Comparison of *Rhizoctonia zeae* Isolates from Florida and Ohio Turfgrasses

M.L. Elliott

Fort Lauderdale Research and Education Center, University of Florida, Fort Lauderdale, FL 33314-7799

Additional index words. *Cynodon* sp., pathogenicity

Abstract. The growth responses of 10 *Rhizoctonia zeae* isolates, obtained from turfgrasses in Florida and Ohio, to four temperatures (20, 25, 30, and 35 °C) and seven fungicides at four concentrations (0, 1, 10 and 100 µg·mL⁻¹ a.i.) were compared. Greenhouse pathogenicity tests were conducted using hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burt-Davy]. Optimal temperature for growth for all isolates was 30 °C. Growth of *R. zeae* isolates from both geographic locations was severely limited (>75%) at 20 °C. All *R. zeae* isolates were insensitive to the benzimidazole fungicides, benomyl and thiophanate methyl. Their sensitivities to iprodione, mancozeb, and quinotzene fungicides were similar. The Florida isolates were more sensitive to chlorothalonil, and the Ohio isolates to thiram. All isolates were pathogenic to hybrid bermudagrass. Chemical names used: methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl); dimethyl 4,4'-*O*-phenylene bis(3-thioallophanate) (thiophanate methyl); pentachloronitrobenzene (quintozone); 3-(3,5-dichlorophenyl)-*N*-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxamide (iprodione); tetrachloroisophthalonitrile (chlorothalonil); tetramethylthiuram disulfide (thiram); manganese ethylenebisdithiocarbamate (mancozeb).

Two turfgrass diseases are caused by *Rhizoctonia* sp., *Rhizoctonia* blight (brown patch) [*Rhizoctonia solani* Kühn/teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk] and *Rhizoctonia* leaf and sheath spot (*R. zeae* Voorhees/teleomorph *Waitea circinata* Warcup & Talbot AG Z, and *R. oryzae* Ryker & Gooch/teleomorph *Waitea circinata* Warcup & Talbot AG O) (Burpee and Martin, 1992; Smiley et al., 1992). For *Rhizoctonia* blight, different symptoms are observed on cool-season turfgrasses (leaf lesions or blight) than on warm-season turfgrasses (basal leaf rot). In addition, different *R. solani* anastomosis groups (AG) are associated with *Rhizoctonia* blight. In general, AG 1 has been isolated from cool-season turfgrass, and AG 2,2 from warm-season turfgrasses. To date, no distinction has been made regarding differences in AG groups or symptoms for cool-season vs. warm-season turfgrasses within *R. zeae* and *R. oryzae*.

The primary warm-season turfgrasses grown in Florida are hybrid bermudagrass, used for golf courses and athletic fields, and St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze], used for recreational, resi-

dential, and commercial landscapes. Although *Rhizoctonia* blight (brown patch) is known to occur on both turf species, the disease occurs more frequently and is more severe on St. Augustinegrass, primarily in the winter and spring (Atilano and Freeman, 1990). *Rhizoctonia* leaf and sheath spot primarily affects bermudagrass, especially during warm humid weather, with *R. zeae* being the predominant species isolated (Elliott, personal observations).

During the summer months, *R. zeae* also has been isolated from, and shown to be pathogenic on, cool-season turfgrasses grown in the northeastern and mid-Atlantic regions of the United States (Martin and Lucas, 1983, 1984; Plumley, 1988). This fungus also has been isolated from symptomatic putting greens in Illinois during cool, wet conditions (Wilkinson and Kane, 1991).

Laboratory and greenhouse studies were initiated to compare *R. zeae* isolates from two geographic regions (Ohio and Florida) to determine if differences in their growth responses exist, as has been observed with *R. solani*. The in vitro response of *R. zeae* isolates to four temperatures and to seven fungicides currently registered for control of *Rhizoctonia* blight were examined. Pathogenicity of these isolates also was compared in the greenhouse using hybrid bermudagrass as the host. A portion of these results has been presented previously (Elliott, 1991).

