Characterization of *Rhizoctonia solani* Isolates Associated with Patch Diseases on Turfgrass

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Cultural characteristics and pathogenicity of *Rhizoctonia solani* isolates obtained from brown patch on creeping bentgrass, *Agrostis pulustris* Huds. and large patch on zoysiagrass, *Zoysia japonica* Steud, were evaluated and compared with known *R. solani* anastomosis groups: AG-2-2III-B, AG-2-2IV, AG-1-IA, AG-4, and AG-5. Bentgrass and zoysiagrass isolates were obtained from infected grass leaf sheaths along disease patch margins. The bentgrass and zoysiagrass isolates differed culturally from one another. The bentgrass isolate and the AG-2-2IIIB tester both showed irregular clusters of mycelia (not sclerotia), concentric zonation, dark brown main hyphae, and sparse aerial hyphae on potato dextrose agar after two weeks of incubation at 22°C, 12h/12h light/dark. These two isolates caused high levels of disease on creeping bentgrass cv. Crenshaw in in vitro pathogenicity tests. The zoysiagrass isolate most closely matched *R. solani* AG-2-2IV in both cultural characteristics and pathogenicity on creeping bentgrass cv. Crenshaw. The zoysiagrass isolate and the AG-2-2IV tester both had abundant aerial hyphal growth, dark brown main hyphae, and no sclerotial formation or zonation on potato dextrose agar after two weeks of incubation. Optimum temperature for growth of both isolates was 25°C but unlike the bentgrass isolate and the AG-2-2IIIB tester, the zoysiagrass isolate and the AG-2-2IV tester did not grow at 35°C. The zoysiagrass isolate and the AG-2-2 IV tester caused low levels of disease on creeping bentgrass cv. Crenshaw in *in vitro* pathogenicity tests. Results indicate that cultural characteristics and host range of the bentgrass isolate and those of the zoysiagrass isolate are different. Isolates representing *R. solani* AG-2-2IIIB and AG-2-2IV were tested for sensitivity to azoxystrobin in *in vitro* tests. Sensitivity to azoxystrobin (effective concentration causing 50% growth inhibition [EC_{50}]) was determined by radial growth on potato dextrose agar amended with 0, 1, 3.2, 10, 31.2, 100, 316, and 1000 mg a.i. azoxystrobin /L after three days incubation at 22°C. EC_{50} values for AG-2-2 IIIB isolates averaged approximately 193 mg a.i. azoxystrobin/L while those for AG-2-2IV isolates averaged approximately <1 mg a.i. azoxystrobin/L. Results suggest there is some variability in fungicide sensitivity between and within *R. solani* AGs and that *R. solani* AG-2-2IIIB isolates may be less sensitive to azoxystrobin fungicide than AG-2-2IV isolates.

INTRODUCTION

Rhizoctonia solani Kuhn is a worldwide, ecologically diverse soilborne fungus belonging to Order Ceratobasidiales of the Basidiomycota and is the mycelial or imperfect state of *Thanatephorus cucumeris* (A. B. Frank) Donk. Relationships within the species *Rhzoctonia solani* are very complex and confusing. Identification is based on anastomoses groups (Ag) among isolates and cultural characteristics. Important taxonomic characteristics of the species include: (1) the absence of asexual spores, or conidia; (2) the absence of clamp connections, or structures involved in genetic recombination; (3) the absence of rhizomorphs; (4)

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small (< 1mm), round, dark brown sclerotia that may or may not be present; (5) multinucleate hyphal cells; (6) pigmented mycelia (shades of brown); (7) right-angled hyphal branching; (8) septum formation in hyphal branches near points of hyphal origin; (9) presence of a dolipore septum; and (10) an optimum growth temperature of 20 to 30°C (Baker 1970, Parmeter and Whitney 1970, Brown and McCarter 1976, Anderson 1982).

