

# Characterization and Pathogenicity of Binucleate *Rhizoctonia* spp. from Azaleas and Other Woody Ornamental Plants with Web Blight

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## ABSTRACT

Frisina, T. A., and Benson, D. M. 1987. Characterization and pathogenicity of binucleate *Rhizoctonia* spp. from azaleas and other woody ornamental plants with web blight. *Plant Disease* 71: 977-981.

Fungi with *Rhizoctonia*-like mycelia were isolated from foliage of azaleas and other woody ornamentals grown in production nurseries in North Carolina and other southeastern states. Isolated fungi were identified as either binucleate *Rhizoctonia* spp. or *Rhizoctonia solani* on the basis of hyphal characteristics and nuclear number. Representative isolates of binucleate *Rhizoctonia* spp. and *R. solani* were characterized for anastomosis, temperature optima in vitro, and virulence on azalea. Isolates of binucleate *Rhizoctonia* spp. (BN8 and BN21) were assigned to *Ceratobasidium* anastomosis group 7 (CAG-7) or were assigned tentatively to CAG-3 (BN2 and BN6) on the basis of hyphal fusion reaction with tester isolates. Three binucleate *Rhizoctonia* spp. (BN10, BN17, and BN22) did not anastomose with any of the tester isolates. Isolates of *R. solani* were assigned to *R. solani* anastomosis group 1 (AG-1). The optimum temperature range for growth of binucleate *Rhizoctonia* spp. and *R. solani* was 24–28 C. Growth rate of *R. solani* at optimum temperatures was more rapid than for binucleate *Rhizoctonia* spp. Most isolates of binucleate *Rhizoctonia* spp. and all isolates of *R. solani* caused a foliar blight on azalea in the greenhouse. However, isolates of binucleate *Rhizoctonia* spp. produced symptoms that more closely resembled typical symptoms of web blight observed on azalea in nurseries. Disease severity was correlated with the growth of aerial mycelia in the foliage.

Web blight is an important foliar disease of azalea (*Rhododendron* sp.) in North Carolina nurseries. The disease consists of leaf blight and leaf lesions on foliage within the internal portions of the plant canopy. Diseased leaves abscise but remain attached to the plant stem by webs of mycelial growth. Severely defoliated plants are not salable, but plants may recover in subsequent seasons with the production of new leaves. Web blight may reach epidemic proportions on azaleas grown in production nurseries or enclosed propagation houses when warm temperatures and high relative humidity prevail. This disease caused severe defoliation of 150,000 azaleas in Florida during periods of warm, humid weather (20).

The causal agent of web blight of azalea in North Carolina has not been identified or characterized, but fungi with mycelia resembling *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris* (Frank) Donk) have been isolated from

symptomatic azaleas and other container-grown woody ornamentals. *Rhizoctonia* spp. have been reported to cause foliar diseases of ornamental plants and other horticultural crops (2,7,19,20). Wehlburg and Cox (20) isolated fungi with mycelial characteristics of *R. solani* from azaleas with leaf blight and concluded that the organism causing the disease was *R.*

*solani*. Weber and Roberts (19) isolated *R. ramicola* Weber & Roberts (*C. ramicola* Tu, Roberts, & Kimbrough) from a variety of woody ornamentals with symptoms of leaf blight. This fungus was later shown to possess a binucleate condition in hyphal cells (18). It is not known if web blight of azalea is caused by *R. solani*, binucleate *Rhizoctonia* spp., or both. This paper reports on the characterization and pathogenicity of some isolates of binucleate *Rhizoctonia* spp. and *R. solani* collected from the foliage of azalea and other woody ornamentals grown in production nurseries in North Carolina and other southeastern states.

## MATERIALS AND METHODS

**Collection and isolation.** Azaleas and other ornamental plants with symptoms of web blight were collected from nurseries in North Carolina or were obtained from the North Carolina Plant Disease and Insect Clinic. Cultures of *Rhizoctonia* spp. isolated from symptomatic plants were obtained from sources listed in Table 1. Diseased plant tissue was surface-disinfested in 0.525% sodium hypochlorite, rinsed with sterile deionized

**Table 1.** Anastomosis group, host, and geographic origin of selected aerial isolates of *Rhizoctonia solani* and binucleate *Rhizoctonia* spp.

