In Vitro Fungicide Sensitivity of *Rhizoctonia* and *Waitea* Isolates Collected from Turfgrasses[1]

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Abstract

Different *Rhizoctonia* species and anastomosis groups (AGs) have been reported to show variable sensitivity to commercial fungicides. Thirty-six isolates of *Rhizoctonia* collected from turfgrasses were tested *in vitro* for sensitivity to commercial formulations of iprodione, triticonazole, and pyraclostrobin. Tested isolates represented *R. solani* AG 1-IB and AG 2-2IIIB; *W. circinata* varieties *zeae* (Wcz) and *circinata* (Wcc); and binucleate *Rhizoctonia*-like fungi (BNR) from different locations in Virginia and Maryland. Each fungicide was added to PDA medium to obtain concentrations at 0, 0.1, 1, 10 and 100 mg a.i.·L^{-1} (0.00001, 0.0001, 0.001 and 0.01 oz a.i.·gal^{-1}). A mycelium plug from each isolate was grown on these plates. The fungicide concentration needed for 50% inhibition of radial growth (EC50) was determined for each isolate by fungicide combination. *Waitea circinata* isolates were moderately sensitive (EC50 = 1 to 10 mg a.i.·L^{-1}) (0.0001 to 0.001 oz a.i.·gal^{-1}) to iprodione while isolates of *R. solani* and BNR were extremely sensitive (EC50 < 1 mg a.i.·L^{-1}). Isolates of AG 2-2IIIB exhibited less sensitivity to triticonazole (mean EC50 = 1.26 mg a.i.·L^{-1}) than AG 1-IB and *W. circinata* (mean EC50 = 0.2, and 0.06 mg a.i.·L^{-1}, respectively). BNR isolates varied in inhibition of growth by triticonazole, exhibiting extreme to moderate sensitivity. Isolates of *W. circinata* were moderately sensitive to pyraclostrobin while most cultures of *R. solani* and BNR were extremely sensitive. Geographic origin of isolates had no influence on the level of fungicide sensitivity. This study demonstrates the importance of accurately identifying the *Rhizoctonia* pathogen causing disease symptoms on a turfgrass for choosing an effective fungicide.

Index words: brown patch, *Rhizoctonia solani*, *Waitea circinata*, iprodione, triticonazole, pyraclostrobin.

Fungicides used in this study: iprodione (Iprodione Pro 2SE); triticonazole (Trinity); pyraclostrobin (Insignia WG).

Significance to the Horticulture Industry

Patch disease caused by different *Rhizoctonia* species and anastomosis groups (AGs) poses a threat to successful maintenance of several important turfgrass species in the southern and transition zones of the United States. Golf courses, sod farms and athletic fields use fungicides to control this disease. Multiple *Rhizoctonia* species and AGs have been isolated from the same diseased patch. Considering the genetic diversity of these pathogens it is important to establish their sensitivity to commonly-used fungicides. This *in vitro* laboratory study revealed that three fungicides commonly used in the turf industry show differential effectiveness in mycelial growth inhibition of fungi responsible for *Rhizoctonia* patch diseases. Therefore, it is important to identify causal pathogens before deciding on which fungicides to use. Additional field tests with these fungicides are needed to determine if *in vitro* results can be replicated under field conditions.

Introduction

Several *Rhizoctonia* species have been known to infect turfgrass species (Burpee and Martin 1992, Smiley et al. 2005). *Rhizoctonia* is a form-genus which includes several anamorphic fungi that rarely produce sexual structures. The induced teleomorphic stages (the sexual fruiting structures and basidiospores) of *Rhizoctonia* species infecting turfgrasses consist of *Thanatephorus*, *Waitea*, and *Ceratobasidium* genera (Smiley et al. 2005). The most abundant and most studied species, *R. solani* Kühn [teleomorph: *Thanatephorus cucumeris* (Frank) Donk], is responsible for brown patch on cool-season turfgrasses and large patch on warm-season turfgrasses (Burpee and Martin 1992). *Rhizoctonia solani* is a genetically diverse species consisting of many anastomosis groups (AGs). Six AGs have been reported to cause blight in turfgrass with AG 1 (-IA and -IB), AG 2 (-2IIIB and -2LP), and AG 4 more commonly isolated from diseased grasses than the other AGs (Zhang and Dernoeden 1995, Smiley et al. 2005). Two closely related species, *R. zeae* Voorhees (teleomorph: *Waitea circinata* var. *zeae* Warcup & Talbot), and *R. oryzae* Ryker & Gooch (teleomorph: *W. circinata* var. *oryzae* Warcup & Talbot) are responsible for leaf and sheath spot of turfgrasses (Smiley et al. 2005). They are presumably less prevalent than *R. solani* but have the capability of causing damaging disease outbreaks. The most recently identified *Rhizoctonia* species, *Waitea circinata* var. *circinata* (proposed anamorph: *R. circinata*) causes brown ring patch on creeping bentgrass (*Agrostis stolonifera L.*) and annual bluegrass (*Poa annua* L.) golf greens (Toda et al. 2005, de la Cerda et al. 2007). *Rhizoctonia cerealis* (teleomorph: *Ceratobasidium cereale* Murray and Burpee; AG-D) may be the most important binucleate *Rhizoctonia* species causing disease on turfgrass. This is a cool-weather pathogen responsible for yellow patch on turfgrasses (Burpee and Martin 1992, Smiley et al. 2005). Other than *R. cerealis*, few studies have been done on binucleate *Rhizoctonia*-like fungi (BNR) from turfgrass swards and soils (Martin and Lucas 1984, Martin, et al. 1984, Burpee and Martin 1992). Taxonomic relationships of these species to binucleate *R. cerealis* are unknown. *Rhizoctonia solani*, *W. circinata* and BNR have different teleomorphs and therefore, represent