The host range of *R. solani* is extensive. The pathogen is capable of causing seedling damping-off, root rot, collar rot, stem canker, crown rot, bud and fruit rots, and foliage blight on a variety of susceptible agriculturally important crops (Baker 1970, Anderson 1982) like soybean (*Glycine max* (L.) Merr.; Liu and Sinclair 1991), cotton (*Gossypium hirsutum* L.; Brown and McCarter 1976), canola (*Brassica campestris* L.; Yitbarek et al 1987), wheat (*Triticum aestivum* L.; Wiseman et al 1995), beet (*Beta vulgaris* L.; Carling et al 1987), potato (*Solanum tuberosum* L. subsp. *tuberosum;* Escande and Echandi 1991), and rosemary (*Rosemarinus officinalis* L.; Conway, et al 1997). *Rhizoctonia solani* also infects a number of turfgrass species (Couch 1995). The fungus was first identified as the causal agent of a disease known as *Rhizoctonia* blight (brown patch) on creeping bentgrass (*Agrostis palustris* Huds.) in 1913 (Burpee and Martin 1992, Couch 1995) and has since become regarded as one of the most destructive diseases of both warm- and cool-season turfgrasses including zoysiagrasses (*Zoysia* spp. Willd; Burpee and Martin 1992, Couch 1995, Aoyagi et al 1998), tall fescue (*Festuca arundinacea* Schreb.; Burpee and Martin 1992, Couch 1995, Aoyagi et al 1998), Kentucky bluegrass (*Poa pratensis* L.; Couch 1995, Aoyagi et al 1998), centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.; Couch 1995, Aoyagi et al 1998), and creeping bentgrass (Burpee and Martin 1992, Couch 1995, Aoyagi et al 1998). *Rhizoctonia* blight on cool-season grasses such as bentgrass is called "brown patch" and on warm-season grasses like zoysiagraass, the disease is referred to as "large patch" (Aoyagi et al 1998, Hyakumachi et al 1998) because of slight differences in symptomatology, time of year of disease outbreaks, and *R. solani* isolate cultural morphology (Couch 1995, Hyakumachi et al 1998).

Variability in disease symptoms, host range, and geographical location of *R. solani* isolates suggests that there are several strains of the species (Burpee and Martin 1992). As of 1994, 12 different strains of the fungus (AG-1 through AG-11 and AG-BI) (Carling et al 1994) have been recognized based on affinities for hyphal fusion (anastomosis), a genetic feature that results in exchange of nuclei and the combining of different genotypes (Kataria et al 1991, Burpee and Martin 1992). Anastomosis groups are categorized based on their mycelial compatibilities for hyphal fusion. Anastomosis occurs between fungal isolates of the same AG but not between isolates of different AG's. Each AG therefore seems to be genetically independent from all others (Parmeter et al 1969, Ogoshi 1985, Burpee and Martin 1992).

Anastomosis groups appear to be fairly host plant specific. For instance, AG-3 occurs commonly on Solanaceae and AG-4 is regularly associated with Pinaceae, Chenopodiaceae, Cruciferae, Leguminosae, Malvaceae, and Solanaceae (Butler 1993). Four anastomosis groups of *R. solani*, AG-1 (specifically, subgroup IA on cool-season turf in Japan), AG-2, AG-4, and AG-5, have been isolated from turfgrasses (Burpee and Martin 1992, Aoyagi et al 1998). Subgroup 2 of AG-2 has been consistently associated with *Rhizoctonia* blight of turfgrasses (Burpee and Martin 1992, Green et al 1993, Zhang and Dernoeden 1995). Reports indicate that brown patch on cool-season turf is typically caused by intraspecific group IIIB of subgroup AG-2-2, while large patch on warm-season turf is incited by intraspecific group IV of subgroup AG-2-2 (Burpee and Martin 1992, Green et al 1993, Zhang and Dernoeden 1995; however, Aoyagi, et al

(1998) established that this isolate should be included in a new group, AG-2-2LP for large patch on Zoysiagrass. Type IIIB is usually associated with infections of foliar portions of family Poaceae, while type IV primarily causes root rots of the Chenopodiaceae (Burpee and Martin 1992).

Several fungicides have been labeled for control of *Rhizoctonia* blight including flutolanil (Prostar, AGREVO Corporation, Wilmington, DE), propiconazole [Banner, Ciba-Geigy Corporation, (Novartis) Greensboro, NC], fenarimol (Rubigan, Dow-Elanco Specialty Products, Indianapolis, IN), iprodione (Chipco 26109, Rhone-Poulenc AG Company, Research Triangle Park, NC), chlorothalonil (Daconil 2787, ISK Biotech Corporation, Mentor, OH), quintozene (Terraclor, Uniroyal Chemical Co., Middlebury, CT), mancozeb (Fore, Rohm and Haas Co., Philadelphia, PA) (Couch 1995), and azoxystrobin (Heritage 50WDG, Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, RG42 6ET, UK). Azoxystrobin is a new beta-methoxyacrylate fungicide that has been used for control of several ascomycete, basidiomycete, and oomycete fungal diseases on such crops as cereals, cucurbits, vegetables, fruits, peanuts, ornamentals, rice, potatoes, and turf. Azoxystrobin is a derivative of the chemically similar strobilurins, a class of naturally occurring fungicides produced by *Strobilurus tenacellus*, a wood-decaying fungus of the mushroom family Tricholomataceae (Dernoeden 1998, Aspinall and Worthington 1999). The fungicide has broad spectrum activity with protectant and acropetal systemic capabilities, meaning the chemical can be taken up by plant xylem and then move upward in the transpiration stream. The chemical is also effective for controlling established infections and can be absorbed by both roots and leaves. Azoxystrobin interferes with cellular respiration in sensitive fungal pathogens by inhibiting transport of mitochondrial electrons (Aspinall and Worthington 1999).

