In Vitro Sensitivity of *Rhizoctonia solani* and Other Multinucleate and Binucleate *Rhizoctonia* to Selected Fungicides

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ABSTRACT

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Isolates of *Rhizoctonia solani, R. zeae, R. oryzae,* and binucleate *Rhizoctonia,* including representatives of 11 anastomosis groups of *R. solani,* were exposed to a range of concentrations (0, 0.01, 0.10, 1.00, or 10.00 mg a.i./L) of five fungicides (benomyl, hexaconazole, iprodione, PCNB, and prochloraz) in vitro. EC_{50} values were determined for each fungus-fungicide combination. All isolates were highly sensitive to hexaconazole (EC_{50} values less than 1 mg a.i./L), and most isolates were moderately sensitive (EC_{50} values between 1 and 10 mg a.i./L) to the other four fungicides. The EC_{50} values for *R. zeae, R. oryzae,* and similar isolates frequently were much lower or much higher than those for isolates of *R. solani* or binucleate *Rhizoctonia,* but they did not follow a consistent pattern. EC_{50} values based on radial growth were as much as 10 times greater than EC_{50} values based on dry weight.

Rhizoctonia solani Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) is a large and heterogeneous collection of predominantly soilborne, primarily plant-pathogenic fungi. *R. solani* has been divided into subspecific groups based on pathogenicity, colony morphology, serology, and other characteristics (11), but perhaps the best way to group isolates of *R. solani* is by anastomosis group (AG) affinity. Currently, *R. solani* is divided into 11 AGs (3,13), and one additional group, AG-10, has been proposed (14).

Division of the species into AGs is based on the ability of hyphae of different isolates to anastomose. When hyphae fuse or connect, parent isolates are said to belong to the same AG. The anastomosis reaction in *R. solani* is a sign of somatic incompatibility (1) and, despite the localized cell death (18) that often accompanies anastomosis, is indicative of relatedness between anastomosing isolates. Members of the same AG are said to be more closely related to each other than to members of other AGs. This presumption has been confirmed by comparative studies using DNA/DNA hybridization techniques (10,24) and molecular markers (25). Because individual isolates in most AGs do not react with isolates from other groups, AGs have been described as isolated populations within *R. solani,* and they may qualify as separate species (21,24).

The reactions of isolates of *R. solani* to many fungicides (6–8,11,12,20,22,23) and at least one antibiotic (9) generally have been reported to vary within and among AGs. Most of these reports evaluated isolates of one to as many as five AGs, although in one report (20) the isolates were not identified as to AG. In this study, we measured and compared the in vitro reactions of isolates of *R. solani*, other multinucleate species of *Rhizoctonia* (with teleomorph *Waitea circinata* Warcup & Talbot), and binucleate *Rhizoctonia* (with teleomorphs *Ceratobasidium* spp.) to a range of concentrations of five fungicides.

MATERIALS AND METHODS

Fifty-seven isolates (Table 1), representing 11 AGs (AG-1, AG-2-1, AG-2-2, AG-3, AG-4, AG-5, AG-6, AG-BI, AG-7, AG-8, and AG-9) of *R. solani, R. zeae* Voorhees (17), *R. oryzae* Ryker & Gooch (17), and binucleate *Rhizoctonia* (AG-C, AG-E [CAG-3], AG-H, and AG-I) (2,15,16), were acquired and maintained on rehydrated potato-dextrose agar (PDA).

The test fungicides were benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate), hexaconazole ((*RS*)-2-(2,4-dichlorophenyl)-1-(IH-1,2,4-triazol-1-yl)hexan-2-ol), iprodione (3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxamide), PCNB (pentachloronitrobenzene), and prochloraz (N-propyl-N-(2-(2,4,6-trichlorophenoxy)-ethyl)-imidazole-1-carboxamide). All 57 fungal isolates were challenged with a range of concentrations of hexaconazole, iprodione, and PCNB; only selected isolates from AG-1, AG-2, AG-3, AG-4, and *R. oryzae* were challenged with benomyl and prochloraz. Based on results with hexaconazole, iprodione, and PCNB, we felt that the shortened list of isolates representing the more common AGs would sufficiently represent the species in tests with benomyl and prochloraz.

Aqueous stock solutions or suspensions of each fungicide except iprodione were prepared. Because of the low solubility of iprodione in water, its stock solution was prepared in acetone. Dilutions of each stock were prepared, and (except for iprodione, which was added before autoclaving) 10 ml of each dilution was added to autoclaved PDA cooled to 50 C to obtain final concentrations of 0, 0.01, 0.10, 1.00, and 10.00 mg a.i./L. The zero-concentration treatments received a quantity of solvent (water or acetone) equivalent to that used in the fungicide treatments. After thorough mixing, fungicide-amended PDA was dispensed into 9-cm petri plates and stored in the dark until use. Plates were used within 1 wk.

