

Characterization of *Rhizoctonia* Species Isolated from Ornamentals in Florida

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ABSTRACT

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Rhizoctonia species were collected from Florida ornamental plants and characterized by nuclear condition and anastomosis grouping. Of 309 isolates, 126 (41%) were collected from roots, 77 (25%) from stems, and 106 (34%) from leaves. Of 129 multinucleate isolates, 43 (33%) were *R. solani* and 4 (3%) were *R. zae* collected from roots, 30 (24%) were *R. solani* from stems, and 52 (40%) were *R. solani* from leaves. Of the isolates of *R. solani*, approximately 60% were from anastomosis group 4 (AG-4), 20% were AG-1 (all from leaves), and 20% could not be typed to the first four anastomosis groups. The remaining 180 isolates were binucleate organisms, 60 (34%) of which were isolated from leaves, 42 (23%) from stems, and 78 (43%) from roots. The majority of isolates of *R. solani* but only a small percentage of binucleate isolates were pathogenic to their hosts of origin. Isolates of *R. solani* AG-4 were pathogenic on a wide range of herbaceous ornamentals.

Before 1980, the majority of diseases on ornamental plants caused by *Rhizoctonia* were identified as *R. solani* Kühn, although little, if any, proof was given to establish their identity (1,2,11,13). Also, few reports of diseases caused by

isolates of *R. solani* have included identification to the level of anastomosis grouping (12,19,20). Recent research on ornamental pathogens has assigned isolates of *R. solani* to anastomosis group 4 (AG-4) (3,18,21). Other reports on several ornamentals show that binucleate isolates of *Rhizoctonia*, such as *R. ramicola* G. F. Weber & D. A. Roberts on *Elaeagnus*, are responsible for some of these diseases (24). Binucleate *Rhizoctonia* spp. have been isolated more frequently than *R. solani* in preliminary studies in Florida (4), but the

role of the former organisms in disease has been determined on only a few hosts, such as azalea (9), longleaf pine (8,10), and *Elaeagnus* (24).

Discrepancies exist among reports of diseases caused by *Rhizoctonia*. Original disease descriptions have identified the pathogen as *R. solani*, but later research has shown that a binucleate organism is the cause or that both binucleate and multinucleate *Rhizoctonia* spp. are involved (7,9,16,17,24). The literature is of little or no help to scientists in diagnostic laboratories when interpreting results of isolations that include *Rhizoctonia* spp. from ornamental plants. While typing to anastomosis group is not usually feasible for a diagnostic laboratory, establishing the nuclear condition of an isolate is quickly and easily accomplished. However, until the major diseases of ornamentals are adequately described, this information is of little help in determining the role of *Rhizoctonia* spp. isolated from a given sample. Therefore, control recommendations must remain broad and will surely result in the use of fungicides that are not always needed. Preliminary collection of isolates of *Rhizoctonia* from Florida ornamentals

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indicates that a variety of species are involved (4).

The following research was conducted to determine which Florida ornamental plants are most commonly infected with *Rhizoctonia*, the tissues that are typically infected, and the characteristics of the isolates recovered from these plants. Isolates were obtained from four laboratories in Florida (private and public) and characterized for *R. solani* by nuclear condition and anastomosis group. Pathogenicity on their host of origin was verified for most groups of strains from a single host genus. In addition, pathogenicity of *R. solani* AG-4 isolates was tested on a single host, pothos (*Epipremnum aureum* (Linden ex André) Bunting).

MATERIALS AND METHODS

Collection of isolates. Isolates of *Rhizoctonia* were obtained from the following Florida laboratories: 1) Division of Plant Industry, Florida Department of Food and Consumer Services, Gainesville; 2) Plant Disease Clinic, Department of Plant Pathology, University of Florida, Gainesville; 3) Central Florida Research and Education Center, University of Florida, Apopka; and 4) Plant Disease Diagnostics, Inc., Apopka. Axenic cultures of the isolates were maintained on Difco potato-dextrose agar (PDA) in slants at 25–28 C. The majority of the isolates were collected between January 1987 and February 1990, although a small percentage was collected in 1986, and isolate 84-12 from pothos was collected in 1984.

