Sensitivity of *Rhizoctonia solani* AG-2-2 from Sugar Beet to Fungicides

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Abstract

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Rhizoctonia damping-off and crown and root rot caused by *Rhizoctonia solani* are major diseases of sugar beet (*Beta vulgaris* L.) worldwide, and growers in the United States rely on fungicides for disease management. Sensitivity of *R. solani* to fungicides was evaluated in vitro using a mycelial radial growth assay and by evaluating disease severity on *R. solani* AG 2-2 inoculated plants treated with fungicides in the greenhouse. The mean concentration that caused 50% mycelial growth inhibition (EC₅₀) values for baseline isolates (collected before the fungicides were registered for sugar beet) were 49.7, 97.1, 0.3, 0.2, and 0.9 $\mu\text{g ml}^{-1}$ and for nonbaseline isolates (collected after registration and use of fungicides) were 296.1, 341.7, 0.9,

0.2, and 0.6 $\mu\text{g ml}^{-1}$ for azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole, respectively. The mean EC₅₀ values of azoxystrobin, trifloxystrobin, and pyraclostrobin significantly increased in the nonbaseline isolates compared with baseline isolates, with a resistant factor of 6.0, 3.5, and 3.0, respectively. Frequency of isolates with EC₅₀ values >10 $\mu\text{g ml}^{-1}$ for azoxystrobin and trifloxystrobin increased from 25% in baseline isolates to 80% in nonbaseline isolates. Although sensitivity of nonbaseline isolates of *R. solani* to quinone outside inhibitors decreased, these fungicides at labeled rates were still effective at controlling the pathogen under greenhouse conditions.

Rhizoctonia solani Kühn (teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk) has been considered to be a major pathogen of sugar beet (*Beta vulgaris* L.) worldwide (Herr 1996; Whitney and Duffus 1986). It is a soilborne pathogen which survives as mycelium or sclerotia in the soil (Menzies 1970). *R. solani* has been considered a major pathogen since the early production of sugar beet crop. Since 2009, growers in North Dakota and Minnesota have listed *Rhizoctonia* spp. as their most important problem and reported that 11% of their fields were infested with *R. solani* (Carlson et al. 2013). *R. solani* has 14 anastomosis groups (AG), AG-1 to AG-13 and AG-B1, which are further divided to subgroups (Carling et al. 2002). AG-1, AG-2-1, AG-2-2, AG-3, AG-4, and AG-5 have been recorded on sugar beet (Windels and Nabben 1989). The destructive AG on both seedling and older sugar beet plants is AG-2-2, which has two subgroups, AG-2-2 IIIB and AG-2-2 IV (Windels and Nabben 1989).

Growers typically use partially resistant cultivars and apply fungicides to manage *R. solani* in sugar beet (Kiewnick et al. 2001; Panella et al. 1994). Fungicides registered in the United States for control of *R. solani* on sugar beet include Quadris (azoxystrobin, active ingredient [a.i.], 22.9%; Syngenta, Greensboro, NC), registered in 1999; Gem (trifloxystrobin, a.i., 42.6%; Bayer, Research Triangle Park, NC), registered in 2002; Headline (pyraclostrobin, a.i., 23.6%; BASF, Research Triangle Park, NC), registered in 2002; Proline (prothioconazole, a.i., 41%; Bayer), registered in 2008; Vertisan (penthiopyrad, a.i., 20.6%; DuPont Crop Protection, Wilmington, DE), registered in 2012 but not available commercially; Kabina 40SC (penthiopyrad, a.i., 40%; Mitsui Chemicals Agro, Inc., Chuo-ku, Japan), which was labeled for use as a seed treatment and became available in 2013; and Priaxor (fluxapyroxad, a.i., 14.33% + pyraclostrobin a.i., 28.58%; BASF), which was labeled and became available in 2012 (Friskop et al. 2014).

