Pathogenicity of *Ceratobasidium cornigerum* and Related Fungi Representing Five Anastomosis Groups

L. L. Burpee, P. L. Sanders, H. Cole, Jr., and R. T. Sherwood

Former research assistant; research associate, and professor, Department of Plant Pathology, The Pennsylvania State University, University Park, 16802; and research plant pathologist, SEA-USDA, U.S. Regional Pasture Research Laboratory, University Park, PA 16802. Present address of senior author: Bermuda Department of Agriculture and Fisheries, Hamilton, BDA.

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ABSTRACT

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Pathogenicity of 40 isolates of *Ceratobasidium cornigerum* and related binucleate *Rhizoctonia solani*-like fungi representing five Ceratobasidium anastomosis groups (CAG 1–5) was evaluated on eight plant species representing six families. Isolates in CAG 1 were pathogenic on gramineous hosts but nonpathogenic or only weakly virulent on species in other families. Isolates in CAG 2 were weakly virulent on pea and French bean seedlings and nonpathogenic on other species. Isolates from CAG 3, 4, and 5 exhibited a wide host range, were more virulent, and caused seed, root, and/or hypocotyl decay. Isolate × soil temperature interactions for disease development were observed in inoculations of wheat and pea seedlings.

At least 20 species of *Rhizoctonia* De Candolle have characteristics which conform to the current concept of *R. solani* Kuhn with the exception of possessing predominantly binucleate hyphal cells (8,9). The few isolates of these fungi that were induced to sporulate were identified as *Ceratobasidium* Rogers spp.

Burpee et al (4) assigned 71 isolates of *C. cornigerum* (Bourd.) Rogers and related fungi to seven Ceratobasidium anastomosis groups (CAG). All isolates in CAG 1 were collected from gramineous hosts, with the majority associated with turfgrasses exhibiting a foliar chlorosis and blight in the northeastern United States. Isolates in the remaining groups were collected from various plant species and geographic regions. Anastomosis group 2 (CAG 2) included isolates of *R. fragariae* Husain & McKeen and *R. ramicola* Weber and Roberts. Four isolates in CAG 2 were induced to sporulate and were identified as *C. cornigerum*.

Several species of binucleate *R. solani*-like fungi have been found associated with diseased plants. Isolates of *R. fragariae* (5) and *R. endophytica* Saksena & Vaartaja (10) cause losses among strawberry plants and seedling pines, respectively. Weber and Roberts (13) identified *R. ramicola* as a cause of silky thread blight of *Elaeagnus pungens* and other species. Isolates of binucleate *R. solani*-like fungi cause a cool-weather blight of creeping bentgrass and Kentucky bluegrass (11). D. K. Bell and D. R. Sumner (*personal communications*) isolated binucleate forms of *Rhizoctonia* from several crop species exhibiting seed, root, and/or hypocotyl decay. In contrast, other studies (Burpee, *unpublished*) indicate a lack of pathogenicity among soilborne isolates of *Ceratobasidium* spp. Similarly, Anderson (2) reported that isolates of *Ceratobasidium* spp. did not cause seedling blight of flax.

In view of the recent discovery of anastomosis groupings among isolates of binucleate *Rhizoctonia* spp. (4) and the general lack of information on their ability to induce disease, an organized assessment of pathogenicity and virulence of these organisms is needed. The present study was designed to evaluate the pathogenicity and host range of 40 binucleate isolates representing five anastomosis groups and to assess the reactions of four turfgrass species to inoculations with binucleate isolates from CAG 1. A preliminary report of these findings has been published (3).

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MATERIALS AND METHODS

Seeds of eight plant species representing six families were surface sterilized in 0.52% sodium hypochlorite for 1 min, rinsed in sterile distilled water for 1 min, and placed on moist filter paper in autoclaved 80 × 80-mm glass jars or 100 × 15-mm plastic petri dishes. Five seeds per jar were incubated at 23 ± 2 C for 24 hr prior to inoculation. Inoculum consisted of mycelial disks, 8 mm in diameter, cut from colonies growing on PDA for 5 days at 23 C. A disk was placed on each seed and incubated at 23 ± 2 C for 8–10 days with ~ 10 hr of fluorescent light, with a mean intensity of 4.6×10^2 lux, and 14 hr dark. The treatments were arranged in a randomized complete block design with four replications. Seedlings were evaluated for disease severity according to the following index: 0 = no disease, 1 = hypocotyl lesions, 2 = root decay, 3 = hypocotyl lesions and root decay, and 4 = seedling necrosis.