It is not known whether these fungicides are effective against all *R. solani* AGs or just a select few. Kataria et al (1991) tested different fungicides against various isolates of several anastomosis groups and found variability in fungicide sensitivity between and within AGs. Knowledge of which AGs are involved in a given *Rhizoctonia* blight outbreak and their sensitivities to different fungicides may help to facilitate selection of the most appropriate fungicide for management of the disease in any particular area or situation.

Because zoysiagrass is often planted around the shoulders of bentgrass greens this study was conducted to classify *R. solani* isolates from brown patch on creeping bentgrass and large patch on zoysiagrass into appropriate AGs based on observations of cultural and pathogenicity characteristics and comparisons with those of known anastomosis testers; and to evaluate the AGs commonly associated with *Rhizoctonia* blight for sensitivity to azoxystrobin in vitro.

MATERIALS AND METHODS

Collection and Isolation. Grass leaf sheaths and blades with symptoms of brown patch and large patch were collected from creeping bentgrass and zoysiagrass, respectively, at the Horticulture Turfgrass Research Center in Stillwater, Oklahoma, in 1997. Samples were taken from the extreme margins of patch areas with forceps and transported to the laboratory in polyethylene bags. Sections of necrotic, straw-colored tissue were removed from infected plant material and were surface sterilized with 10% Clorox (The Clorox Company, Oakland, CA) for 1 min, plated onto potato dextrose agar (PDA, Difco Laboratories, Detroit, MI) amended with 0.3 g / L streptomycin sulfate (Sigma Chemical Co., St. Louis, MO) contained in petri dishes, and incubated at 22°C (12-h light / 12-h dark regime). After 48 h of incubation, hyphal tips from each isolate were transferred to fresh PDA petri dishes (100 x 15 mm). Following another 48 h of

incubation, hyphal tips were transferred to PDA slants and stored in a fungal culture collection at 11°C. The bentgrass isolate designated as #345, and the one from zoysiagrass as #414. *Rhizoctonia solani* isolates #96, #300, #309 were obtained from stock slant cultures from Dr. Kenneth E. Conway's laboratory (Oklahoma State University, NRC Rm 326, Department of Entomology and Plant Pathology, Stillwater, OK, 74078) fungal culture collection. These isolates were originally obtained from R. J. Cook (United States Department of Agriculture, Agricultural Research Service Pullman, WA) and were chosen for the experiment because they are representatives of the following AGs: AG-4, AG-1-IA, and AG-5, respectively. Also chosen for the experiment were *R. solani* isolates #410, a cool season isolate from zoysiagrass and #411, isolated from warm season zoysiagrass and in Kansas and presumably representing anastomosis intraspecific groups AG-2-2IV, and AG-2-2IIIB, respectively. These two isolates were obtained from N. A. Tisserat (Kansas State University, Manhattan, KS). According to Dr. Tisserat (pers. comm.), isolate #410 was not associated with warm-season patch disease and isolate #411 was isolated from zoysiagrass in the Kansas City area and was associated with cool weather (fall and spring) injury to Zoysiagrass. Mycelial fragments were removed from the slant cultures, plated onto PDA, and were incubated as above for use in subsequent experiments.

Cultural characteristics. *R. solani* isolates #345 and #414 were identified based on comparisons of cultural characteristics, hyphal anastomosis, and number of nuclei per hyphal cell with those of the known AG testers *R. solani* #96, *R. solani* #300, *R. solani* #309, *R. solani* #410, and *R. solani* #411. Five PDA cultures per isolate were observed for colony color, sclerotial formation, growth zonation, and aerial mycelium after incubation for two weeks at 22°C, 12-h light / 12-h dark and the experiment was repeated once. To confirm the multinucleate condition, mycelia from each isolate were removed from PDA cultures and teased apart on a clean glass slide. Hyphae were stained with acridine orange (Sigma Chemical, St. Louis, MO; Dhringra and Sinclair 1985) and observed using epifluorescence under an ultraviolet microscope. Following a modified procedure described by Aoyagi et al (1998), we observed 15 hyphal cells per isolate for the multinucleate condition in two separate experiments.

Hyphal anastomosis. Hyphal anastomosis reactions were observed by removing mycelial plugs (0.75 cm in diameter) of isolates #345 and #414 from actively growing week old PDA cultures and pairing them with mycelial plugs of tester isolates of known AG having the same cultural characteristics as isolates #345 and #414. Tests were conducted on 2% reverse osmosis (RO) water agar in 100 x 15 mm petri dishes following modified procedures of Parmeter et al (1969), and Zhang and Dernoeden (1995). Petri dishes were incubated at 22°C until hyphae from paired isolates began to overlap (two or three days). Overlapping hyphae were then stained with lacto-fuchsin red (Carmichael 1955, Escande and Ecandi 1991) and were observed for two types of anastomosis reactions by using light microscopy at 400X magnification. Perfect anastomosis occurs between hyphae from the same isolate or a genetically identical isolate (clone) and is characterized by complete fusion of cell walls and cytoplasm. Imperfect anastomosis is the result of cell wall fusion but exchanged cytoplasm does not remain viable as with perfect anastomosis (Yokoyama et al 1985a, 1984b, Wilkinson 1988, Aoyaki et al 1998).

