Comparison of Rhizoctonia zeae Isolates from Florida and Ohio Turfgrasses

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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. x C. transvaalensis Burtt-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 (quinotzene); 3-(3,5-dichlorophenyl)-N-(1-methylpropyl)-2,4-dioxo-1-imidazolidinocarbamide (iprodione); tetrachloroisophtalonitrile (chlorothalonil); tetramethylthiuram disulfide (thiram); manganese ethylenebisdithiocarbamate (mancozeb).

Two turfgrass diseases are caused by Rhizoctonia sp., Rhizoctonia blight (brown patch) [Rhizoctonia solani (Kühn/teleomorph Thamnophorus 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 I 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, residential, 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 (Attilano 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 (Eragrostioideae and Panicioideae). 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, and septa. For PDA, and production of small (<1 mm diameter) red-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), quinotzene (Terracol 50% DF), iprodione (Chipco 26019 50% WP), chlorothalonil (Daconil 2787 40% F), thiram (Spotrem 75% WD), and mancozeb (Dithane M-45 80% WP). Fungicides were incorporated into autoclaved and cooled PDA at concentrations of 0, 1, 10, and 10 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-
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 ≤ 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 ≤ 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 (Carlino 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

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

| Isolate | Grass host | Temp °C | Florida | Ohio |
|---------|------------|---------|---------|------|
|         |            | 20      | 25      | 30   | 35   |
| Rz-1    | Bermuda    | 6.3 e   | 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     |

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

| Fungicide | Florida | Ohio | F | P > F |
|-----------|---------|------|---|------|
| Benomyl   | 99.6    | 99.6 | 0.00  | 0.9773 |
| Thiophanate methyl | 99.7 | 99.0 | 0.81  | 0.3723 |
| Quinozline | 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 |

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*Values are means (N = 8) obtained after 72 h.
*Mean separations within columns by Waller–Duncan k ratio test, P ≤ 0.05.
*Annual bluegrass and perennial ryegrass mixture.
*NS = Nonsignificant or significantly different from means of Florida isolates by linear contrasts at P ≤ 0.05 or 0.001, respectively, N = 40.
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.

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