Cultural Characteristics and Host Range of *Codinaea fertilis*

C. Lee Campbell

Assistant professor, Department of Plant Pathology, North Carolina State University, Raleigh 27650.
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ABSTRACT

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Optimum temperature for radial growth of *Codinaea fertilis* isolates ranged from 24 to 28 C on potato-dextrose agar (PDA). On 13 agar media, radial growth rate among four isolates differed significantly at 28 C; growth on PDA was the most rapid and produced the most dense mycelial growth. Light did not affect the rate of radial mycelial growth on PDA at 23–24 C; however, continuous fluorescent light generally enhanced sporulation (10 to 10^6 times greater per petri plate) compared to cultures grown in continuous darkness on potato-dextrose, V-8 juice, distilled water, oatmeal, and Czapek-dox agar media. In greenhouse tests, *C. fertilis* induced root rot in Persian clover, arrowleaf clover, subterranean clover, red clover, crimson clover, white clover, alsike clover, white sweet clover, hop clover, hairy vetch, crown vetch, alfalfa, Korean lespedeza, Kobe striate lespedeza, corn, snap bean, soybean, and garden pea. *C. fertilis* was isolated from roots of these plants except crown vetch and corn 77 or 82 days after planting into infested soil. The fungus induced no visible symptoms on common bermudagrass, orchardgrass, tall fescue grass, or birdsfoot trefoil; of these, *C. fertilis* was isolated only from roots of tall fescue grass.

Additional key words: clover root rot, forage legumes, pathogenicity.

Codinaea fertilis Hughes and Kendrick induces a root rot of ladino clover (*Trifolium repens* L.) (1,6,7) and has also been isolated from red clover (*T. pratense* L.) (7; W. A. Cope and R. E. Welty, *unpublished*), subterranean clover (*T. subterraneum* L.), and alfalfa (*Medicago sativa* L.) (7). Isolates of *C. fertilis* obtained from ladino clover in North Carolina (NC) are morphologically similar to those obtained by Menzies (6) in New Zealand (NZ) and correspond with the description given by Hughes and Kendrick (3). Menzies (7) examined the effect of temperature on the growth of NZ isolates on potato-dextrose agar, but additional information is unavailable concerning the in vitro growth characteristics of *C. fertilis*. The pathogenicity of *C. fertilis* on hosts other than white clover has not been evaluated.

The present study was undertaken to determine the effects of

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medium, temperature, and light on growth and sporulation of isolates of *C. fertilis* and to determine the susceptibility of selected plants to this pathogen.

MATERIALS AND METHODS

Isolates of *C. fertilis*. Cultures of *C. fertilis* were isolated on water agar from roots of ladino clover plants growing in a mixture of clover and tall fescue grass (*Festuca arundinacea* Schreb.) in Wake County, NC. Cultures were maintained on potato-dextrose agar (PDA) slants at 4–8 C. Pathogenicity of each isolate of *C. fertilis* used in this study on ladino clover was previously established (1).

Effect of temperature on fungal growth. Plugs (4–5 mm in diameter) from an actively growing culture of *C. fertilis* on PDA were placed "right side"-up in the center of petri dishes containing 25 ml of PDA. Petri dishes were enclosed in plastic bags and incubated in the dark at 4, 8, 12, 16, 20, 24, 28, 32, and 36 C. Four isolates and four replications per treatment were used. Daily radial growth rate at each temperature was calculated as an overall average from two perpendicular colony diameter measurements made every 2 days for 10 days.

