death occurred only a few weeks after the onset of symptoms. Removal of bark from the root collar of trees with even the earliest crown symptoms revealed wedge-shaped areas of dead cambium pointed upward (Fig. 1). Beneath the dead cambium were blue-stained areas that penetrated the stem in pie-shaped sectors almost to the pith. The stain pattern in cross section at the base of the stem indicated that the blue-stained areas extended upward from major lateral roots. When most of these roots were affected, the cambium around the entire base of the tree was killed.

Root systems of several trees adjacent to dead or dying trees but showing no top symptoms were excavated and observations were made of roots and resin exudation. The major lateral roots and vertical secondary roots ("sinkers") were severed, removed, split, and examined for internal symptoms. Several large living roots were found with isolated areas of blue stain that apparently originated in small dead roots, which were usually resin-soaked and stained. Isolated patches of stain were seen in roots as deep as 30 cm in the soil.

After the crown turned red, the stain moved rapidly up the stem to within the crown. Concomitantly, numerous dense stromatic masses of black mycelium developed between bark scales along the trunk. These masses later developed pycnidial cavities.

Although the root excavations occasionally indicated what appeared to be root contact infection between two adjacent trees, the general pattern of mortality did not appear to be one of radial spread from discrete loci. Many adjacent trees often had symptoms in the same stage of development, indicating more or less simultaneous infection.

ISOLATIONS

Roots of various sizes showing resin exudation and/or blue stain were selected from trees with slight or no top symptoms. The roots were washed to remove soil and loose bark. They were then carefully split and small cores removed aseptically with a Swedish increment hammer at regular intervals across the cut surface of one-half of each root. The cores were placed on water, malt extract, or potato-dextrose agar and incubated at room temperature for 2 wk. Isolations using the same procedure were also made from split stem sections taken from the blue-stained areas in the vicinity of the root collar. Finally, cultures were made from the black stromatic masses in the bark scales and from single conidia taken from locules in the stromata.

Fig. 1. Wedge-shaped areas of blue stain in wood at base of tree infected by *Botryosphaeria dothidea*.

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A black fast-growing fungus was recovered from more than 70% of cores from roots that showed staining, resin exudation, or both and from more than 90% of the cores taken from stem sections. The same fungus was also isolated from the masses of stromatic tissue from bark and from single conidia.

Morphological and cultural characteristics of the fungus indicated it was the *Dothiorella* state of *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & de Not. (= *B. ribis* Gross. & Dug.). This identification was confirmed by Dr. Brian Sutton of the Commonwealth Mycological Institute. The perfect state of the fungus was not seen on infected trees nor was it produced in culture.

INOCULATIONS

Preliminary inoculations with *Dothiorella* were made in June 1978. The fungus was grown on sterilized pine sawdust in Erlenmeyer flasks for 1 mo. Two lateral roots 6–8 cm in diameter on each of six 18-yr-old trees were carefully uncovered about 30 cm from the root collar. A small area was cleaned with 75% alcohol and a 1-cm-diameter hole was drilled about 3 cm deep with a sterilized drill. The hole was filled with sawdust inoculum, immediately sealed with melted paraffin, and re-covered with soil. After 2 mo, the roots were dug and split lengthwise through the point of inoculation. The split roots were examined for staining and resin infiltration and isolations made as described earlier.

Resin infiltration occurred in all roots and was present 8–20 cm proximally and distally from the point of inoculation. The extent of staining was difficult to determine because of the heavy resin infiltration, but the cambium was killed and *Dothiorella* was isolated from all roots 6–15 cm (avg. 13 cm) on both sides of the point of inoculation.

In November 1978, two roots on each of 15 18-yr-old trees were inoculated in a similar manner and an additional root on each tree was inoculated with sterile sawdust as a control. A single inoculation was also made on each stem just above the root collar with a control on the opposite side of the tree. After 7 wk, observations and isolations were made as described previously. At this time, the control roots showed resin infiltration about 3 cm on each side of the point of inoculation. Fourteen of the 30 roots inoculated with *Dothiorella* showed a similar condition and the fungus could be recovered only immediately adjacent to the point of inoculation. The other 16 roots had resin infiltration for a distance of 6–20 cm (avg. 8.2 cm) and *Dothiorella* was reisolated within 1 cm of the limit of resin infiltration in most cases. The fungus was isolated only from immediately around points of inoculation on root collars.

