Sensitivity of Binucleate *Rhizoctonia* spp. and *R. solani* to Selected Fungicides In Vitro and on Azalea Under Greenhouse Conditions

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ABSTRACT

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Benomyl, benodanil, chlorothalonil, and iprodione were evaluated in vitro for inhibition of linear growth of binucleate *Rhizoctonia* spp. (BN2 and BN8) from *Rhododendron* sp. with web blight and *Rhizoctonia solani* (RS15) from *Ilex crenata* 'Helleri' with leaf blight. Isolates of binucleate *Rhizoctonia* spp. and *R. solani* were sensitive ($ED_{50} < 1.1$ μg a.i./ml) to all fungicides tested in vitro. Dosage-response curves were similar for isolates of binucleate *Rhizoctonia* spp. and *R. solani* grown on potato-dextrose agar (PDA) amended with benodanil and iprodione. Dosage-response curves were steeper, on a log-probit basis, for *R. solani* grown on PDA amended with benomyl or chlorothalonil than for binucleate *Rhizoctonia* spp. Levels of inhibition of 14 selected isolates of binucleate *Rhizoctonia* spp. and four isolates of *R. solani* were normally distributed at ED_{50} concentrations of the fungicides. Benomyl, benodanil, and iprodione were evaluated under greenhouse conditions for efficacy of control of web blight (suppression of aerial mycelium) on azaleas inoculated with virulent isolates of binucleate *Rhizoctonia* spp. (BN2) and *R. solani* (RS25). Both binucleate *Rhizoctonia* spp. and *R. solani* responded similarly to fungicide treatments; the drenches of iprodione and the sprays of benomyl, benodanil, and iprodione effectively limited aerial mycelial growth.

Web blight is a serious foliar disease of azaleas (*Rhododendron* spp.) and other ornamental plants in North Carolina and other southeastern states. The disease can cause severe defoliation of container-grown ornamentals when conditions of warm temperature and high relative humidity prevail. A previous report (14) indicated that the causal agent of web blight of azalea was *Rhizoctonia solani* Kühn, and recommendations for chemical control of the disease have been based on this assumption. Benomyl, iprodione, and chlorothalonil are currently labeled for control of web blight on ornamentals.

Recently, fungi with mycelium typical of the form genus *Rhizoctonia* (9), but possessing predominantly binucleate hyphal cells instead of multinucleate hyphal cells characteristic of *R. solani*, were isolated from azaleas and other ornamentals with symptoms of web blight (6). Pathogenicity of these isolates on azalea has been verified (6). Binucleate *Rhizoctonia* spp. are taxonomically distinct from *R. solani*. The perfect state of some isolates of binucleate *Rhizoctonia* spp. has been identified as *Ceratobasidium* Rogers (2,10). The perfect state of *R. solani* is *Thanatephorus cucumeris* (Frank) Donk (13).

Differences in cultural morphology, growth rate, and virulence exist between binucleate *Rhizoctonia* spp. and *R. solani* (6). Differences also may exist in sensitivity to the fungicides labeled for control of web blight. Although chemical control of web blight has been investigated (1), the isolate used in the study was reported to be *R. solani* and not binucleate *Rhizoctonia* spp. Therefore, further studies are needed to determine if the fungicides recommended to control web blight are effective against binucleate *Rhizoctonia* spp.

Our studies were conducted to determine the sensitivity of binucleate *Rhizoctonia* spp. and *R. solani* to selected fungicides in vitro and on inoculated azalea under greenhouse conditions.

MATERIALS AND METHODS

Laboratory studies. Growth inhibition of binucleate *Rhizoctonia* spp. (BN2 and BN8, *Ceratobasidium* anastomosis groups 3 and 7, respectively), isolated from azalea with web blight, and *R. solani* (RS15, anastomosis group 1), isolated from the foliage of *Ilex crenata* Thunb. 'Helleri' with leaf blight, was determined on Difco potato-dextrose agar (PDA) amended with benomyl (Benlate 50WP), iprodione (Rovral 50WP), chlorothalonil (Bravo 500), and benodanil (Benefit 50WP). Fungicides were suspended in sterile deionized H_2O, then added to cooled (50 C) PDA at concentrations of 0.15, 0.30, 0.60, 1.25, and 2.50 μg a.i./ml (w/v). A nonamended check was also prepared for each isolate. The amended PDA (20 ml) was dispensed into 100-mm-diameter petri dishes. Mycelial plugs (6 mm diameter) cut from the margins of 3- to 4-day-old cultures of binucleate *Rhizoctonia* spp. and *R. solani* on PDA were placed onto three replicate plates of PDA at each fungicide concentration. Radial growth of each replicate was measured after 48 hr at 25 C, and percent inhibition of radial growth for each isolate was calculated based on the nonamended treatment for each isolate. Linear regression equations were fitted to logarithmic-probability data of fungicide concentration and percent growth inhibition for each fungicide-isolate treatment so that slope values and ED_{50} values (concentration giving 50% linear growth inhibition) could be interpolated (3). The experiment was repeated three times.