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non-interbreeding populations. These species have distinct morphology, physiology, virulence and genetic constitution (Kataria et al. 1991, Wong and Kaminski 2007). Some researchers consider *R. solani* to be a species complex with several genetically different subpopulations (Cubeta and Vilgalys 1997, Lübeck and Poulsen 2001, Sharon et al. 2006).

On golf greens and fairways, fungicides are routinely used to control *Rhizoctonia* diseases. To a lesser extent, sod farms, athletic fields and home lawns may also use fungicides in disease management programs. Since *Rhizoctonia* blight is caused by several species and AGs, it is possible for turfgrasses to be infected simultaneously by more than one *Rhizoctonia* group. In a case of infection by multiple *Rhizoctonia* species and AGs, successful control of *Rhizoctonia* blight requires fungicides with activity against all species and AGs that are present. Therefore, information on effectiveness of recommended fungicides is as important as accurate identification of the pathogen.

In the United States, triticonazole, pyraclostrobin, and iprodione are commonly used to control turfgrass patch diseases caused by *Rhizoctonia* species. These fungicides belong to different fungicide groups with different modes of action. Iprodione, an older product, is a dicarboximide fungicide [fungicide resistance action committee (FRAC) code = 2]. Pyraclostrobin and triticonazole are relatively new fungicides to the turf industry and they belong to the quinone outside inhibitor (QoI) group (FRAC code = 11) and demethylation inhibitor (DMI) group (FRAC code = 3), respectively. Triticonazole was introduced by BASF chemical company (Research Triangle Park, NC) to the turf market in early 2007. It is a penetrant fungicide with a triazole moiety and once absorbed by the plant, has an upward or acropetal movement through the xylem. Pyraclostrobin was first marketed in 2002 by BASF (Bartlett et al. 2002) and belongs to the strobilurin or QoI fungicide group. QoI fungicides inhibit mitochondrial respiration within fungal cells by blocking electron transport at the cytochrome bc-1 complex, resulting in fungal cells starved of ATP, inhibiting growth and disease development (Bartlett et al. 2002, Gisi and Sierotzki 2008). Pyraclostrobin has a translaminar movement in plants and no records of resistance have been reported from *R. solani* or *Waitea* species to this fungicide. Pyraclostrobin is recommended for control of brown patch, large patch, and leaf and sheath spot diseases of turfgrasses.

There are no previous reports of triticonazole and pyraclostrobin fungicides being tested on *Rhizoctonia* isolates *in vitro*. Although the level of sensitivity of *W. circinata* var. *zeae* and *oryzae* to iprodione has been documented, no similar studies have been conducted with the newly emerged *W. circinata* var. *circinata* isolates. Therefore, we tested 36 isolates of *Rhizoctonia* representing different species and AGs collected from turfgrasses for their *in vitro* growth responses to the above three fungicides which represent the dicarboximide, QoI, and DMI fungicide families.