Azoxystrobin, trifloxystrobin, and pyraclostrobin are quinone outside inhibitors (QoI), which block electron transfer between cytochrome b and cytochrome c1 by binding to cytochrome b and lead to halting the ATP production (Balba 2007). Penthiopyrad is a succinate dehydrogenase inhibitor (SDHI), which stops ATP production

by binding to succinate dehydrogenase enzyme located in mitochondrial membrane (Hägerhäll 1997). Prothioconazole is a sterol biosynthesis demethylation inhibitor (DMI), which disturbs plasma membrane structure and results in abnormal fungal growth and death (Georgopapadakou 1998). These groups of fungicides were classified by the Fungicide Resistance Action Committee as medium to high risk with regard to resistance evolution (Anonymous 2016).

Growers in the United States have been using azoxystrobin for control of *R. solani* in sugar beet since it was labeled in 1999. In 2013, growers in Minnesota and North Dakota, responsible for producing 57% of the U.S. sugar beet crop (Anonymous 2014), listed azoxystrobin, followed by pyraclostrobin, and prothioconazole, as the fungicides most commonly used to control *R. solani* (Carlson et al. 2013). Although fungicides have been used annually to manage *R. solani*, we are not aware of any peer review article which has evaluated sensitivity of *R. solani* AG 2-2 isolates from sugar beet to fungicides.

R. solani isolates from several different plants and regions were evaluated for their sensitivity to fungicides. For example, *R. solani* isolates from potato (*Solanum tuberosum* L.) (Campion et al. 2003), rice (*Oryza sativa* L.) (Chen et al. 2012, 2014; Olaya et al. 2012; Sundravandana et al. 2007; Zhang et al. 2009), grasses (Blazier and Conway 2004; Martin et al. 1984), tobacco (*Nicotiana tabacum* L.) (Csinos and Stephenson 1999; LaMondia 2012), soybean (*Glycine max* (L.) Merr.) (Ajayi and Bradley 2014), and table beet (Olaya et al. 1994) were evaluated for their sensitivity to different group of fungicides. Some studies evaluated fungicide sensitivity of *R. solani* isolates from soil (Carling et al. 1990; Goll et al. 2014; Jin et al. 2009; Kataria et al. 1991). Some of these previous studies showed that *R. solani* has the ability to develop resistance to QoI fungicides, as was demonstrated by *R. solani* AG-1-1A from rice (Olaya et al. 2012) and AG-2-2 IIIB from turf grass (Blazier and Conway 2004).

It would be useful to determine baseline sensitivity of *R. solani* isolates from sugar beet to fungicides so that any changes in the pathogen's sensitivity could be monitored over time to determine how best to manage the fungus while preserving the utility of fungicides. This research was conducted to (i) develop baseline sensitivity data of *R. solani* AG-2-2 from sugar beet for QoI (i.e., azoxystrobin, trifloxystrobin, and pyraclostrobin), SDHI (i.e., penthiopyrad), and DMI (i.e., prothioconazole) fungicides and determine whether a shift in sensitivity to any or all of the tested fungicides has occurred since 1999; (ii) determine whether there was cross resistance between QoI fungicides; and (iii) evaluate the efficacy of the tested fungicides at controlling *R. solani* isolates in the greenhouse.

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Materials and Methods

Source of *R. solani* isolates. *R. solani* AG-2-2 isolates were obtained from the University of Minnesota, Northwest Research and Outreach Center, Crookston (Dr. Carol Windels and Jason Brantner). These isolates were collected from sugar beet samples submitted by growers from Minnesota and North Dakota. In all, 105 *R. solani* AG-2-2 isolates were used in this study; of these, 27 isolates were used to establish a baseline for sensitivity because they were collected prior to 1999, before the labeled use of any foliar fungicide for control of *R. solani* in sugar beet. The remaining 78 isolates, collected between 2005 and 2012 (Table 1), after fungicides were labeled and widely used for control of *R. solani*, were used to determine whether a shift in sensitivity has occurred.

