Comparative Sensitivity of *Rhizoctonia solani* and Rhizoctonia-like Fungi to Selected Fungicides In Vitro

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ABSTRACT

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Benomyl, carboxin, PCNB, iprodione, chlorothalonil, and triadimefon were added to potato-dextrose agar at 0, 1, 10, and 100 mg a.i./L. The in vitro growth response (inhibition of linear growth) of 16 isolates of *Rhizoctonia solani*, binucleate Rhizoctonia-like fungi, and *R. zeae* from several sources were tested on fungicide-amended media. *Rhizoctonia solani* and binucleate Rhizoctonia-like fungi were sensitive to benomyl (EC_{50} [effective concentration for 50% inhibition of linear growth] <10 mg a.i./L), whereas isolates of *R. zeae* were tolerant to benomyl ($EC_{50} >50$ mg a.i./L) but sensitive to the other fungicides. Fungi were most sensitive to iprodione (EC_{50} generally <1 mg a.i./L), but growth inhibition in response to other fungicides (especially PCNB) differed considerably. Anastomosis group tester isolates of *R. solani* were variable in response to carboxin, PCNB, chlorothalonil, and triadimefon.

The form-genus *Rhizoctonia* presently contains nearly 100 species including basidiomycetes, ascomycetes, and imperfect fungi (17). Several of these species have been shown to induce various diseases on turfgrasses and other crops (4,6). The most-studied and well-known of these is *Rhizoctonia solani* Kühn (perfect state, *Thanatephorus cucumeris* [Frank] Donk) which induces brown patch on many cool- and warm-season turfgrasses (9). The major identifying characteristics of *R. solani* include: branching of lateral hyphae at right or acute angles, branching near the distal septum of cells in young vegetative hyphae, constriction of the branch and formation of a septum in the branch near the point of origin, some shade of brown pigmentation, and multinucleate hyphal cells (17).

Certain fungi closely resemble *R. solani* in mycelial characteristics but possess predominantly binucleate hyphal cells (18). The perfect states of some of these binucleate Rhizoctonia-like fungi (RLF) have been identified as species of *Ceratobasidium* Rogers (4–6,18). This group of binucleate RLF has been shown to possess discrete anastomosis groups similar to those within *R. solani* (5). One anastomosis group, Burpee's CAG 1 (5), includes isolates of *Rhizoctonia cerealis* van der Hoeven which induces sharp eyespot in cereals (1) and "yellow patch" on some turfgrasses (4).

Other binucleate RLF and *Rhizoctonia* spp. may also induce diseases of turfgrasses. Campbell (7) isolated several binucleate RLF from roots of ladino clover (*Trifolium repens* L.) from fescue-clover pastures and was able to induce severe foliar blight on tall fescue (*Festuca arundinacea* Schreb.) in greenhouse inoculations with some isolates. These isolates differed in several characteristics from the present species criteria for *R. cerealis* (1,4,7).

Another species, *Rhizoctonia zeae* Voorhees, may cause foliar blight on some grasses during hot and humid conditions in North Carolina (15). Isolates of *R. zeae* are quite distinct from *R. solani* and binucleate RLF (26). The isolates studied from turfgrasses consistently possessed multinucleate hyphal cells (15), in disagreement with another report on the nuclear condition of *R. zeae* (24).

Because of the diversity of species of *Rhizoctonia* and RLF which may induce turfgrass diseases, this study was initiated to establish possible differences among pathogenic isolates of *R. solani*, binucleate RLF, and *R. zeae* in response to several fungicides. The information gained by this, and a companion study (14) on in vivo differences among blights induced by some isolates of *Rhizoctonia* spp. following fungicide treatments could be used as additional criteria for establishment of biological relatedness among these fungi, as well as be useful in development of control measures.