In vitro characterization of isolates. The nuclear condition of isolates was examined on receipt, after a 3- to 5-day-old PDA culture was stained with 3% KOH and aqueous safranin O (25). Anastomosis group typing for multinucleate isolates was performed as previously described (14). Anastomosis groups 1–4 were used; tester isolates were AG-1 (ATCC 42128), AG-2 (ATCC 42126), AG-3 (Butler 141), and AG-4 (ATCC 42127) (18).

Pathogenicity tests on the host of origin. Groups of three or more isolates from a single genus were included in pathogenicity tests on that genus. Whenever possible, the species from which the isolate originated was employed. Plants were produced from seedlings or rooted in steam-treated (1.5 hr at 90 C) potting medium (50% Canadian peat and 50% pine bark). The steamed medium was amended with 3.4 kg of dolomitic lime, 0.45 kg of Micromax (micronutrient source), and 4 kg of Osmocote (19-6-12 slow-release fertilizer) per cubic meter. Plants were grown for 4–8 wk (depending on plant type), and they were determined to be free of symptoms before use in pathogenicity tests. Three plants were used for each isolate in addition to a set of uninoculated controls. Each isolate was included in at least two tests.

Inoculum was produced on PDA plates for 3 days at 25–28 C under cool-white fluorescent lights at $8 \mu\text{E}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$. Each plate of inoculum was blended with 200 ml of tap water in a Stomacher Lab Blender-400 (Tekmar Company, Cincinnati, OH) for 2 min before use. Plants were inoculated by adding 10 ml (10-cm-square pots) or 20 ml (15-cm-diameter pots) of the mycelial slurry to the medium surface before being lightly watered. Environmental conditions were those suited for production of the host genus. Symptoms of infection in leaves, stems, and/or roots were observed for 2 wk to 6 mo depending on the host genus. Root quality was determined by carefully inverting the pot to remove it and examining the roots on the potting medium surface. Reisolation from symptomatic tissues on PDA amended with streptomycin sulfate (100 ppm) was attempted in at least one test for each isolate. Tests were performed between December 1988 and April 1990.

Pathogenicity of *R. solani* AG-4 isolates on pothos. Pothos was used as a standard plant to test pathogenicity of isolates of *R. solani* AG-4 from a variety of hosts because it was easily obtained and developed obvious symptoms rapidly. Forty-three isolates of *R. solani* AG-4 from 19 species of plants were inoculated to pothos. Not all of the isolates tested were chosen for their pathogenicity on their original host. Each test employed an isolate of *R. solani* AG-4 (84-12 or 86-91) that was obtained from pothos and had previously been demonstrated as pathogenic on pothos as a control. Inoculations of pothos were performed as described earlier. Each isolate was included in three tests between May and October 1989. Plants were maintained in a greenhouse with a maximum light level of $400 \mu\text{E}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ and temperatures between 18 and 33 C after inoculation. The number of dead leaves (attributable to petiole rot) per plant after 2 wk was recorded. The mean number of