In vitro sensitivity of *R. solani* to azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole. A mycelial radial growth assay was used to evaluate *R. solani* sensitivity to the above-listed fungicides as described by Kataria et al. (1991), with slight modifications. A cork borer (number 3) was used to cut 5-mm-diameter mycelial plugs from the actively growing margin of 4-day-old cultures of *R. solani*. The plugs were inverted onto the fungicide-amended (described later) and nonamended media and incubated at $22 \pm 2^\circ\text{C}$ in the dark for 72 h. After incubation, the colony diameters were measured in two perpendicular directions. The percentage of mycelium growth reduction for each isolate and concentration was calculated as $[100 - (\text{diameter in amended media}/\text{diameter in nonamended media} \times 100)]$ and analyzed by regression analysis against the log fungicide concentration. The concentration that caused 50% mycelial growth inhibition (EC_{50}) was determined by interpolation of the 50% intercept (Russell 2004) using SAS (version 9.3; SAS Institute, Inc.; Cary, NC). A completely randomized design was used, with two replications per fungicide concentration. The experiment was repeated once.

Technical grades of azoxystrobin (96% a.i.; Syngenta), trifloxystrobin (98.8% a.i.; Bayer), pyraclostrobin (98% a.i.; BASF), penthiopyrad

(95% a.i.; DuPont), and prothioconazole (99.4% a.i.; Bayer) were used to prepare stock solutions of $100,000 \mu\text{g ml}^{-1}$ in acetone (EM Science, Gibbstown, NJ). The 10-fold serial dilutions were prepared to have fungicide concentrations of 0, 10, 100, 1,000, and $10,000 \mu\text{g ml}^{-1}$. Salicylhydroxamic acid (SHAM; Sigma-Aldrich, St. Louis) was dissolved in methanol (Sigma-Aldrich) to obtain a stock solution of $10,000 \mu\text{g ml}^{-1}$. One liter of half-strength potato dextrose agar (PDA; HIMEDIA) medium was amended with 1 ml of one of the fungicide concentrations and 1 ml of SHAM, which was used to prevent an alternative oxidation respiration pathway (Wood and Hollomon 2003). Isolates that showed an EC_{50} value $\geq 100 \mu\text{g ml}^{-1}$, as was the case for azoxystrobin and trifloxystrobin, were tested again using fungicide concentrations of 0, 1, 10, 100, 500, and $1,000 \mu\text{g ml}^{-1}$. Due to low solubility of the technical grades in water, formulated products of azoxystrobin (Quadris, 22.9% a.i.) and trifloxystrobin (Gem, 42.6% a.i.) were used to prepare fungicide concentrations as was reported by Kataria et al. (1991).

Efficacy of azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole in controlling *R. solani* AG-2-2. Efficacy of the above-listed fungicides for controlling *R. solani* AG-2-2 was evaluated in the Agricultural Experiment Station greenhouse at North Dakota State University in Fargo. Eight *R. solani* isolates were chosen based on the AG-2-2 subgroups and their EC_{50} values for azoxystrobin and trifloxystrobin. Four isolates of AG-2-2 IIIB (two isolates [22-1 and 571] with high EC_{50} values [$\geq 100 \mu\text{g ml}^{-1}$] and two isolates [850 and 946] with low EC_{50} values [$< 10 \mu\text{g ml}^{-1}$]) and four isolates of AG-2-2 IV (isolates 393 and 40-2 with high EC_{50} values and isolates 60 and 31-1 with low EC_{50} values) were chosen. Because of space limitation and the time required to prepare and inoculate the high number of treatments ($n = 960$), the isolates were divided and evaluated for all fungicides in two studies, and each study was repeated once.