Isolate	Anastomosis group	Host	Geographic origin <sup>a</sup>
<i>R. solani</i>			
RS3	AG-1 <sup>b</sup>	<i>Impatiens</i> sp.	North Carolina
RS15	AG-1	<i>Ilex crenata</i>	North Carolina
RS25	AG-1	<i>Pittosporum</i> sp.	Florida
Binucleate <i>Rhizoctonia</i> spp.			
BN2	CAG-3? <sup>c</sup>	<i>Rhododendron</i> sp.	North Carolina
BN6	CAG-3?	<i>Ilex crenata</i>	North Carolina
BN8	CAG-7	<i>Rhododendron</i> sp.	North Carolina
BN10	?	<i>Pittosporum</i> sp.	North Carolina
BN17	?	<i>Cotoneaster</i> sp.	North Carolina
BN21	CAG-7	<i>Raphiolepis indica</i>	Alabama
BN22	?	<i>Juniperus chinensis</i>	Florida

<sup>a</sup> Isolate RS3 provided by D. Strider, North Carolina State University; RS25 provided by A. Chase, Apopka Agricultural Experiment Station, Apopka, FL; BN8 and BN17 provided by R. Jones, North Carolina State University; BN21 provided by J. Mullen, Alabama Cooperative Extension Service, Auburn; and BN22 (P85-285) provided by the Florida Division of Plant Industry, Gainesville.

<sup>b</sup> Anastomosis groups for *Rhizoctonia solani*. Tester isolates included: AG-1 (Butler Cal43), AG-2 type 1 (Herr Rhs 50), AG-2 type 2 (Herr 52), AG-4 (Campbell), and AG-5 (Burpee), all provided by B. Martin, Connecticut Agricultural Experiment Station, and AG-3 (American Type Culture Collection 14006).

<sup>c</sup> *Ceratobasidium* anastomosis group. Tester isolates included: CAG-1 (Bn-1), CAG-2 (BN-2), CAG-3 (BN-6), CAG-4 (BN-9), and CAG-5 (BN-14a), all provided by D. R. Sumner, Coastal Plains Agricultural Experiment Station, Tifton, GA, CAG-6 (American Type Culture Collection 13244), and CAG-7 (Florida Type Culture Collection 585).

Paper 10985 of the journal series of the North Carolina Agricultural Research Service, Raleigh 27695-7601.

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Accepted for publication 9 June 1987 (submitted for electronic processing).

water, and placed on acidified potato-dextrose agar (APDA) or on modified (0.5  $\mu\text{g}$  a.i./ml benomyl) Flowers's media (8,10). Fungi with mycelia typical of the form-genus *Rhizoctonia* were transferred, after 24 hr of growth, onto potato-dextrose agar (PDA) for further observation. Of 31 isolates of *Rhizoctonia* spp. obtained, 24 were from the foliage of azalea with symptoms of web blight.

**Hyphal characterization.** Hyphal characteristics and the nuclear condition of hyphal cells of all 31 isolates of *Rhizoctonia* spp. were determined by staining hyphal tips or 2- to 4-day-old moniloid cells, in small plugs of PDA, with 0.05% aniline blue in lactophenol (5). The slide preparation was heated gently over a flame until the agar melted and uniform staining developed, then observed at 400 $\times$ . The nuclear condition in hyphal tip cells was confirmed using fluorescent DNA-binding fluorochrome 4',6'-diamidino-2-phenylindol (DAPI) according to the procedure of Martin (11). As a result of nuclear staining procedures, seven binucleate *Rhizoctonia* spp. and three isolates of *R. solani* were selected for characterization and pathogenicity studies on azalea (Table 1).

**Production of sexual state.** The modified soil-over-culture technique of Tu and Kimbrough (17) and the nutrient stepdown technique of Adams and Butler (1) were used to induce fruiting. For the soil-over-culture technique, representative isolates of *R. solani* and *Rhizoctonia* spp. were grown on yeast extract agar in 100-mm-diameter petri dishes for 3-5 days. The cultures were covered with 90 g of sterile soil at 30% moisture content and incubated at room temperature in the direct light with the petri dish covers removed. Cultures were moistened once or twice a day with deionized water. For the nutrient stepdown technique, isolates were placed on glucose nitrate agar of the following formulations (g  $\text{NaNO}_3$ /g glucose per liter): 6/15, 3/15, and 0.25/20.