MATERIALS AND METHODS

Sixteen fungal isolates representing at least three *Rhizoctonia* spp. were tested for sensitivity to several fungicides in vitro. Isolates of *R. solani* included anastomosis group (AG) tester isolates AG 1, 2, 3, and 4 (16), two isolates (RS 96 and RS 132) from diseased Bermuda grass (*Cynodon dactylon* L.), and one isolate (RS 44) from diseased foliage of tall fescue. The AG tester isolates of *R. solani* were not previously evaluated for pathogenicity to tall fescue or other turfgrasses, although similar isolates in AG 1, 2, and 4 are known turfgrass pathogens. The remaining *R. solani*, the binucleate RLF, and isolates of *R. zeae* were pathogens of tall fescue. The isolates from Bermuda grass fused with AG tester isolate AG 4 of *R. solani*, but a killing reaction resulted; RS 44 from tall fescue was assignable to AG 1. Binucleate RLF included one isolate (Bn 55) of *R. cerealis* from diseased bentgrass (*Agrostis palustris* Huds.), five isolates (Bn 15, Bn 83, Bn 89, Bn 109, and Bn 133) from ladino clover roots (7), and one isolate (Bn 110) from organic debris from soil in tall fescue turf. Two isolates (RZ 42 and RZ 197) of *R. zeae* originally isolated from foliar lesions in tall fescue were also tested.

Dilutions of each of the six fungicides were prepared (w/v) by dissolving appropriate amounts of each fungicide (based on the active ingredient) in 10 ml of 95% ethanol. Equal volumes of ethanol containing diluted fungicides were added to sterile, cool (50 C) potato-dextrose agar (PDA) to get concentrations of 1, 10, and 100 mg a.i./L of PDA for each fungicide. A zero (0) concentration treatment was prepared for each isolate, and contained 1% (v/v) of 95% ethanol to ensure equivalent ethanol concentrations in all treatments. Fungicide-amended PDA was dispensed aseptically into 9-cm-diameter plastic petri dishes (~ 20 ml per dish). The fungicides tested included: benomyl [methyl 1-(butyl-carbamoyl)-2-benzimidazole carbamate], carboxin (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin), PCNB (pentachloronitrobenzene), triadimefon [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone], iprodione [3-(3,5-dichlorophenyl)-*N*-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxamide], and

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chlorothalonil (tetrachloroisophthalonitrile).

Plugs of mycelium (4-mm diameter) were cut from the margins of actively growing PDA cultures of the isolates of *Rhizoctonia* spp. and inverted in the center of fungicide-amended and unamended PDA plates with three replicate plates for each isolate-fungicide combination. Colony diameters (in millimeters) were measured after plates were incubated ~48 hr at 28 C in the dark. Relative proportion of growth by fungicide treatments for each combination was calculated based on the unamended treatment for each isolate. Two separate tests were conducted because of the large number of isolates and fungicides tested. Benomyl, carboxin, and PCNB were tested together as were triadimefon, iprodione, and chlorothalonil. Each experiment was repeated with similar results; the data reported herein were from the second run of each experiment.

Effects of fungicide treatments were compared by analysis of variance and by pertinent single-degree-of-freedom linear contrasts (planned comparisons) between isolate and fungicide treatment combinations (23). The estimated effective concentration of each fungicide to give 50% inhibition of radial growth (EC_{50}) for each isolate was determined, whenever possible, by interpolation from computer-generated log-probit plots of fungicide concentration and relative inhibition for each isolate-fungicide combination (13). Whenever possible, slopes of the lines were also determined. For purposes of comparison, isolates were considered tolerant of a fungicide if the EC_{50} exceeded 50 mg a.i./L, moderately sensitive if the EC_{50} was 1–10 mg a.i./L, and extremely sensitive if the EC_{50} was less than 1 mg a.i./L.

RESULTS

There were significant first-order interactions (of isolates × fungicides, and fungicides × concentrations), and significant second-order interactions for both experiments. The most important interaction was that isolates responded differently depending on the fungicide in both tests. Therefore, isolates within a species (eg, isolates of *R. solani* or *R. zeae*) and/or between species responded differently to the fungicides. The first-order interactions of isolates × concentrations were not significant for both experiments. Major groupings of isolates (eg, isolates of binucleate RLF versus those of *R. solani*) were compared for response to specific fungicides using contrasts (planned comparisons); these comparisons indicated that isolates of *R. zeae* differed significantly from those of *R. solani* in response to benomyl (Table 1). Isolates of *R. zeae* were less sensitive to benomyl than isolates of *R. solani* and the binucleate RLF. As a group, isolates of *R. zeae* did not differ significantly from isolates of the binucleate RLF at $P = 0.05$ in response to benomyl (Table 1), but they were significantly different at $P = 0.10$ (*unpublished*). Isolates of binucleate RLF and *R. solani* in general did not differ in response to benomyl or the other fungicides when compared over all fungicide concentrations (Table 1).