DISCUSSION

B. dothidea is a cosmopolitan fungus with a wide host range. It was first reported in 1929 from Hawaii (4), where it was found on 14 species of plants belonging to 13 different genera. No mention was made of its possible pathogenic relationship with any of the hosts. Kliejunas (2) reported the *Dothiorella* state of *B. dothidea* associated with a bleeding canker of *Araucaria heterophylla* (Salis.) Franco on the island of Hawaii and typical symptoms were reproduced by inoculation through wounds. The *Dothiorella* state was found fruiting on naturally occurring cankers but the perfect state was not seen.

B. dothidea commonly causes basal and trunk cankers on *Casuarina* spp. on the island of Oahu, and on several occasions, it has been found associated with girdling lesions on young plants of *Eucalyptus saligna* Sm. and *E. globulus* Labill. on the island of Hawaii (*unpublished*). Both the perfect and imperfect states are commonly produced on these hosts.

Under the imperfect state name of *Fusicoccum tingens* Goid., *B. dothidea* was reported to cause dieback of several *Pinus* spp., including slash pine, in East Africa (1). The fungus produced a dark stain in the wood and pycnidial stromata in the bark similar to those found in Hawaii.

B. dothidea as a pathogen is generally associated with branch and trunk cankers and is not believed to have been reported previously to infect roots directly from the soil as apparently occurred in this case. The Hawaiian situation is almost identical to one recently reported from South Africa (5), in which *Diplodia pinea* (Desm.) Kickx. was found to cause a root disease of *Pinus* spp. This fungus is also usually associated with diseases of stems, twigs, or buds but was never previously reported to cause a root disease of older trees. The description and illustrations of the symptoms and mode of infection in South Africa were identical to those given for the Hawaiian problem.

The average annual rainfall for the past 14 yr in the area of pine mortality was 91.2 cm. This is considered near the lower limit of precipitation for commercial forest land classification in Hawaii and is well below the normal precipitation rate in the natural ranges of loblolly and slash pines. An analysis of the yearly rainfall patterns since 1968 showed that in 1975, the total was only 66.2 cm and the monthly rainfall from April to October averaged only 0.9 cm compared with a normal 3.6 cm. Average rainfall for 1976, 1977, and 1978 was also below normal. Since 1979, rainfall has been above normal. A year of rainfall considerably below normal (68.5 cm) also occurred in 1970, but during that year, no long period of low rainfall occurred and 1971 rainfall was considerably above normal. The trees at that time also were much smaller and competition was less than in 1975–1977.

The onset of the problem appeared to coincide with low rainfall (27% below normal) in 1975. Mortality was first noted in 1976 and reached a maximum in 1977. Since 1979, rainfall has been above normal, and currently, there is only a scattering of dying trees except in one location where rainfall is believed to be lower; however, no measurements are available.

Association of *B. dothidea* with drought-induced stress is consistent with other reports of this fungus on pine (1) and other species (3). With *Cornus stolonifera* Michx. and *Liquidambar styraciflua* L., for example, stem water potentials greater than −12 bars increased the extent of cankers induced by inoculation with *B. dothidea* (3). No cankers were formed at less than −12 bars. Cankers ceased to expand within 1 wk after stem turgidity was restored by watering, indicating that increased susceptibility after exposure to stress was reversible. This is similar to observations made in this study, where inoculations in pine roots were most successful during the summer when rainfall was low and where mortality in plantations ceased with the return of normal rainfall.

The trees were under additional stress because of overstocking of the stands. Examination of stem cross sections showed that radial growth had been steadily decreasing for several years, indicating a need for thinning. Thinning is recommended as a means for decreasing competition for water during future drought periods.

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