Table 1. Characterization of *Rhizoctonia* species isolated from ornamentals

Plant (genus)	Isolates (no.)	Tissue type(s)	Identification ^a	
			Type	No.
<i>Aglaonema</i>	8	Stems/roots	Bi	8
<i>Araucaria</i>	2	Leaves	Bi	2
	4	Roots	Bi/M	3/1
<i>Azalea</i>	8	All types	Bi	8
<i>Begonia</i>	5	All types	AG-4	5
<i>Caladium</i>	3	Leaves	Bi	3
<i>Chrysalidocarpus</i>	4	Leaves/stems	AG-4/Bi	2/2
	2	Roots	Bi	2
<i>Epipremnum</i>	8	Leaves	AG-4	8
<i>Euphorbia</i>	4	Stems	AG-4/M	1/3
<i>Ficus</i>	6	All types	AG-1/AG-4	1/1
			M/Bi	1/3
<i>Fittonia</i>	3	Leaves	AG-4	3
<i>Ilex</i>	4	All types	Bi	4
<i>Impatiens</i>	7	Stems	Bi/M	2/5
	2	Roots	M	2
<i>Juniperus</i>	15	All types	Bi	15
<i>Liatis</i>	8	Roots/bulbs	M	8
<i>Ligustrum</i>	3	All types	Bi/M	2/1
<i>Magnolia</i>	3	Leaves	Bi	3
<i>Nephrolepis</i>	3	Leaves	M	3
	2	Roots	M	2
<i>Nerium</i>	4	Leaves	AG-1/M	1/3
	1	Stems	Bi	1
<i>Peperomia</i>	3	All types	Bi/M	2/1
<i>Petunia</i>	1	Stems	Bi	1
	2	Roots	AG-4/Bi	1/1
<i>Philodendron</i>	6	All types	Bi	6
<i>Pilea</i>	3	Leaves	AG-1/AG-4	1/1
			M	1
<i>Pinus</i>	8	Leaves	Bi/M	4/4
		Stems	Bi/M	1/1
<i>Pittosporum</i>	9	Leaves	Bi	9
	4	Roots	Bi	4
<i>Polyscias</i>	3	All types	Bi	3
<i>Radermachera</i>	5	All types	AG-4/Bi	4/1
<i>Raphiolepis</i>	3	Leaves	Bi	3
	2	Roots	Bi/M	1/1
<i>Rosa</i>	4	Stems/roots	Bi/M	2/2
<i>Rumohra</i>	11	Leaves	Bi/M	6/5
<i>Spathiphyllum</i>	3	Leaves	AG-4/Bi	1/2
	8	Stems/roots	Bi	8
<i>Vinca</i>	2	Leaves/stems	Bi/M	1/1
	7	Roots	Bi/M	2/5

^aIsolates were designated as AG-1, AG-4, or M (undetermined multinucleate) for *R. solani* or as Bi for binucleate *Rhizoctonia* spp.

dead leaves was ranked on the following scale: slight (one or two leaves), moderate (three or four leaves), or strong (five or more leaves).

RESULTS

Collection and characterization of isolates. Of 309 isolates, 126 (41%) were collected from roots, 77 (25%) from stems, and 106 (34%) from leaves. Of 129 multinucleate isolates, 43 (33%) were *R. solani* and 4 (3%) were *R. zea* Voorhees collected from roots, 30 (24%) were *R. solani* from stems, and 52 (40%) were *R. solani* from leaves. Of the isolates of *R. solani*, approximately 60% were from AG-4 (from all tissue types), 20% were AG-1 (all from leaves), and 20% could not be typed to the first four anastomosis groups. The remaining 180 isolates were binucleate organisms, 60 (34%) of which were isolated from leaves, 42 (23%) from stems, and 78 (43%) from roots.

One hundred and sixty-two isolates from hosts in 31 genera, involving at least three isolates from each genus, were studied (Table 1). A total of 76 other genera of ornamental plants were represented in the collection. Isolates from leaves were obtained from pothos, *Fittonia verschaffeltii* (Hort. ex Lem.) Coem. (nerve plant), *Pilea* spp., and *Rumohra adiantiformis* (G. Forster) Ching (leatherleaf fern). Isolates from stems were obtained from *Euphorbia pulcherrima* Willd. ex Klotzch (poinsettia) (Table 1). Other isolates from various tissue types were collected from hosts such as *Azalea* spp., *Begonia* spp., *Ficus* spp., and *Juniperus* spp. *Aglaonema* spp., *Azalea* spp., *Ilex* spp., and *Philodendron* spp. yielded only binucleate *Rhizoctonia*. *R. solani* AG-4 was recovered from many hosts and was the only species isolated from begonia, pothos, and nerve plant (Table 1).

general, more binucleate species were recovered from woody ornamentals (especially leaf tissue) than from herbaceous ornamentals.