Analysis of specific isolate response to separate fungicides revealed a variability in response to the various fungicides (Tables 2 and 3), and explained the significant isolate × fungicide interaction in the overall analyses of variance. Isolates of *R. solani* differed in sensitivity to benomyl, with AG 2 and the Bermuda grass isolate, RS 96, being less sensitive than the other isolates (Table 2). Binucleate RLF were similarly sensitive or extremely sensitive to benomyl, as most isolates were completely inhibited at 10 and 100 mg a.i./L. Isolates RZ 197 and RZ 42 of *R. zeae* were tolerant to benomyl at the concentrations tested (Table 2).

Isolate responses to carboxin also varied (Table 2). Isolates of *R. solani* were generally extremely sensitive (EC_{50} <1) with the exception of the *R. solani* AG 3 tester isolate and (to a lesser degree) RS 96 and RS 132, the Bermuda grass isolates. Binucleate RLF varied in response to carboxin from moderate to extreme sensitivity, whereas the isolates of *R. zeae* were extremely sensitive (Table 2).

Fungi were more variable in response to PCNB than to the other fungicides tested; isolates exhibited extreme sensitivity, moderate sensitivity, and tolerance (Table 2).

Most isolates were extremely sensitive to iprodione. Isolates RZ 197 and RZ 42 of *R. zeae* were moderately sensitive (Table 3).

Chlorothalonil severely inhibited growth of isolates AG 3, RS 44, RS 132, and RS 96 of *R. solani*. The anastomosis group testers AG 1, 2, and 4 were moderately sensitive (Table 3). The response of binucleate RLF isolates to the chemical also varied, with approximately half of the isolates tested being sensitive and the remainder being tolerant (eg, BN 83) or moderately sensitive (Table 3). Isolates of *R. zeae* differed in response to chlorothalonil, with RZ 197 moderately sensitive and RZ 42 extremely sensitive. This was the only fungicide tested for which the response of the isolates of *R. zeae* differed (Tables 2 and 3).

Triadimefon was somewhat less effective than the other fungicides in inhibiting growth of isolates of *Rhizoctonia* (Tables 2 and 3). The isolates of *R. zeae* and *R. solani* AG 1 were the only isolates that were extremely sensitive to triadimefon; the others were all moderately sensitive (Table 3).

DISCUSSION

When isolates of *R. solani*, binucleate RLF, and *R. zeae* were compared as groups, the only obvious difference in response to fungicides was the tolerance of the isolates of *R. zeae* to benomyl in

TABLE 1. Single degree-of-freedom linear contrasts between major *Rhizoctonia* spp. and binucleate Rhizoctonia-like fungi (RLF) groupings and their response to individual fungicides[a]

Fungicide treatment	Mean squares for isolate group contrasts: Binucleate RLF vs. *R. solani*	Binucleate RLF vs. *R. zeae*	*R. solani* vs. *R. zeae*
Benomyl	0.0009 ns[b]	0.4821 ns	0.9875 **
Carboxin	0.0053 ns	0.0743 ns	0.1030 ns
PCNB	0.1571 ns	0.2723 ns	0.0309 ns
Iprodione	0.0623 ns	0.3562 ns	0.2690 ns
Chlorothalonil	0.0296 ns	0.0496 ns	0.1391 ns
Triadimefon	0.0096 ns	0.0160 ns	0.0449 ns

[a]Response over all concentrations (no significant isolate × concentration interaction) based on a random model for general application to other similar isolates not used in this study.
[b]ns and ** indicate not significant and significant at $P = 0.01$, respectively.