Disks of mycelium 6 mm in diameter cut from the growing edge of cultures of fungal isolates were placed in the center of plates amended with one of the five concentrations of fungicide. Each fungus-fungicide combination was replicated three times, and the study was repeated with selected isolates and selected fungicides. In addition, because the large size of the experiment required that it be done in sections, one isolate (W14L) was repeated with each section to ensure consistency of results.

Radial growth was recorded after 96 hr at 20–22 C. The agar containing the fungal colony was then melted in an autoclave. The mycelial mat was separated from the molten agar by suction filtration and then air-dried to a constant weight. In a separate experiment conducted to determine whether autoclaving significantly affected the mass of harvested mycelium, dry weights of autoclaved and nonautoclaved colonies of *R. solani* produced in liquid shake cultures were measured. No significant differences were observed.

Relative growth of each isolate was evaluated based on mycelial growth (dry weight and radial growth) as a percentage of the control (zero-concentration) treatment for each fungus-fungicide combination. The concentration of fun-

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gicide required to give 50% inhibition of growth (EC_{50}), based on changes in radial growth and mycelial dry weight, was estimated, when possible, by computer-generated log-probit plots (5,19) for each fungus-fungicide combination. Whenever the estimated EC_{50} fell within the range of test concentrations, the exact figure is reported. Occasionally, EC_{50} values fell outside the range of test concentrations. In these cases, the estimated EC_{50} is reported as greater than 10 or less than 0.01 mg a.i./L.

RESULTS

The reactions of all 57 isolates, by groups, to in vitro exposure to increasing concentrations of hexaconazole, iprodione, and PCNB are summarized in Table 2. All multinucleate and binucleate species or groups were highly sensitive to hexaconazole, as indicated by EC_{50} values, based on reductions in dry weight, of 0.12 mg a.i./L or less (Table 2). *R. zeae, R. oryzae,* and the other multinucleate isolates possessing similar characteristics (*Rhizoctonia* sp. [AK] and [X-1]) all had EC_{50} values below 0.01 mg a.i./L.

Isolates of *Rhizoctonia* generally were less sensitive to iprodione and PCNB (Table 2); EC_{50} values based on reduction in dry weight ranged from 0.42 mg a.i./L with PCNB to more than 10 mg a.i./L with iprodione. All multinucleate and binucleate groups reacted in a generally similar way to PCNB, but the *R. zeae-R. oryzae* group of isolates appeared generally less sensitive to iprodione than either *R. solani* or the binucleates.

The reactions of isolates to benomyl and prochloraz are summarized in Table 3. EC_{50} values for each fungicide based on dry weights were generally comparable to those for iprodione and PCNB. *R. oryzae,* though sensitive to prochloraz, appeared relatively insensitive to benomyl.

EC_{50} values based on radial growth were consistently higher (as much as 10 times) than the corresponding EC_{50} values based on dry weight (Tables 2 and 3). Changes in dry weight and radial growth in response to increasing concentrations of the various fungicides followed the same trend but differed greatly in magnitude.

DISCUSSION

Varying reactions to fungicides and other substances within and among groups of fungi that fall under the collective label of *Rhizoctonia* have been reported (4,8,9,12,20,23). However, studies to date have included only representatives of the four most common AGs of *T. cucumeris* (*R. solani*), along with selected representatives of *Ceratobasidium* (binucleate *Rhizoctonia*) and *Waitea* (*R. oryzae, R. zeae,* and *Rhizoctonia* sp. AK and X-1). The results of this study, which evaluated representatives of 11 AGs of *T. cucumeris,* support the earlier conclusions. Using the sensitivity scale described by Martin et al (12), all isolates of all AGs of *T. cucumeris* can be called extremely sensitive to hexaconazole, with EC_{50} values below 1 mg a.i./L. At the same time, isolates of *T. cucumeris* collectively

Table 1. Anastomosis group, geographic origin, and source of isolates of *Rhizoctonia solani* and other *Rhizoctonia* used in this study