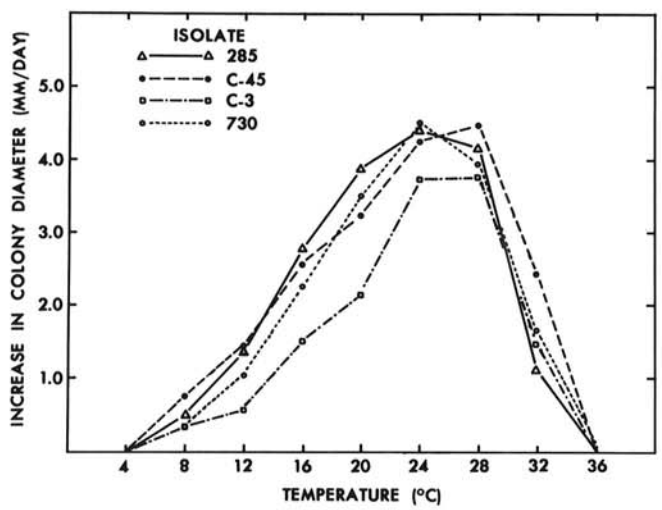

Fig. 1. Growth rates of four isolates of *Codinaea fertilis* on potato-dextrose agar at nine temperatures in the dark. Data are the average of four replications.

TABLE 1. Radial growth rate of *Codinaea fertilis* on 13 agar media at 28 C in the dark

Medium	Increase in colony diameter (mm/day)[y] of isolate: 730	C-45	C-3	280	Mean[z]
Czapek-dox	4.8	5.4	4.8	4.5	4.9 a
Soil extract	5.3	4.8	4.6	4.3	4.7 ab
Potato-dextrose	5.0	4.6	4.5	4.2	4.6 b
Cornmeal	4.9	4.1	3.8	3.8	4.2 c
Acidified potato-dextrose	4.3	4.2	4.2	4.1	4.2 cd
Oatmeal	4.6	4.1	3.9	3.8	4.1 cde
Beef-extract	4.4	4.1	4.3	3.5	4.1 cde
Lima bean	4.5	4.2	3.9	3.7	4.1 cde
Potato dextrose + yeast extract	4.2	4.1	4.1	3.9	4.1 cde
Bean pod	4.4	4.5	4.0	2.8	3.9 de
Malt extract	4.0	4.4	3.4	3.8	3.9 e
Nutrient	3.7	3.6	3.3	3.1	3.4 f
Water	2.7	1.8	1.7	1.7	2.0 g
Mean[z]	4.4 a	4.3 b	3.9 c	3.6 d	

Coefficient of variation = 6.8%

[y] Each value is calculated as the overall average of two perpendicular colony diameter measurements taken at 2-day intervals for 10 days for each of three replications.

[z] Means with the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

Effect of medium on fungal growth. The following agar media were prepared and 25 ml was dispensed into sterile petri dishes (85 mm in diameter): bean pod, beef extract, cornmeal, Czapek-dox, lima bean, malt-extract, nutrient, oatmeal, potato-dextrose, potato-dextrose acidified (1 ml 50% lactic acid per liter), potato-dextrose + 1 g of yeast extract per liter, soil extract, and distilled water (4). Purified agar (Difco-Bacto Agar, Detroit, MI 48232) was used in the preparation of all media. Plugs (4–5 mm diam) from an actively growing culture of *C. fertilis* on PDA were placed in the center of each petri dish. Four isolates of *C. fertilis* (280, 730, C-3, and C-45) were used in a completely randomized design with three replications. Petri dishes were enclosed in plastic bags and were placed in a 28 C constant-temperature, unlighted incubator. Daily radial growth rate on each medium was calculated as an overall average of two perpendicular colony diameter measurements made every 2 days for 10 days.

Effects of light on fungal growth and sporulation. Colonies of two *C. fertilis* isolates (C-45 and C-6) were established on PDA with eight replicates per treatment to determine the effect of light on growth. The effect of light on sporulation was evaluated by using the same two isolates and Czapek-dox, oatmeal, potato-dextrose, V-8 juice, and distilled water agar media at 20–23 C with four replicates per treatment. Petri dishes were enclosed in clear plastic bags; darkness was insured by further placing petri dishes in two layers of brown paper sacks. Continuous light was from cool-white (high-output 96T12) fluorescent bulbs (average luminance 1.13 Cd cm^{-2}, measured incidence = 269 lux) 20 cm above petri dishes. Relative sporulation was determined by flooding colonies after 3 wk with 10 ml of distilled water plus one drop of Tween-20 per 100 ml and counting the number of phialospores in six aliquot samples per petri dish by means of a hemacytometer. Spore counts were averaged to a per petri dish basis.