**Anastomosis tests.** Anastomosis groups were determined using the procedure of Parmeter et al (13). Isolates of *R. solani* were paired with five tester isolates of *R. solani* (4,13). Binucleate isolates were paired with the *R. solani* tester isolates and with seven tester isolates of *Ceratobasidium* spp. (6). Overlapping hyphae of paired isolates on agar coated glass slides were stained with 0.05% aniline blue in

lactophenol and observed at 400 $\times$  for anastomosis. Hyphal strands were traced back to their origins to ascertain that anastomoses were between paired isolates. Isolates were placed in the anastomosis group of the tester isolate when cell wall and cytoplasmic fusion occurred or when hyphal fusion resulted in a subsequent killing reaction of hyphal cells (9).

**Temperature studies.** Mycelial plugs (4 mm diameter) of each isolate, taken from near the margin of 3- to 5-day-old cultures on PDA, were placed on PDA in 100-mm-diameter petri plates. Five cultures of each isolate were stacked together randomly and were incubated in the dark at each of the following temperatures: 11, 16, 20, 24, 28, 32, and 36 C. Two radial measurements of each replicate were taken at 2-day intervals until colonies reached the edges of the plates or until 10 days had elapsed. The experiment was repeated three times.

**Pathogenicity tests.** Pathogenicity of the isolates was evaluated on 6-mo-old *R. obtusum* (Lindl.) Planch. cv. Gumpo grown in pine bark-sand (3:1, v/v) in 11.5-cm-diameter clay pots. Plants were fertilized weekly with 1.8  $\mu\text{g}$ /ml of water-soluble 21-7-7 (Peter's fertilizer products, W. R. Grace, Fogersville, PA). Two days before inoculation, 1-2 cm of the potting medium was removed from the surface of each pot and replaced with autoclaved sand (121 C, 15 psi, 60 min). The sand layer and potting medium were then drenched with 80 ml of a 2.5-ml/L solution of chlorothalonil (Daconil 2787 4.17F). Twenty-four hours after drenching, a second layer of autoclaved sand 1-2 cm thick was added to each pot to cover the sand exposed to the fungicide. About 1.5 g of 14-day-old cultures of *R. solani* or binucleate *Rhizoctonia* spp. grown on autoclaved bran in 250-ml Erlenmeyer flasks (20 g bran, 20 ml  $\text{H}_2\text{O}$ , 121 C, 15 psi, 60 min, for two consecutive days) was used as inoculum. Inoculum was placed on the soil surface at the base of each of five replicated plants per treatment. Each of the five plants per treatment was placed in a separate humidity chamber (1.01- $\text{m}^3$  capacity). Relative humidity in each chamber was kept above 90% by saturating pads at the base of each chamber with water two to three times a day. Plants were removed from the chambers after 5 days and rated for disease severity. Symptomatic foliage was rated on a scale of 1-5, where 1 = 0%, 2 = 1-25%, 3 = 26-50%, 4 = 51-75%, and 5 = 76-100% of the foliage diseased. The height that aerial mycelia grew from the soil surface (point of inocula placement) into the foliage was measured for each isolate. The experiment was repeated three times.