Isolate	Anastomosis group	Geographic origin	Collector and/or provider
R. solani (*Thanatephorus cucumeris*)			
43	1-1C	Canada	N. A. Anderson
7317	1-1A	W. Java	N. A. Anderson
7341	1-1A	E. Java	N. A. Anderson
F56L	2-1	Alaska	D. E. Carling and R. H. Leiner
KHP1	2-1	Alaska	D. E. Carling and R. H. Leiner
HV1	2-1	Japan	A. Ogoshi
F6M	2-1	Alaska	D. E. Carling and R. H. Leiner
V3L	2-1	Alaska	D. E. Carling and R. H. Leiner
BS11	2-1	Alaska	D. E. Carling and R. H. Leiner
BR2	2-2 IV	Japan	S. Kuninaga
GAR2	2-2 ?	New York	G. S. Abawi
B60	2-2 IIIB	Japan	S. Kuninaga
RI64	2-2 IV	Japan	A. Ogoshi
W14L	3	Alaska	D. E. Carling and R. H. Leiner
T141	3	Idaho	J. R. Davis
R542	3	Japan	A. Ogoshi
1330	3	Australia	S. M. Neate
M2	3	Alaska	D. E. Carling and R. H. Leiner
BS5	3	Alaska	D. E. Carling and R. H. Leiner
KHP17	3	Alaska	D. E. Carling and R. H. Leiner
L22	3	Alaska	D. E. Carling and R. H. Leiner
SCL13	3	Alaska	D. E. Carling and R. H. Leiner
P42	3	?	N. A. Anderson
RH75	4	Japan	A. Ogoshi
SN1	4	Japan	A. Ogoshi
T183	4	Idaho	J. R. Davis
GM-10	5	Japan	A. Ogoshi
ST-6-1	5	Japan	A. Ogoshi
HAM11	6	Japan	S. Kuninaga
NTA31	6	Japan	S. Kuninaga
1529	7	Japan	S. Kuninaga
1556	7	Japan	S. Kuninaga
811	8	Australia	S. M. Neate
C1	8	Washington	E. N. Bassett
H1	8	Washington	E. N. Bassett
S21	9	Alaska	D. E. Carling and R. H. Leiner
S9R1	9	Alaska	D. E. Carling and R. H. Leiner
AI14	BI	Japan	S. Kuninaga
TE24	BI	Japan	S. Kuninaga
R. zeae (*Waitea circinata*)			
C504		Japan	A. Ogoshi
590		Japan	A. Ogoshi
N101		Japan	A. Ogoshi
R. oryzae (*W. circinata*)			
CAL1		California	P. S. Gunnell
321		Washington	A. Ogoshi
231		Washington	A. Ogoshi
541		Japan	A. Ogoshi
161		Washington	A. Ogoshi
Rhizoctonia sp. (*W. circinata*)			
Z16[a]		Alaska	D. E. Carling and R. H. Leiner
Z4[a]		Alaska	D. E. Carling and R. H. Leiner
26b1[a]		Washington	A. Ogoshi
81b2[a]		Washington	A. Ogoshi
Rhizoctonia sp. (*Ceratobasidium* sp.)			
S1212	E	Alaska	D. E. Carling and R. H. Leiner
Bn180	E	Alaska	D. E. Carling and R. H. Leiner
Bn31	E	Alaska	D. E. Carling and R. H. Leiner
Bn184	I	Alaska	D. E. Carling and R. H. Leiner
T46	H	Alaska	D. E. Carling and R. H. Leiner
S99	C	Alaska	D. E. Carling and R. H. Leiner

[a] *Rhizoctonia* isolates with anamorphs similar but not identical to *R. zeae* and *R. oryzae* and with a *Waitea* teleomorph.

Table 2. EC_{50} values (mg a.i./L) for groups of isolates of *Rhizoctonia solani* and other multinucleate and binucleate *Rhizoctonia* exposed to hexaconazole, iprodione, and PCNB