Sunshine Mix LC1 (73 to 83% Canadian sphagnum peat moss, perlite, and dolomite lime; Sun Gro Horticulture Distribution, Inc., Agawam, MA) was used to fill plastic trays (25 by 14 by 13 cm; T. O. Plastic, Inc., Clearwater, MN) into which 10 sugar beet seeds of 'Crystal 539RR', susceptible to *R. solani* (Niehaus 2011), were planted in a 2-cm-deep furrow made in the middle of each tray. Serial fungicide dilutions (0, 0.1, 1, 10, 100, 1,000, and $10,000 \mu\text{g ml}^{-1}$) as well as one of the widely recommended field application rates (Khan 2000) of azoxystrobin at $3,292 \mu\text{g ml}^{-1}$ (672 ml ha^{-1}), trifloxystrobin at $2,330 \mu\text{g ml}^{-1}$ (256 ml ha^{-1}), pyraclostrobin at $3,392 \mu\text{g ml}^{-1}$ (672 ml ha^{-1}), penthiopyrad at $9,656 \mu\text{g ml}^{-1}$ ($2,192 \text{ ml ha}^{-1}$), and prothioconazole at $3,652 \mu\text{g ml}^{-1}$ (417 ml ha^{-1}) were prepared using water. Fungicides were applied as an in-furrow application using a Generation III Research Sprayer (DeVries Manufacturing, Hollandale, MN) through a 4001E flat-fan nozzle calibrated to deliver the solutions at 138 kPa and 6.3 km h^{-1} . The order of treatments was started with the control (distilled water) and then with the fungicides from the lowest to highest concentrations. Distilled water was used to rinse the sprayer three times between fungicides. After applying the fungicides, one *R. solani*-inoculated barley grain (Gaskill 1968) was placed 1 cm to the side of each sugar beet seed. After inoculation, sugar beet seed and inocula were covered with LC1 mix. Trays were placed under greenhouse conditions at $20 \pm 2^\circ\text{C}$ and a 12-h photoperiod (Argus Control Systems, Ltd., Surrey, British Columbia, Canada) and irrigated daily. Three weeks after treatments, plants were removed from trays and roots were washed carefully under running tap water and evaluated using a 0-to-7 scale, where 0 = no disease, 1 = crown area slightly scurfy, 2 = $< 5\%$ symptoms, 3 = 6 to 25% symptoms, 4 = 26 to 50% symptoms, 5 = 51 to 75% symptoms, 6 = $> 75\%$ symptoms, and 7 = root completely deteriorated (Scholten et al. 2001). To confirm that the symptoms were caused by *R. solani*, the fungus was reisolated from symptomatic plants by cutting three small pieces (2 mm each) of the infected roots that were immersed in 0.5% NaOCl for 10 s, rinsed twice in sterilized distilled water, and plated on 1.5% water agar media. Morphological characteristics were used to identify the pathogen (Parmeter 1970). A randomized complete block design with three replicates was used with isolates, fungicides, and concentrations as the factors.

Table 1. Year of collection, state of origin, and number of *Rhizoctonia solani* AG-2-2 isolates (*N*) and their subgroups from sugar beet used for mycelial radial growth assay and in greenhouse studies

Isolates, year	State	N	AG-2-2 subgroups (N) ^a	
			IIIB	IV
Baseline ^b				
1986	Minnesota	5	1	4
1987	Minnesota	2	0	2
	North Dakota	4	1	3
1988	Minnesota	3	0	3
1987–1988	Minnesota	3	0	3
	North Dakota	4	0	4
1989	Minnesota	1	0	1
1993	Minnesota	5	2	3
Subtotal	...	27	4	23
Nonbaseline ^c				
2005	Minnesota	1	0	1
	North Dakota	3	0	3
2006	Minnesota	14	5	9
	North Dakota	6	2	4
2007	Minnesota	12	9	3
2008	Minnesota	9	6	3
	North Dakota	2	1	1
2012	Minnesota	30	15	15
	North Dakota	1	0	1
Subtotal	...	78	38	40
Total	...	105	42	63

^a *R. solani* AG-2-2 subgroups were determined following Sneh et al. (1991).

^b *R. solani* isolates collected before 1999, prior to the use of any foliar fungicide labeled for control of *R. solani* in sugar beet.

^c *R. solani* isolates were collected from sugar beet fields during the period when quinone outside inhibitor and demethylation inhibitor fungicides were labeled and used in sugar beet.