Linear growth response of an additional 14 isolates of binucleate *Rhizoctonia* spp., from the foliage of azalea and other ornamentals with web blight, and four isolates of *R. solani*, from the foliage of ornamentals with leaf blight, was evaluated on PDA amended with benomyl, benodanil, chlorothalonil, and iprodione, at concentrations found in the previous experiments to inhibit the mycelial growth of binucleate isolates BN2 and BN8 or of *R. solani* isolate RS15 by 50% (ED_{50} concentrations). Binucleate isolates were plated onto three replicated plates of PDA amended with benomyl at 0.7 μg a.i./ml, chlorothalonil at 0.9 μg a.i./ml, benodanil at 0.7 μg a.i./ml, and iprodione at 0.7 μg a.i./ml. Isolates of *R. solani* were plated onto three replicated plates of PDA amended with benomyl at 0.7 μg a.i./ml, chlorothalonil at 0.9 μg a.i./ml, benodanil at 0.3 μg a.i./ml, and iprodione at 0.6 μg a.i./ml. Percent radial growth inhibition of the 18 isolates was calculated on the basis of untreated controls for each isolate, and levels of growth inhibition were compared to levels of inhibition (50%) observed for isolates BN2, BN8, and RS15 at similar fungicide concentrations. The experiment was repeated three times.

Greenhouse studies. Control of web blight of azalea with benomyl, benodanil, and iprodione was evaluated on 6-mo-

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old Satsuki azalea (*Rhododendron* sp. 'Gumpo') grown in pine bark-sand (3:1, v/v) in 11.5-cm-diameter clay pots and fertilized weekly with 1.8 μg/ml of water-soluble 21-7-7 Peters fertilizer (W. R. Grace and Co., Fogersville, PA). Isolates BN2 and RS25 were selected for use in the test because they are virulent on azalea (6). Approximately 2.0 g of 14- to 21-day-old cultures of binucleate *Rhizoctonia* spp. (BN2, from azalea with web blight) or *R. solani* (RS25, from *Pittosporum tobira* (Thunb.) Aiton with foliar blight) grown on autoclaved bran (50 g of bran and 50 g of H_2O, 121 C, 15 psi, 60 min, for two consecutive days) was used as inoculum. Inoculum was placed on the container surface at the base of the plant stem, then covered with approximately 1.0 cm of autoclaved pine bark (121 C, 15 psi, 60 min). Fungicides were applied to the inoculated plants as soil drenches or foliar sprays. Soil treatments consisted of 100-ml drenches of benomyl at 300 μg a.i./ml, iprodione at 900 μg a.i./ml, or benodanil at 75, 150, or 300 μg a.i./ml. Foliar sprays were applied to the upper and lower leaf surface of each plant to the point of runoff with a 1-L hand-held pressure sprayer and consisted of benomyl at 300 μg a.i./ml, iprodione at 900 μg a.i./ml, or benodanil at 600, 1,200, or 1,800 μg a.i./ml. To prevent fungicide sprays from contacting inoculum on the container surface, a sheet of aluminum foil and a layer of sterile cotton were placed over the inoculum in each pot before application. This protective layer was removed immediately after plants were sprayed. Two replicate plants of each fungicide treatment were placed into humidity chambers (1.01 m^3 capacity) in a split-plot design with random main plots. Subplots were the fungicide treatments for each isolate main plot treatment factor. Inoculated plants were removed from the chambers after 6 days, and disease severity was rated by measuring the growth of aerial mycelium of *Rhizoctonia* spp. from the container surface (point of inoculum placement) into the foliage of the plant. The height that aerial mycelium grew from the container surface into the foliage of the plant was highly correlated (P = 0.001) with disease severity in previous tests (6). The experiment was conducted four times.