Host range. Twenty-seven plant species or cultivars were tested for susceptibility to *C. fertilis* under greenhouse conditions. Inoculum was prepared on 5% cornmeal-sand medium as previously described (1), except the inoculum was grown in the dark at 28 C and was not stirred during incubation. *C. fertilis* inoculum was mixed for 3–5 min with a steamed sand and sandy loam soil mixture (1:3, v/v) to give a total inoculum to sand-soil mixture of 1:6 (v/v) in test i and 1:7 (v/v) in test ii. In test i, the soil mixture was infested with isolate 280 and C-45 as separate treatments; in test ii, isolates C-3 and C-45 were thoroughly mixed prior to soil infestation. Autoclaved inoculum was used for control treatments. Infested soil was placed in 10.2-cm-diameter clay pots. In test i, two 6-wk-old seedlings of each potential legume host grown in a steamed soil mixture were transplanted into each pot of infested and control soil. Grasses were direct-seeded. Two pots were established for each isolate × host combination and two control (uninoculated) pots were established for each host. In test ii, four pots of infested soil and one control pot were prepared for each host. Additionally, all plants were direct-seeded in test ii. All pots were placed in a greenhouse where daily mean temperatures ([minimum temperature + maximum temperature]/2) were 24–32 C.

Plants were grown in infested or control soil for 77 days in test i and 82 days in test ii. Plants then were removed from the soil mixture by washing under running tap water and the roots were rated for disease according to the following scale: 1 = apparently healthy, no discoloration of root tissue; 2 = discoloration present only on taproot (where present) or only on fibrous roots, less than 25% of root tissue discolored; 3 = discoloration present on taproot and fibrous roots, 25–50% root tissue discolored; 4 = extensive discoloration present on taproots and/or on many fibrous roots, greater than 50% of root tissue discolored and tissue often rotting away; and 5 = plant dead. Isolations were made on water agar from each host to assay for the presence of *C. fertilis*.

RESULTS AND DISCUSSION

Cultural characteristics. Radial growth rate (mm/day) of each of the four isolates of *C. fertilis* on PDA was greatest at 24–28 C (Fig. 1). These optimum temperatures for mycelial growth are similar to

the 26 C growth optimum reported for NZ isolates (6). Radial growth rate declined as temperature increased or decreased and no measureable growth occurred at 4 or 36 C. Growth of isolate C-3 was somewhat slower than the other three isolates at 28 C or below. The range at which growth occurred (8–32 C) includes temperatures outside the growth limits of 15.5 and 30.0 C reported for isolates of *C. fertilis* in NZ (6).

Growth rate was significantly ($P = 0.05$) different among the four isolates of *C. fertilis* on 13 agar media (Table 1), which indicates variability among isolates. Growth rate on the 13 agar media averaged over the four isolates also was significantly ($P = 0.05$) different. Czapek-dox, soil extract, and potato-dextrose agars produced the most rapid growth, while nutrient and water agars produced the slowest growth. Mycelial growth was least dense on water, soil extract, and Czapek-dox agars (Fig. 2; soil-extract agar not shown, but similar in mycelial density to water agar). Of the media tested, PDA was the most suitable for mycelial production by *C. fertilis* in vitro.