Pathogenicity tests on the host of origin. Twenty-four of the genera listed in Table 1 were included in pathogenicity tests with *Rhizoctonia* spp. originally isolated from these hosts (Table 2). Most isolates of *R. solani* were pathogenic on their host of origin, with the exception of those from poinsettia. Many of the binucleate isolates, such as those obtained from species of *Aglaonema*, *Araucaria*, *Caladium*, and *Philodendron*, were not pathogenic to their host of origin. Binucleate isolates from leaves of species of *Juniperus* and *Pittosporum* were pathogenic to those hosts. On some plants, the tissues attacked were not those listed in the original isolations. For example, binucleate isolates from *Spathiphyllum* spp. roots caused petiole/leaf rot when inoculated back to the host. Pothos developed symptoms typical of foot rot (petiole rot) (17), although the isolates were originally recovered from leaves.

Pathogenicity of *R. solani* AG-4 isolates on pothos. Thirty-three isolates caused symptoms of foot rot on pothos, which ranged from slight to strong and were relatively consistent among the three tests for each isolate. No pattern could be detected with respect to either host, tissue of origin, or pathogenicity to that host when inoculated to pothos. Of the 10 isolates that were not pathogenic to pothos, only three were not pathogenic to their host of origin (Table 3). Two isolates of *R. solani* AG-4 (from *Radermachera sinica* (Hance) Hemsl. and pothos) were inoculated to pothos, *R. sinica*, *Hedera helix* L. (English ivy), *Impatiens wallerana* J. D. Hook., and *Pittosporum tobira* (Thunb.) W. T. Aiton and caused aerial blight on each plant species (*unreported data*).

DISCUSSION

The partial characterization of *Rhizoctonia* species from ornamentals grown in Florida has led to a series of conclusions regarding these organisms. First, the major anastomosis group represented is AG-4. This group has been reported previously as the primary anastomosis group infecting bedding plants such as impatiens, salvia, petunia, and vinca (21). These isolates were also shown to be pathogenic to a wide range of plants including cabbage, celosia, impatiens, and pepper. Our results also indicate that many isolates of *R. solani* AG-4 are pathogenic on a wider range of hosts than the host genus of origin and closely related plants. *Rhizoctonia* crown rot of New Guinea impatiens in New Jersey was caused by *R. solani* AG-4 (3). Trujillo et al found that although AG-4 could be recovered from carnations in Hawaii, AG-2-2 was more

Table 2. Pathogenicity of *Rhizoctonia* fungi from ornamentals

Original plant genus	Species tested	Symptom expression	Pathogenic to original host ^a
<i>Aglaonema</i>	<i>commutatum</i> Schott 'Silver Queen'	None	0/8(Bi)
<i>Araucaria</i>	<i>Heterophylla</i> (Salisb.) Franco	None	0/6(Bi)
<i>Begonia</i>	<i>semperflorens-cultorum</i> Hort.	Stem/root rot	5/5(M)
<i>Caladium</i>	× <i>hortulanum</i> Hort.	None	0/3(Bi)
<i>Chrysalidocarpus</i>	<i>lutescens</i> H. Wendl.	Root rot	2/2(M) 1/4(Bi)
<i>Epipremnum</i>	<i>aureum</i> (Linden ex André) Bunting 'Golden pothos'	Petiole rot	8/8(M)
<i>Euphorbia</i>	<i>pulcherrima</i> Willd. ex Klotzsch	None	0/4(M)
<i>Ficus</i>	<i>benjamina</i> L.	Root rot	2/3(Bi) 2/3(M)
<i>Fittonia</i>	<i>verschaffeltii</i> (Hort. ex Lem.) Coem.	Leaf blight	3/3(M)
<i>Impatiens</i>	<i>wallerana</i> J. D. Hook	Stem rot	7/11(M)
<i>Juniperus</i>	<i>chinensis</i> L. 'Shore'	Needle blight	6/15(Bi)
<i>Liatris</i>	<i>spicata</i> (L.) Willd.	Leaf blight	6/8(M)
<i>Nephrolepis</i>	<i>exaltata</i> (L.) Schott 'Fluffy Ruffle'	Leaf blight	4/5(M)
<i>Peperomia</i>	<i>obtusifolia</i> (L.) A. Dietr.	None	0/3(M,Bi)
<i>Petunia</i>	× <i>hybrida</i> Vilm.-Andr.	Stem rot/leaf spot	1/2(Bi) 1/1(M)
<i>Philodendron</i>	<i>scandens</i> C. Koch & H. Sello subsp. <i>oxycardium</i> (Schott) Bunt.	None	0/6(Bi)
<i>Pilea</i>	<i>cadierii</i> Gagnep. & Guillaumin	Leaf blight	3/3(M)
<i>Pittosporum</i>	<i>tobira</i> (Thunb.) W. T. Aiton	Leaf blight	4/13(Bi)
<i>Polyscias</i>	<i>fruticosa</i> (L.) Jaeger		0/3(Bi)
<i>Radermachera</i>	<i>sinica</i> (Hance) Hemsl.	Stem rot	4/5(M)
<i>Raphiolepis</i>	<i>indica</i> (L.) Lindl.	Leaf blight	1/5(M)
<i>Rumohra</i>	<i>adiantiformis</i> (G. Forst) Ching	Leaf blight	5/11(M)
<i>Spathiphyllum</i>	Schott 'Petite'	Petiole/leaf rot	10/10(Bi) 1/1(M)
<i>Vinca</i>	<i>minor</i> L.	Stem rot	1/3(Bi) 2/6(M)

