Root Rot of Ladino Clover Induced by *Codinaea fertilis*

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ABSTRACT

CAMPBELL, C. L. 1980. Root rot of ladino clover induced by *Codinaea fertilis*. Plant Disease 64: 959-960.

Codinaea fertilis was consistently isolated from roots of ladino clover 'Tillman' in Wake County, NC. In 10 samples from four locations, *C. fertilis* comprised 6.1–29.1% of isolates obtained from clover roots. Isolates of *C. fertilis* were grown on cornmeal and sand medium and incorporated into pasteurized or field soil in two greenhouse studies. A light to dark brown surface necrosis developed on many tap roots and fibrous roots within 9 wk of transplantation into infested soil. *C. fertilis* was consistently reisolated from symptomatic roots and from some healthy roots but was not isolated from plants grown in noninfested soil. *C. fertilis* has not previously been reported in the United States.

Ladino clover (*Trifolium repens* L.) is the most important perennial legume in North Carolina legume-grass pastures. Stands usually decline rapidly 2–3 yr after seeding. Poor persistence results from root system degeneration, which has been associated with disease, insect damage, adverse environmental conditions, and management practices (1–4).

Although many fungi have been reported to induce root rot on ladino clover, *Fusarium* spp. predominate. In an assay conducted during the 1979 growing season, *Codinaea fertilis* Hughes & Kendrick was consistently isolated from ladino clover roots with typical root rot symptoms. *C. fertilis* has previously been isolated in North Carolina from ladino clover (L. T. Lucas, *personal communication*) and red clover (W. A. Cope and R. E. Welty, *unpublished*). Although *C. fertilis* has been reported to cause a root rot of white clover on North Island, New Zealand (5), it has not been implicated as a pathogen in the United States. This paper reports the occurrence of similar symptoms associated with *C. fertilis* on ladino clover in North Carolina (NC) and describes procedures for isolation, inoculum production, and pathogenicity tests of NC isolates of this fungus.

MATERIALS AND METHODS

Isolate collection. Samples were taken at irregular intervals throughout the 1979 growing season from tall fescue (*Festuca arundinacea* Schreb.)-ladino clover and pure ladino clover plantings on research farms in Wake County, NC. Samples consisted of all plants and soil in an area 10–20 cm in diameter and 15–20 cm deep. Clover plants were washed from samples in running tap water, and roots were surface-disinfested in 0.5% sodium hypochlorite solution for 2–3 min. Subsamples of roots (5–15 mm sections) were taken without regard to visual symptoms, placed on 2% water agar in petri dishes, and incubated 4–8 days at 23–25 C. Hyphae that grew from root subsamples were transferred to culture tubes containing potato-dextrose agar (PDA). Fungi were identified after 14–21 days on PDA.

Greenhouse pathogenicity tests. Inoculum of *C. fertilis* was prepared by growing fungus on a cornmeal and sand medium (CSM). The medium was prepared by mixing 57 g of silica sand and 2.8 g of cornmeal in a 9-cm petri plate, adding 16 ml of distilled water, and autoclaving for 20–25 min at 20 lb pressure. A small piece (4–5 mm diameter) of *C. fertilis* mycelium from an actively growing PDA culture was placed on the cooled CSM in a petri dish and incubated at room temperature for 21 days with 12 hr supplemental fluorescent light daily. The CSM in each plate was stirred with a sterile glass rod 14 days after inoculation to enhance even colonization. Twenty-four different isolates were used.

C. fertilis was mixed for 3–5 min with a mixture of steamed sand and sandy loam soil (1:3 v/v) to give an inoculum to sand-soil mixture ratio of 1:6 v/v. The infested soil mixture was placed in 10.2-cm-diameter clay pots, and two 3-mo-old ladino clover 'Tillman' seedlings were transplanted into each pot. Enough inoculum was prepared for one pot for each of 17 isolates and two pots for each of the other seven isolates. Ten control pots were prepared as above, except the CSM-*C. fertilis* plates were autoclaved before incorporation.

Plants were placed in a greenhouse at 23–30 C. Nine weeks after transplantation, plants were removed from the soil mixture by washing under running tap water. Roots were rated as healthy or diseased.

Similar procedures were followed in a second greenhouse study, except 1) 10 isolates of *C. fertilis* were used; 2) three to five pots of infested soil mixture were prepared with steamed soil for each isolate; 3) three to five pots of infested soil mixture were prepared with natural, sandy loam field soil for each isolate; and 4) 20 pots each of steamed and field soil mixture were included as controls.