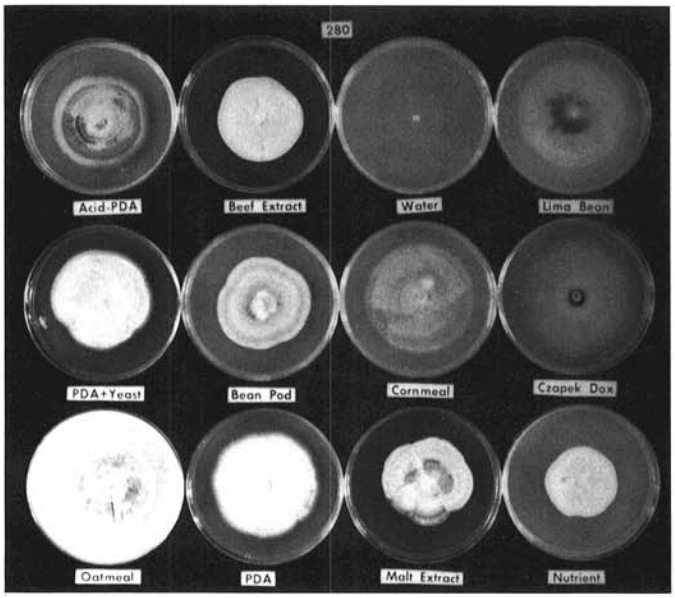

Fig. 2. Appearance of isolate 280 of *Codinaea fertilis* on 12 agar media after 12 days incubation at 28 C in the dark.

Fig. 3. Root rot symptoms (discoloration) induced by *Codinaea fertilis* on subterranean clover (*Trifolium subterraneum*) after 82 days in infested soil in the greenhouse. Healthy root systems were not discolored.

Growth of *C. fertilis* on agar media is slow relative to other fungi (*Fusarium* spp., *Rhizoctonia solani* Kühn, and Rhizoctonialike fungi) isolated from the roots of white clover. Addition of yeast extract to PDA did not enhance radial mycelial growth, but significantly ($P = 0.05$) decreased it compared with unamended PDA (Table 1). Growth rate of *C. fertilis* on acidified PDA was signficantly ($P = 0.05$) less than on unamended PDA (Table 1) which may indicate an effect of pH on growth of this fungus.

Continuous light or darkness did not affect the growth rate of *C.*

TABLE 2. Effect of light[x] on sporulation of two isolates of *Codinaea fertilis* on five agar media at 20–23 C

Isolate[z]		Average number of spores petri dish[y]				
		V-8	Potato-dextrose	Water	Oatmeal	Czapek-dox
C-6	Light	1.08×10^7	5.86×10^6	1.52×10^5	1.54×10^6	7.25×10^4
	Dark	2.61×10^5	1.75×10^4	2.33×10^4	8.46×10^5	0
C-45	Light	4.41×10^7	1.76×10^5	1.56×10^6	2.00×10^5	0
	Dark	5.50×10^4	1.88×10^4	0	6.25×10^3	0

[x] Light = 24 hr cool-white fluorescent light 20 cm above petri dishes enclosed in clear plastic bags; dark = petri dishes enclosed in clear plastic bags and then enclosed in two layers of brown paper.
[y] Values are the mean of six aqueous aliquot samples from each of four replicate petri dishes per treatment; an average of 90% of the total medium surface areas was covered by fungal growth in each treatment.
[z] Isolates derived from single-spore culture.

TABLE 3. Disease rating for greenhouse-grown plants grown in soil infested with *Codinaea fertilis*[y]

Plant	Disease rating[z]	
	Test i	Test ii
Germineae		
Cynodon dactylon (common bermudagrass)	1.0	1.0
Dactylis glomerata (orchardgrass)	1.0	1.0
Festuca arundinacea 'KY 31' (tall fescue)	1.0	1.0
Zea mays 'Pioneer 3369A' (corn)	···	2.0
Leguminosae		
Glycine max 'Lee' (soybean)	1.1	3.9
Lespedeza cuneata 'Common' Sericea lespedeza	1.0	1.5
Lespedeza stipulacea 'Yadkin' Korean lespedeza	2.8	
Lespedeza striata 'Common' Kobe striate lespedeza	1.6	2.7
Lotus corniculatus 'Viking' birdsfoot trefoil	1.0	1.0
Medicago lupulina hop clover (black medic)	2.0	1.3
M. sativa 'Apalachee' alfalfa	···	1.6
M. sativa 'Liberty' alfalfa	1.0	1.6
M. sativa 'NCS 18' alfalfa	1.1	···
M. sativa 'Weevil Check' alfalfa	1.0	···
Melilotus alba 'Floranna' white sweet clover	2.3	1.9
Phaseolus vulgaris 'Tendergreen' snap bean	3.5	3.1
Pisum sativum L. 'Alaska' garden pea	···	2.2
Trifolium hybridum alsike clover	2.4	2.1
T. incarnatum 'Common' crimson clover	3.0	2.4
T. pratense 'Kenland' red clover	3.2	1.6
T. repens 'Regal' white clover	1.9	1.9
T. repens 'Tillman' white clover	2.5	2.0
T. resupinatum Persian clover	3.4	2.8
T. subterraneum 'Mt. Barker' subterranean clover	3.3	3.1
T. vesiculosum 'Yuchi' arrowleaf clover	3.4	2.5
Coronilla varia 'Chemung' crown vetch	1.0	1.5
Vicia villosa hairy vetch	3.0	2.8

