Characterization of *Rhizoctonia* Isolates Associated with Damping-Off of Bedding Plants

C. T. STEPHENS, Former Research Associate, L. J. HERR and A. F. SCHMITTHENNER, Professors, and C. C. POWELL, Associate Professor, Department of Plant Pathology, The Ohio State University, Columbus 43210 and the Ohio Agricultural Research and Development Center, Wooster 44691

ABSTRACT

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Forty-six *Rhizoctonia* isolates from greenhouse-grown bedding plants and field-grown nonbedding plants were evaluated for pathogenicity on six bedding plant hosts. More isolates from bedding plants were pathogenic and generally more virulent than those from nonbedding plants. Hosts differed significantly in their susceptibility to Rhizoctonia damping-off. Impatiens was the most susceptible; cabbage and celosia were somewhat less susceptible; ageratum was moderately susceptible; and tomatoes and peppers were the least susceptible hosts. All bedding plant isolates were *Rhizoctonia solani*, anastomosis group 4 (AG-4). Some nonbedding plant isolates were other fungi, and those that were *R. solani* were of several anastomosis groups.

Fungi tentatively identified as *Rhizoctonia solani* Kuehn cause damping-off and severe losses in the Ohio bedding plant industry. Because mycelium of some ascomycetous fungi, as well as of species of *Rhizoctonia* other than *R. solani*, is morphologically similar to that of *R. solani*, errors in identification are common (5,13). Recent evidence indicates that some binucleate *Rhizoctonia* spp. resemble *R. solani* so closely that they have frequently been studied as *R. solani* (7,9). Consequently, the *Rhizoctonia* isolates from bedding plants must be characterized more fully.

Because of difficulties in producing the perfect state, *Thanatephorus cucumeris* (Frank) Donk, the vegetative characteristics proposed by Parmeter and coworkers (5,6) are often used to identify *R. solani*. These characteristics include presence of multinucleate cells, a prominent septal pore apparatus, branching near the distal septum of cells, constriction of the branch and formation of a septum in the branch near the point of origin, and some shade of brown pigmentation in cultures. Other characteristics usually present are monilioid cells, undifferentiated sclerotia, hyphae greater than 5 μm in diameter, rapid growth in culture, and some degree of pathogenicity. Anastomosis has also been used as a criterion for identification. Although failure to anastomose is not exclusionary, successful anastomosis with a known isolate of *R. solani* can be considered as evidence that an isolate is *R. solani* (6).

The objectives of this study were to ascertain the identity and anastomosis grouping of *Rhizoctonia* isolates cultured from bedding plants grown in the greenhouse and to compare their virulence on bedding plants with that of *Rhizoctonia* isolates cultured from field-grown nonbedding plant hosts.

MATERIALS AND METHODS

Isolates. Of the 46 isolates investigated, 22 were from damped-off greenhouse bedding plants. The remaining 24 came from a variety of nonbedding plant field hosts and included four tester isolates representative of anastomosis groups AG-1, AG-2, AG-3, and AG-4 (courtesy of Neil Anderson, Department of Plant Pathology, University of Minnesota). Isolates were assigned consecutive numbers to simplify record keeping. Isolates 1–4 were the AG tester cultures; isolates 5–18 were originally isolated from nine different nonbedding plant field hosts; isolates 19–40 were from 12 different greenhouse bedding plant hosts; and isolates 41–46 were *R. solani* AG-4 isolates from five nonbedding plant hosts. Cultures were maintained on potato-dextrose agar (PDA) slants.

Plants assayed. The following bedding plants were used in the pathogenicity and host range assays: tomato (*Lycopersicon esculentum* Mill. 'Early Girl'), pepper (*Capsicum frutescens* L. 'California Wonder'), cabbage (*Brassica oleracea* var. *capitata* L. 'Golden Acre'), celosia (*Celosia argentea* L. 'Red Fox'), ageratum (*Ageratum houstonianum* Mill. 'Blue Blazer'), and impatiens (*Impatiens wallerana* Hook. 'Dwarf Blaze').

