Root Decay Caused by Kretzschmaria clavus: Its Relation to Macadamia Decline

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ABSTRACT

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Colonies similar to those originating from ascospores of *Kretzschmaria clavus* were isolated consistently from advancing margins of decay in macadamia roots. Like naturally infected roots, tissues were decayed and black lines were formed when healthy roots were inoculated with root tissues colonized by *K. clavus* originating from both decayed tissue and ascospores. The pathogen was reisolated from diseased tissues of artificially inoculated roots. At Hilo and vicinity more than 80% of declining macadamia trees had extensive root decay caused by *K. clavus*. Severity of tree decline was positively correlated with amount of root decay.

Additional key words: *Macadamia integrifolia*, Xylariaceae.

Macadamia (*Macadamia integrifolia* Maiden & Betche) is a commercially important crop in Hawaii. The macadamia industry has expanded rapidly in recent years. Unthrifty appearance of trees followed by defoliation and death (Fig. 1-A) was noticed in macadamia orchards more than 10 years ago. Such decline of macadamia trees was thought to be due to deficiency of certain essential elements (6). It was observed recently that declining trees occasionally fell to the ground, even when there was no strong wind. Most of their roots were decayed and all decayed roots had black lines which consisted of pigmented fungal hyphae (Fig. 1-B, C). The decay extended into the main trunk. We report here the isolation of the pathogen and its relation to macadamia decline. A brief account of this work has been published (3).

MATERIALS AND METHODS

Isolation and inoculation.—Discolored root and trunk tissue samples (ca. 20 × 10 × 4 mm) taken immediately adjacent to healthy tissues were surface-sterilized with 1% sodium hypochlorite solution for 5 minutes, rinsed with sterile distilled water, and plated on acidified potato-dextrose agar (100 ml agar plus 0.3 ml of 50% lactic acid). Four pieces of tisses were plated on each of ten plates and incubated at 24 C. Because of the slow growth of the pathogen, tissues and the surrounding nonpathogenic fungi on agar were discarded if they appeared during the first 3 days of incubation at 24 C.

Stromata produced on the surfaces of diseased roots and trunks (Fig. 1-D) were collected from the field. Ascospores were released by crushing the brittle stromata in water with a glass rod. The spore suspension was filtered through two layers of cheesecloth and centrifuged at 3,000 *g* for 5 minutes. After the supernatant liquid was discarded, spores were surface-sterilized with 0.8% sodium hypochlorite solution for 5 minutes and rinsed three times with sterile distilled water by centrifugation. Spores then were plated on acidified potato-dextrose agar.

Root segments (40×20 mm) and root chips (30×10×5 mm) in 500-ml wide-mouth bottles containing 15 ml of distilled water were autoclaved for 15 minutes, and then inoculated with the fungal cultures isolated from diseased tissue or with ascospores, and incubated at 24 C for 6 weeks. Roots (40- to 60-mm diameter) of 14-year-old healthy macadamia trees in the field were exposed by removal of aa lava rocks round the trunks. Root barks (ca. 30 × 100 mm) were removed with a chisel and replaced with colonized root tissues. The inoculum was

TABLE 1. Relationship between age of macadamia trees and severity of decline

Age of trees[a] (years)	Trees with dieback		
	Total (%)	Crown dieback <30% (%)	Crown dieback 30-100% (%)
3	0	0	0
6	9	2	7
19	81	63	18
25	87	55	32

[a]One hundred trees were surveyed for each age category.