RESULTS

In 10 samples from four locations in Wake County, NC, *C. fertilis* comprised 6.1–29.1% of isolates obtained from

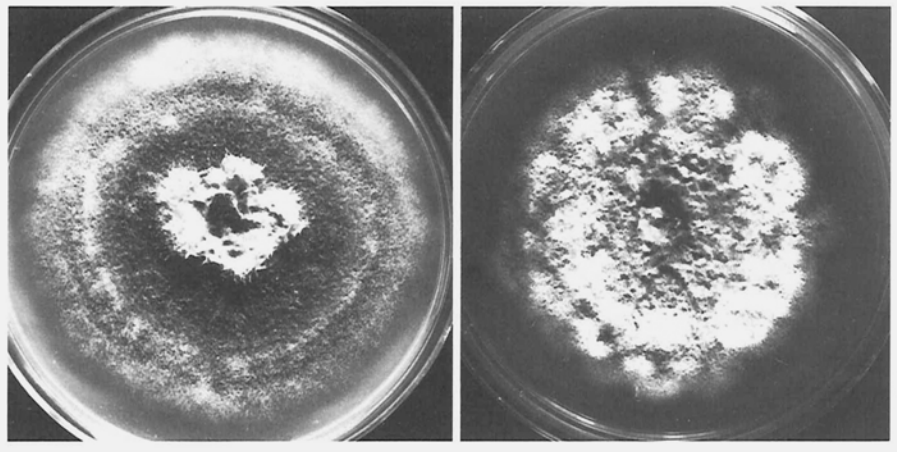

Fig. 1. Colony morphology of *Codinaea fertilis* on potato-dextrose agar after 24 days at 28 C.

Paper 6461 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh.

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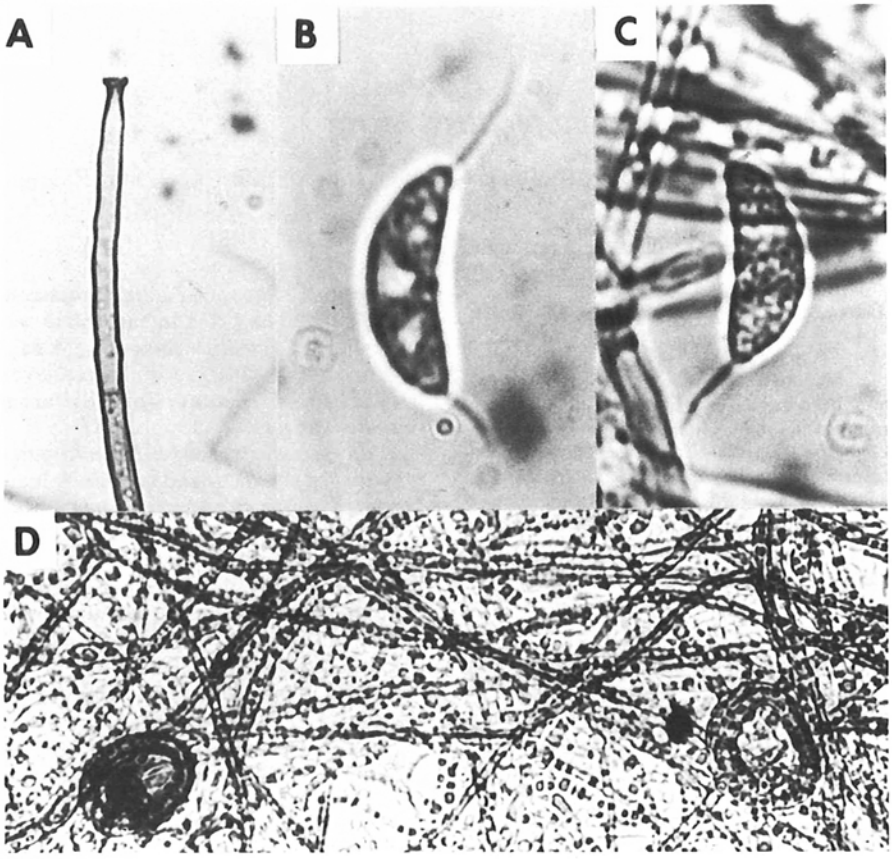

Fig. 2. *Codinaea fertilis*: **(A)** conidiophore with collarette (× 960); **(B)** and **(C)** phialospores with setulae (× 2,400); and **(D)** mycelium with typical rings (× 960).