Pathogenicity assays. Each of the vegetable and floral bedding plant hosts was assayed by a postemergence damping-off method. Seeds were sown in double rows in flats 16 cm long × 12 cm wide × 6 cm deep containing a peat-vermiculite medium (Jiffy Mix) watered to container capacity (12). Seed species that germinated at about the same time were planted in the same flat, one row of each species. Celosia and cabbage emerged in 3 days, tomato and ageratum in 5 days, and impatiens and peppers in 7 days. Flats were enclosed in plastic bags (92 × 183 cm), 16 per bag, to maintain high humidity. Two 15-cm wooden stakes were placed in each flat to support the plastic bag above emerging seedlings. Planted flats were then incubated in a growth chamber with 12-hr days at 27 C and 25,000 lux and 21 C nights. After emergence, seedlings were inoculated by burying colonized PDA disks (12 mm in diameter) 0.5 cm deep at the end of each seedling row, immediately beside the first seedling. Inoculum disks were obtained from cultures grown on 20 ml of PDA in 90-mm petri plates for 4 days at 24 C. After incubation for 6 days, the length of row with damped-off seedlings was measured.

The isolates were assayed in two series of tests. Test series 1 involved 40 isolates, 22 from bedding plants and 18 from field-grown nonbedding plants. Test series 2 involved the assay of six selected AG-4 field isolates to evaluate more fully the pathogenicity and range of virulence of nonbedding plant *R. solani* AG-4 isolates.

Characterization of isolates. The 46 isolates were examined initially for conformance with certain of the criteria set forth by Parmeter (5) and Parmeter et al (6) for distinguishing *R. solani*. Isolates that possessed these characteristics were stained by nuclear staining procedures to determine the numbers of nuclei in vegetative cells. Two nuclear staining procedures were used: the aniline blue rapid-staining method of Sanders et al (9) and Herr's modified Giemsa staining procedure (2). Numbers of nuclei per vegetative cell and nature of the septal pore apparatus were ascertained.

All multinucleate isolates were cultured

Present address of senior author: Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824.

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for 2 wk at 24 C on PDA supplemented with 1 g of yeast extract per liter (8). The isolates were then assigned to cultural type groups (CTG) by Ruppel's cultural typing method (8) for distinguishing tentative anastomosis groups.

To substantiate the results of the culture typing, 10 of the 22 bedding plant isolates were paired with the AG tester isolates on sterile glass slides coated with 2% water agar. A 2-mm agar disk of a tester culture was placed about 3 cm from a similar disk of an unknown isolate. The slides were then placed on moistened filter paper in petri dishes and examined after 2–3 days at 24 C for hyphal anastomosis (6).

RESULTS

The 40 isolates of test series 1 differed significantly in pathogenicity and virulence as judged by the isolate means for all six hosts (Table 1). As shown by Duncan's test of significance, the 40 isolate means spanned, more or less, a continuum ranging from highly virulent to nonvirulent on all six hosts. To simplify interpretation, we arranged the isolate means into three virulence categories (Table 1): highly virulent, moderately virulent, and slightly virulent to nonvirulent, representing 9.1–6.3, 6.2–3.2, and 3.1–0 cm of damped-off seedling row, respectively.

Most isolates in the highly virulent category were bedding plant isolates (14 of 16 isolates); all five isolates in the moderately virulent category were bedding plant isolates; and only three of 19 isolates in the slightly virulent to nonvirulent category were bedding plant isolates. In contrast, the nonbedding plant field isolates were, as a group, much less virulent on all six bedding plant hosts. Sixteen nonbedding plant field isolates were categorized as slightly virulent to nonvirulent, none was moderately virulent, and only two were highly virulent (Table 1).

Because these isolate means represent average reactions of each isolate on all six hosts, a pathogenicity index (PI) (number

Table 1. Pathogenicity and virulence of 40 *Rhizoctonia* isolates on six bedding plant hosts as determined by a postemergence damping-off assay