Materials and Methods

Fungal isolates. Ten *R. zeae* isolates were used in this study. These isolates were from two distinct geographic regions, Florida and

Ohio, and included two distinct subfamilies of turfgrasses, cool-season (Festucoideae) and warm-season (Eragrostoideae and Panicoideae). Four Florida isolates were from bermudagrass and one was from St. Augustinegrass. One of the five Ohio isolates was from bentgrass (*Agrostis palustris* Huds.) and four were from a mixture of annual bluegrass (*Poa annua* L.) and perennial ryegrass (*Lolium perenne* L.). Isolates were stored on Difco potato-dextrose agar (PDA) slants at room temperature and in 100% glycerol at -70 °C.

Isolates were initially identified as *R. zeae* based on the presence of multinucleate hyphae, pink- or salmon-colored mycelia appressed to PDA, and production of small (<1 mm diameter) reddish-brown sclerotia in the PDA. Results from the temperature and fungicide studies described below were used to confirm this identification (Burpee and Martin, 1992).

Temperature study. For each isolate, plugs (10 mm diameter) of mycelia were cut from colonies actively growing on PDA and transferred to fresh PDA. One plug was centered on each plate with four replicate plates per isolate-temperature combination. Radial mycelial growth was measured after 48, 72, and 96 h incubation at 20, 25, 30 or 35 °C. Each isolate was evaluated twice at each temperature. Since similar results were obtained for both evaluations, data were pooled and analysis of variance and linear contrasts (SAS Institute, 1985) were used to compare growth response among all isolates.

Fungicide study. The basal medium used was PDA. The fungicides evaluated were benomyl (Tersan 1991 50% WP), thiophanate methyl (Fungo 50% WP), quintozone (Terraclor 50% DF), iprodione (Chipco 26019 50% WP), chlorothalonil (Daconil 2787 40.4% F), thiram (Spotrete 75% WDG), and mancozeb (Dithane M-45 80% WP). Fungicides were incorporated into autoclaved and cooled PDA at concentrations of 0, 1, 10, and 100 µg·mL⁻¹ a.i.. Fungicides were sterilized by stock solutions or suspensions in 95% ethanol or 50% ethanol with sterile deionized water. Preliminary studies indicated that these amounts of ethanol did not affect fungal growth. Plugs of mycelia were transferred to the fungicide-amended and nonamended PDA plates, with three replicate plates per isolate-fungicide concentration combination. Plates were incubated at 30 °C and radial mycelial growth was measured after 72 and 96 h.

Effects of fungicides on growth of the isolates were determined by calculating the percent radial growth on the fungicide-amended PDA as compared to growth on the nonamended PDA. This experiment was conducted twice. Since similar results were obtained, the results from both experiments were pooled for statistical analysis (SAS Institute, 1985). Linear contrasts were used to compare growth responses among *R. zeae* isolates from Florida and Ohio.

Pathogenicity study. Rootless sprigs of 'Tifgreen' hybrid bermudagrass were planted into 10-cm square pots containing a root-zone mix composed of 80% sand and 20% Cana-

Received for publication 15 June 1998. Accepted for publication 6 Oct. 1998. Florida Agricultural Experiment Station Journal Series R-06531. Appreciation is expressed to Dr. A.R. Chase for providing two of the fungal isolates from Florida and Dr. A. O'Leary for providing the isolates from Ohio. The excellent technical support of Scott Chasse is also gratefully acknowledged. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

dian sphagnum peat. The mix was autoclaved for 90 min before planting the sprigs. Pots with sprigs were placed in a greenhouse under intermittent mist for initial rooting. The pots were then placed outdoors on raised benches and were maintained at 7.5-cm height, fertilized every 2 weeks with 2.5 g of 16N-4.3P-8.3K (Vigoro Par Ex, Winter Haven, Fla.) per pot, and irrigated as needed. Plants were maintained under these conditions for at least 6 months, then acclimated in a greenhouse for 5 d prior to inoculation with *R. zeae*.