Group	No. of isolates	Hexaconazole				Iprodione				PCNB			
		Dry weight		Radial growth		Dry weight		Radial growth		Dry weight		Radial growth	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
R. solani													
AG-1	3	<0.01	0.002–0.004	0.03	0.02–0.04	1.51	1.02–1.99	>1, <10		0.83	0.40–1.25	5.74	1.87–8.38
AG-2-1	6	0.03	0.01–0.10	0.27	0.05–0.40	1.43	0.58–2.94	4.69	1.11–7.08	1.77	0.42–2.80	>10	1.15–50.11
AG-2-2	4	0.10	0.05–0.17	0.61	0.14–1.12	5.80	1.00–19.93	5.79	1.90–12.35	0.96	0.23–2.38	1.38	0.51–2.94
AG-3	10	0.08	0.02–0.23	0.43	0.16–0.79	0.97	0.40–1.70	1.88	1.50–2.32	1.66	0.38–3.35	8.82	3.66–11.70
AG-4	3	0.05	0.03–0.07	0.54	0.29–0.73	1.11	1.01–1.23	2.63	2.12–3.28	5.75	3.08–8.17	>10	9.05–17.96
AG-5	2	0.03		0.31	0.21–0.40	2.58	2.52–2.63	3.06	2.12–3.99	2.43	1.71–3.15	7.16	7.04–7.29
AG-6	2	0.06	0.05–0.06	0.22	0.11–0.32	2.44	2.21–2.66	>1, <10		0.69	0.65–0.72	2.01	1.72–2.29
AG-7	2	<0.01	0.005–0.007	0.04	0.03–0.05	>1, <10		>1, <10		5.51	1.51–9.50	>10	
AG-8	3	0.12	0.07–0.16	0.26	0.15–0.43	1.79	1.24–2.07	2.79	1.96–3.21	2.18	1.87–2.33	>10	11.63–15.64
AG-9	2	0.02	0.01–0.02	0.10	0.06–0.14	1.02	0.92–1.11	2.15	1.37–2.92	0.94	0.81–1.06	1.78	1.33–2.23
AG-BI	2	0.03	0.02–0.03	0.07	0.04–0.09	1.07	0.77–1.37	1.90	1.89–1.90	0.42	0.40–0.44	2.64	1.40–3.88
R. zeae	3	<0.01	0.002–0.02	0.01	0.01–0.02	>10	6.91–10.71	>10		2.29	1.44–3.57	2.90	2.64–3.17
R. oryzae	5	<0.01	0.001–0.01	0.01	0.005–0.01	6.84	4.22–9.46	>10	9.98–14.79	1.10	0.65–1.40	2.69	2.34–3.17
Rhizoctonia sp. (AK)	2	<0.01	0.002–0.006	0.01	0.007–0.02	>10		>10		2.86	2.81–2.90	3.95	3.19–4.71
Rhizoctonia sp. (X-1)	2	<0.01	0.004–0.006	<0.01	0.003–0.007	>10	15.37–26.57	>10	36.53–39.05	2.01	1.73–2.29	2.97	2.76–3.17
Binucleate *Rhizoctonia*	6	0.08	0.02–0.17	0.69	0.23–1.82	1.32	0.99–1.95	6.59	2.23–22.95	4.48	0.90–11.00	3.75	1.52–6.49

Table 3. EC_{50} values (mg a.i./L) for groups of isolates of *Rhizoctonia solani* and *R. oryzae* exposed to benomyl and prochloraz

Group	No. of isolates	Benomyl				Prochloraz		
		Dry weight		Radial growth		Dry weight		Radial growth
		Mean	Range	Mean	Range	Mean	Range	Mean
R. solani								
AG-1	3	0.21	0.14–0.26	>0.1, <1		0.30	0.21–0.46	>10
AG-2-1	3	1.49	1.19–1.88	3.26	2.91–3.71	2.21	1.37–3.52	>10
AG-2-2	3	0.83	0.57–1.16	5.05	3.30–6.08	>10		>10
AG-3	5	1.42	1.08–1.69	3.62	3.14–4.07	5.91	4.91–7.98	>10
AG-4	3	0.28	0.24–0.31	>1, <10		0.74	0.64–0.91	>10
R. oryzae	3	>10		>10		0.62	0.55–0.68	1.49

appear to be less sensitive (moderately sensitive on the Martin et al [12] scale) to iprodione, PCNB, benomyl, and prochloraz.

Greater differences appear on an irregular basis when genera are compared. Compared to isolates of *Thanatephorus* and *Ceratobasidium,* isolates of *Waitea* (*R. zeae, R. oryzae,* and *Rhizoctonia* sp. AK and X-1) appear generally more sensitive to hexaconazole and less sensitive to iprodione and benomyl and display a similar range of sensitivity to PCNB and prochloraz (Tables 2 and 3). Although it is beyond the scope of this study to explain these differences, it is useful to note them and to be cautious when generalizing about the reactions of multinucleate and binucleate *Rhizoctonia* to fungicides.

The magnitude of the difference between EC_{50} values based on radial growth and those based on dry weight for a given fungus-fungicide combination suggests that radial growth may be a less accurate indicator of sensitivity to fungicides than is dry weight. These data suggest that in most cases fungicide-amended media permit growth on the medium to a greater extent than in the medium, and therefore radial growth data could be misleading. However, these quantitative differences in EC_{50} values based on radial growth and on dry weight may have little significance because the in vitro assay is of comparative rather than absolute value.

These data do confirm a range of reactions among isolates of *R. solani* and related groups to five fungicides in vitro. Previous studies indicate that isolates also differ when fungicides are evaluated under greenhouse and field conditions (11,22). Additional field and greenhouse studies are needed to establish the practical value of these fungicides against isolates of multinucleate and binucleate *Rhizoctonia.* Also, expanded studies with more isolates from diverse geographic and ecological settings representing each group would provide additional useful information on ranges in sensitivity.

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