Materials and Methods

Isolates for this experiment were selected from a larger collection maintained for a genetic diversity study of *Rhizoctonia* species infecting cool-season turfgrass in Maryland (MD), and Virginia (VA) (Amaradasa et al. 2013). Six sites in VA and two sites in MD (Table 1) were used to collect *Rhizoctonia* isolates during the summers of 2007, 2008, and 2009. Diseased turfgrass leaf samples included less intensively managed tall fescue (*Festuca arundinacea* Schreb.) lawns and golf roughs, and more intensively managed creeping bentgrass/annual bluegrass golf greens. *Rhizoctonia* isolation, maintenance, and identification by performing anastomosis reactions and ITS sequence analysis were done according to the methods described by Amaradasa et al. (2013). Thirty six isolates (Table 1) from the larger collection were tested for sensitivity on the formulated fungicides triticonazole (Trinity), iprodione (Iprodione Pro 2SE) and pyraclostrobin (Insignia WG) all of which are manufactured by BASF Corporation. *Rhizoctonia* isolates consisted of 10 isolates of *R. solani* AG 1-IB and seven isolates of AG 2-2IIIB, six isolates of binucleate *Rhizoctonia*-like fungi (BNR), nine isolates of *W. circinata* var. *zeae* (Wcz), and four isolates of *W. circinata* var. *circinata* (Wcc). As per label recommendations, water was used to prepare different concentrations of fungicide suspensions. Concentrated fungicide suspensions of each fungicide were pipetted into flasks containing autoclaved ¼ strength PDA cooled to 50C (122F) to obtain the following concentrations: 0.1, 1, 10 and 100 mg a.i.·L^{-1} (0.00001, 0.0001, 0.001 and 0.01 oz a.i.·gal^{-1}). Fungicide-amended media were then dispensed at 15 ml (0.9 in^{-3}) per 9 cm (3.5 in) diameter petri dish. Concentrated fungicide suspensions as well as fungicide amended PDA media were continuously stirred to ensure uniform mixing during the process. Control petri plates were not amended with fungicide. Fungicide-amended and control petri plates were inoculated with 6 mm (0.24 in) mycelial plugs cut from the margin of actively growing PDA cultures of the *Rhizoctonia* isolates. Mycelial plugs were inverted and placed at the center of each petri plate. Four replicate petri plates per isolate for each fungicide concentration and controls were incubated in the dark at 27C (80.6F). Colonies of fungicide-amended plates were measured along two right angle diameters just before the mycelial mat of the control reached the edge of the petri plate. The diameter of the mycelial plug was subtracted and the average growth of each isolate was determined using the two growth measurements. The percent growth inhibition for each isolate by fungicide combination was calculated using the following formula.

% Inhibition = 100 × (diameter of control – diameter of treated) / diameter of control

The experiment was repeated once and chi-square test was performed for the two data sets of each fungicide using the PROC GLIMMIX procedure of SAS ver. 9.3 (SAS Institute, Cary, NC). Since the two data sets were not different at $p = 0.05$, they were pooled for the analysis. The percent growth inhibition of each isolate in response to the different fungicide concentrations were used in a standard curve to determine the effective concentration that caused 50% growth inhibition (EC50) by log-probit analysis in SAS. Probit analysis was employed to calculate EC50 values because it transforms the sigmoid dose-response curve to a straight line that can be analyzed by regression through maximum likelihood or least squares. Probit method was introduced by Finney (1952) and it allows more accurate estimation of EC50 than the untransformed data. The SAS program statements obtained from Hsiang et al. (1997) were slightly modified to analyze our data. The EC50 data were log transformed to correct for lognormal distribution and subjected to analysis of variance (ANOVA) using JMP® version 11 (SAS Institute

Table 1. Geographic origin, host, management type, and anastomosis group of *Rhizoctonia* and *Waitea* isolates used in this study[z].

Isolate	Host	Origin	Management type	Species acronym	Anastomosis group
BELT 114	Tall fescue	Beltsville, MD	Lawn	Rs	AG 2-2IIIB
BLBG 06	CBG/ABG	Blacksburg, VA	Lawn	Rs	AG 2-2IIIB
BLBG 32C	CBG/ABG	Blacksburg, VA	Golf green	Rs	AG 2-2IIIB
BSF 42	Tall fescue	Richmond, VA	Lawn	Rs	AG 2-2IIIB
BSF 90	Tall fescue	Richmond, VA	Lawn	Rs	AG 2-2IIIB
LB 312	Tall fescue	Leesburg, VA	Lawn	Rs	AG 2-2IIIB
LB 4303	Tall fescue	Leesburg, VA	Golf rough	Rs	AG 2-2IIIB
BELT 26	Tall fescue	Beltsville, MD	Lawn	Rs	AG 1-IB
BELT 02	Tall fescue	Beltsville, MD	Lawn	Rs	AG 1-IB
BLBG 320	Tall fescue	Blacksburg, VA	Lawn	Rs	AG 1-IB
BLBG430	Tall fescue	Blacksburg, VA	Lawn	Rs	AG 1-IB
LB 123	Tall fescue	Leesburg, VA	Lawn	Rs	AG 1-IB
LB 234	Tall fescue	Leesburg, VA	Lawn	Rs	AG 1-IB
PW3 326	Tall fescue	Woodbridge, VA	Lawn	Rs	AG 1-IB
HDN 111A	Tall fescue	Herndon, VA	Golf rough	Rs	AG 1-IB
LB 4217A	Tall fescue	Leesburg, VA	Golf rough	Rs	AG 1-IB
ANP 301B	Tall fescue	Annapolis, MD	Lawn	Rs	AG 1-IB
BELT 122	Tall fescue	Beltsville, MD	Lawn	BNR	unknown
BELT 17	Tall fescue	Beltsville, MD	Lawn	BNR	unknown
LB 226	Tall fescue	Leesburg, VA	Lawn	BNR	unknown
HDN 325B	CBG/ABG	Herndon, VA	Golf green	BNR	unknown
ANP 107	Tall fescue	Annapolis, MD	Lawn	BNR	unknown
LB 4202A	Tall fescue	Leesburg, VA	Golf rough	BNR	unknown
BELT 05	Tall fescue	Beltsville, MD	Lawn	Wcz	WAG-Z
LB 319	Tall fescue	Leesburg, VA	Lawn	Wcz	WAG-Z
PW 220	Tall fescue	Woodbridge, VA	Lawn	Wcz	WAG-Z
PW 119	Tall fescue	Woodbridge, VA	Lawn	Wcz	WAG-Z
BELT 159	Tall fescue	Beltsville, MD	Lawn	Wcz	WAG-Z
LB 228	Tall fescue	Leesburg, VA	Lawn	Wcz	WAG-Z
LB 4116	Tall fescue	Leesburg, VA	Golf rough	Wcz	WAG-Z
VABCH 08	Tall fescue	Virginia Beach, VA	Lawn	Wcz	WAG-Z
VABCH 10	Tall fescue	Virginia Beach, VA	Lawn	Wcz	WAG-Z
BLBG 211	CBG/ABG	Blacksburg, VA	Golf green	Wcc	WAG
BLBG 216	CBG/ABG	Blacksburg, VA	Golf green	Wcc	WAG
BLBG 202	CBG/ABG	Blacksburg, VA	Golf green	Wcc	WAG
BLBG 08	CBG/ABG	Blacksburg, VA	Golf green	Wcc	WAG