Ten water agar plates per pairing were observed for 25 points of contact each. Pairings of mycelial plugs from the same isolate were designated as control reactions. A pair of separate isolates was considered genetically identical if more than 80% of fusion contacts were perfect anastomoses (Aoyagi et al 1998).

Temperature-growth experiment. Mycelial plugs (0.75 cm in diameter) were

cut from actively growing 2-d-old PDA cultures of isolates #345 (brown patch), #414 (large patch), #411 (AG-2-2IIIB), and #410 (AG-2-2IV) and plated onto new PDA petri dishes. Five replicate petri dishes for each isolate were incubated in the dark at 21, 25, 26, 30, and 35°C. Two perpendicular colony diameters were measured on the bottom of each plate after 24-h incubation. Agar plug diameters were subtracted from every measurement. The two colony diameters for each plate were averaged, and a mean growth rate was calculated from the five replicate plates for each temperature. The test was conducted twice with similar results.

Pathogenicity tests using Conetainers. All *R. solani* isolates were tested for pathogenicity to creeping bentgrass cv. Crenshaw. Inoculum was produced by adding one 100 x 15 mm PDA culture, chopped into approximately 1 cm^2 pieces, to 250-ml Erlenmeyer flasks containing 20 g oat seed and 3 ml RO water that had been autoclaved (121°C, 1.05 kg / cm^2, 20 min) on each of three consecutive days. Flasks containing oat seed were incubated at 22°C (12-h light / 12-h dark regime) and after 14 da the colonized seed was removed and dried overnight under a laminar-flow hood.

Creeping bentgrass seed was obtained from Lofts Great Western Seed Company (Albany, OR). Pathogenicity of *R. solani* isolates on creeping bentgrass was tested in plastic Conetainers (Stuewe & Sons, Inc., Corvallis, OR) 3 cm in diameter and 21 cm deep following a modified procedure previously described by Wilkinson (1988). Conetainers were filled with 100 ml of autoclaved vermiculite (W. R. Grace & Co., Cambridge, MA) and seeded with 0.25 g bentgrass seed that had been surface sterilized with 10% NaOCl for 1 min. Seeds were covered with a thin layer of vermiculite, watered every other day with a Peter's 20-20-20 solution (W. R. Grace & Co., Fogelsville, PA), and maintained in a growth chamber (12-h light / 12-h dark, 20 to 22°C). Conetainers were covered with plastic wrap to maintain 100% relative humidity.

Two weeks after planting, the bentgrass was cut to a height of 1 cm with sterile scissors. One infested oat seed was then introduced aseptically to the vermiculite surface of each conetainer except for the controls, which were inoculated with noninfested oat seed. Inoculated bentgrass was then placed in a growth chamber (15-h light / 9-h dark, 20 to 25°C) and kept moist (100% relative humidity) with plastic covering. Inoculated bentgrass was watered every other day with Peter's 20-20-20 solution. Disease was rated 2 wk after inoculation using the disease index described by Aoyagi et al (1998), where 0 = healthy, 1 = 1 to 25% diseased, 2 = 26 to 50% diseased, 3 = 51 to 75% diseased, and 4 = 76 to 100% diseased. Disease severity was calculated as

$$\Sigma \left[\frac{\text{disease index x no. of inoculated grass samples in each index}}{\text{maximum index x total no. of inoculated grass samples}} \right] \text{x } 100$$

All treatments consisted of four replicates and tests were conducted two times with similar results.

Fungicide variability tests. Aliquots of a stock solution of azoxystrobin dissolved in molten 3 / 4 strength PDA were added to subsequent molten 3 / 4 strength PDA to obtain final concentrations of 1, 3.2, 10, 31.2, 100, 316, and 1000 mg a.i. azoxystrobin per L medium (ppm). These concentrations resulted in equal spacing on a $\log_{10}$ scale (Keinath and Zitter 1998). Three-quarter strength PDA plates without fungicide were used as controls. Mycelial plugs 0.75 cm in diameter were cut from actively growing cultures of the fungal isolates and placed inverted either onto control or fungicide-amended plates, each containing 20 ml of agar medium. Eight replicate plates were used for each concentration. After 3 da of growth at 22°C (12-h light / 12-h dark regime), two perpendicular colony diameters

were measured on the bottom of each plate. Agar plug diameters were subtracted from every measurement. The two colony diameters for each plate were averaged and a mean diameter was calculated from the eight replicate plates. Percent radial growth inhibition was calculated as

$$\left[\frac{\text{mean dia. on unamended PDA} - \text{mean dia on fungicide – amended PDA}}{\text{mean dia on unamended PDA}}\right] \text{X } 100$$