[y] In test i, two 6-wk-old seedlings of each potential host were transplanted into two pots each of infested and control soil and grown for 77 days; in test ii, four pots of infested soil and one control pot were direct-seeded with each potential host and grown for 82 days. Greenhouse temperatures ranged from 25 to 32 C. All plants in control pots were apparently healthy with no discoloration of root tissue.
[z] Rating scale: 1 = apparently healthy, no discoloration of root tissue; 2 = discoloration present only on taproot (where present) or only on fibrous roots; 3 = discoloration present on taproot and fibrous roots; 4 = extensive discoloration on tap root and many fibrous roots, tissue often rotting away; and 5 = plant dead.

fertilis on PDA; however, light did affect its sporulation (Table 2). With an average of 90% of the agar surface covered by fungal growth, number of spores produced by isolates C-6 and C-45 ranged from 10 to 10^6 times greater in light than in the dark, with the exception of C-45 on Czapek-dox agar which produced no spores in either light or dark (Table 2). Sporulation of both isolates was greatest on V-8 juice agar in the presence of light.

Host range. *C. fertilis* induced some root discoloration or rot on, and was isolated from 18 of 20 legume species or cultivars tested; no symptoms were induced on the roots of the pasture grass species tested (Table 3), although *C. fertilis* was recovered from tall fescue roots. Generally, less root rot was observed in the second test than in the first test.

C. fertilis induced symptoms in some hosts in test ii, but not in test i. Plants were exposed to inoculum beginning with seeding in test ii, whereas seedlings were transplanted in test i. This suggests that juvenile tissue in these hosts was susceptible, while more mature plant tissues were resistant. Symptoms on all legume hosts were similar and included light to dark brown surface discoloration of taproots and/or fibrous roots over large root areas. Based on visual evaluations, the stelar region of only subterranean clover in both tests and soybeans in test ii was symptomatic. The following legumes appeared to be most susceptible in greenhouse tests: snap bean, Persian clover, arrowleaf clover, subterranean clover, red clover, crimson clover, and hairy vetch. Others considered to be hosts were: Korean lespedeza, ladino white clover, alsike clover, white sweet clover, hop clover, alfalfa, Kobe striate lespedeza, soybeans, and garden pea. Common bermudagrass, orchardgrass, tall fescue, and birdsfoot trefoil lacked symptoms and were not considered to be hosts. Although some root discoloration was present on corn and crown vetch plants in test ii, *C. fertilis* could not be recovered from roots of these species. Corn and crown vetch probably are not hosts for *C. fertilis*; however, work should be undertaken to determine if a toxin might be produced in the inoculum-substrate mix that could induce such discoloration in these plants. The organism was isolated from all species considered as hosts, but was not recovered from control plants.

Root diseases, often of complex etiology, are important in yield reduction and lack of persistence of forage legumes (2,4,5). In this study, a preliminary host range of *C. fertilis* was established that includes many forage legumes in the southeastern United States. In relatively short greenhouse tests, the forage legumes varied in susceptibility to *C. fertilis* from very susceptible (eg, subterranean clover) to apparently not susceptible (eg, birdsfoot trefoil). The geographic range of *C. fertilis* should be determined and a quantitative assessment made of the damage induced by this pathogen under a wide range of field conditions.

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