[z]CBG/ABG = creeping bentgrass/annual bluegrass; Rs = *R. solani*; BNR = binucleate *Rhizoctonia*-like fungi; Wcz = *W. circinata* var. *zeae*; Wcc = *W. circinata* var. *circinata*.

Inc., Cary, NC). The EC50 values of isolates were grouped by their AGs or species for comparison. Since the EC50 values showed a significant effect, mean separation was performed using Fisher's least significant difference at $p = 0.05$. The sensitivity scale based on EC50 values adopted by Martin et al. (1984b) and described below was used to compare the sensitivity of the isolates to various fungicides. Isolates were considered extremely sensitive if the EC50 of a fungicide was less than 1 mg a.i.·L^{-1} (0.0001 oz a.i.·gal^{-1}), moderately sensitive if the EC50 was 1 to 10 mg a.i.·L^{-1} (0.0001 to 0.001 oz a.i.·gal^{-1}), and tolerant if EC50 exceeded 50 mg a.i.·L^{-1} (0.006 oz a.i.·gal^{-1}).

Results and Discussion

All isolates of *R. solani* (AG 1-IB, and AG 2-2IIIB) and binucleate *Rhizoctonia*-like fungi (BNR) except for LB 4202A were extremely sensitive to iprodione (Table 2). The average EC50 values for these three groups were < 1 mg a.i.·L^{-1} (< 0.0001 oz a.i.·gal^{-1}) (Table 3). The LB 4202A isolate showed an EC50 value slightly above 1 mg a.i.·L^{-1} (0.0001 oz a.i.·gal^{-1}) and would be considered moderately sensitive. All *W. circinata* isolates were moderately sensitive to iprodione [EC50 = 1.72 to 2.83 mg a.i.·L^{-1} (0.0002 to 0.0003 oz a.i.·gal^{-1}); Tables 2 and 3]. Isolates of AG 1-IB, and *W. circinata* were extremely sensitive to triticonazole [EC50 ≤ 0.5 mg a.i.·L^{-1} (≤ 0.00006 oz a.i.·gal^{-1}); Tables 2 and 3]. Isolates of AG 2-2IIIB appeared to be moderately sensitive to triticonazole and had a mean EC50 value of 1.26 mg a.i.·L^{-1} (0.0002 oz a.i.·gal^{-1}). The EC50 values of BNR isolates were variable and ranged from 0.09 to 3.78 mg a.i.·L^{-1} (0.0001 to 0.0005 oz a.i.·gal^{-1}) (Table 2). However, the majority of EC50 values were ≥ 1 mg a.i.·L^{-1} (≥ 0.0001 oz a.i.·gal^{-1}), indicating moderate sensitivity to triticonazole. Isolates of *W. circinata* var. *zeae* and var. *circinata* were moderately sensitive to pyraclostrobin with average EC50 values of 2.29 and 1.4 mg a.i.·L^{-1} (0.0003 and 0.0002 oz a.i.·gal^{-1}) respectively (Tables 2 and 3). With the exception of AG 2-2IIIB isolates BSF 42 and BSF 90, which were moderately sensitive to pyraclostrobin, isolates of all other groups were extremely sensitive [EC50 < 1 mg a.i.·L^{-1} (< 0.0001 oz a.i.·gal^{-1})] to this fungicide.

