Cylindrocladium Root and Crown Rot of Alfalfa in Hawaii

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ABSTRACT

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Calonectria crotalariae (anamorph: *Cylindrocladium crotalariae*) and *Cylindrocladium clavatum* were consistently isolated from crown and root lesions of young alfalfa (*Medicago sativa*) plants. Retarded growth, chlorosis, and death of plants were characteristic of this disease. Black, slightly sunken lesions 2–10 mm in their greatest dimension often girdled the stems, leading to a rapid collapse and death of plants. Seedling damping-off was also associated with infection. The disease was reproduced by artificial inoculations with conidia from both fungi.

Additional key words: *Cylindrocladium floridanum, C. scoparium*, lucerne

During March 1981, serious losses of a large number of alfalfa (*Medicago sativa* L.) seedlings in experimental plots at Waimanalo, Oahu, and Kohala, HI, were observed. The diseased seedlings were stunted, and chlorotic plants collapsed before dying. Damping-off of seedlings shortly after emergence was also observed. Black sunken lesions 2–10 mm in size were observed on the roots and in the crown area. Girdling appeared to be associated with the rapid collapse of aerial portions of the plant. *Cylindrocladium* spp. were isolated in very high frequencies from the necrotic tissues.

Although *Cylindrocladium* spp. have been reported as pathogens of numerous plants in the Leguminosae, this appears to be the first report of *Cylindrocladium* causing root or crown rot of alfalfa in the United States.

MATERIALS AND METHODS

Isolation and identification. Necrotic tissue from diseased or killed plants was washed, excised, and rinsed for 20 min under running water; it was then dipped into 0.5% sodium hypochlorite, blotted momentarily on paper towels, and plated on 2% water agar or a selective medium (8). Isolation plates were incubated at 25 ± 1 C for 48 hr. Hyphal-tip transfers were made to 10% V-8 juice agar (VJA) or acidified potato-dextrose agar. Stock cultures were maintained on VJA slants and on VJA disks in sterile distilled water at 25 ± 1 C.

Isolates used for this study were established from single-conidial transfers grown on VJA under continuous cool-white fluorescent illumination of 2,700 lux at 24 C. Isolate 849 of *Calonectria crotalariae* (Loos) Bell & Sobers (anamorph: *Cylindrocladium crotalariae* (Loos) Bell & Sobers) and isolate 850 of *Cylindrocladium clavatum* Hodges & May were grown on VJA for 5 and 10 days, respectively, and examined for morphological characteristics. Ascospores of isolate 849 were examined after 15 days.

Pathogenicity trials. The trials were conducted on alfalfa cultivar Ranger sown in sterilized potting medium containing three parts soil, one part vermiculite, and one part peat moss (v/v). Thirty seeds were planted in 225-cc pots. Three days after sowing, plants were thinned to 20 per pot. Two days thereafter, plants were recounted and any late germinating seeds were removed. Each pot of seedlings was then inoculated by drenching the soil with 5 ml of a conidial suspension containing 10^4 conidia per milliliter. Conidia were produced on VJA as described above. Inoculated plants were not watered for 24 hr and were maintained in the greenhouse throughout the test. After 18 days, plants were counted, examined, and assayed for *Calonectria crotalariae*; plants inoculated with *Cylindrocladium clavatum* were similarly examined. Each test consisted of 10 pots per isolate and was done three times. Ten uninoculated pots served as controls for each test.

RESULTS

Isolation and identification. Initial sampling of diseased plants at Waimanalo yielded only *Cylindrocladium clavatum* and *Calonectria crotalariae*. Subsequent sampling of 60 more plants collected at three different times and representing nine sites in the field yielded 45% *Calonectria crotalariae*, 40% *Cylindrocladium clavatum*, and 2% *C. scoparium* Morgan. Other fungi isolated at Waimanalo included *Rhizoctonia solani* Kühn, *Rhizoctonia* sp. (binucleate vegetative mycelium), *Fusarium* spp., *Sclerotium* sp., and *Pythium* sp.