Table 1. ED_{50} values[x] (μg a.i./ml) for isolates of binucleate *Rhizoctonia* spp. and *R. solani* exposed to benomyl, benodanil, chlorothalonil, and iprodione

Isolate[y]	ED_{50} (μg a.i./ml)			
	Benomyl	Chlorothalonil	Iprodione	Benodanil
BN2	1.02	1.03	0.75	0.97
BN8	0.77	0.91	0.66	0.47
RS15	0.78	0.86	0.62	0.32
LSD[z]	ns	ns	ns	0.33*

[x] Interpolated from log-probit plots of percent inhibition and fungicide concentration.
[y] Binucleate *Rhizoctonia* spp. (BN2 and BN8) from *Rhododendron* sp. with web blight; *R. solani* (RS15) from foliage of *Ilex crenata* 'Heller' with leaf blight.
[z] Least significant difference $P = 0.05$, $n = 3$; ns = not significant.

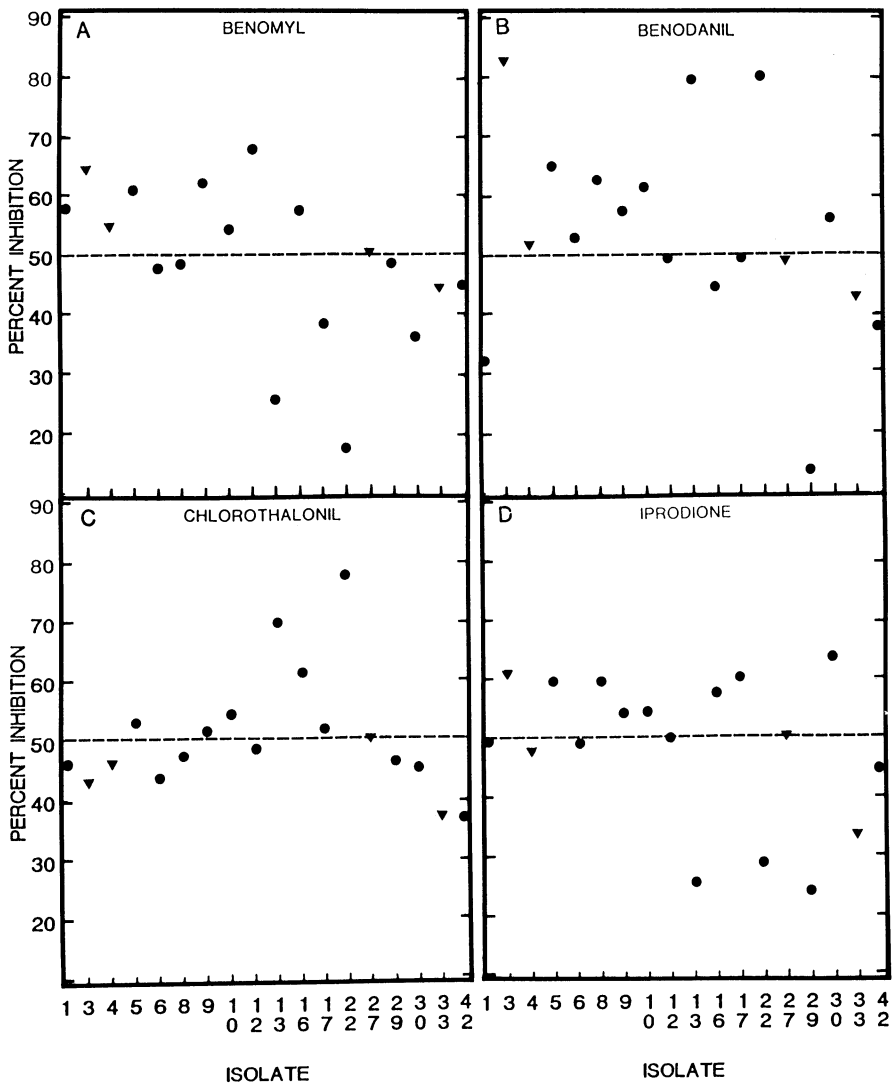

Fig. 1. Percent inhibition of mycelial growth of binucleate *Rhizoctonia* spp. and *R. solani* on potato-dextrose agar amended with benomyl at 0.7 μg a.i./ml, benodanil at 0.7 or 0.3 μg a.i./ml, chlorothalonil at 0.9 μg a.i./ml, and iprodione at 0.7 or 0.6 μg a.i./ml. The horizontal line at 50% represents the level of mycelial growth inhibition of isolates BN2, BN8, and RS15 at similar (ED_{50}) concentrations. • = Binucleate *Rhizoctonia* spp., ▼ = *R. solani*.