There was significant difference ($p \leq 0.5$) among the mean EC50 values of *Rhizoctonia* groups for all three fungicides. Mean separation of EC50 values of different *Rhizoctonia* groups (Table 3) largely agreed with the sensitivity scale of Martin et al. (1984b). For instance, average EC50 values for iprodione were significantly higher for the two *W. circinata* groups than the rest (Table 3). AG 1-IB, and *W. circinata* groups showed significantly lower average EC50 for triticonazole than BNR and AG 2-2IIIB. According to the Martin

Table 2. ***In vitro*** **impact of pyraclostrobin, iprodione, and triticonazole on mycelial growth of** ***Rhizoctonia*** **species[z].**

Group	Isolate	Pyraclostrobin				Iprodione				Triticonazole			
		EC50	95% CI Sig. (+/–)	Slope	Int	EC50	95% CI Sig. (+/–)	Slope	Int	EC50	95% CI Sig. (+/–)	Slope	Int
AG 2-2IIIB	BELT 114	0.48	18.06 – <0.001	0.41	0.13	0.48	1.59 – 0.14	1.75	0.55	2.33	n.c. – n.c.	0.44	–0.16
	BLBG 06	0.14	1.94 – <0.001	0.51	0.43	0.46	1.52 – 0.13	1.72	0.58	1.10	n.c. – n.c.	0.40	–0.02
	BLBG 32C	0.24	9.75 – <0.001	0.48	0.30	0.51	1.68 – 0.15	1.72	0.51	1.15	n.c. – n.c.	0.51	–0.03
	BSF 42	1.65	n.c. – n.c.	0.39	–0.09	0.43	1.34 – 0.13	1.98	0.73	1.39	>100 – <0.001	0.36	–0.05
	BSF 90	1.39	n.c. – n.c.	0.51	–0.07	0.48	1.62 – 0.13	1.64	0.52	0.73	n.c. – n.c.	0.49	0.07
	LB 312	0.64	59.24 – <0.001	0.37	0.07	0.60	1.76 – 0.18	2.41	0.53	1.37	n.c. – n.c.	0.47	–0.06
	LB 4303	0.88	>100 – <0.001	0.39	0.02	0.60	1.94 – 0.19	1.87	0.42	0.73	n.c. – n.c.	0.48	0.07
AG 1-IB	BELT 26	0.05	0.69 – <0.001	0.51	0.66	0.39	1.24 – 0.12	1.89	0.77	0.14	2.26 – <0.001	0.49	0.42
	BELT 02	0.04	0.64 – <0.001	0.49	0.67	0.42	1.24 – 0.12	2.12	0.81	0.34	7.85 – <0.001	0.51	0.24
	BLBG 320	0.35	20.57 – <0.001	0.55	0.25	0.46	1.42 – 0.14	1.95	0.66	0.18	1.62 – <0.001	0.65	0.49
	BLBG430	0.08	0.66 – <0.001	0.56	0.63	0.46	1.56 – 0.07	1.43	0.48	0.12	3.04 – <0.001	0.41	0.38
	LB 123	0.52	8.68 – <0.001	0.74	0.21	0.42	1.17 – 0.14	2.35	0.88	0.21	1.70 – <0.001	0.69	0.47
	LB 234	0.22	1.89 – <0.001	0.87	0.57	0.41	1.32 – 0.12	1.86	0.71	0.18	2.73 – <0.001	0.51	0.38
	PW3 326	0.18	0.99 – <0.001	0.78	0.59	0.36	1.08 – 0.11	1.98	0.89	0.20	2.78 – <0.001	0.54	0.38
	HDN 111A	0.08	1.34 – <0.001	0.63	0.69	0.41	1.41 – 0.05	1.19	0.46	0.10	1.90 – <0.001	0.49	0.50
	LB 4217A	0.11	2.63 – <0.001	0.55	0.52	0.49	1.10 – 0.10	6.15	0.94	0.05	0.50 – <0.001	0.56	0.74
	ANP 301B	0.17	1.61 – <0.001	0.50	0.38	0.48	1.28 – 0.15	2.48	0.8	0.50	20.30 – <0.001	0.48	0.15
BNR	BELT 122	0.10	1.10 – <0.001	0.53	0.52	0.93	2.83 – 0.27	1.71	0.05	0.17	12.34 – <0.001	0.41	0.32
	BELT 17	0.46	4.85 – <0.001	0.48	0.16	0.37	0.96 – 0.13	2.63	1.12	0.09	0.47 – <0.001	1.04	1.09
	LB 226	0.30	19.55 – <0.001	0.45	0.24	0.96	2.86 – 0.29	1.99	0.03	1.99	n.c. – n.c.	0.28	–0.08
	HDN 325B	0.09	0.86 – <0.001	0.50	0.53	0.78	2.40 – 0.09	1.96	0.21	3.78	n.c. – n.c.	0.29	–0.16
	ANP 107	0.26	2.08 – <0.001	0.54	0.31	0.85	2.56 – 0.25	2.14	0.15	1.25	>100 – <0.001	0.34	–0.03
	LB 4202A	0.17	2.08 – <0.001	0.53	0.42	1.15	3.86 – 0.18	1.39	–0.08	1.10	>100 – <0.001	0.44	–0.02
R. zeae	BELT 05	0.15	1.11 – <0.001	0.63	0.52	2.08	6.41 – 0.68	1.77	–0.56	0.05	0.42 – <0.001	0.66	0.87
	LB 319	1.30	23.11 – 0.01	0.46	–0.05	1.84	5.93 – 0.62	1.92	–0.51	0.03	0.25 – <0.001	0.66	1.00
	PW 220	1.79	n.c. – n.c.	0.51	–0.13	2.25	7.07 – 0.74	1.74	–0.61	0.06	0.38 – <0.001	0.83	1.02
	PW 119	1.35	>100 – <0.001	0.47	–0.06	2.52	7.62 – 0.83	1.87	–0.75	0.05	0.58 – <0.001	0.53	0.67
	BELT 159	2.22	>100 – 0.02	0.41	–0.14	1.88	6.07 – 0.66	1.91	–0.53	0.08	0.60 – <0.001	0.66	0.71
	LB 228	3.79	>100 – 0.06	0.42	–0.24	2.83	8.18 – 0.95	2.04	–0.92	0.04	0.33 – <0.001	0.68	0.92
	LB 4116	3.81	>100 – 0.03	0.36	–0.21	1.72	5.50 – 0.57	1.75	–0.41	0.11	1.57 – <0.001	0.52	0.5
	VABCH 08	2.63	>100 – 0.02	0.43	–0.18	2.51	7.49 – 0.86	2.04	–0.81	0.14	2.59 – <0.001	0.54	0.47
	VABCH 10	3.60	>100 – 0.02	0.36	–0.20	2.46	7.43 – 0.86	2.05	–0.8	0.15	1.60 – <0.001	0.57	0.47
R. circinata	BLBG 211	1.14	21.49 – 0.006	0.45	–0.03	2.17	7.35 – 0.70	1.54	–0.52	0.04	0.32 – <0.001	0.67	0.92
	BLBG 216	0.60	5.63 – 0.003	0.52	0.11	2.71	8.80 – 0.83	1.48	–0.64	0.04	0.27 – <0.001	0.89	1.22
	BLBG 202	1.58	84.92 – <0.001	0.44	–0.09	2.63	7.48 – 0.94	2.3	–0.97	0.03	0.30 – <0.001	0.63	0.95
	BLBG 08	2.30	n.c. – n.c.	0.59	–0.21	2.71	9.17 – 0.78	1.34	–0.58	0.05	0.38 – <0.001	0.66	0.85