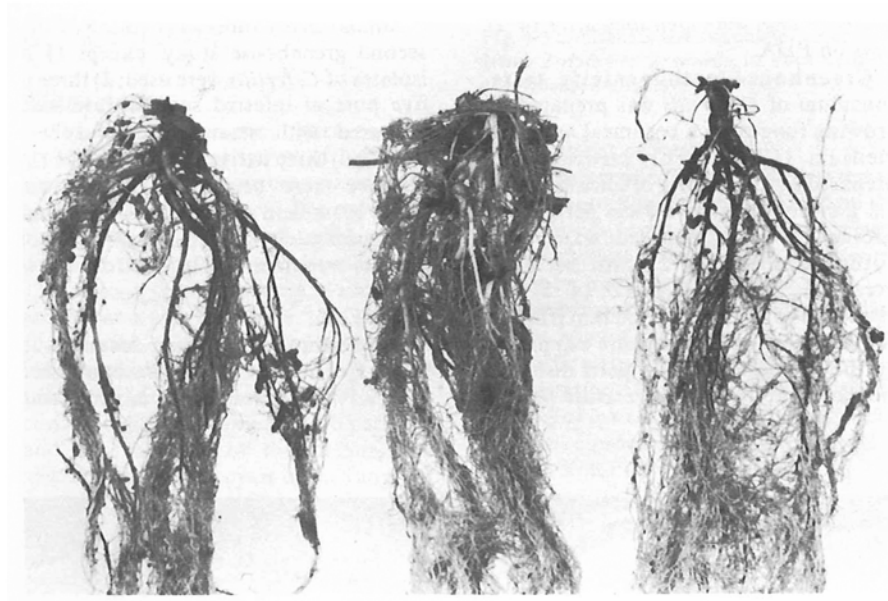

Fig. 3. Root systems of ladino clover 'Tillman' from soil infested with *Codinaea fertilis* (left and right) and from control soil (center). Note characteristic discoloration (darkening) of tissue in roots from infested soil.

clover roots. Other fungi isolated, in order of decreasing frequency, were *Fusarium oxysporum* (Schlecht.) Snyd. & Hans., *F. solani* (Mart.) Appel & Wr., *Rhizoctonia solani* Kühn, binucleate *Rhizoctonia*-like fungi, *F. roseum* Lk. emend. Snyd. & Hans., *Gliocladium* sp., *Trichoderma* sp., *Pythium* sp., *Nigrospora* sp., *Alternaria* sp., *Curvularia* sp., *Colletotrichum* sp., and *Cladosporium* sp.

Colony morphology of *C. fertilis* on PDA is shown in Fig. 1. A typical conidiophore with collarette and typical phialospores with setulae are presented in Fig. 2A and 2B–C, respectively. Hyphal rings observed frequently in cultures of *C. fertilis* on PDA are presented in Fig. 2D. Conidiophore, phialospore, and setula measurements for NC isolates of *C. fertilis* grown on PDA were similar to those obtained by Menzies (5). Setae were not distinguishable from conidiophores in NC isolates, and ropelike aggregates of hyphae were frequently observed in colonies growing on PDA. Single-spore cultures of *C. fertilis* isolates from clover roots in North Carolina have been deposited in the American Type Culture Collection.

Root rot symptoms were present in 81, 58, and 51% of plants grown in infested steamed soil in experiments 1 and 2 and infested natural soil in experiment 2, respectively. Light to dark brown surface discoloration of tap roots and fibrous roots was the most common symptom. In several cases, tap roots were rotted away and fibrous roots were often necrotic. Distinct lesions were not observed, but relatively large areas of root surfaces were discolored (Fig. 3). *C. fertilis* was consistently reisolated from symptomatic roots and from some roots without symptoms from infested soil but was not reisolated from roots of plants grown in noninfested soil.

DISCUSSION

Decline of ladino clover in clover-grass pastures has been associated with interactions among diseases, insects, and environment (1–4). *C. fertilis* has not previously been reported as a component in this complex in the United States. *C. fertilis* was readily isolated from roots of field-grown ladino clover and from roots with rot symptoms induced in pasteurized and field soil in the greenhouse. The proportion of ladino clover decline attributable to root rot induced by *C. fertilis* is not known and probably varies in relation to other factors. Root infection by *C. fertilis* may be favored by insect damage and is probably influenced to a large extent by genetic variability in ladino clover populations. *C. fertilis* infection may also predispose roots to infection by other fungi. The means of survival and dispersal of this pathogen are not known.

ACKNOWLEDGMENTS

I wish to thank Charles R. Harper for his valuable technical assistance and Marvin Williams for preparing the photographs.

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