RESULTS

Laboratory studies. Isolates of binucleate *Rhizoctonia* spp. were similar to isolate RS15 of *R. solani* ($P = 0.05$) in sensitivity to benomyl, chlorothalonil, and iprodione (ED_{50} < 1.1 g a.i./ml) (Table 1). Although isolates of binucleate *Rhizoctonia* spp. and *R. solani* were sensitive to benodanil (ED_{50} < 1.0 μg a.i./ml) (Table 1), binucleate *Rhizoctonia* spp. (BN2) was significantly less sensitive ($P = 0.05$) than *R. solani* (RS15).

The 14 selected isolates of binucleate *Rhizoctonia* spp. and the four isolates of *R. solani* were sensitive to benomyl, benodanil, chlorothalonil, and iprodione at concentrations that inhibited BN2, BN8, and RS15 by 50%. Levels of inhibition for most of these isolates were 40–65% (Fig. 1). When frequency tables were constructed for these data, levels of inhibition of the additional isolates appeared normally distributed about the 50% level.

Significant interaction among isolate, fungicide, and concentration was apparent when data for growth response were plotted on a log-probit basis (Fig. 2). Slope values for both binucleate *Rhizoctonia* spp. and *R. solani* exposed to benomyl (Fig. 2A) were higher than slope values for the isolates treated with the other fungicides (Fig. 2B, C, D), indicating greater linear growth inhibition of both binucleate *Rhizoctonia* spp. and

R. solani with benomyl than with benodanil, chlorothalonil, or iprodione as fungicide concentration increased. Slopes for both binucleate *Rhizoctonia* spp. and *R. solani* exposed to chlorothalonil were lowest (Fig. 2C), indicating a slower rate of growth inhibition as fungicide concentration increased, relative to the other fungicides. Dosage-response curves for isolates exposed to either benodanil or iprodione appeared similar (Fig. 2B, D), indicating a similar rate of growth inhibition as fungicide concentration increased.

When dosage response data were plotted on a log-probit basis for isolates exposed to benomyl and chlorothalonil, a significant interaction between isolate and concentration was apparent (Fig. 2A,C). Dosage-response curves for *R. solani* (RS15) exposed to benomyl or chlorothalonil had 1.5- to 1.6-fold higher slope values than dosage-response curves for binucleate *Rhizoctonia* spp. (BN2, BN8) treated similarly, indicating that benomyl and chlorothalonil were approximately 1.5 times more active against *R. solani* than against binucleate *Rhizoctonia* spp. as fungicide concentration increased. Dosage-response curves for binucleate *Rhizoctonia* spp. and *R. solani* treated with benodanil or iprodione appeared parallel when plotted on a log-probit basis (Fig. 2B, D), indicating that both binucleate *Rhizoctonia* spp. and *R. solani* were inhibited at similar rates by benodanil and iprodione as fungicide concentration increased.

Greenhouse studies. Analysis of isolate response to benomyl, benodanil, and iprodione under greenhouse conditions showed that binucleate *Rhizoctonia* spp. (BN2) was similar ($P = 0.05$) to *R. solani* (RS25) in response to the fungicides tested. The main effects were highly significant ($P = 0.001$), indicating that the efficacy of control of web blight (suppression of aerial mycelium) was influenced by the fungicide used and method of application (spray or drench). When data of isolate response to the fungicides were analyzed separately by method of application, no significant differences were found between binucleate *Rhizoctonia* spp. and *R. solani* in response to the fungicide treatments, so data for binucleate *Rhizoctonia* spp. and *R. solani* were combined for analysis of fungicide main effects. Benomyl (300 μg a.i./ml), iprodione (900 μg a.i./ml), and benodanil (600 μg a.i./ml) applied as foliar sprays were equivalent in limiting aerial mycelium relative to the untreated control (Table 2). Mycelial growth was unaffected by increasing concentrations of benodanil (Table 2), and regression analysis of these data showed no dosage response with benodanil as fungicide concentration increased. Iprodione soil drench (900 μg a.i./ml) was highly effective in limiting aerial mycelium of *Rhizoctonia* spp., compared with benomyl or benodanil soil drenches and the untreated control (Table 3).