Azoxystrobin dose response curves were constructed for the *R. solani* isolates by plotting probit-transformed (Zadoks and Schein 1979) percent radial growth inhibition against log-transformed fungicide concentration. The concentration of azoxystrobin causing 50% growth inhibition compared to growth on unamended PDA (EC_{50}) was estimated for each isolate by interpolation from the fitted regression line (second-degree polynomial) using SAS regression. The activity of azoxystrobin fungicide was considered to be strong if the EC_{50} was <10 mg a.i./L, moderate if the EC_{50} was 11 - 100 mg a.i./L, weak if the EC_{50} was 101-1000 mg a.i./L, and ineffective if the EC_{50} was >1000 mg a.i./L. The experiment was repeated once with similar results.

Statistical analysis. Data from the pathogenicity experiments were subjected to analysis of variance using a general linear model (GLM), and mean separation was determined with Duncan's multiple range test ($P \leq 0.05$) (SAS, version 6.10, SAS Institute Inc., Cary, NC). Fungicide variability data were analyzed using SAS regression.

RESULTS

Cultural characteristics. *R. solani* isolate #345 obtained from brown patch on creeping bentgrass in Stillwater, Oklahoma was most similar to the AG-2-2IIIB tester isolate #411. Both isolates were buff in color early in the growth development stage but turned to a dark brown color within two weeks. Isolates #345 and #411 also had irregular clusters of mycelia (not sclerotia), zonation or concentric rings, and sparse aerial hyphae on PDA after two weeks of incubation (Table 1). *Rhizoctonia solani* isolate #414 obtained

Table 1. Cultural characteristics of *Rhizoctonia solani* isolates commonly associated with turfgrasses

Rhizoctonia isolate	Anastomosis group	Colony Color[1]	Nuclear condition[2]	Aerial mycelium	Sclerotia	Zonation
#96 (T)[4]	AG-4	B-DB[3]	>2	Absent	Present	No
#300 (T)	AG-1-IA	C	>2	Absent	Present	No
#309 (T)	AG-5	B	>2	Absent	Present	No
#345 (BG)	AG-2-2IIIB	B-DB	>2	Absent	Absent	Yes
#410 (ZG)	AG-2-2IV	DB	>2	Present	Absent	No
#411 (BG)	AG-2-2IIIB	B-DB	>2	Absent	Absent	Yes
#414 (ZG)	AG-2-2IV	DB	>2	Present	Absent	No

[1]Cultures grown on PDA at 22°C for two weeks.
[2]Multinucleate condition is a distinguishing characterisitic of *R. solani*.
[3]Colony color designations: C = cream, B = buff, DB = dark brown.
[4]BG = bentgrass isolate, ZG = zoysiagrass isolate, T = tester culture.

from large patch on zoysiagrass most closely resembled the AG-2-2IV tester isolate #410. Both isolates were dark brown early in growth and remained that color after two wk. These two isolates exhibited abundant aerial mycelia and neither had sclerotial formation or zonation patterns (Table 1).

Hyphal anastomosis. Known anastomosis testers #410 and #411 were chosen for hyphal anastomosis tests with unknown AG isolates #345 and #414, r espectively, based on identical characteristics in culture. When isolates were paired with self as control reactions, 100% perfect anastomosis was observed (Fig. 1). It was determined that *R. solani* isolates #345 (from creeping bentgrass) and #414 (from zoysiagrass) are not identical strains (perfect fusion frequency of only 8%) but belong to separate anastomosis intraspecific groups AG-2-2 IIIB and AG-2-2 IV, respectively. Perfect fusion was observed among 94.4% of hyphal fusions between isolates #345 and #411 (AG-2-2 IIIB). When #345 was paired with the representative AG- 2-2 IV tester #410, mean perfect anastomosis frequency of 3.33% was obtained. Pairings between isolates #414 and #410 (AG-2-2 IV) resulted in a mean perfect fusion frequency of 84.6%. Pairings between isolate #414 and the representative AG-2-2 IIIB tester #411 resulted in a mean perfect fusion frequency of only 4.4%.