Data Analysis. Levene's homogeneity of variance test was used to determine whether the runs for each study could be combined for analysis. Student's *t* test ($P = 0.05$) was used to test differences between mean EC₅₀ of baseline and nonbaseline isolates. Correlation between EC₅₀ values for QoI fungicides for *R. solani* isolates were determined using Pearson correlation analyses ($\alpha = 0.05$). The non-parametric Kruskal-Wallis test was used to analyze the greenhouse data, as described by Shah and Madden (2004). The disease severity median value was calculated for each tray, and the respective mean rank for all isolates, fungicides, and concentrations was calculated using PROC RANK in SAS. For each treatment, the ranked disease severities were used to estimate relative effects, standard errors, and the 95% confidence intervals using the SAS macro LD-CI (<http://www.ams.med.uni-goettingen.de/sasmakr-de.shtml>) to compare among different treatments (Shah and Madden 2004).

Results

In vitro sensitivity of *R. solani* to azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole. The two trials for each experiment were combined based on Levene's test for homogeneity of variance. Baseline isolates (isolates collected before 1999) showed EC₅₀ values $\leq 3 \mu\text{g ml}^{-1}$ for pyraclostrobin, penthiopyrad, and prothioconazole and a wide range of EC₅₀ values for azoxystrobin and trifloxystrobin (Table 2). The mean EC₅₀ values were 49.7, 97.1, 0.32, 0.2, and $0.9 \mu\text{g ml}^{-1}$ and the ranges of EC₅₀ values were 0.43 to 597.43, 0.14 to 823.54, 0.04 to 2.70, 0.04 to 2.27, and 0.11 to $2.40 \mu\text{g ml}^{-1}$ for azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole, respectively (Table 2).

Nonbaseline isolates collected in 2005 through 2012 showed also a wide range of EC₅₀ values for azoxystrobin and trifloxystrobin, with means of 269.1 and $341.7 \mu\text{g ml}^{-1}$, respectively (Table 2). Mean EC₅₀ values were $0.9 \mu\text{g ml}^{-1}$ for pyraclostrobin, $0.2 \mu\text{g ml}^{-1}$ for penthiopyrad, and $0.6 \mu\text{g ml}^{-1}$ for prothioconazole (Table 2). The ranges of EC₅₀ values were 0.18 to 876.58, 0.09 to 888.41, 0.02 to

6.43, 0.02 to 0.61, and 0.03 to $0.56 \mu\text{g ml}^{-1}$ for azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole, respectively (Table 2). When the nonbaseline isolates were compared with the baseline isolates, there were significant ($P \leq 0.05$) increases in the mean EC₅₀ values for azoxystrobin, trifloxystrobin, and pyraclostrobin (Table 2), with a resistant factor (mean EC₅₀ value of nonbaseline isolates/mean EC₅₀ of baseline isolates) of 6.0, 3.5, and 3, respectively (Table 2). The frequency of isolates with EC₅₀ values $>10 \mu\text{g ml}^{-1}$ for azoxystrobin and trifloxystrobin increased from 25% in baseline isolates to $>80\%$ in nonbaseline isolates (Table 3). There was no shift in mean EC₅₀ values between baseline and nonbaseline *R. solani* isolates evaluated for sensitivity to penthiopyrad and prothioconazole (Table 2).

There was a significant positive correlation in baseline isolates between EC₅₀ values of pyraclostrobin and EC₅₀ values of trifloxystrobin ($r = 0.8$; $P < 0.0001$). In nonbaseline isolates, a significant positive correlation was found between EC₅₀ values of azoxystrobin and trifloxystrobin ($r = 0.65$; $P < 0.0001$), EC₅₀ values of azoxystrobin and pyraclostrobin ($r = 0.3$; $P = 0.01$), and EC₅₀ values of trifloxystrobin and pyraclostrobin ($r = 0.38$; $P < 0.0006$).

Efficacy of different concentrations of azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole at controlling *R. solani* in the greenhouse. For all isolates, except isolate 31 (AG-2-2 IV; low EC₅₀ value), the low fungicide concentrations ($\leq 10 \mu\text{g ml}^{-1}$) were not effective at controlling *R. solani* and the ranked mean disease severities were significantly different from the noninoculated control (no symptoms). Isolate 31 was effectively controlled by azoxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole at concentrations $\leq 10 \mu\text{g ml}^{-1}$ because they did not result in disease severities that were significantly different from the noninoculated controls (Figs. 1 and 2).