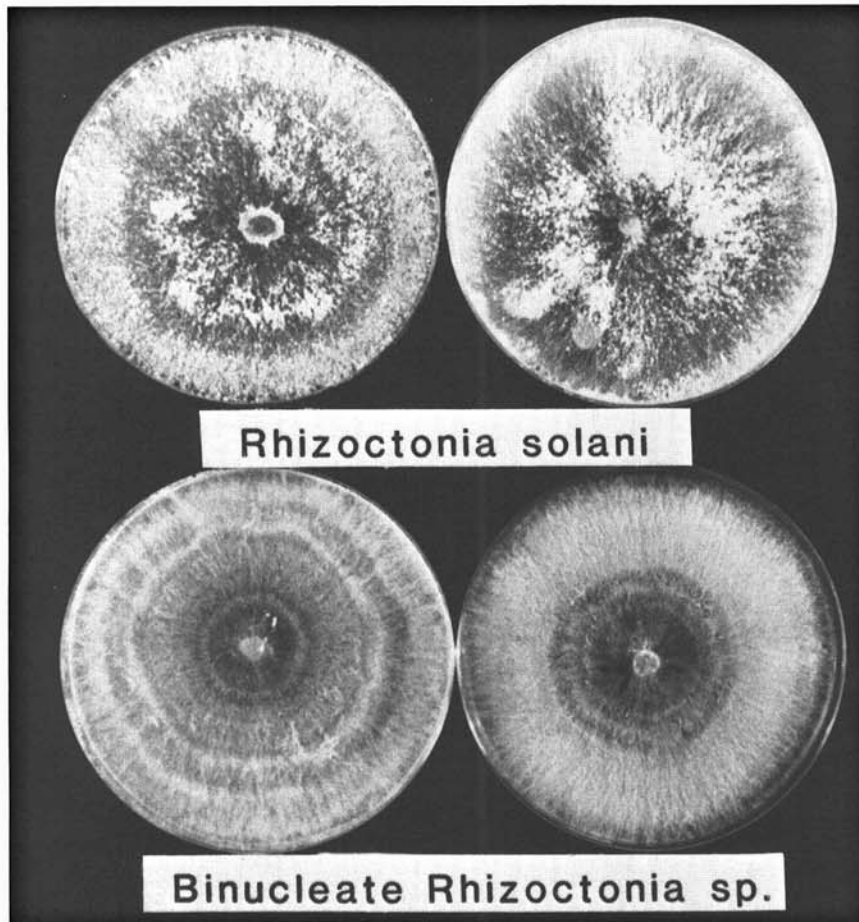


Fig. 1. Four-day-old cultures of *Rhizoctonia solani* and binucleate *Rhizoctonia* spp. on potato-dextrose agar. After 10-14 days, mycelia of *R. solani* became darkly pigmented and mycelia of binucleate *Rhizoctonia* spp. were white or light brown.

## RESULTS

**Isolation and identification.** Isolates recovered from the foliage of azalea and

other ornamental plants with symptoms of web blight possessed mycelia with characteristics typical of the form-genus *Rhizoctonia* (15). Vegetative hyphae had dolipore septa, branching near distal septa, and constrictions of the branch base (15). Most isolates formed darkly pigmented sclerotia in culture after 7–10 days, and mycelia in culture were typically light tan to dark brown. Twenty-six of the 31 *Rhizoctonia*-like isolates possessed a binucleate condition in hyphal cells and were designated binucleate *Rhizoctonia* spp. Binucleate isolates occasionally possessed hyphal cells with three or four nuclei per cell. Binucleate *Rhizoctonia* spp. were recovered from all 24 azaleas sampled with symptoms of web blight. Binucleate *Rhizoctonia* spp. were also isolated from the foliage of *Ilex crenata* cv. Helleri, *Pittosporum* sp., and *Cotoneaster* sp. from North Carolina, *Raphiolepis indica* (L.) Lindl. from Alabama, and *Juniperus chinensis* L., *Pittosporum tobira* Ait., and *Ligustrum* sp. from Florida.

Colonies of binucleate *Rhizoctonia* spp. were typically zonate after 4 days of growth on PDA (14; Fig. 1), and mycelia were white or light brown. Binucleate isolates BN2, BN8, BN10, and BN21 (Table 1) typically produced light to darkly pigmented sclerotia in culture after 10–14 days. Binucleate isolates BN6 and BN17 (Table 1) produced tufts of aerial mycelium in culture, but sclerotia were absent or very sparsely produced after 10–14 days. Isolate BN22 (Table 1) did not produce sclerotia after 10–14 days of growth in culture.