Temperature-growth of *R. solani* isolates. *Rhizoctonia solani* isolates #345 and #411 grew at all five temperatures tested . The optimum temperature of these two isolates was 25°C, with mean colony diameters of 1.52 cm and 1.67 cm, respectively. This evidence lends support to the earlier conclusion made by observations of cultural

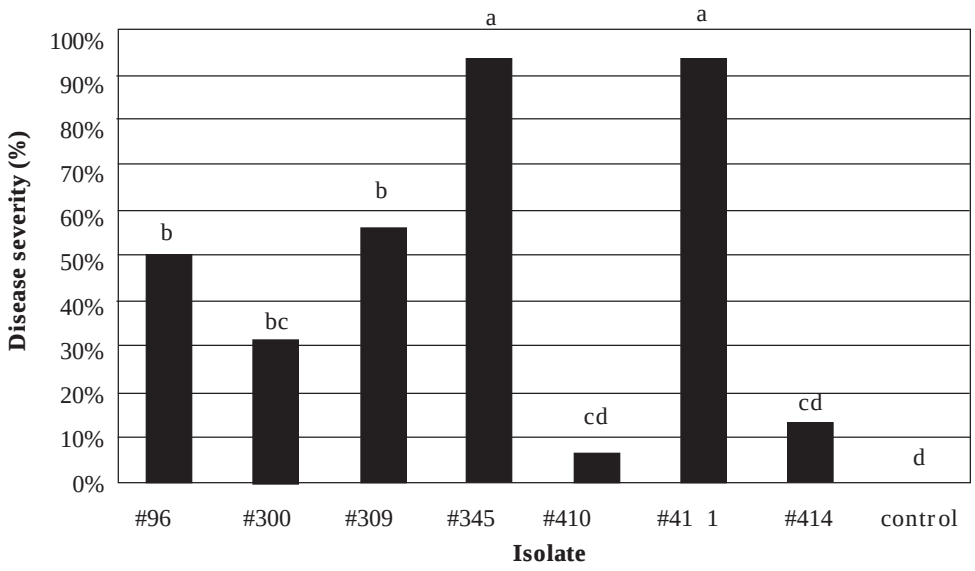

Figure. 1. Pathogenicity of *Rhizoctonia solani* turfgrass isolates on creeping bentgrass cv. Crenshaw grown in conetainers. Disease severity = S (disease index x the number of grass samples in each index)/(maximum index x the total number of grass samples) x 100 (Aoyagi et al 1998). Disease index of brown patch was rated two weeks after incubation at 20 to 25°C using a scale of 0 to 4, where 0 = no symptoms and 4 = dead grass. Isolate #96 = AG-4; #300 = AG-1-IA; #309 = AG-5, #345 = AG-2-2IIIB; #410 = AG-2-2IV ; #411 = AG-2-2IIIB; #414 = AG-2-2IV. Column values having the same letter(s) do not differ significantly ($P \leq 0.05$) according to Duncan's multiple range test.

characteristics and anastomosis reactions that isolate #345, like #411, belongs to AG-2-2IIIB. Isolates #414 and #410 also had growth rate optima at 25°C, with mean colony diameters of 0.94 cm and 1.30 cm, respectively. This evidence further confirmed conclusions from earlier experiments that both isolate #414 and #410 were representatives of AG-2-2IV.

Pathogenicity tests using Conetainers. *Rhizoctonia solani* isolates #345 (bentgrass isolate) and #411 (warm season zoysia isolate) caused the highest levels of disease on creeping bentgrass compared to all of the other isolates (Fig. 1). Initial leaf symptoms observed were small, tan lesions that enlarged and became surrounded by reddish brown margins over time. Eventually grass leaves became necrotic and brown in color. These symptoms were similar to symptoms of brown patch on creeping bentgrass under field conditions. Zoysiagrass isolates #410 and #414 were the least aggressive pathogens to bentgrass. Moderate levels of disease were produced by the tester isolates #96, #300, and #309. All uninoculated control bentgrass remained healthy. Koch's postulates were tested and *R. solani* was isolated from all treatments except the control.

Fungicide variability tests. The four *R. solani* isolates from AG-2-2 IIIB and AG-2-2IV grew at all seven azoxystrobin concentrations after 3 da (Fig. 2). Isolates #345 and #411 (both AG-2-2 IIIB) had similar responses to azoxystrobin. These isolates were slightly less sensitive to the fungicide than isolates #410 and #414 (AG-2-2IV). At 1 mg a.i. azoxystrobin / L, isolate #345 growth was inhibited by only 16% (probit = 4.01) and isolate #411 was inhibited by 40% (probit = 4.75). Isolates #410 and #414 had similar responses to azoxystrobin. Isolate #410 growth was inhibited by 51% (probit = 5.03) while isolate #414 growth was inhibited by 61% (probit = 5.28). At 1000 mg a.i. azoxystrobin / L, isolates #345 and #411