For each *R. zeae* isolate, one PDA plate culture, chopped into 5-mm² pieces, was used to inoculate a 1-L flask containing 125 mL of potato-dextrose broth. Flasks were incubated in a 30 °C shaking (50 rpm) water bath. After 4 d of growth, the contents of each flask were homogenized in a Waring blender. This technique of using a mycelial slurry for inoculation was used previously for examining pathogenicity of *Rhizoctonia* isolates on ornamentals grown in pots in Florida (Chase, 1991); it eliminates the need to produce and store inoculum that uses grain or grass seed as a substrate.

The basic technique of Martin and Lucas (1983) was followed for the inoculation of the pots. Just prior to inoculation, bermudagrass leaves were trimmed to 6.5 cm and misted with deionized water. About 30 mL of the mycelial slurry was used to drench each pot, which was then covered with a plastic bag. The control pots received only water. All pots (four per *R. zeae* isolate or control treatment) were randomly arranged in a tray and subirrigated during the course of the experiment.

After 7 d, plants were evaluated for disease based on percentage of blighted leaf tissue in each pot. After evaluation, symptomatic leaf tissue from each treatment was removed, surface-sterilized in sodium hypochlorite, blotted dry, and placed on PDA supplemented with benomyl (Martin et al., 1984). Leaf tissue from the control treatment was treated similarly. Plates were examined for *R. zeae* growth for up to 14 d. The experiment was conducted twice. For the first experiment, the mean high and low temperatures in the greenhouse were 36 and 21 °C, respectively; for the second experiment, they were 34 and 19 °C, respectively.

Results and Discussion

Temperature study. Mycelial growth differed significantly ($P \leq 0.05$) among individual *R. zeae* isolates from both Florida and Ohio at the four temperatures evaluated (Table 1). However, at 30 °C, the average growth for all the *R. zeae* isolates from warm-season turfgrasses grown in a subtropical climate (Florida) was the same as the average growth for all *R. zeae* isolates from cool-season turfgrasses grown in a temperate climate (Ohio). The greatest difference among isolates from the two geographic locations was observed at 25 °C; average mycelial growth for the Ohio isolates was 6.1 mm greater than that for the Florida isolates. *Rhizoctonia zeae* is considered to be the dominant *Rhizoctonia*

pathogen in Florida during the summer months, while in the Midwest and other northern temperate regions, *R. solani* is dominant. However, it was reported that 25% of the *Rhizoctonia*-like fungi isolated from New Jersey golf courses were *R. zeae* isolates (Plumley, 1988).

Rhizoctonia solani may be the dominant pathogen in northern temperate regions because night temperatures are cooler there than in Florida during the summer months. It was demonstrated in Georgia that both *R. solani* (AG 2) and *R. zeae* caused a root disease of corn at 34 °C day/20 °C night temperatures, but only *R. solani* caused significant disease at 28/16 or 21/8 °C (Sumner and Bell, 1982). Night temperatures rarely drop below 21 °C during the summer months in Florida, but do so in northern temperate climates. Perhaps the high night (>20 °C) and day (>30 °C) temperatures experienced may explain why *Rhizoctonia* blight is not observed in Florida during the summer months, but *Rhizoctonia* leaf and sheath spot is. It may also simply be due to the differences in the two *R. solani* AG groups associated with *Rhizoctonia* blight.