In the 85 diseased plants assayed from Kohala, *Calonectria crotalariae* was isolated from 85% of the plants, whereas *Cylindrocladium floridanum* Sobers & Seymour and *C. scoparium* were isolated from 6 and 4% of the plants, respectively. All three species were isolated from black, necrotic root lesions, brown root rots, and necrotic crowns. *Cylindrocladium clavatum* was not found at Kohala, although more than 100 subcultures of *Cylindrocladium* sp. were established from isolation plates and identified. Other fungi isolated at Kohala included *R. solani, Rhizoctonia* sp. (binucleate vegetative mycelium), *Mycoleptodiscus* sp., *Pythium* spp., and *Fusarium* spp.

Isolate 849 of *Calonectria crotalariae* was characterized by one- to three-septate, straight, cylindric conidia averaging 70.8 μm in length and 7.45 μm in width. Stipes averaged 294 μm in length and terminated in globose vesicles 12.9 μm in diameter. Ascospores were one to three septate, averaging 44.5 μm in length and 5.0 μm in diameter.

Isolate 850 of *Cylindrocladium clavatum* produced one-septate, cylindric conidia, averaging 51.5 μm in length and 5.1 μm in width. Stipes averaged 174.2 μm long and terminated in a clavate vesicle, 4.2 μm wide and 37.4 μm long. Perithecia were not observed.

Pathogenicity trials. Both *Calonectria crotalariae* and *Cylindrocladium clavatum* were pathogenic to alfalfa seedlings, and symptoms on inoculated plants were similar to those observed in the field during the first 3 wk of growth. Infection commonly began on the hypocotyl as slightly depressed lesions. Necroses starting in these areas extended to the cotyledons, killing the seedling. Roots were also infected, and necrotic tissue was frequently dark brown to black.

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All seedlings inoculated with conidia of *Calonectria crotalariae* were infected after 18 days, with an average of 60% of the seedlings killed in three replicates (range, 52–65%). *Cylindrocladium clavatum* was less virulent, killing an average of 15% of the seedlings in three replicates. Both fungi were reisolated from 10 plants randomly selected from each replicate. Control plants remained healthy.

DISCUSSION

Cylindrocladium root and crown rot of alfalfa in Hawaii was caused by *Cylindrocladium clavatum* and *Calonectria crotalariae* and appeared to involve two other *Cylindrocladium* spp., *C. scoparium* and *C. floridanum*. This disease may pose significant limitations to the establishment of alfalfa in the tropics and subtropics.

Calonectria crotalariae is apparently widespread in Hawaii with a wide host range, causing collar and root rot of *Carica papaya* L. (Caricaceae) (11), *Acacia koa* L. (Leguminosae) (1), and *Leea coccinea* Planch (Leeaceae) (9). In unpublished studies (J. Y. Uchida and M. Aragaki), the fungus caused severe diseases on *Dendrobium* sp. (Orchidaceae), *Phalaenopsis* sp. (Orchidaceae), *Anthurium andraeanum* Linden ex André (Araceae), *Cissus rhombifolia* Vahl (Vitaceae), and *Schefflera arboricola* Hayata (Araliaceae). Other important diseases caused by *C. crotalariae* are black root rots of soybean (*Glycine max* (L.) Merr.) and peanut (*Arachis hypogaea* L.) in the southeastern United States (6,14–16) and Japan (10). *Cylindrocladium clavatum* causes diseases on peanut and soybean (3). Soybean and peanut are also susceptible to *Cylindrocladium scoparium* and *C. floridanum* (16).

The susceptibility of alfalfa to *Cylindrocladium scoparium* was previously demonstrated in artificial inoculations (2,5); this susceptibility to *C. scoparium* has been useful in the detection of propagules in soil by seedling infection bioassay (4,17). An alfalfa seedling bioassay to detect *Cylindrocladium clavatum* in stands of *Pinus* sp. has also been useful (C. S. Hodges, *personal communication*). Naturally occurring leaf spot and stalk blight of alfalfa attributed to *C. scoparium* was first recorded from India (13). However, the species identification of the pathogen is questionable because these authors describe the conidia as having two to four septa instead of being uniseptate. A root and crown rot disease of alfalfa caused by a fungus tentatively identified as *Calonectria theae* Loos was also previously reported from India (7). The occurrence and pathogenicity of *C. theae* on *A. koa* (Leguminosae) have been documented in Hawaii (12), but the fungus was not found on alfalfa in present studies.

This report is apparently the first record of *Calonectria crotalariae* and *Cylindrocladium clavatum* on alfalfa, and for *C. clavatum* this appears to be the first record of the fungus in the United States.

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