Single degree-of-freedom linear contrasts between spray treatments and drench treatments of each fungicide showed that spray treatments of benodanil were more effective ($P = 0.01$) than the drench, the drench treatment of iprodione

Table 2. Height of aerial mycelium[w] of binucleate *Rhizoctonia* spp. and *R. solani* after 6 days of incubation on azalea treated with foliar sprays[x] of benomyl, benodanil, and iprodione

		Benomyl (μg a.i./ml)	Benodanil (μg a.i./ml)			Iprodione (μg a.i./ml)
Isolate[y]	Untreated	300	600	1,200	1,800	900
BN2	3.14	2.15	1.95	1.26	1.15	1.56
RS25	4.91	2.66	1.98	1.62	1.71	2.66
Mean[z]	4.16 a	2.41 b	1.96 bc	1.44 c	1.43 c	2.11 bc

[w] Mean mycelial growth (cm) measured from point of inoculum placement (container surface) into foliage, $n = 8$.
[x] Applied to foliage to point of runoff.
[y] Binucleate *Rhizoctonia* spp. (BN2) from azalea with web blight; *R. solani* (RS25) from *Pittosporum tobira* with foliar blight.
[z] Means followed by same letter are not significantly different by Waller-Duncan's *k*-ratio *t* test (*k*-ratio = 100, $P = 0.05$, $n = 16$).