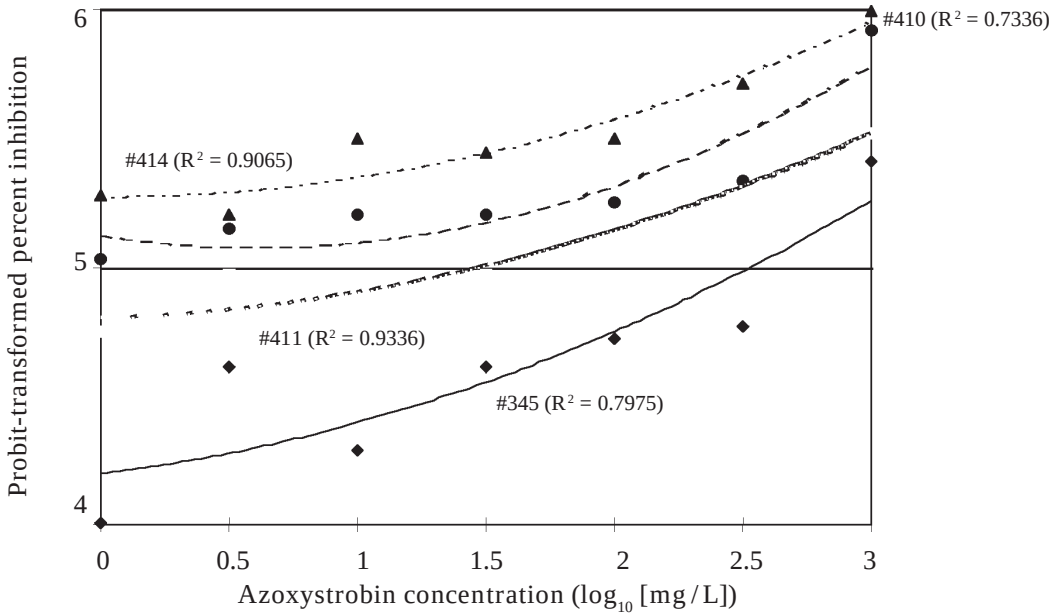

Figure 2. Dose-response curves for four isolates of *Rhizoctonia solani* to azoxystrobin fungicide. Percent inhibition (relative colony diameter) = (diameter on unamended medium – diameter on azoxystrobin – amended medium)/(diameter on unamended medium) X 100. *R. solani* isolates are as follows: #345 = AG-2-2IIIB; #411 = AG-2-2IIIB; #410 = AG -2-2IV; and #414 = AG-2-2IV. The 50% effective concentrations were approximately 355 mg a.i. azoxystrobin/L and 31.2 mg a.i. azoxystrobin/L for isolates #345 and #411, respectively, and <1 mg a.i. azoxystrobin/L for isolates #410 and #414.

(AG-2-2IIIB) again demonstrated slightly lower sensitivity than the AG–2-2IV isolates (#410 and #414). Isolates #345 and #411 were inhibited by 66% (probit=5.41) and 70% (probit = 5.52), respectively. Isolates #410 and #414 wer e inhibited by 82% (probit = 5.92) and 84% (probit = 5.99) at 1000 mg a.i. azoxystrobin / L, respectively. The fungicide concentration in the recommended label rate is approximately 1500 mg a.i. azoxystrobin / L. Our results indicate that the four *R. solani* isolates are sensitive to fungicide at 1000 mg a.i. azoxystrobin / L, all showing >60% growth inhibition; therefore, in field situations, the label rate should be effective for inhibiting of the growth of these four isolates.

The azoxystrobin concentrations that reduced radial growth of isolates by 50% (EC_{50}) were determined to be approximately 355 mg a.i. azoxystrobin / L and 31.2 mg a.i. azoxystrobin / L for isolates #345 and #411 (both AG-2-2 IIIB), respectively, and <1 mg a.i. azoxystrobin / L for isolates #410 and #414 (both AG-2-2 IV). There is some variability in fungicide sensitivity between strains of *R. solani* and between isolates of AG-2-2 IIIB. In this case, AG-2-2 IIIB isolates from brown patch were less sensitive to azoxystrobin than AG-2-2IV isolates from large patch.

DISCUSSION

Rhizoctonia blight on cool- and warm-season turfgrasses may be caused by two separate intraspecific groups of *R. solani*, specifically AG-2-2 IIIB and AG-2-2 IV, respectively (Burpee and Martin 1992, Zhang and Dernoeden 1995, Aoyaki et al 1998, Hyakumachi et al 1998). However, three other strains of *R. solani* (AG-1-IA, AG-4, and AG-5) have also been associated with *Rhizoctonia* blight infections but with less consistency than strains AG-2-2IIIB and AG-2-2IV (Anderson 1982, Burpee and Martin 1992). We wanted to confirm AGs of *R. solani* isolates, #345 (BG) and #414 (Z), fr om brown patch on creeping bentgrass and large patch on zoysiagrass in Oklahoma, respectively, by comparing their cultural and pathogenicity characterisitics with those of known anastomosis turfgrass isolates.