Seventeen of the binucleate isolates, with the highest virulence in the laboratory pathogenicity test, and three pathogenic isolates of *R. solani* were selected for pathogenicity evaluation on *Triticum aestivum* L. 'Chancellor' and *Pisum sativum* L. 'Green Arrow' grown in an aerated steam-treated (85 C for 30 min) soil mix consisting of one part Hagerstown silty clay loam and one part sand (1:1, v/v) supplemented with 1.6 g of dolomitic limestone per liter of mix. Inoculum was produced by introducing mycelial disks of isolates into 1-L Erlenmyer flasks containing 600 g of autoclaved sand cornmeal mixture (500 cm^3 sand, 15 cm^3 cornmeal, 100 ml H_2O). The flasks were placed in the dark at 25 C for 2 wk and the infested substrates were then removed and individually added to separate portions of the soil mix (1:4, v/v). Aliquots of infested soil mix were placed in 10-cm-diameter plastic pots, and five pea or wheat seeds were planted ~ 1 cm below the soil surface. The soil was watered daily throughout the study. The pots were placed in growth chambers at 16 ± 2 C, 22 ± 2 C, and 28 ± 2 C. Soil temperatures were recorded with a thermograph equipped with a soil probe. The lighting regime consisted of 14 hr fluorescent and incandescent light, with a mean intensity of 1.68×10^4 lux, and 10 hr dark. Treatments were arranged in a completely randomized design with three replications. After 2–3 wk of incubation, emergence was recorded and seedlings were evaluated according to the aforementioned disease index.

Pathogenicity of 10 binucleate isolates from CAG 1 was evaluated on pot-grown creeping bentgrass (*Agrostis palustris* Huds. 'Penncross'), Kentucky bluegrass (*Poa pratensis* L. 'Merion'), perennial ryegrass (*Lolium perenne* L. 'Pennfine') and tall fescue (*Festuca arundinacea* Schreb. 'Kentucky 31'). Inoculum was prepared by growing the test isolates on autoclaved rye grain. The grasses were inoculated 3–4 wk after seeding by placing 10–20 kernels of inoculum on the foliage in the center of the pot area. After inoculation, the pots were placed under individual transparent plastic covers and incubated in a growth chamber at 20 ± 2 C. Treatments were arranged in a completely randomized design with three replications. The lighting regime consisted of 14 hr of fluorescent and incandescent light, with a mean intensity of 1.56×10^4 lux, and 10 hr of dark. Two weeks after inoculation the covers were removed and disease severity was evaluated according to a 0–10 visual rating scale, with 0 = no disease, 1 = 1–10% of the grass blighted, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, 9 = 81–90%, 10 = 91–100%, or essentially complete blighting of the foliage.

RESULTS AND DISCUSSION

The majority of isolates in CAG 1 and 2 were either nonpathogenic or only weakly virulent (disease index < 1) on most of the plant species tested (Tables 1 and 2). Wheat was the only species that was susceptible to the majority of CAG 1 isolates tested. The mean disease indices for CAG 3, 4, and 5 were higher ($P = 0.05$)

TABLE 1. Mean disease indices for French bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), radish (*Raphanus sativus*), and onion (*Allium cepa*) seedlings inoculated with isolates of binucleate *Rhizoctonia solani*-like fungi representing five Ceratobasidium anastomosis groups or with isolates of *R. solani* from four anastomosis groups[a]

Anastomosis group	Isolates tested	Mean disease indices[b,c]			
		P. vulgaris	*P. sativum*	*R. sativus*	*A. cepa*
CAG 1	9	0.1 A	0.1 A	0.0 A	0.3 A
CAG 2	12	0.8 B	0.8 B	0.1 A	0.1 A
CAG 3	9	2.4 C	2.2 C	0.8 B	1.8 C
CAG 4	4	2.0 C	2.2 C	1.7 C	1.3 B
CAG 5	6	2.1 C	3.0 D	2.0 CD	1.9 C
R. solani	5	1.6 BC	1.7 BC	2.5 D	0.8 B
Check		0.0 A	0.0 A	0.0 A	0.0 A