[z]EC50 = concentration (mg a.i.·L^{-1}) required to inhibit radial growth by 50%; BNR = binucleate *Rhizoctonia*-like fungi; Slope = slope of the log-probit dose response curve; Int = intercept of the log-probit dose response curve; n.c. = not calculated. Confidence intervals (CI) could not be calculated by probit analysis for that isolate. Sig. = significance level; n.c. = not calculated.

Table 3. Fifty percent growth reduction (EC50) values (mg a.i.·L^{-1}) for isolates of *R. solani*, *W. circinata* and binucleate *Rhizoctonia*-like fungi exposed to pyraclostrobin, iprodione, and triticonazole in petri dish trials[z].

Group	No. of isolates	Pyraclostrobin		Iprodione		Triticonazole	
		Mean EC50[x]	Range[y]	Mean EC50	Range	Mean EC50	Range
AG 1-IB	10	0.18^{c} ± 0.15	0.04 – 0.52	0.43^{c} ± 0.04	0.36 – 0.49	0.20^{b} ± 0.13	0.05 – 0.50
AG 2-2IIIB	7	0.77^{b} ± 0.57	0.14 – 1.65	0.51^{c} ± 0.07	0.43 – 0.60	1.26^{a} ± 0.54	0.72 – 2.33
Wcz	9	2.29^{a} ± 1.28	0.15 – 3.81	2.23^{a} ± 0.38	1.72 – 2.83	0.08^{c} ± 0.04	0.03 – 0.15
Wcc	4	1.40^{ab} ± 0.72	0.60 – 2.30	2.56^{a} ± 0.26	2.16 – 2.71	0.04^{c} ± 0.01	0.03 – 0.05
BNR	6	0.23^{c} ± 0.14	0.09 – 0.46	0.84^{b} ± 0.26	0.37 – 1.15	1.40^{a} ± 1.37	0.09 – 3.78

[z]Wcz = *W. circinata* var. *zeae*; Wcc = *W. circinata* var. *circinata*; BNR = binucleate *Rhizoctonia*-like fungi.

[y]Ranges show the lowest and highest EC50 values for individual isolates within each group.