TABLE 2. EC_{50} values and slopes[a] of fungicide dosage-linear growth response curves for *Rhizoctonia* spp., binucleate Rhizoctonia-like fungi (RLF), and *R. zeae* in fungicide-amended PDA after 48 hr of growth at 28 C. Test 1

Isolate[b]	Fungicide: Benomyl EC_{50}	Benomyl Slope	Carboxin EC_{50}	Carboxin Slope	PCNB EC_{50}	PCNB Slope
AG 1	1.48	−0.87	0.13	−0.11	2.03	−0.19
AG 2	5.60	−0.60	0.31	−0.52	0.19	−0.19
AG 3	<1[c]	···	38.80	−0.46	0.83	−0.35
AG 4	<1	···	0.01	−0.10	>100.00	···
RS 44	<1	···	<1	···	0.11	−0.30
RS 132	<1	···	1.46	−0.48	<1	···
RS 96	4.64	−0.77	1.98	−0.53	<1	···
BN 110	1.40	−1.10	4.00	−0.24	41.0	−0.12
BN 55	<1	···	1.00	−0.09	<1	···
BN 15	<1	···	<1	···	137.0	−0.26
BN 83	<1	···	13.50	−0.17	<1	···
BN 89	<1	···	3.40	−0.27	68.90	−0.04
BN 109	<1	···	<1	···	215.50	−0.17
BN 133	<1	···	1.05	−0.36	0.20	−0.45
RZ 197	114.30	−0.34	0.06	−0.23	2.12	−0.55
RZ 42	57.90	−0.54	0.06	−0.26	1.28	−0.40

[a]EC_{50} values (milligrams a.i./L) and slopes were determined by computer interpolation or extrapolation from log-dosage probit-inhibition plots for each isolate-fungicide combination.
[b]RS, BN, and RZ refer to *Rhizoctonia solani*, binucleate RLF, and *R. zeae*, respectively.
[c]Growth was completely halted at 10 and 100 mg a.i./L, but growth was inhibited at <50% at 1 mg a.i./L.

comparison to those of *R. solani* and binucleate RLF, which were sensitive or extremely sensitive to benomyl. In these experiments, we used only two isolates of *R. zeae*, but subsequent tests with other isolates indicated consistent tolerance of *R. zeae* to this fungicide (Martin, *unpublished*).

Windham and Lucas (27) recently identified the perfect state of *R. zeae* as a *Waitea* sp. and the description of mycelium of isolates of *Waitea circinata* Warcup and Talbot as pathogens of turfgrasses in New Zealand by Christensen (8) is similar to that of North Carolina isolates. Christensen (8) also tested isolates of *R. solani*, a binucleate RLF, and *W. circinata* for in vitro response to benomyl, and reported tolerance of *W. circinata* isolates to benomyl. The similar mycelial characteristics, pathogenicity to turfgrasses, and tolerance to benomyl of *R. zeae* and *W. circinata* may suggest relatedness between these fungi. The significance of benomyl tolerance of *R. zeae* in turfgrass pathology cannot yet be fully evaluated since *R. zeae* has only recently been shown to induce foliar blight on tall fescue and other turfgrasses (15) and its full impact as a turfgrass pathogen awaits further evaluation.

Benomyl has been shown to be effective against most diseases induced by *R. solani* but it is not effective against all basidiomycetes (10). The relative sensitivity of *R. solani* and binucleate RLF to benomyl is in agreement with results of other workers (2,12,25). Bollen and Fuchs (2) reported an ED_{50} of 1–5 mg/L for one isolate of *R. solani*. Edgington et al (12) reported an ED_{50} of 3.0 mg/L for *Thanatephorus cucumeris* [Frank (Donk)] (imperfect state = *R. solani*) and van der Hoeven and Bollen (25) reported ED_{50} values for *R. cerealis* between 2.2 and 3.1 mg a.i./L.

Most isolates of *R. solani* were sensitive or moderately sensitive to PCNB, but some binucleate RLF were tolerant. Isolate variability in sensitivity to PCNB was not unexpected since Shatla and Sinclair (21) demonstrated that isolates of *R. solani* from cotton exhibited a range of sensitivity to PCNB that approximated a normal distribution.

Results indicated that there may be differences in isolate response to fungicides within the group characterized by *R. solani*. The differential response of AG tester isolates of *R. solani* to several fungicides is noteworthy. In other fungicide tests with isolates of *R. solani*, AG affinities have not been reported. A group of isolates of *R. solani* within specific anastomosis groups should be investigated for variation within and among AG groups. Similarly, groups of isolates within CAG groups for pathogenic binucleate *Rhizoctonia* spp. (5) and within other *Rhizoctonia* spp. (eg, *R. zeae*) should be tested for variability in response to fungicides.