[a] Surface-sterilized seeds were inoculated with 8-mm-diameter mycelial disks and incubated for 8–10 days at 23 ± 2 C with 10 hr light and 14 hr dark.
[b] Seedlings were evaluated according to disease index values defined: 0 = no disease, 1 = hypocotyl lesions, 2 = root decay, 3 = hypocotyl lesions and root decay, and 4 = seedling necrosis.
[c] Within a column, values followed by the same letter are not significantly different according to Duncan's modified (Bayesian) least significant difference, $P = 0.05$.

TABLE 2. Mean disease indices for wheat (*Triticum aestivum*), peanut (*Arachis hypogaea*), lettuce (*Lactuca sativa*), and tomato (*Lycopersicon esculentum*) seedlings inoculated with isolates of binucleate *Rhizoctonia solani*-like fungi representing five Ceratobasidium anastomosis groups or with isolates of *R. solani* from four anastomosis groups[a]

Anastomosis group	Isolates tested	Mean disease indices[b,c]			
		T. aestivum	*A. hypogaea*	*L. sativa*	*L. esculentum*
CAG 1	9	0.7 A	0.0 A	0.5 B	0.0 A
CAG 2	12	0.3 A	0.3 B	0.3 B	0.7 B
CAG 3	9	0.7 A	1.6 C	0.7 B	1.4 C
CAG 4	4	0.6 A	1.3 BC	0.7 B	2.8 D
CAG 5	6	0.7 A	1.6 C	0.9 BC	2.7 DE
R. solani	5	0.6 A	0.7 BC	1.5 C	2.3 E
Check		0.0 B	0.0 A	0.0 A	0.0 A

[a] Surface sterilized seeds were inoculated with 8-mm-diameter mycelial disks and incubated for 8–10 days at 23 ± 2 C with 10 hr light and 14 hr dark.
[b] Seedlings were evaluated according to disease index values defined: 0 = no disease, 1 = hypocotyl lesions, 2 = root decay, 3 = hypocotyl lesions and root decay and 4 = seedling necrosis.
[c] Within a column, values followed by the same letter are not significantly different according to Duncan's modified (Bayesian) least significant difference, $P = 0.05$.

than those for CAG 1 and 2 for each plant species except wheat and lettuce. Several binucleate isolates from CAG 3, 4, and 5 were more virulent than were isolates of *R. solani* on the host species tested.

The mean disease indices for wheat and pea grown in infested soil at 16 ± 2 C, 22 ± 2 C, and 28 ± 2 C are presented in Table 3. The six CAG 1 isolates tested were pathogenic, but only weakly virulent, on wheat at each temperature. The mean disease index for CAG 1 decreased with an increase in soil temperature. The mean disease index of 0.7 for CAG 1, on wheat, grown at a soil temperature of 16 ± 2 C, was greater ($P = 0.05$) than the disease indices for the other groups of binucleate isolates. Few isolates from the other groups were pathogenic on wheat, regardless of soil temperature. Emergence of wheat was not reduced ($P = 0.05$) by the binucleate or *R. solani* isolates tested (Table 4).

All isolates from CAG 2–5, except Bn 57 at a soil temperature of 16 ± 2 C, caused hypocotyl lesions and/or root decay of pea seedlings at each soil temperature. The mean disease indices for CAG 3, 4, and 5 on pea were greater ($P = 0.05$) than for CAG 1 and 2 at all soil temperatures (Table 3). At 22 ± 2 C, the mean disease indices for CAG 3 and 5 were greater ($P = 0.05$) than that for *R. solani*. All isolates of *R. solani* and isolates in CAG 3, 4, and 5, except Bn 31 and Bn 50, caused preemergence damping-off ($P = 0.05$) of pea at each soil temperature (Table 4). The majority of isolates in CAG 1 and 2 did not cause a reduction in pea seedling emergence at any of the soil temperatures (Table 4).

All CAG 1 isolates, except Bn 75, caused a foliar blight of each grass species tested (Fig. 1). The mean disease indices for creeping bentgrass, perennial ryegrass, tall fescue, and Kentucky bluegrass were 8.0, 4.7, 5.0, and 1.5, respectively. These data, along with pathogenicity data reported here for other species, denotes host specificity for gramineous hosts within CAG 1. This is supported by the fact that all CAG 1 isolates were collected from members of the Gramineae.