Category / Isolate	Source[u]	Group[v]	Length of seedling row damped-off (cm)[w]						Isolate means[x] (cm)	PI[y]/VI[z]
			Celosia	Cabbage	Ageratum	Tomato	Impatiens	Pepper		
Highly virulent										
1	AG tester (F)	AG-1	9.6	11.1	11.0	7.8	12.5	2.8	9.1 a	6/5
18	Potato (F)	AG-4	9.4	9.8	0.8	3.4	8.8	2.8	7.0 efgh	6/4
19	Pansy (B)	AG-4	5.1	7.0	7.5	5.4	9.9	5.1	6.7 ghi	6/6
21	Impatiens (B)	AG-4	9.8	10.0	8.5	3.5	8.5	1.9	7.0 efgh	6/5
22	Impatiens (B)	CTG-4	8.6	9.5	5.6	2.1	8.0	5.6	6.6 ghi	6/4
23	Dusty miller (B)	AG-4	10.0	8.5	9.6	3.0	9.8	3.1	7.3 cdefg	6/4
24	Verbena (B)	CTG-4	11.6	10.0	8.3	3.3	10.8	6.6	8.4 abc	6/6
27	Salvia (B)	AG-4	10.4	9.9	7.3	2.4	10.0	8.5	8.1 bcde	6/5
28	Portulaca (B)	CTG-4	5.9	8.6	8.9	2.4	10.3	5.3	6.9 fghi	6/5
32	Pepper (B)	CTG-4	8.0	7.7	8.6	7.6	7.6	4.1	7.3 cdefg	6/6
33	Coralbells (B)	AG-4	9.0	9.6	9.1	7.3	9.3	3.1	7.9 bcdef	6/5
35	Impatiens (B)	AG-4	9.8	9.4	10.4	7.9	10.6	3.0	8.5 ab	6/5
37	Salvia (B)	CTG-4	9.4	7.1	9.0	2.0	9.6	4.0	6.9 fghi	6/5
38	Petunia (B)	CTG-4	7.8	9.5	9.5	8.5	8.5	6.0	8.3 abcd	6/6
39	Salvia (B)	AG-4	9.4	8.0	7.0	5.9	8.4	3.0	7.0 efgh	6/6
40	Verbena (B)	AG-4	7.5	8.6	8.8	6.9	8.9	4.8	7.6 bcdefg	6/6
Moderately virulent										
25	Salvia (B)	CTG-4	8.5	9.1	6.1	2.3	10.0	0.0	6.2 ghi	5/4
26	Vinca (B)	CTG-4	9.7	9.0	0.0	0.0	7.5	0.0	4.4 k	3/3
30	Impatiens (B)	AG-4	9.1	6.4	7.3	2.3	5.3	0.6	5.1 jk	6/4
31	Impatiens (B)	CTG-4	4.4	5.9	6.6	3.0	9.4	3.6	5.5 j	6/5
34	Impatiens (B)	CTG-4	10.0	9.0	2.5	0.6	8.9	3.8	5.8 ij	6/4
Slightly virulent to nonvirulent										
2	AG tester (F)	AG-2	0.0	2.1	0.0	0.0	0.4	0.0	0.4 no	2/0
3	AG tester (F)	AG-3	1.4	1.3	0.0	0.0	0.3	0.0	0.5 no	3/0
4	AG tester (F)	AG-4	3.8	1.8	0.0	0.0	5.9	1.0	2.1 lm	4/2
5	Cucumber (F)	NR	0.0	0.0	0.5	0.0	0.0	0.0	0.1 o	1/0
6	Melon (F)	NR	0.0	0.0	0.0	0.0	0.0	0.0	0.0 o	0/0
7	Melon (F)	NR	0.0	0.0	0.0	0.0	0.0	0.0	0.0 o	0/0
8	Cucumber (F)	AG-2	2.5	5.3	2.8	2.0	5.0	0.5	3.0 l	6/2
9	Melon (F)	NR	0.0	0.0	0.0	0.0	0.0	0.0	0.0 o	0/0
10	Sugar beet (F)	AG-2	2.4	0.5	0.0	0.4	3.0	0.0	1.1 no	4/0
11	Sugar beet (F)	AG-2	2.1	0.9	0.4	0.0	2.4	0.0	1.0 no	4/0
12	Sugar beet (F)	AG-4	0.5	1.6	0.0	0.0	0.9	0.0	0.5 no	3/0
13	Turf (F)	CTG-4	3.0	0.6	0.0	0.0	4.4	0.0	1.3 mn	3/1
14	Turf (F)	NR	0.0	0.0	0.0	0.0	0.0	0.0	0.0 o	0/0
15	Turf (F)	NR	0.3	0.3	0.0	0.0	0.0	0.0	0.1 o	2/0
16	Potato (F)	BN	0.0	0.0	0.0	0.0	0.0	0.0	0.0 o	0/0
17	Potato (F)	CTG-3	0.0	0.0	0.0	0.0	0.0	2.0	0.3 no	1/0
20	Verbena (B)	CTG-4	4.0	2.0	0.0	0.0	6.9	0.0	2.1 lm	3/2
29	Cabbage (B)	AG-4	0.1	3.4	0.0	0.0	0.5	0.0	0.7 no	3/1
36	Chrysanthemum (B)	CTG-4	0.0	0.0	0.0	0.0	0.0	0.0	0.0 o	0/0
Plant means[x]			5.1 b	5.1 b	4.1 c	2.2 d	5.5 a	2.0 d		

[u] AG testers courtesy of Neil Anderson, Department of Plant Pathology, University of Minnesota. F = nonbedding plant field isolate; B = bedding plant isolate.