Five of the 31 *Rhizoctonia*-like isolates possessed a multinucleate condition in hyphal cells and were identified as *R. solani*. Isolates of *R. solani* were recovered from the foliage of *Impatiens* sp. and *I. crenata* cv. Helleri from North Carolina, *P. tobira* and *Ficus* sp. from Florida, and *Pinus strobus* L. from Tennessee. *R. solani* was not isolated from azalea. Isolates of *R. solani* typically produced an abundance of darkly pigmented sclerotia after 10–14 days of growth on PDA, and mycelia was darkly pigmented.

Attempts to produce the perfect states of isolates of binucleate *Rhizoctonia* spp. or *R. solani* were not successful.

**Anastomosis tests.** Ten isolates of binucleate *Rhizoctonia* spp. and three isolates of *R. solani* were characterized to anastomosis group (Table 1). Two isolates of binucleate *Rhizoctonia* spp. were assigned to Ceratobasidium anastomosis group 7 (CAG-7) on the basis of cell wall and cytoplasmic fusion with the CAG-7 tester isolate, and two isolates were tentatively assigned to CAG-3 (Table 2). Isolates tentatively assigned to CAG-3 appeared to anastomose by cell wall fusion with the CAG-3 tester isolate, but no killing reaction occurred, and the sites of anastomosis were few (one to four per slide). Numerous sites of anastomosis (>10/slide) were observed with other binucleate and multinucleate isolates. Three isolates of binucleate *Rhizoctonia* spp. were not assigned to an anastomosis group because of failure to anastomose with any of the CAG tester isolates (Table 1). None of the binucleate *Rhizoctonia* spp. anastomosed with tester isolates of *R. solani*.

Isolates of *R. solani* were assigned to *R. solani* anastomosis group 1 (AG-1) on the basis of hyphal anastomosis with the AG-1 tester isolate (Table 1).

**Temperature effects and growth characteristics.** The optimal temperature range for growth of both binucleate and multinucleate isolates was between 24 and 28 C (Fig. 2). Growth of all isolates was minimal at C and 32 C. No isolates grew at 36 C. Growth rate at optimal temperature for *R. solani* was about 18–19 mm/day and was significantly more rapid than the growth rate of 15–17 mm/day at optimal temperatures for binucleate *Rhizoctonia* spp. (Fig. 2).

**Pathogenicity.** Disease severity ratings on azaleas caused by isolates of *R. solani* were significantly higher than those caused by isolates of binucleate *Rhizoctonia* spp. (Fig. 3). Isolates of *R. solani* grew to a greater height within the canopy than isolates of binucleate *Rhizoctonia* spp. (Table 2). Isolates of *R. solani* typically caused brown-black necrotic lesions on leaves that coalesced after 48 hr of incubation in the humidity

chambers. After 5 days of incubation in the humidity chambers, a general necrosis of the foliage occurred with an average of >50% of the foliage diseased. Most isolates of binucleate *Rhizoctonia* spp. caused both discrete leaf lesions (Fig. 4) and irregularly shaped necroses of the foliage that remained confined to foliage within the interior portion of the plant canopy. After a 5-day incubation period, plants inoculated with the binucleate *Rhizoctonia* spp. averaged 20–30% of the foliage diseased.

Disease severity ratings for both binucleate *Rhizoctonia* spp. and *R. solani* were significantly correlated with the height that aerial mycelium extended into the foliage of the plants (Table 2).

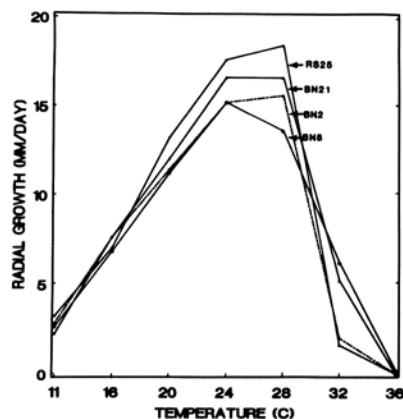


Fig 2. Radial growth of representative isolates of *Rhizoctonia solani* (RS25) and binucleate *Rhizoctonia* spp. (BN21, BN2, and BN8) at 11, 16, 24, 28, 32, and 36 C. LSD at  $P=0.05$  was 0.7 at 11 C, 1.0 at 16 C, 1.0 at 20 C, 0.8 at 24 C, 1.02 at 28 C, 2.6 at 32 C, and 0.6 at 36 C.