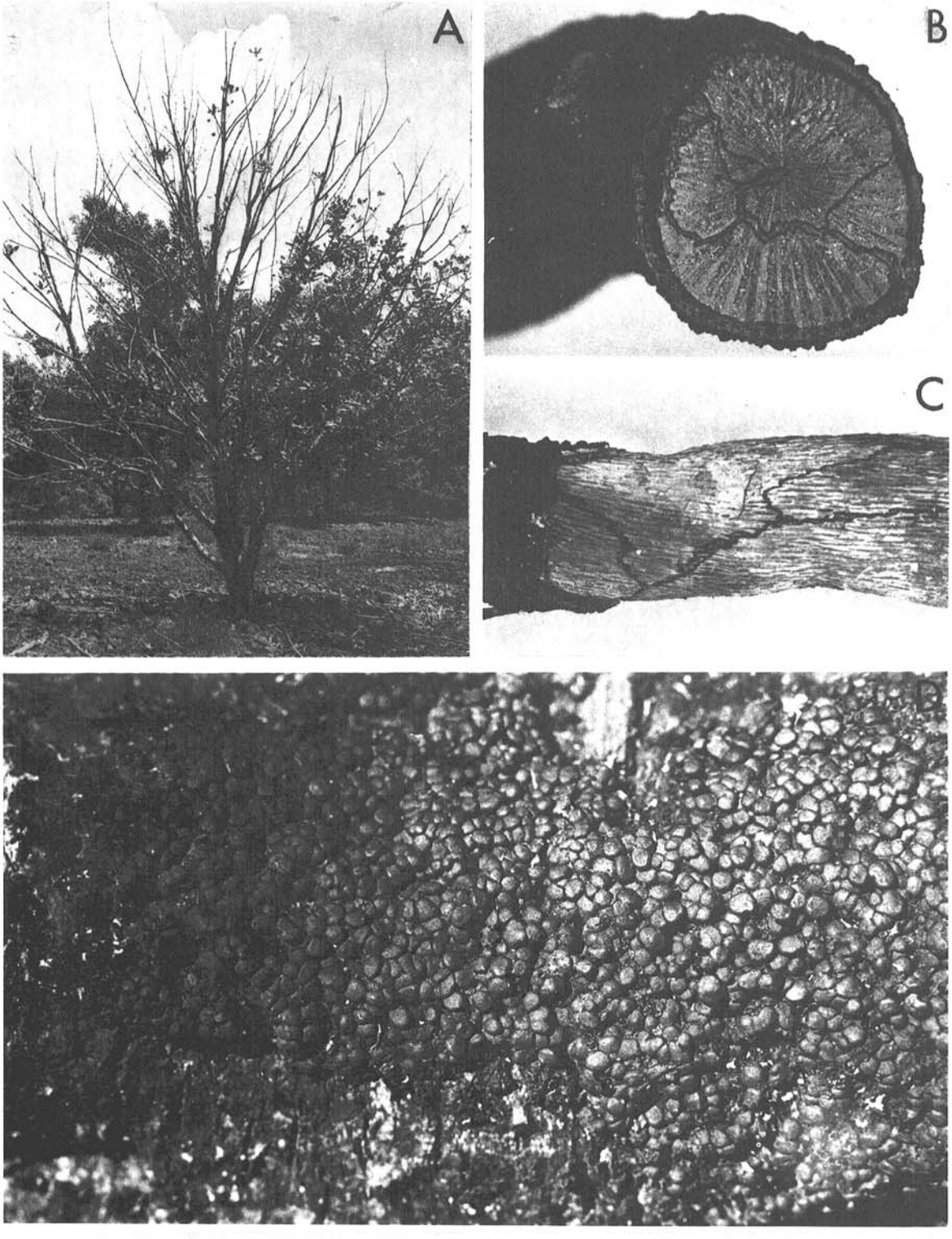

Fig. 1-A to D). A) A declining macadamia tree with most of its large roots decayed by *Kretzschmaria clavus*. The lower part of the trunk of this tree also is decayed by the fungus. **B)** Cross-section of the decayed macadamia root. Note the black lines which consist of pigmented *K. clavus* hyphae in the tissue. **C)** View of a decayed root with the bark removed. Black lines are visible on the surface of the wood tissue. **D)** Small mushroom-shaped carbonaceous stromata of *Kretzschmaria clavus* produced on the surface of diseased trunk. Figures 1-(B to D) are natural size.

covered with moistened paper towels, and bound to the root with vinyl flagging. Control roots were similarly treated, but with noncolonized root tissues. Data were taken 3-5 months after inoculation.

Survey.—To determine the relationship between age of trees and severity of decline, 100 trees per age class randomly selected at Keaau were surveyed by visual estimation for percentage of crown with dieback. Roots of 62 declining trees at Hilo and vicinity were exposed for study of the association of root decay with tree decline. The amount of root decay of 32 trees was studied in detail.

RESULTS

Pathogenicity tests.—A fungus with white mycelia on the margins of the colonies appeared from the edges of the diseased tissues after incubation for 5 days. The colonies became grayish black on the surface behind the advancing margin and developed blackish sterile stromatic aggregates below the surface. Colonies from ascospores were similar in appearance to those isolated from diseased tissues. The stromata (2-5 mm in diameter) were carbonaceous and mushroom-shaped (Fig. 1-D). Ascospores (8-13 μm $\times$ 23-38 μm) were navicular and light- to dark-brown. The fungus was identified as *Kretzschmaria clavus* (Fr.) Sacc. of the Xylariaceae by J. A. von Arx. The stromata were identical with those of *K. clavus* deposited in the herbaria of the National Fungus Collections, U. S. Department of Agriculture, Beltsville, Maryland.