[v] AG = anastomosis group determined by pairing with AG testers; CTG = cultural type group (tentative anastomosis group) determined by Ruppel's method (8). NR = not a *Rhizoctonia*. BN = a binucleate *Rhizoctonia* sp., not *R. solani*.

[w] Mean of four replications measured after 6 days; isolate × plant interaction means, least significant difference (0.05) = 3.12 cm.

[x] Values followed by the same letter are not significantly different from each other at the 5% level according to Duncan's new multiple range test.

[y] PI = pathogenicity index, the number of hosts with length of seedling row damped-off exceeding 0 cm.

[z] VI = virulence index, the number of hosts with length of seedling row damped-off exceeding 3.12 cm.

of hosts with length of damping-off in the seedling row exceeding zero) and a virulence index (VI) (number of hosts with length of seedling row damping-off exceeding the least significant difference [$P = 0.05$] of 3.12 cm of the isolate × plant means) were used to summarize the component host reactions (Table 1). For example, isolate 1 (PI/VI of 6/5), with the most damping-off (9.1 cm) of any isolate, was pathogenic on all six hosts but significantly virulent on only five. Isolate 26 (PI/VI of 3/3) was unusual among the moderately virulent isolates in that it was pathogenic on only three of the six hosts. Among the slightly virulent to nonvirulent isolates, isolate 8 (PI/VI of 6/2) was unique in being pathogenic on all six hosts.

The six bedding plant hosts differed significantly in susceptibility to damping-off, as judged by the individual plant means for all 40 isolates (Table 1). The hosts fell into four categories of susceptibility according to Duncan's test of significance. Impatiens was most susceptible; cabbage and celosia were next, followed by moderately susceptible ageratum; and tomato and pepper were the least susceptible of the six hosts to all 40 isolates. However, the significant isolate × plant interaction indicated that not all hosts were equally susceptible to given isolates (Table 1). Tomato and pepper plant means did not differ, yet their reactions to specific isolates (isolate × plant means) differed significantly. Tomato was more susceptible to isolates 1, 32, 33, and 35 than pepper and less susceptible to isolates 22, 24, 27, and 34 (Table 1). Impatiens was less susceptible to isolate 30 than celosia, even though the plant mean for impatiens was significantly higher than that of celosia. Cabbage was more susceptible to isolate 29 than celosia, although their plant means were similar.

In test series 2, six additional *R. solani* AG-4 isolates, all obtained from field-grown nonbedding plants, were tested for pathogenicity and virulence on the six bedding plant hosts. The six isolates fell into five virulence groups based on Duncan's multiple range test of isolate means (Table 2). Host plants differed significantly in susceptibility to damping-off as judged by the plant means. Impatiens and cabbage were the most susceptible, followed by celosia, then ageratum; tomato and pepper were the least susceptible. The three isolates least virulent on all hosts (isolates 41, 42, and 43) were avirulent on ageratum, tomato, and pepper (Table 2). The virulence of isolates 43 and 44, both from beet, differed significantly on each host. Isolate 45 (from radish) was nonpathogenic on tomato. The sugar beet and baby's tears isolates (isolates 44 and 46) were similar on each of the hosts except pepper. Clearly, these nonbedding plant isolates of *R. solani* AG-4 differed significantly in host range and virulence on the six bedding plant hosts.

Isolate characteristics. Because they lacked the commonly recognized features of *Rhizoctonia* spp. (4–6), field isolates 5, 6, 7, 9, 14, and 15 were eliminated from further study; all were nonpathogenic except isolate 5. The remaining isolates were all multinucleate except isolate 16, which was binucleate and a *Rhizoctonia* sp., but not *R. solani* (4,7). When plated on Ruppel's medium (8), the remaining 11 field-grown nonbedding plant host isolates were found to be of several cultural type groups, while the 22 bedding plant isolates all were assignable to CTG-4. The 10 bedding plant isolates that were paired with AG tester isolates were all found to belong in AG-4.