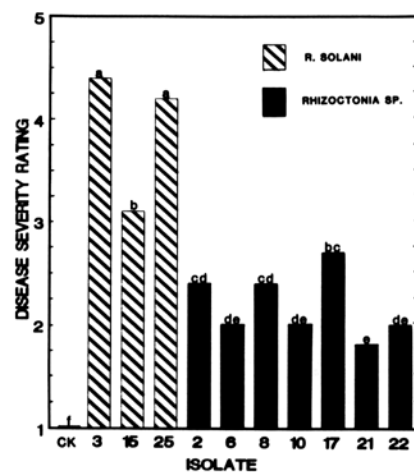


Fig 3. Virulence of *Rhizoctonia solani* and binucleate *Rhizoctonia* spp. on *Rhododendron obtusum* cv. Gumpo 5 days after inoculation. Bars with the same letter are not significantly different ( $k$ -ratio = 100) by Waller-Duncan  $k$ -ratio  $t$  test. Disease severity rating: 1 = 0%, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100% diseased foliage. CK is the uninoculated control. Coefficient of variation = 14.4.

Table 2. Correlation coefficients and levels of significance for disease severity rating and height of aerial mycelium of *Rhizoctonia solani* and binucleate *Rhizoctonia* spp. that grew from the soil surfaces of container-grown azaleas

Experiment	Correlation coefficient <sup>a</sup>	Significance level <sup>b</sup>	Mean height of aerial mycelium (cm) <sup>c</sup>	
			Binucleate	<i>R. solani</i>
1	0.7624	0.0001	6.90	9.80
2	0.6135	0.0001	4.43	8.10
3	0.6766	0.0001	6.06	9.53

<sup>a</sup> Pearson product-moment.

<sup>b</sup>  $P > R$  (H:Rho = 0),  $n = 55$ .

<sup>c</sup> Mean of five replicates each of seven isolates of binucleate *Rhizoctonia* spp. and five replicates each of three isolates of *R. solani*.

## DISCUSSION

Results of initial isolations from the foliage of diseased plants showed that only binucleate *Rhizoctonia* spp. were isolated from azaleas with web blight and that *R. solani* was isolated only from ornamentals other than azalea. Results of the pathogenicity study indicate that binucleate *Rhizoctonia* spp. cause web blight of azalea. Although both *R. solani* and binucleate *Rhizoctonia* spp. caused leaf blight symptoms on azalea, the symptoms of web blight produced by the binucleate isolates more closely resembled symptoms of the disease that are observed in the nursery. Wehlburg and Cox (20) reported that *R. solani* caused leaf blight of azaleas in Florida; however, they did not report on the nuclear condition of the isolates they recovered. Cultures of their isolates were not available for examination, but it is possible that these isolates from azalea were binucleate and, therefore, not *R. solani*.

Binucleate *Rhizoctonia* spp. were also isolated from the foliage of other container-grown woody ornamentals with leaf lesions or foliar blight, indicating a relatively wide host range for binucleate *Rhizoctonia* spp.

The assignment of some isolates of binucleate *Rhizoctonia* spp. to CAG-3 or CAG-7 agreed with that of Burpee et al (6), who placed binucleate isolates from foliage of woody ornamentals into CAG-3 or CAG-7. Some isolates of binucleate *Rhizoctonia* spp. did not anastomose with any of the tester isolates in this study. Although CAG tester isolates 1-7 were used in these studies, Ogoshi et al (12) described 15 anastomosis groups for binucleate *Rhizoctonia* spp. Binucleate

isolates that did not anastomose with CAG tester isolates 1-7 in this study may be members of one of Ogoshi's 15 CAG or may have lost or never had the ability to anastomose (6).

Results of anastomosis testing with isolates of *R. solani* agree with the assignment of aerial isolates of *R. solani* to AG-1 by Anderson (3) and Parmeter et al (13).