The cool-weather blight of fine turf caused by CAG 1 isolates (11) has been observed primarily on bentgrasses and bluegrasses (H. Cole, Jr., *unpublished*). Results reported here indicate that perennial ryegrass and tall fescue are also susceptible and must be considered to be potential hosts.

TABLE 3. The influence of soil temperature on disease indices for wheat (*Triticum aestivum*) and pea (*Pisum sativum*) seedlings harvested from soil infested with isolates of binucleate *Rhizoctonia solani*-like fungi representing five Ceratobasidium anastomosis groups or with isolates of *R. solani*[a]

Anastomosis group	Isolates tested	Mean disease indices[b,c]					
		T. aestivum			*P. sativum*		
		16 ± 2 C	22 ± 2 C	28 ± 2 C	16 ± 2 C	22 ± 2 C	28 ± 2 C
CAG 1	6	0.7 A	0.5 A	0.3 A	1.5 A	0.8 A	0.3 A
CAG 2	3	0.0 B	0.0 A	0.0 A	0.5 B	0.8 A	0.7 A
CAG 3	4	0.1 B	0.1 A	0.5 A	3.5 C	3.9 B	4.0 B
CAG 4	3	0.0 B	0.0 A	0.0 A	3.9 C	2.8 C	3.4 B
CAG 5	4	0.1 B	0.2 A	0.2 A	3.5 C	4.0 B	3.7 B
R. solani	3	0.5 AB	0.5 A	0.2 A	3.7 C	2.6 C	3.6 B
Check		0.0 B	0.0 A	0.0 A	0.0 D	0.1 A	0.0 A

[a] Seedlings were harvested from infested soil after 3 wk of growth with 14 hr light and 10 hr dark.
[b] Seedlings were evaluated according to disease index values defined: 0 = no disease, 1 = hypocotyl lesions, 2 = root decay, 3 = hypocotyl lesions and root decay, and 4 = seedling necrosis.
[c] Within a column, values followed by the same letter are not significantly different according to Duncan's modified (Bayesian) least significant difference, $P = 0.05$.

TABLE 4. The influence of soil temperature on emergence of wheat (*Triticum aestivum*) and pea (*Pisum sativum*) seedlings harvested from soil infested with isolates of binucleate *Rhizoctonia solani*-like fungi representing five Ceratobasidium anastomosis groups (CAG 1–5) or with isolates of *R. solani*

Anastomosis group	Isolates	Emergence (%)[a,b]					
		T. aestivum			*P. sativum*		
		16 ± 2 C	22 ± 2 C	28 ± 2 C	16 ± 2 C	22 ± 2 C	28 ± 2 C
CAG 1	Bn 1	92 A	100 A	100 A	58 ABC	83 AB	67 A
	Bn 2	100 A	100 A	92 A	67 AB	75 BC	67 A
	Bn 20	92 A	92 A	100 A	75 A	83 AB	92 B
	Bn 21	75 A	92 A	100 A	58 ABC	58 CD	83 AB
	Bn 97	100 A	92 A	92 A	42 ABCD	100 A	83 AB
	Bn 98	83 A	75 AB	92 A	75 A	83 AB	92 B
CAG 2	Bn 43	100 A	100 A	100 A	75 A	83 AB	92 B
	Bn 57	92 A	100 A	100 A	70 A	75 BC	92 B
	Bn 73	100 A	100 A	100 A	70 A	100 A	83 AB
CAG 3	Bn 28	100 A	83 AB	100 A	0 D	0 E	0 E
	Bn 31	100 A	92 A	100 A	83 A	0 E	0 E
	Bn 35	83 A	92 A	92 A	0 D	17 E	0 E
	Bn 36	86 A	100 A	92 A	25 BCD	0 E	0 E
CAG 4	Bn 32	92 A	83 AB	92 A	8 D	0 E	0 E
	Bn 38	100 A	100 A	92 A	0 D	58 CD	42 C
	Bn 45	83 A	92 A	92 A	17 CD	17 E	0 E
CAG 5	Bn 24	75 A	100 A	92 A	17 CD	0 E	0 E
	Bn 49	75 A	75 AB	92 A	0 D	0 E	33 CD
	Bn 50	83 A	83 AB	83 A	42 ABCD	0 E	0 E
	Bn 59	75 A	83 AB	100 A	0 D	0 E	0 E
R. solani	Rh 1	75 A	100 A	92 A	33 BCD	50 D	17 DE
	Rh 4	75 A	58 B	92 A	0 D	0 E	0 E
	Rh 7	92 A	92 A	100 A	8 D	55 D	17 DE
Check		92 A	100 A	100 A	70 A	100 A	83 AB