Fungicide study. The growth response to the fungicides, across all fungicide concentrations, for the *R. zeae* isolates from Florida and those from Ohio are listed in Table 2. Significant differences ($P \leq 0.05$) between *R. zeae* isolates from Florida vs. Ohio were observed

for two of the seven fungicides evaluated. Ohio isolates were more sensitive to thiram but less sensitive to chlorothalonil. As expected, none of the *R. zeae* isolates was sensitive to the benzimidazole fungicides, benomyl and thiophanate methyl, in agreement with reports on other *R. zeae* turfgrass isolates (Burpee and Martin, 1992; Christensen, 1979; Martin et al., 1984) and with another report in which the plant hosts were not specified (Carling et al., 1990). However, these results do not agree with a report on *R. zeae* and *R. oryzae* isolates, obtained from rice, that were sensitive to benzimidazole fungicides (Kataria et al., 1991). As this latter study indicated, genetic or physiological differences among isolates from different hosts (e.g., corn, rice, and turfgrasses) might account for the conflicting results. In the present study, individual isolates differed in response to both fungicides and temperature. When the *R. zeae* isolates were compared as two distinct groups based on geographic origin, growth response differences were significant only for thiram and chlorothalonil.

Pathogenicity study. All four *R. zeae* isolates were pathogenic to 'Tifgreen'. Although a few individual leaf lesions were observed, the primary symptom was a total blight of the leaf blades. Across all four isolates in both experiments, inoculated plants had 78% blighted leaf tissue, whereas control plants

Table 1. Effect of temperature on radial mycelial growth (mm) of *Rhizoctonia zeae* isolates obtained from Florida and Ohio turfgrasses.

		Temp ^z (°C)			
Isolate	Grass host	20	25	30	35
Florida					
Rz-1	Bermuda	6.3 e ^y	17.6 e	39.0 b–d	29.0 g
Rz-2	Bermuda	7.5 de	18.6 e	40.0 a	36.5 b–d
Rz-4	Bermuda	9.1 a–c	24.4 d	40.0 a	33.8 ef
87-254	St. Augustine	8.0 cd	25.0 cd	38.5 d	34.0 d–f
88-222	Bermuda	10.1 a	27.4 bc	39.6 ab	39.3 a
Mean		8.2	22.6	39.4	34.5
Ohio					
293A	Bent	8.5 b–d	32.0 a	39.1 a–d	31.9 f
295	Blue/Rye Mix ^x	8.5 b–d	24.4 d	40.0 a	39.6 a
410A	Blue/Rye Mix	9.1 a–c	29.8 ab	38.6 cd	37.1 a–c
410B	Blue/Rye Mix	9.5 ab	30.4 a	39.5 a–c	35.9 c–e
899	Blue/Rye Mix	8.9 a–c	27.1 c	40.0 a	38.6 ab
Mean		8.2 ^{NS}	28.7 ^{****}	39.4 ^{NS}	36.6 [*]
F value		5.32	21.84	3.33	11.97
P > F		0.0001	0.0001	0.002	0.0001

^zValues are means (N = 8) obtained after 72 h.

^yMean separations within columns by Waller-Duncan k ratio *t* test, $P \leq 0.05$.

^xAnnual bluegrass and perennial ryegrass mixture.

^{ns, *, ****}Nonsignificant or significantly different from means of Florida isolates by linear contrasts at $P \leq 0.05$ or 0.001, respectively. N = 40.

Table 2. Linear contrasts between in vitro growth responses to fungicides for *Rhizoctonia zeae* isolates from Florida and Ohio turfgrasses.

Fungicide	Florida ^z	Ohio ^z	F	P > F
Benomyl	99.6	99.6	0.00	0.9773
Thiophanate methyl	99.7	99.0	0.81	0.3723
Quintozene	44.6	42.3	1.38	0.2468
Iprodione	59.6	56.1	1.85	0.1802
Chlorothalonil	76.2	89.6	18.98	0.0001
Thiram	77.1	70.8	4.33	0.0433
Mancozeb	71.4	74.8	0.48	0.4937

^zMean (N = 90) percent radial growth (mm) across all three concentrations (1, 10, and 100 mg·mL⁻¹ a.i.) of each fungicide relative to growth on unamended media.