Zhang and Dernoeden (1995) suggested that anastomosis classification by the traditional means of microscopic observation of hyphal pairings is tedious and time-consuming and that simple observations of cultural characteristics such as colony color, presence or absence of sclerotia, presence or absence of zonation patterns, and type of mycelial growth are usually reliable enough to tentatively classify isolates into anastomosis groups. In this study, we found that observations of cultural characteristics of our isolates and comparisons of those characteristics with isolates of known anastomosis grouping were quite dependable in classifying our isolates (#345 and #414) into their respective AGs. Of the five known anastomosis tester turfgrass isolates used (AG-2-2IIIB, AG-2-2IV, AG-1-IA, AG-4 and AG-5), isolate #345 from brown patch most closely matched the AG-2-2IIIB (isolate #411) tester in cultural characteristics while isolate #414 from large patch most closely matched the AG-2-2IV (isolate #410) tester. To further confirm our AGs, we chose to observe hyphal anastomosis reactions between pairings of the brown patch isolate (#345) and the large patch isolate (#414) with the tester isolates AG-2-2 IIIB (#411) and AG-2-2IV (#410), respectively, using light microscopy.

In this study, imperfect fusion was not observed in positive control pairings between identical isolates and was observed only infrequently between different isolates of the same anastomosis grouping. Following the relationship defined by Aoyagi et al, (1998), we considered the relationship between isolates as clonal (i.e., identical AG) if the frequency of perfect fusion was greater than 80%. In pairings of brown patch isolate #345 with the AG-2-2IIIB tester (#411) and of large patch isolate #414 with the AG-2-2IV tester (#410), we observed >80% perfect fusion frequency, lending support to our

earlier conclusions of AG of isolates #345 and #414 based on cultural characteristics alone.

Butler (1993) stated that AGs appear to be plant host specific. We wanted to examine whether there were any differences in pathogenicity to creeping bentgrass cv. Crenshaw between the *R. solani* turfgrass isolates. Our results showed that pathogenicity varies with AG. We found that isolate #345 (AG-2-2IIIB) and isolate #411 (AG-2-2IIIB tester) were most pathogenic on creeping bentgrass cv. Crenshaw while isolates #414 (AG-2-2IV) and #410 (AG-2-2IV tester) were least pathogenic to the grass. We observed moderate pathogenicity by isolates #96 (AG-4 tester), #300 (AG-1-IA tester), and #309 (AG-5 tester). Even though AG-4, AG-1-IA, and AG-5 may be capable of causing average levels of disease, AG-2-2 IIIB isolates appear to be more pathogenic on creeping bentgrass cv. Crenshaw.

Kataria et al (1991) have documented and demonstrated that there is variability in fungicide sensitivity within and between AGs because of differences in molecular and biochemical characteristics. It has been suggested that knowledge of fungicide sensitivity levels between and within AGs is useful in selecting appropriate fungicides for reliable and efficient control of *R. solani* diseases (Kataria et al 1991, Zhang and Dernoeden 1995). An analysis of AG sensitivity to fungicides allows us to draw firm conclusions about the consistency or variability of performance of a fungicide both within and between AGs. We wanted to determine if such variations in sensitivity to azoxystrobin, a common fungicide used to control *Rhizoctonia* blight on cool- and warm-season turfgrasses, were evident between strains AG-2-2IIIB from brown patch and strains AG-2-2IV from large patch and within isolates of strains AG-2-2IIIB and AG-2-2IV. We found that fungal isolates #410 and #414 belonging to AG-2-2IV (large patch) were more sensitive to azoxystrobin fungicide than fungal isolates #345 and #411 belonging to AG-2-2IIIB (brown patch). The AG-2-2IV isolates (#410 and #414) demonstrated consistent azoxystrobin sensitivity, both having EC_{50} values of <1 mg a.i. azoxystrobin / L. There was, however, variability in fungicide sensitivity between isolates #345 and #411 representing AG-2-2IIIB. Isolate #345 was less sensitive (EC_{50} = 355 mg a.i. azoxystrobin / L) than isolate #411 (EC_{50} =3 1.2 mg a.i. azoxystrobin / L). Our results indicate that azoxystrobin fungicide may be more effective in controlling *Rhizoctonia* blight (large patch) on warm-season turfgrasses than in controlling *Rhizoctonia* blight (brown patch) on cool-season turfgrasses because isolates representing AG-2-2IV were more sensitive to azoxystrobin than isolates representing AG-2-2IIIB. However, our results from isolates of two major turfgrass anastomosis groups only approximate azoxystrobin sensitivity levels of representative *R. solani* populations; therefore, it may not be safe to draw accurate conclusions about the specificity of these AGs to azoxystrobin fungicide. We can only speculate that there is variability in azoxystrobin sensivity within and between entire AGs. Additional in vitro testing with greater numbers of isolates for each anastomosis group, and *in vivo* tests on diseased turfgrass in growth chambers would be necessary to confirm our findings.

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