DISCUSSION

Damping-off attributed to *Rhizoctonia* in the Ohio bedding plant industry appears to be caused primarily by isolates within *R. solani* AG-4. Most AG-4 bedding plant isolates were highly virulent to moderately virulent; only three were in the slightly virulent to nonvirulent category when tested on six bedding plant hosts. However, only one (isolate 18) of four *R. solani* AG-4 or CTG-4 isolates from field-grown nonbedding plants was highly virulent; the remaining three (isolates 20, 29, and 36) were slightly virulent to nonvirulent (Table 1). The highly virulent isolate 18 was originally isolated from potato. This was surprising because solanaceous isolates frequently attack only other solanaceous crops (1,11) and potato isolates, generally AG-3, reportedly attack only potato (10). However, isolate 18 was an AG-4 rather than an AG-3. This example demonstrates the dangers of characterizing *R. solani* isolates based only on their source.

Six additional AG-4 isolates from field plants were tested in a second assay to more fully explore the pathogenicity and virulence of AG-4 isolates from nonbedding plants. These six field isolates varied widely in host range and virulence. Despite the obvious variation in host range and virulence encountered within the AG-4 isolates tested, a tendency for AG-4 isolates from bedding plants (as a group) to be more pathogenic and virulent on bedding plant hosts than were AG-4 isolates from field plants was apparent. In addition, evidence of specificity of particular AG-4 isolates for certain bedding plant hosts was found. For example, isolate 29 from cabbage was virulent only on cabbage (Table 1). In the case of tomato and pepper, several isolates differed significantly in their ability to cause damping-off on one or the other of these solanaceous hosts. Thus, this study supplies some evidence supporting Sherwood's (10) contention that host specificity occurs within as well as among anastomosis groups.

The AG-1 tester isolate (isolate 1) was highly virulent on the bedding plant hosts (isolate means, Table 1), but no AG-1 isolates were collected in Ohio from diseased bedding plants. Whether this failure indicates simply that AG-1 has not been introduced into the bedding plant greenhouses or that it does not survive under greenhouse conditions is unknown.

The "root-rotting" AG-2 field isolates, not generally very active in damping-off of seedlings (3,10), were all in the slightly virulent to nonvirulent category (Table 1), as were the "potato-attacking" AG-3 isolates (10). The discovery of some non-*Rhizoctonia* isolates among the nonbedding plant field isolates demonstrates the necessity for applying the *R. solani* identification criteria of Parmeter et al (5,6) and, if need be, the *Rhizoctonia* spp. criteria of Ogoshi (4) to all isolates, even those provided by other researchers. Parmeter's (5) contention that the widely divergent results reported for *R. solani* investigations is in part the result of misidentification of *R. solani* seems well founded.

Although significant differences in host susceptibility were evident from the plant means for all 40 isolates, examination of the individual isolate × plant interaction means reveals that in the case of tomato and pepper (the least susceptible hosts), 11 bedding plant

Table 2. Pathogenicity and virulence of *Rhizoctonia solani* AG-4 isolates on six bedding plant hosts as determined by a postemergence damping-off assay

Isolate	Source	Length of seedling row damped-off (cm)[x]						Isolate means[y]
		Celosia	Cabbage	Ageratum	Tomato	Impatiens	Pepper	
41	Alfalfa (146[z])	6.7	7.8	0.5	0.0	7.6	0.0	3.8 d
42	Alfalfa (140[z])	6.1	8.9	0.0	0.0	7.9	0.0	3.8 d
43	*Beta* sp.	4.4	5.3	0.7	0.0	4.3	0.0	2.5 e
44	Sugar beet	8.4	12.4	7.8	7.4	13.8	6.8	9.4 a
45	Radish	7.3	7.1	9.5	0.0	11.8	2.4	6.3 c
46	Baby's tears	8.3	12.4	6.6	6.2	12.9	4.8	8.5 b
Plant means[y]		6.8 b	9.0 a	4.2 c	2.3 d	9.7 a	2.3 d	

[x] Mean of four replications measured after 6 days; isolate×plant interaction means, least significant difference (0.05) = 1.47 cm.

[y] Means followed by the same letter are not significantly different according to Duncan's new multiple range test ($P = 0.01$).

[z] Isolate designations of Neil Anderson, Department of Plant Pathology, University of Minnesota.

isolates for each host were significantly virulent. Thus, even if it were practical, cropping shifts to a host judged less susceptible on the basis of plant means would not likely reduce damping-off losses appreciably in the long run.

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