had only 7%. For individual isolates, the average percentages of blighted leaves were 73% for Rz-2, 84% for 88-222, 70% for 293A, and 84% for 899. When only the data for the inoculated plants were analyzed, differences among the isolates were nonsignificant at $P \leq 0.05$. *Rhizoctonia zeae* was reisolated from blighted leaves in all fungal treatments, but not from the control. *Rhizoctonia zeae* was previously shown to be pathogenic on creeping bentgrass, Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass, tall fescue (*Festuca arundinacea* Schreb.), centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], and common bermudagrass (Martin and Lucas, 1983). Our study demonstrates that the fungus is also pathogenic on hybrid bermudagrass. Turfgrass *R. zeae* isolates from one region are not necessarily host specific for pathogenicity (Haygood and Martin, 1990; Martin and Lucas, 1983, 1984). Our results, using only four isolates, suggest this is also true among isolates obtained from two distinctly different geographic regions.

Literature Cited

- Atilano, R. and T.E. Freeman. 1990. Brown patch of turfgrasses. Plant Pathology Fact Sheet No. 20. Univ. of Florida, Gainesville.
- Burpee, L. and B. Martin. 1992. Biology of *Rhizoctonia* species associated with turfgrasses. Plant Dis. 76:112-117.
- Carling, D.E., D.J. Helm, and R.H. Leiner. 1990. In vitro sensitivity of *Rhizoctonia solani* and other multinucleate and binucleate *Rhizoctonia* to selected fungicides. Plant Dis. 74:860-863.
- Chase, A.R. 1991. Characterization of *Rhizoctonia* species isolated from ornamentals in Florida. Plant Dis. 75:234-238.
- Christensen, M.J. 1979. *Rhizoctonia* species associated with diseased turf grasses in New Zealand. N.Z. J. Agr. Res. 22:627-629.
- Elliott, M.L. 1991. Comparison of *Rhizoctonia zeae* isolates from turfgrass in Ohio and southern Florida. Phytopathology 81:811. (Abstr.)
- Haygood, R.A. and S.B. Martin. 1990. Characterization and pathogenicity of species of *Rhizoctonia* associated with centipedegrass and St. Augustinegrass in South Carolina. Plant Dis. 74:510-514.
- Kataria, H.R., U. Hugelshofer, and U. Gisi. 1991. Sensitivity of *Rhizoctonia* species to different fungicides. Plant Path. 40:203-211.
- Martin, S.B. and L.T. Lucas. 1983. Pathogenicity of *Rhizoctonia zeae* on tall fescue and other turfgrasses. Plant Dis. 67:676-678.
- Martin, S.B. and L.T. Lucas. 1984. Characterization and pathogenicity of *Rhizoctonia* spp. and binucleate *Rhizoctonia*-like fungi from turfgrasses in North Carolina. Phytopathology 74:170-175.
- Martin, S.B., L.T. Lucas, and C.L. Campbell. 1984. Comparative sensitivity of *Rhizoctonia solani* and *Rhizoctonia*-like fungi to selected fungicides in vitro. Phytopathology 74:778-781.
- Plumley, K.A. 1988. *Rhizoctonia* spp. associated with golf course turfgrass in southern New Jersey. Phytopathology 78:1510. (Abstr.)
- SAS Institute. 1985. SAS user's guide: Statistics, ver. 5 ed. SAS Inst., Cary, N.C.
- Smiley, R.W., P.H. Dernoeden, and B.B. Clarke. 1992. Compendium of turfgrass diseases, 2nd ed. APS Press, St. Paul, Minn.
- Sumner, D.R. and D.K. Bell. 1982. Root diseases induced in corn by *Rhizoctonia solani* and *Rhizoctonia zeae*. Phytopathology 72:86-91.
- Wilkinson, H. and R. Kane. 1991. Prime conditions lead to disease outbreaks. Lawn Landscape Maint. Dec.:8, 10.