All fungicides applied at concentrations greater than $1,000 \mu\text{g ml}^{-1}$, which included the recommended field application rates for all tested fungicides, were able to control all *R. solani* isolates because disease

Table 2. Mean effective fungicide concentration that inhibited mycelial radial growth by 50% (EC₅₀) values of azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole for *Rhizoctonia solani* AG-2-2 isolates collected before 1999 (baseline isolates) and from 2005 to and including 2012 (non-baseline isolates) from sugar beet fields in Minnesota and North Dakota^a

		EC ₅₀ (µg ml ^{−1}) ^b				
		Baseline isolates		Nonbaseline isolates		
MOA ^c	Fungicide	Mean	Range	Mean	Range	RF ^d
QoI	Azoxystrobin	49.7 (65.43)	0.43–597.43	296.1 (127.75)*	0.18–876.58	6.0
QoI	Trifloxystrobin	97.1 (58.43)	0.14–823.54	341.7 (94.28)*	0.09–888.41	3.5
QoI	Pyraclostrobin	0.3 (0.64)	0.04–2.70	0.9 (1.13)*	0.02–6.43	3.0
SDHI	Penthiopyrad	0.2 (0.21)	0.04–2.27	0.2 (0.12)	0.02–0.61	1.0
DMI	Prothioconazole	0.9 (0.35)	0.11–2.40	0.6 (0.25)	0.03–0.56	0.7

^a Mycelial radial growth assay was conducted using potato dextrose agar media. Cultures were kept in darkness at $22 \pm 2^\circ\text{C}$ for 3 days.

^b Values in parenthesis denote standard deviation; * indicates EC₅₀ values significantly different from baseline isolates at $P = 0.05$ following Student's *t* test.

^c Mode of action (MOA): QoI = quinone outside inhibitors, SDHI = succinate dehydrogenase inhibitor, and DMI = demethylation inhibitor.

^d Resistant factor (RF) was derived from mean EC₅₀ value of nonbaseline isolates/mean EC₅₀ value of baseline isolates.

Table 3. Frequency of effective fungicide concentration that inhibited mycelial radial growth by 50% (EC₅₀) values of azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole for 27 baseline *Rhizoctonia solani* isolates collected before 1999 and 78 nonbaseline isolates collected from 2005 through 2012 from sugar beet fields in Minnesota and North Dakota using a mycelial radial growth assay^a

Active ingredient	Isolate group	Isolate frequency (%) at EC ₅₀ range ($\mu\text{g ml}^{-1}$)				
		0.01–0.1	0.1–1	1–10	10–100	100–1,000
Azoxystrobin	Baseline	0.00	7.14	67.86	17.86	7.14
	Nonbaseline	0.00	7.69	11.54	32.05	48.72
Trifloxystrobin	Baseline	0.00	32.14	42.86	3.57	21.43
	Nonbaseline	1.28	2.56	12.82	6.41	76.92
Pyraclostrobin	Baseline	25.00	67.86	7.14	0.00	0.00
	Nonbaseline	6.41	73.08	20.51	0.00	0.00
Penthiopyrad	Baseline	64.29	32.14	3.57	0.00	0.00
	Nonbaseline	19.23	80.77	0.00	0.00	0.00
Prothioconazole	Baseline	0.00	57.14	42.86	0.00	0.00
	Nonbaseline	3.85	82.05	14.10	0.00	0.00

^a Cultures were grown on potato dextrose agar and kept at $22 \pm 2^\circ\text{C}$ in darkness for 3 days.

severities of plants treated with these fungicide concentrations were not significantly greater from those of noninoculated controls (Figs. 1 and 2).