[x]Log EC50 values were subjected to analysis of variance. Means were separated using the test of Fisher's least significant difference at $p = 0{\cdot}05$, and inverse log transformed means are shown. Means not connected by same letter are significantly different. The ± value immediately following each mean is the standard deviation.

et al. (1984b) scale, moderate pyraclostrobin sensitivity was displayed by *W. circinata* isolates, while other groups were extremely sensitive to this fungicide. However, ANOVA results for pyraclostrobin showed EC50 values of AG 2-2IIIB isolates [average EC50 = 0.77 mg a.i.·L^{-1} (0.00009 oz a.i.·gal^{-1})] and Wcc [average EC50 of 1.40 mg a.i.·L^{-1} (0.0002 oz a.i.·gal^{-1})] were similar (Table 3).

On a few occasions, the probit model could not calculate confidence intervals of EC50 values due to too much variability in data (Table 2). Similar results have been reported by Jaspers (2001). The slopes and intercepts of response curves shown in Table 2 give an indication of fungicide efficacy. Both of these parameters are inversely proportional to the EC50 value.

Iprodione was introduced almost three decades ago to control a wide variety of fungal diseases including brown patch of turfgrasses (Radice et al. 2001). Previous studies have shown *R. solani* and binucleate *Rhizoctonia* are more sensitive to iprodione than are Wcz and *W. circinata* var. *oryzae* (Wco) (Martin et al. 1984a and 1984b, Carling et al. 1990, Kataria et al. 1991). However, these studies did not include isolates of Wcc. The results of our study indicated not only Wcz and Wco but also Wcc isolates are moderately sensitive to iprodione. The isolates of AG 1-IB, AG 2-2IIIB, BNR and Wcz represent several different geographic locations throughout VA and MD (Table 1). In this study, geography did not influence EC50 values of the isolates since there were no large deviations of sensitivity among different isolates. However, it would be beneficial to study differences in sensitivity of *Rhizoctonia* isolates from differently managed turf areas. Bentgrass putting greens are extensively managed with routine fungicide applications while lawns and athletic fields are rarely exposed to fungicides. Although we would expect higher EC50 values for isolates from putting greens, we did not have the fungicide application details to effectively test the impact of turf management regime on EC50.

Triticonazole is a second generation DMI fungicide which disrupts sterol biosynthesis in fungal cell membranes, leading to alterations of the structure and disturbances in the division and development of cells (Mueller and Bradley 2008, Pfeufer and Ngugi 2012). Studies on DMI fungicides such as propiconazole, fenarimol, and triadimefon have shown variable results *in vitro* and in field studies for different species and AGs of *Rhizoctonia* (Kataria and Gisi 1996, Vargas 2005, Meyer et al. 2006). Because of the variability in effectiveness within the DMI group, it is important to understand the sensitivity of *Rhizoctonia* isolates to triticonazole since it is a newer active ingredient. There are no previous reports of *Rhizoctonia* isolates challenged with this fungicide *in vitro*. Our study indicated AG 1-IB, and all varieties of *W. circinata* were extremely sensitive to triticonazole [EC50 < 1 mg a.i.·L^{-1} (< 0.0001 oz a.i.·gal^{-1})], while isolates of AG 2-2IIIB displayed moderate sensitivity.

There seems to be no association between EC50 variability and geography. For example, AG 2-2IIIB isolates BSF 42 and BSF 90 from Richmond, VA showed moderate sensitivity [EC50: 1.39 mg a.i.·L^{-1} (0.0002 oz a.i.·gal^{-1})] and extreme sensitivity [EC50: 0.73 mg a.i.·L^{-1} (0.00009 oz a.i.·gal^{-1})], respectively for triticonazole. Similarly, LB 312 showed a moderate EC50 value of 1.37 mg a.i.·L^{-1} (0.0002 oz a.i.·gal^{-1}) while LB 4303 had a comparatively low EC50 value of 0.73 mg a.i.·L^{-1} (0.00009 oz a.i.·gal^{-1}) for triticonazole. Both of these isolates were collected from Leesburg, VA. Kataria et al. (1991) tested a single isolate of AG 1, Wco, and Wcz against different DMI fungicides *in vitro*. Their results showed all three isolates were extremely sensitive to cyproconazole. However in this study, propiconazole, another DMI fungicide, was less efficacious for the AG 1 isolate [EC90 = 47 mg a.i.·L^{-1} (0.006 oz a.i.·gal^{-1})] while both Wcz and Wco isolates showed extreme sensitivity [EC90 ≤ 2 mg a.i.·L^{-1} (0.0002 oz a.i.·gal^{-1})]. These results indicate that different DMI fungicides can have different efficacies against different *Rhizoctonia* (*Thanatephorus* and *Waitea*) genera and AGs. The variation of EC50 values within a *Rhizoctonia group* (e.g. AG 2-2IIIB and BNA with triticonazole) may be attributed to the inherent genetic variation among those fungal isolates or previous exposure to the tested fungicide. Hsiang et al. (2007) calculated EC50 values for 186 and 279 isolates of *Sclerotinia homoeocarpa* F.T. Bennett obtained from turfgrass plots treated with propiconazole or left untreated, respectively. For both populations, EC50 values had a wide distribution with the majority of values falling around the mean. Also, they observed mean EC50 of treated populations showing a shift towards decreased sensitivity compared to the untreated populations. In our study, BNR isolate LB 325B with less sensitivity to triticonazole (Table 2) was isolated from a golf green exposed to fungicide treatments. However, we did not have fungicide application details to

make accurate correlations and the number of isolates from a single *Rhizoctonia* group was not large enough to make statistically sound inferences.