Although attempts at producing perfect states of either binucleate or multinucleate isolates were not successful, some researchers (6,16) have designated binucleate isolates as species of *Ceratobasidium*, even in the absence of a perfect state. Some isolates of binucleate *Rhizoctonia* spp. were found to possess hyphal cells with predominantly two but occasionally three or four nuclei per cell. Isolates with one to three nuclei per cell have been treated as members of binucleate *Rhizoctonia* spp. (12). However, Adams (1) reported that a trinucleate isolate of *Rhizoctonia* formed heterokaryons with an AG-4 tester of *R. solani*, proving that the trinucleate isolate was *R. solani* and not a binucleate *Rhizoctonia* spp. Although none of the binucleate isolates anastomosed with tester isolates of *R. solani* in this study, indicating a taxonomic separation from *R. solani*, taxonomic placement of fungi with mycelial states similar to *R. solani* is difficult in the absence of the perfect state. Attempts to produce the perfect state of the web blight isolates should be continued.

The optimum growth temperature of 24-28 C for isolates of both binucleate *Rhizoctonia* spp. and *R. solani* in vitro are within the ranges of summertime temperatures when web blight is most

severe in production nurseries in North Carolina. Web blight of azalea is not typically a problem in the nursery during seasons when temperatures are cool and under greenhouse conditions when temperatures are above 32 C (T. A. Frisina, unpublished). Future studies should include tests to determine the effects of temperature on disease progression.

Differences in virulence existed among isolates of *Rhizoctonia* spp. used in this study. Isolates of *R. solani* were more virulent on azalea than any of the binucleate *Rhizoctonia* spp., and in some cases, isolates of binucleate *Rhizoctonia* spp. were avirulent on azalea. Disease severity ratings for all isolates were positively correlated with the height that the mycelium extended from the container surface into the foliage. At optimal temperatures in vitro, isolates of *R. solani* grew more rapidly than isolates of binucleate *Rhizoctonia* spp. This suggests that the greater virulence of isolates of *R. solani* on azalea may be a function of a more rapid rate of growth of aerial mycelium from the soil surface into the canopy of diseased plants.

## ACKNOWLEDGMENTS

We wish to thank Billy I. Daughtry for technical assistance, Bruce Martin for help with the DAPI nuclear staining procedure, and Marvin Williams for photographic assistance.

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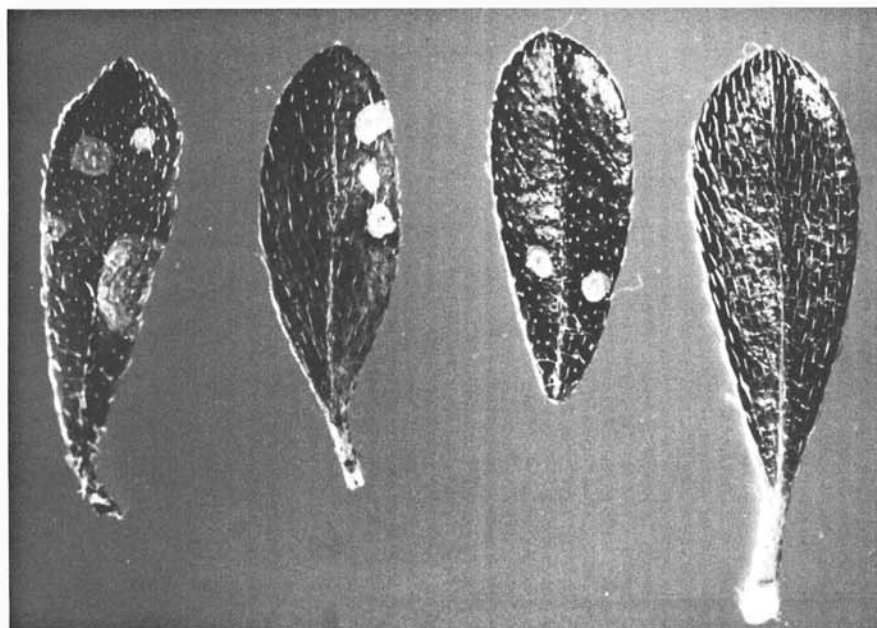


Fig. 4. Leaf lesions on *Rhododendron obtusum* cv. Gumpo caused by binucleate *Rhizoctonia* spp. Uninoculated leaf on the right.

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