In preliminary tests, neither naturally-decayed root tissues nor stromata of *K. clavus* collected from the field caused infection on roots of healthy macadamia trees. Successful inoculation was obtained only when artificially colonized tissues were used.

Roots of healthy macadamia trees became diseased 3 months after inoculation with root tissues colonized by *K. clavus*. Just as in naturally infected roots, black lines were formed in decayed root tissues. In one experiment five of eight roots inoculated with the isolate from diseased tissue and seven of 13 roots inoculated with that from an ascospore developed the disease. In another experiment, inoculation was successful in 10 of 30 cases for the former and nine of 22 for the latter. None of 12 control roots developed the disease. *Kretzschmaria clavus* was reisolated from artificially-inoculated diseased tissues.

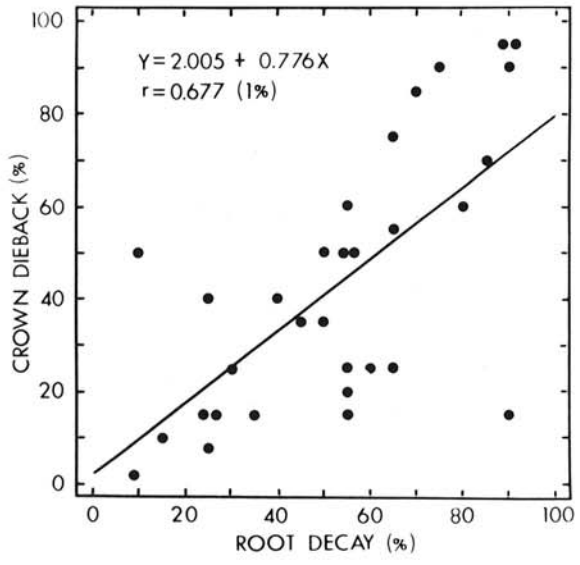

Fig. 2. Relation between severity of macadamia decline and the amount of roots decayed by *Kretzschmaria clavus*.

Survey.—Incidence and severity of macadamia decline were positively correlated with age of tree (Table 1). All 3-year-old trees that were surveyed were healthy. Percentages of trees with crown dieback were 9, 81, and 87% for 6, 19, and 25 years of age, respectively. The percentage of trees with 30-100% crown dieback were 7% for age 6, 18% for age 19, and 32% for age 25. On the island of Hawaii, root decay of macadamia caused by *K. clavus* was found only at Hilo and vicinity. Of 62 declining trees that were surveyed, 51 (82%) had root decay. The correlation between percentage of crown dieback and percentage of root decayed by *K. clavus* was highly significant (Fig. 2). When roots of five healthy appearing macadamia trees were exposed and examined, only one lateral root was decayed by *K. clavus*. Declining trees with root decay typical of that caused by *K.* clavus were not found at Honomalino, Kohala, Pahala, Kalopa, or Kona.

DISCUSSION

Phytophthora cinnamomi commonly is present in ohia (*Metrosideros collina* subsp. *polimorpha*) forests on the island of Hawaii (2). The fungus causes trunk canker (1, 5, 7) and rootlet necrosis (4) of macadamia. However, the number of macadamia trees with *Phytophthora* canker at any given time is very small and the disease is not associated with macadamia decline in Hawaii. Although *P. cinnamomi* was present in several orchards, the effect of infection of macadamia rootlets by that fungus on tree health was negligible (4).

Our data suggest that root decay caused by *K. clavus* is the major cause of macadamia decline at Hilo and vicinity. Most macadamia orchards in these areas were ohia forests previously. It is not known if this fungus exists in forests as a saprophyte or parasite. Efforts to locate stromata of *K. clavus* in an ohia forest near a macadamia orchard have been unsuccessful. The lack of declining trees with typical root decay caused by *K. clavus* in the other parts of the island also is unexplained.

Absence of decline among young macadamia trees may be related to slowness in growth of *K. clavus* and in disease development caused by the fungus. It also is possible that only mature trees are susceptible to *K. clavus*.

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