Sensitivity of *Rhizoctonia solani* to Succinate Dehydrogenase Inhibitor and Demethylation Inhibitor Fungicides

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Abstract

Soybean seedling diseases are caused by *Rhizoctonia solani* and can be managed with seed-applied fungicides that belong to different chemistry classes. To provide a benchmark for assessing a decline in sensitivities to these fungicide classes, *R. solani* isolates collected prior to 2001 were evaluated for their sensitivities to succinate dehydrogenase inhibitor (SDHI) (penflufen and sedaxane) and demethylation inhibitor (DMI) fungicides (ipconazole and prothioconazole). The effective concentration of each fungicide that reduced mycelial growth by 50% (EC₅₀) was determined in vitro and compared with those of isolates recovered after 2011 from soybean plants with damping off and hypocotyl and root rot symptoms across different soybean-growing regions in the United States and Canada. All isolates, regardless of collection date, were extremely sensitive (EC₅₀ < 1 µg/ml) to the SDHI fungicides but were either extremely sensitive or moderately sensitive (1 ≤ EC₅₀ ≤ 10 µg/ml) to

the DMI fungicides. For all four active ingredients, variation in sensitivities was observed within and among the different anastomosis groups composing both isolate groups. Isolates collected after 2011, which also had varying in vitro sensitivities, were further evaluated for in vivo sensitivity to the four fungicides in the greenhouse. In vitro fungicide sensitivity did not always coincide with fungicide efficacy in vivo because all isolates tested, regardless of in vitro sensitivity, were effectively controlled by the application of the seed treatment fungicides in the greenhouse. Overall, our results indicate no shift in sensitivity to the fungicide classes evaluated, although considerable variability in the sensitivities of the two groups of isolates examined was present. Based on this research, continued monitoring of fungicide sensitivities of *R. solani* populations should occur to determine whether sensitivities become further reduced in the future.

Rhizoctonia solani (syn. *Thanatephorus cucumeris*), a ubiquitous necrotrophic fungus, causes economically important diseases of soybean worldwide. In the north-central United States, seedling diseases caused by *R. solani*, including seed decay, pre- and postemergence damping off, hypocotyl rot, and root rot, can suppress yield (Doupnik 1993), and losses of up to 48% have been reported on small research plots in Iowa (Tachibana 1968). Although considered to be members of the same species, isolates of *R. solani* exhibit marked genetic diversity, leading to their classification into anastomosis groups (AG) and subgroups within certain AG. To date, 14 AG (AG 1 to 13 and AG-B1) have been identified (Carling 1996; Carling et al. 1999; Ogoshi 1987). Five AG have been associated with soybean seedling disease: AG-2-2IIIB (Chela Fenille et al. 2002; Liu and Sinclair, 1991; Muyolo et al. 1993), AG-3 (Nelson et al. 1996), AG-4 (Bolkan and Ribeiro 1985; Chela Fenille et al. 2002; Muyolo et al. 1993; Nelson et al. 1996; Ploetz et al. 1985; Rizvi and Yang 1996; Zhao et al. 2005), AG-5 (Nelson et al. 1996), AG-7 (Baird et al. 1996), and AG-11 (Carling et al. 1994). Isolates of AG-2-2IIIB are highly aggressive on soybean roots (Muyolo et al. 1993; Nelson et al. 1996) and are generally more aggressive than the hypocotyl-infecting isolates of AG-4 (Chela Fenille et al. 2002; Muyolo et al. 1993; Zhao et al. 2005). AG-5 isolates have been reported to be pathogenic on soybean seedlings (Nelson et al. 1996; Zhao et al. 2005) and adult plants (Nelson et al. 1996), while isolates of AG-3 (Nelson et al. 1996), AG-7 (Baird et al. 1996), and AG-11 (Carling et al. 1994) are mildly aggressive on soybean seedlings.