The other fungicides tested, especially iprodione, were generally effective in inhibiting the *Rhizoctonia* spp. in vitro. Sanders et al (20) investigated the efficacy of iprodione for control of several turfgrass pathogens for in vitro growth inhibition. They reported ED_{50} values of <10 mg/L for *R. solani* and a probable *Ceratobasidium* sp. (*R. cerealis* ?). Results in the present study indicated extreme sensitivity, with EC_{50} values of <1 mg/L for most *R. solani* and binucleate RLF and EC_{50} values of <5 mg/L for the isolates of *R. zeae* that were studied. Sanders et al (19) similarly investigated triadimefon and reported that four of five isolates of *R. solani* were sensitive with an ED_{50} <10 mg/L; however, one isolate was tolerant (ED_{50} <100 mg/L). Our results indicated that isolates of *R. solani* were less sensitive to triadimefon than to other fungicides but were still moderately sensitive (EC_{50} usually <10 mg/L).

The inhibitory effects of carboxin on *R. solani* and other basidiomycetes are well documented (3,11,22) with ED_{50} values of <5 mg/L. Results from this study are in agreement with the exception of those for the AG 3 tester isolate of *R. solani* (EC_{50} = 38 mg/L). Again, studies of within-species and among-AG-group sensitivities should clarify this indication of possible differential sensitivities to fungicides.

Results of these experiments indicated that different *Rhizoctonia* spp. and isolates within species may respond differentially to fungicides in vitro. These results indicate only direct effects of fungicides on the pathogens tested and the data may not correlate with disease control in the field. Such a situation has been reported by Van der Hoeven and Bollen (25) in which sharp eyespot (induced by *R. cerealis*) was enhanced by benomyl applications, even though *R. cerealis* was sensitive to benomyl in vitro. We have further evaluated the effect of these fungicides for reduction of foliar blights induced by some of these isolates of *Rhizoctonia* spp. (14) to better establish differences among isolates and disease response induced by such isolates in relation to fungicide applications.

TABLE 3. EC_{50} values and slopes[a] of fungicide dosage-linear growth response curves for *Rhizoctonia* spp., binucleate Rhizoctonia-like fungi (RLF), and *R. zeae* in fungicide-amended PDA after 48 hr of growth at 28 C. Test 2

	Fungicide					
	Iprodione		Chlorothalonil		Triadimefon	
Isolate[b]	EC_{50}	Slope	EC_{50}	Slope	EC_{50}	Slope
AG 1	<1[c]	···	1.86	−0.25	0.80	−0.51
AG 2	<1	···	9.80	−0.20	9.85	−0.47
AG 3	<1	···	0.24	−0.20	4.17	−0.40
AG 4	<1	···	4.74	−0.27	14.89	−0.55
RS 44	<1	···	0.30	−0.25	2.97	−0.45
RS 132	<1	···	0.16	−0.27	1.61	−0.33
RS 96	<1	···	0.02	−0.15	2.03	−0.38
BN 110	<1	···	3.74	−0.17	3.06	−0.59
BN 55	<1	···	<1	···	5.70	−0.75
BN 15	<1	···	0.11	−0.24	4.99	−0.47
BN 83	<1	···	112.68	−0.20	14.10	−0.52
BN 89	0.85	−0.99	4.64	−0.18	2.65	−0.40
BN 109	<1	···	0.76	−0.28	7.67	−0.48
BN 133	<1	···	1.23	−0.23	3.65	−0.55
RZ 197	2.53	−0.90	10.33	−0.27	0.15	−0.30
RZ 42	2.20	−0.97	0.69	−0.24	0.19	−0.36

[a] EC_{50} values (milligrams a.i./L) and slopes were determined by computer interpolation or extrapolation from log-dosage probit-relative growth plots for each isolate-fungicide combination.

[b] RS, BN, and RZ refer to *Rhizoctonia solani*, binucleate RLF, and *R. zeae*, respectively.

[c] Growth completely halted at 10 and 100 mg a.i./L, but was inhibited <50% at 1 mg a.i./L.

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