[a] Emergence recorded 3 wk after planting.
[b] Within a column, values followed by the same letter are not significantly different according to Duncan's modified (Bayesian) least significant difference, $P = 0.05$.

Most isolates in CAG 1 and 2 could be distinguished from those in CAG 3, 4, and 5 by differences in cultural morphology (4). This situation, in addition to the apparent specificity of CAG 1 isolates for members of the Gramineae and the nonpathogenicity or weak virulence of CAG 2 isolates, suggests that there is a tendency toward morphologic and physiologic homogeneity within CAG. This may be supported by physiological and serological studies simlar to those carried out with *R. solani* AG (1,7,12).

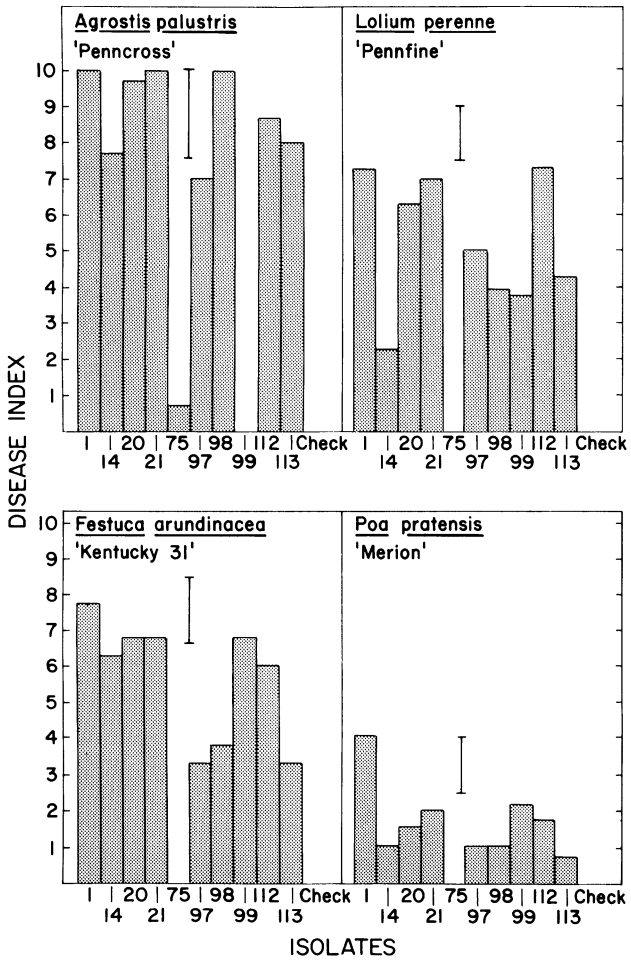

Fig. 1. Disease indices (0–10 scale, with 0 = no disease, 1 = 10% disease, and 10 = 100% disease) for creeping bentgrass (*Agrostis palustris*), perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinacea*), and Kentucky bluegrass (*Poa pratensis*) 2 wk after inoculation with isolates of binucleate *Rhizoctonia solani*-like fungi from Ceratobasidium anastomosis group 1. Vertical lines represent LSDs.

The pathogenicity data from the soil infestation study were similar to those of the seed inoculation study. The increase in virulence of CAG 1 isolates on wheat with decreasing soil temperature correlates well with temperature-growth relations of these fungi in culture (11). The high incidence of preemergence damping-off of pea caused by isolates from CAG, 3, 4, and 5 at each soil temperature demonstrates the potential importance of these fungi as soilborne pathogens. The widespread geographic distribution of the isolates and the frequency with which they are found in diseased plants (5,6,10) further emphasizes this point.

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