Pyraclostrobin was very effective against all *Rhizoctonia* groups other than isolates of *W. circinata*, which were moderately sensitive (Table 3). An *in vitro* study conducted by Meyer et al. (2006) found an isolate of *R. solani* AG 1-IA was extremely sensitive to pyraclostrobin [EC50 = 0.094 mg a.i.·L^{-1} (0.00001 oz a.i.·gal^{-1})]. The closely related AG 1-IB isolates used in this study gave similar results for pyraclostrobin sensitivity by displaying EC50 values of 0.02 to 0.59 mg a.i.·L^{-1} (0.000002 to 0.00007 oz a.i.·gal^{-1}) which fall within the extremely sensitive category. Several QoI fungicides have shown reduced effectiveness *in vitro* for some pathogens due to site specific mutations (Chin et al. 2001, Kim et al. 2003, Ma et al. 2003). Ma et al. (2003) reported resistance to azoxystrobin by *Alternaria* species isolated from pistachio in California. Azoxystrobin and trifloxystrobin-resistant isolates of *Pyricularia grisea*, the causal agent of grey leaf spot, have been identified from perennial ryegrass (*Lolium perenne* L.) turf in Illinois (Kim et al. 2003). They found that resistance was due to a small number of specific mutations in cytochrome b, the target site for this fungicide class (Chin et al. 2001, Kim et al. 2003, Ma et al. 2003).

Cross resistance has been shown by other fungal organisms for all the fungicides tested in this study (Leroux et al. 1999, Karaoglanidis and Thanassoulopoulos 2003, Fernandez-Ortun 2006). Cross resistance occurs when a fungus resistant to one fungicide in a chemical family can compromise all fungicides in that family. An important study would be to test sensitivity of *Waitea* species to vinclozolin which is the only other dicarboximide fungicide approved for turf. This would help to elucidate the level of cross resistance in this fungicide family. This is useful since the cross resistance relationship between fungicides within the same family can vary greatly (Leroux et al. 1999). In the same way, our research data can be used to identify *Rhizoctonia* groups less sensitive to pyraclostrobin and triticonazole and test for cross resistance with other turf fungicides within a single family.

Some fungal organisms are capable of using alternative respiration pathways in response to QoI fungicides tested *in vitro* (Wise et al. 2008). The chemical salicylhydroxamic acid (SHAM) is used to prevent a fungus from taking an alternative respiration pathway during *in vitro* tests. Since the ability of *Rhizoctonia* species to use alternative respiration is not well documented, we conducted an initial test incorporating SHAM with three representative isolates viz. PW 220 (Wcz), BSF 90 (AG 2-2IIIB), and LB 226 (BNR) and found no synergistic growth inhibition in SHAM plus pyraclostrobin amended plates (data not shown). Therefore, SHAM was not added to the pyraclostrobin-amended PDA plates for determining effectiveness against *Rhizoctonia* isolates.

The primary objective of this fungicide sensitivity study was to test if *Rhizoctonia* groups have different levels of sensitivity to the three fungicides tested. The *in vitro* sensitivity of different *Rhizoctonia* species, AGs, and subgroups varied for each fungicide. However, it is necessary to replicate fungicide tests in the field to determine the level of correlation with *in vitro* results. In fact, certain diseases have been reported as not being controlled, or disease incidence increased, when fungicides are applied in greenhouse or field trials (Van der Hoeven and Bollen 1980, Martin, et al. 1984a, Sumner 1987). Van der Hoeven and Bollen (1980) observed an increase of sharp eye spot of rye caused by *R. cerealis* in response to benomyl in the field even though the pathogen was sensitive to this fungicide *in vitro*. While one explanation may be a decrease in antagonistic microflora, *in vitro* and *in vivo* differences in efficacy may be due to fungicide metabolism by the plant. Environmental conditions also greatly influence the effectiveness of a fungicide by favoring either the plant or the pathogen (Blandino et al. 2011, Koch 2012).

Based on the results from this study, it is apparent that individually none of the three fungicides tested is capable of controlling all *Rhizoctonia* groups infecting cool-season turfgrasses. If the *Rhizoctonia* pathogen is not known, better results may be obtained by applying triticonazole in combination with either iprodione or pyraclostrobin. Field trials are needed to assess the practical application of these *in vitro* results.

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