^aPathogenicity on original host is given as the number of multinucleate (M) and/or binucleate (Bi) isolates of those tested.

common and virulent (22).

Binucleate species of *Rhizoctonia* from ornamentals are recovered frequently from aerial blights of Florida woody ornamentals. In the past, some of these diseases have been attributed to binucleate species of *Rhizoctonia* (8,9,24), while others have been attributed to *R. solani* (1,16) or *Rhizoctonia* sp. (7,11, 12,15). Although in some cases confusion is attributable to poorly described fungi, other cases have shown that binucleate rather than multinucleate species of *Rhizoctonia* are the predominant incitants of a given disease (8,9). Silky thread blight (web or aerial blight) of *Pittosporum* is caused by a binucleate fungus originally called *R. ramicola*, which has been placed in *Ceratobasidium* anastomosis group 3 (CAG-3) (23). Web blight of azalea is caused by fungi in CAG-3 and CAG-7, although some isolates of *R. solani* AG-4 can also cause these symp-

toms (9). Finally, seedling blight of longleaf pine is primarily caused by CAG-3, but in some years, the major pathogen recovered is *R. solani* AG-4 (8).

Symptom expression differed frequently between those described by the laboratory making the original isolation and those obtained in these pathogenicity tests. This is probably attributable to differences in environmental conditions, because potting medium and air temperatures have been shown to influence development of *Rhizoctonia* aerial blight on *Nephrolepis* (Boston fern) and foot rot on pothos (5,6). Although many ornamentals grown in Florida are occasional hosts of *Rhizoctonia* spp., over a 3-yr period only 31 genera were commonly affected by this group of fungi, and not all isolates could be confirmed as pathogens. The role of many of the binucleate fungi remains uncertain. This may in part be attributable to the lack

of appropriate test conditions for confirmation of pathogenicity. Also, it is rare to isolate a single potential pathogen from the root system of a rotted plant, and interactions between other root pathogens and *Rhizoctonia* species may exist. We recommend that diagnostic laboratories routinely determine the nuclear condition of these organisms; typing to anastomosis group is of limited value at this time. Binucleate *Rhizoctonia* spp. from roots should not be considered strong suspects in root diseases unless they are recovered from specific hosts and tissues for which pathogenicity data are available. To date, little information regarding control strategies for multinucleate vs. binucleate species of *Rhizoctonia* is available. Until such time as differences in efficacy of fungicides or other control strategies are determined, diseases caused by isolates of *Rhizoctonia* spp. should be treated similarly.