Discussion

In this study, 105 isolates of *R. solani* AG-2-2 were tested for their sensitivity to azoxystrobin, trifloxystrobin, pyraclostrobin, penthiopyrad, and prothioconazole. Azoxystrobin, trifloxystrobin, and pyraclostrobin

showed mean EC_{50} values of 49.7, 97.1, and 0.3 $\mu\text{g ml}^{-1}$, respectively, for *R. solani* AG-2-2 baseline isolates. The mean EC_{50} values for azoxystrobin, trifloxystrobin, and pyraclostrobin increased to 296.1, 341.7, and 0.9 $\mu\text{g ml}^{-1}$, respectively, in the AG-2-2 nonbaseline *R. solani* isolates. Although azoxystrobin, trifloxystrobin, and pyraclostrobin belong to the same fungicide group (QoI) (Balba 2007), the mean EC_{50} values of baseline and nonbaseline isolates suggested that pyraclostrobin had the greatest intrinsic activity. Pasche et al.

Fig. 1. Efficacy of five fungicides at controlling four *Rhizoctonia solani* isolates: two AG-2-2 IIIB isolates (22-1, with high concentration that caused 50% mycelial growth inhibition [EC_{50}] value $\geq 100 \mu\text{g ml}^{-1}$) and isolate 850, with low EC_{50} value [$<10 \mu\text{g ml}^{-1}$]) and two AG-2-2 IV isolates (393, with high EC_{50} value and isolate 60, with low EC_{50} value [$<10 \mu\text{g ml}^{-1}$]) for azoxystrobin and trifloxystrobin using susceptible Crystal 539RR sugar beet in the greenhouse. Plants were kept at $20 \pm 2^\circ\text{C}$ and a 12-h photoperiod for 3 weeks. Nonparametric analysis was used to analyze relative effects of each treatment on disease severity symptoms. There were three replicates for each treatment and the experiment was repeated once.

(2004) and Wise et al. (2008) also reported similar results for *Alternaria solani* on potato (*S. tuberosum* L.) and *Ascochyta rabiei* on chick pea (*Cicer arietinum* L.), respectively.

In this study, azoxystrobin showed a high mean EC_{50} value ($\geq 100 \mu\text{g ml}^{-1}$) for *R. solani* AG-2-2 (IIIB and IV, data not shown)

isolates whereas Blazier and Conway (2004) reported a high mean EC_{50} value ($\geq 100 \mu\text{g ml}^{-1}$) for AG-2-2 IIIB and low mean EC_{50} value for AG-2-2 IV, probably because of the low number ($n = 4$) of isolates evaluated. Jin et al. (2009) and Sundravadana et al. (2007) also reported low EC_{50} values for azoxystrobin but for just

Fig. 2. Efficacy of five fungicides at controlling four *Rhizoctonia solani* isolates: two AG-2-2 IIIB isolates (571, with high concentration that caused 50% mycelial growth inhibition [EC_{50}] value [$\geq 100 \mu\text{g ml}^{-1}$] and isolate 946, with low EC_{50} value [$< 10 \mu\text{g ml}^{-1}$]) and two AG 2-2 IV isolates [40-2, with high EC_{50} value and isolate 31-1, with low EC_{50} value [$< 10 \mu\text{g ml}^{-1}$]] for azoxystrobin and trifloxystrobin using susceptible Crystal 539RR sugar beet in the greenhouse. The inoculated and treated seeded trays were kept at $20 \pm 2^\circ\text{C}$ and a 12-h photoperiod, and watered daily for 3 weeks. Nonparametric analysis was used to analyze relative effects of each treatment on disease severity symptoms evaluated using a scale of 0 (no disease) to 7 (root completely deteriorated). There were three replicates for each treatment and the experiment was repeated once.

one tested *R. solani* isolate. These studies which reported low EC₅₀ values for azoxystrobin did not have the isolates exposed to the fungicide as long as in our study.