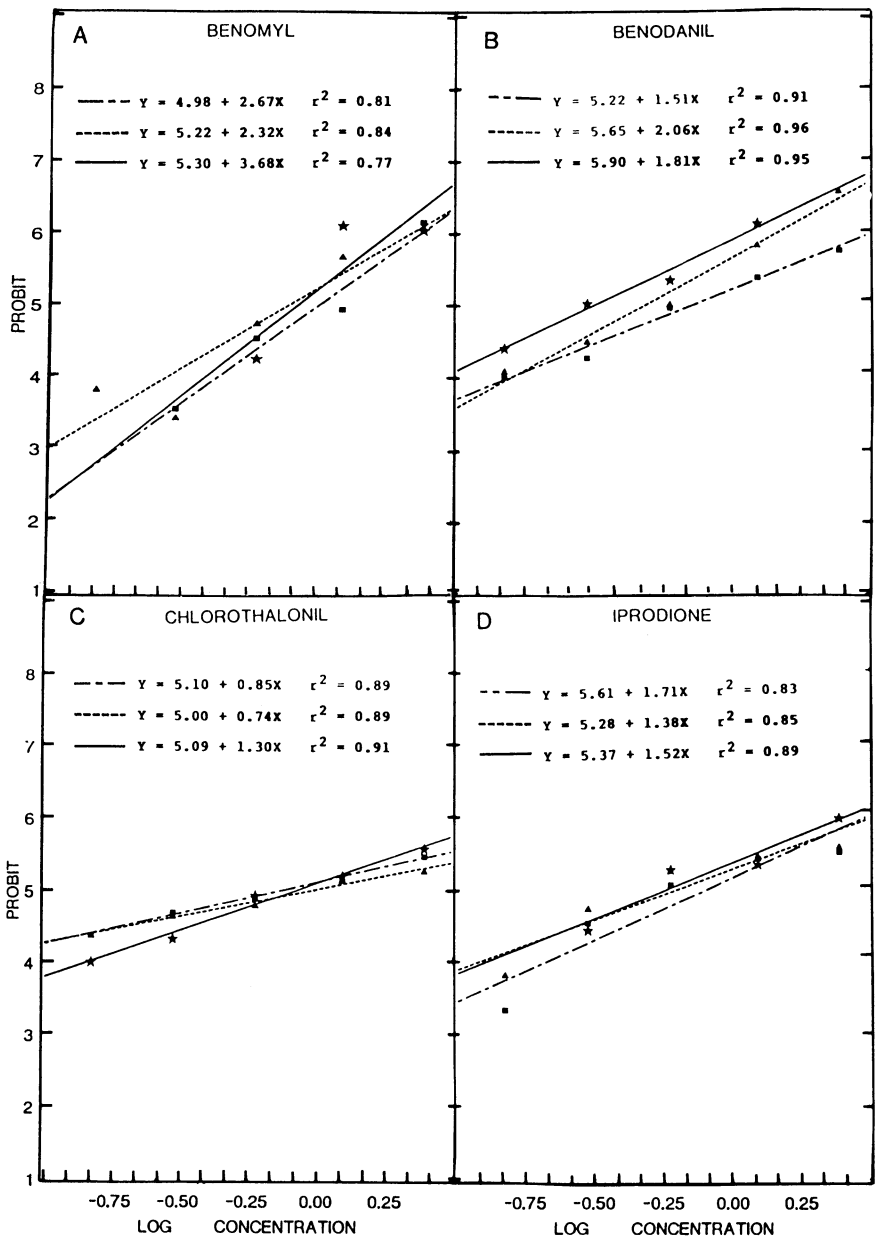

Fig. 2. Dosage-response curves for mycelial growth inhibition of binucleate *Rhizoctonia* spp. (BN2, BN8) and *R. solani* (RS15) with **(A)** benomyl, **(B)** benodanil, **(C)** chlorothalonil, and **(D)** iprodione. ■ = BN2, ▲ = BN8, and ★ = RS15.

Table 3. Height of aerial mycelium[x] of binucleate *Rhizoctonia* spp. and *R. solani* after 6 days of incubation on azalea treated with soil drenches (100 ml) of benomyl, benodanil, and iprodione

		Benomyl (μg a.i./ml)	Benodanil (μg a.i./ml)			Iprodione (μg a.i./ml)
Isolate[y]	Untreated	300	75	150	300	900
BN2	3.14	2.40	2.04	1.58	1.99	0.30
RS25	4.91	4.14	4.10	3.29	2.83	0.00
Mean[z]	4.16 a	3.27 b	3.07 bc	2.43 c	2.40 c	0.15 d

[x] Mean mycelial growth (cm) measured from point of inoculum placement (container surface) into foliage, $n = 8$.

[y] Binucleate *Rhizoctonia* spp. (BN2) from azalea with web blight; *R. solani* (RS25) from *Pittosporum tobira* with foliar blight.

[z] Means followed by same letter are not significantly different by Waller-Duncan's k-ratio t test (k-ratio = 100, $P = 0.05$, $n = 16$).

was more effective ($P = 0.0001$) than the spray, and the spray and drench treatments of benomyl were equivalent ($P = 0.10$) in limiting mycelium of binucleate *Rhizoctonia* spp. and *R. solani*.

DISCUSSION

Binucleate *Rhizoctonia* spp. did not differ from *R. solani* in sensitivity to benomyl, benodanil, chlorothalonil, or iprodione for control of web blight, either in vitro or under greenhouse conditions. The sensitivity of both binucleate *Rhizoctonia* spp. and *R. solani* to benomyl, chlorothalonil, and iprodione agree with other published accounts (4,7,8,11,12). However, insensitivity of some isolates of binucleate *Rhizoctonia* spp. to selected fungicides has been reported (8,11). Sanders et al (11) indicated that although most isolates of binucleate *Rhizoctonia*-like fungi from turfgrasses were sensitive to iprodione in vitro ($ED_{50} < 10$ μg a.i./ml), one binucleate isolate was resistant to iprodione (ED_{50} 1,000 μg a.i./ml). Martin et al (8) and Sanders et al (11) showed that some isolates of binucleate *Rhizoctonia* spp. from turfgrasses were insensitive (ED_{50} 100–1,000 μg a.i./ml) to chlorothalonil. Although no fungicide insensitivity was observed for isolates of binucleate *Rhizoctonia* spp. or *R. solani* in this study, there was a significant threefold difference between binucleate *Rhizoctonia* spp. (BN2) and *R. solani* (RS15) in sensitivity to benodanil. ED_{50} values for binucleate *Rhizoctonia* spp. and *R. solani* treated with benodanil, however, were less than 1.0 μg a.i./ml, and the reduced sensitivity of the binucleate isolate did not affect control when benodanil was applied to inoculated azaleas under greenhouse conditions.

Screening the 14 additional isolates of binucleate *Rhizoctonia* spp. and four isolates of *R. solani* at ED_{50} concentrations of the fungicides provided a rapid assay to screen for resistance to the fungicides. None of the additional isolates were resistant to the fungicides. Levels of mycelial inhibition of the additional isolates appeared normally distributed.

Binucleate *Rhizoctonia* spp. cause foliar diseases on a number of plant species (2) and are the causal agents of some diseases originally reported to be caused by *R. solani* (5,6). Although differential response to fungicides has been reported for species of *Rhizoctonia* (8), results of this study indicate a similarity in fungicide sensitivity between binucleate *Rhizoctonia* spp. and *R. solani* and imply that web blight of azalea, caused by binucleate *Rhizoctonia* spp., can be controlled by benomyl, benodanil, chlorothalonil, or iprodione.

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