Table 3. Pathogenicity of *Rhizoctonia solani* AG-4 isolates on *Epipremnum aureum* (pothos)

Plant species	Tissue type	Isolate no.	Pathogenicity on pothos ^a	Pathogenic on host of origin
<i>Araucaria heterophylla</i>	Roots	87-256	Not pathogenic	No
<i>Begonia semperflorans-cultorum</i>	Leaves	87-77	Slight to Moderate	Yes
<i>Begonia</i> × <i>rex-cultorum</i>	Leaves	87-122	Strong	Yes
<i>Begonia</i> × <i>rex-cultorum</i>	Roots	87-124	Not pathogenic	Yes
<i>Begonia</i> sp.	Stems	87-139	Moderate	Yes
<i>Begonia</i> sp.	Roots	87-140	Strong	Yes
<i>Chrysalidocarpus lutescens</i>	Leaves	86-113	Strong	No
<i>Chrysalidocarpus lutescens</i>	Stems	87-267	Moderate	Yes
<i>Epipremnum aureum</i>	Leaves	84-12	Moderate	Yes
<i>Epipremnum aureum</i>	Leaves	86-91	Strong	Yes
<i>Euphorbia pulcherrima</i>	Stems	87-111	Slight	No
<i>Euphorbia pulcherrima</i>	Stems	88-217	Not pathogenic	No
<i>Euphorbia pulcherrima</i>	Stems	88-293	Slight	No
<i>Ficus benjamina</i>	Stems	86-169	Not pathogenic	Yes
<i>Fittonia verschaffeltii</i>	Leaves	87-258	Strong	Yes
<i>Fittonia verschaffeltii</i>	Leaves	87-263	Strong	Yes
<i>Fittonia verschaffeltii</i>	Leaves	88-200	Moderate	Yes
<i>Impatiens wallerana</i>	Roots	87-46	Moderate	Yes
<i>Impatiens wallerana</i>	Stems	87-148	Not pathogenic	Yes
<i>Impatiens wallerana</i>	Stems	88-69	Not pathogenic	Yes
<i>Impatiens wallerana</i>	Roots	88-72	Strong	Yes
<i>Impatiens wallerana</i>	Roots	88-289	Strong	Yes
<i>Impatiens wallerana</i>	Stems	88-290	Slight	Yes
<i>Impatiens wallerana</i>	Stems	89-68	Moderate	Yes
<i>Nephrolepis exaltata</i>	Leaves	86-149	Moderate	Yes
<i>Nephrolepis exaltata</i>	Leaves	87-210	Moderate	Yes
<i>Nephrolepis exaltata</i>	Roots	88-219	Moderate	Yes
<i>Nephrolepis exaltata</i>	Leaves	88-261	Not pathogenic	Yes
<i>Petunia</i> × <i>hybrida</i>	Roots	87-142	Strong	Yes
<i>Pilea cadierei</i>	Leaves	87-205	Slight	Yes
<i>Pilea spruceana</i>	Leaves	87-136	Strong	Yes
<i>Radermachera sinica</i>	Stems	86-159	Slight to Moderate	Yes
<i>Radermachera sinica</i>	Stems	87-37	Slight to Moderate	Yes
<i>Radermachera sinica</i>	Roots	87-216	Slight to Moderate	Yes
<i>Radermachera sinica</i>	Stems	87-265	Slight to Moderate	Yes
<i>Rumohra adiantiformis</i>	Leaves	87-23	Not pathogenic	Yes
<i>Rumohra adiantiformis</i>	Leaves	89-21	Slight	Yes
<i>Rumohra adiantiformis</i>	Leaves	89-45	Not pathogenic	Yes
<i>Spathiphyllum</i> sp.	Leaves	87-268	Strong	Yes
<i>Vinca minor</i>	Roots	86-131	Slight	Yes
<i>Vinca minor</i>	Leaves	89-149	Strong	Yes
<i>Vinca minor</i>	Roots	89-151	Strong	Yes
<i>Vinca minor</i>	Roots	88-286	Not pathogenic	No

^aThe mean number of dead leaves per test was ranked on the following scale: slight (one or two leaves), moderate (three or four leaves), or strong (five or more leaves). Mixed results were obtained for some isolates that caused slight disease in one test but were not pathogenic in the other tests.

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