The high EC₅₀ values of azoxystrobin and trifloxystrobin in vitro could be a result of *R. solani* using an additional mechanism of alternative oxidation besides the alternative respiration pathway which is inhibited by SHAM, as was suggested by LaMondia, (2012) and Jin et al. (2009). In this study, *R. solani* isolates showed a low mean EC₅₀ value for prothioconazole, similar to that reported by Ajayi and Bradley (2014). Other triazoles, including cyproconazole (Kataria et al. 1991), ipconazole (Ajayi and Bradley 2014), triadimefon (Martin et al. 1984), and hexaconazole (Carling et al. 1990), were also effective at reducing mycelial radial growth of *R. solani*. It was not surprising that there was no increase in prothioconazole mean EC₅₀ value because this fungicide became available in 2006 and it was not widely used for control of *R. solani* in sugar beet (Carlson et al. 2013). In this study, penthiopyrad showed a low mean EC₅₀ value for *R. solani* isolates collected prior to 1999 as well as isolates collected from 2005 to 2012 (Table 1), most likely because they were never exposed to the fungicide in the field since penthiopyrad was first used, in the form of a seed treatment, in 2014. Similarly, other baseline studies showed that SDHI, including thifluzamide, boscalid (Chen et al. 2012), and fluxapyroxad (Chen et al. 2014), had low EC₅₀ values for *R. solani*.

In the in vitro study, *R. solani* AG-2-2 isolates showed a shift in sensitivity to the tested QoI fungicides. The resistant factor was 6.0, 3.5, and 3 for azoxystrobin, trifloxystrobin, and pyraclostrobin, respectively. Azoxystrobin, since becoming labeled in 1999, was recommended and was most commonly used for control of *R. solani* in the United States, including North Dakota and Minnesota (Khan 2000). Pyraclostrobin was labeled in 2002, became available in 2003, and, since then, was the most used fungicide (on 60 to 90% of planted acreage) for control of *Cercospora beticola* in Minnesota and North Dakota (Carlson et al. 2013). Around 2009, growers in this bistate area started using pyraclostrobin as an in-furrow application for the control of *Rhizoctonia* damping-off and root rot; however, it was not as widely used as azoxystrobin (Carlson et al. 2013). Trifloxystrobin was labeled for use on sugar beet in 2002 and used for control of *C. beticola* but in a relatively small area (1 to 10% of the sugar beet acreage) in southern Minnesota (Carlson et al. 2013). Growers have not listed trifloxystrobin (Gem) as a product they have used for control of *R. solani* in sugar beet (Carlson et al. 2013). The decrease in sensitivity of *R. solani* to azoxystrobin was probably due to its prolonged exposure to this fungicide with little or no rotation with other fungicide groups (Carlson et al. 2013); thus, high selection pressure on *R. solani* isolates could have occurred. It should be noted that one of the authors who works directly with growers and agriculturists in Minnesota and North Dakota did not observe or receive any report of a field failure of azoxystrobin for control of *R. solani* in sugar beet. It is also possible that resistance to QoI can occur over a short period of time (Pasche et al. 2004). Similarly, the increased usage of pyraclostrobin for control of *R. solani* could be driving the selection pressure for isolates with reduced sensitivity to pyraclostrobin. Because trifloxystrobin was not used as the other QoI for controlling *R. solani* (Carlson et al. 2013), the increase in its mean EC₅₀ value could be due to its positive cross resistance with azoxystrobin, as was reported in this study and another study by Pasche et al. (2004).

In the in vivo study, AG-2-2 isolates with low and high EC₅₀ values were controlled effectively by all tested fungicides at one of the recommended application rates (azoxystrobin at 672 ml ha⁻¹, trifloxystrobin at 256 ml ha⁻¹, pyraclostrobin at 672 ml ha⁻¹, penthiopyrad at 2,192 ml ha⁻¹, and prothioconazole at 417 ml ha⁻¹). Similarly, LaMondia (2012) reported that *R. solani* isolates with a high EC₅₀ value for azoxystrobin could be easily controlled with the same fungicide under greenhouse conditions.

In conclusion, *R. solani* isolates shifted to reduced sensitivity (high EC₅₀ values) for QoI fungicides. Although *R. solani* isolates had high EC₅₀ values, they were still controlled by tested fungicides at labeled rates in vivo. It is possible that, with continued use of QoI fungicides,

selection pressure for isolates with high EC₅₀ values may result in populations of the pathogen which might not be controlled in the field, as occurred in rice fields in Louisiana (Olaya et al. 2012). In areas where *R. solani* isolates had high EC₅₀ values to QoI fungicides, it will be necessary to develop, implement, and evaluate different management strategies that will provide effective disease control and manage fungicide resistance.

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