With the unavailability of commercial soybean cultivars marketed for resistance (Bradley et al. 2001), soybean growers in the north-central

United States rely mostly on seed treatment fungicides to protect soybean seedlings against *R. solani* infections. Two of the seed treatment fungicides commonly used to manage seedling diseases of soybean caused by *R. solani* belong to two different fungicide chemistry classes: demethylation inhibitors (DMI) and succinate dehydrogenase inhibitors (SDHI). The DMI inhibit fungi by targeting C14-demethylase, an important enzyme required for the biosynthesis of sterols (Koller 1988; Ragsdale 1975). DMI are effective against a wide range of fungi and offer preventive, curative, and eradication functions (Kuck et al. 2012; Mueller et al. 2013). SDHI inhibit respiration in target organisms by altering the activity of the succinate dehydrogenase complex, which is part of the respiratory chain involved in ATP generation (Avenot and Michailides 2010; Yankovskaya et al. 2003). Although earlier generations of the SDHI such as carboxin provided excellent protection against basidiomycetes (Hewitt 1998), newer members of this class possess a broad spectrum of activity against a wide range of fungal pathogens (Sierotzki and Scalliet 2013) and are thereby becoming more important as a disease control option in the crop protection industry. Members of these two fungicide chemistries exhibit specificity in their mode of action and have the potential to select for resistant genotypes, and many fungal pathogens (e.g., *Alternaria alternata*, *Puccinia striiformis*, *Blumeria graminis*, *Cercospora beticola*, *Ustilago maydis*, *Mycosphaerella graminicola*, and *Ucinula necator*) have been reported to show reduced sensitivities to active ingredients in both fungicide classes under field and laboratory conditions (Avenot and Michailides 2007; Bayles et al. 2000; de Waard et al. 1986; Karaoglanidis et al. 2000; Keon et al. 1991; Mavroeidi and Shaw 2005; Miller and Gubler 2003).

Penflufen and sedaxane, which belong to the SDHI fungicide class, and ipconazole and prothioconazole, which belong to the DMI group, are four important active ingredients of seed-treatment fungicides used for controlling seedling diseases of soybean caused by *R. solani*. Penflufen and sedaxane became available to the U.S. soybean industry in 2012. Ipconazole received approval as a soybean seed treatment from the U.S. Environmental Protection Agency in 2008, while prothioconazole became available as a seed treatment in 2013. Generally, seed treatment fungicides marketed for managing soybean seedling diseases, including those caused by *R. solani*, are either formulated as mixtures of different active ingredients with the potential to control a broad range of seedling pathogens, or sold

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as solo products with specificity for a particular pathogen. Despite the potential risk for resistance development with the single-site-inhibitor individual products, there are currently no reports documenting the sensitivity of soybean-infecting isolates of *R. solani* to these active ingredients in historical and recently collected isolates. In addition to the fungicide mode of action, genetic variation within the pathogen population is another factor that can generally increase the risk of fungicide resistance. *R. solani* is considered as a primarily asexual organism (Adams 1996) and fungicide resistance is not expected to be a problem in this species. However, population genetics studies of *R. solani* have revealed high genotypic diversity among populations of AG-1A (Bernardes de Assis et al. 2008, 2009), AG-2-IIIB (Taheri and Tarighi 2012; Zheng et al. 2013), AG-3 (Ferrucho et al. 2013), and AG-4 (Haratian et al. 2013). These findings, together with the observation of the sexual stage of certain AG (including AG-1, AG-2-2, AG-3, and AG-4) in agricultural fields (Cubeta and Vilgalys 1997; Olaya and Abawi 1994; Toda and Hyakumachi 2006), suggest a role for sexual reproduction as a source of genetic variation in *R. solani* and the potential for fungicide resistance development. In fact, resistance to the quinone outside inhibitors, another single-site inhibitor, has been reported in AG-1 populations of *R. solani* causing foliar diseases of rice and soybean (Olaya et al. 2012). Although resistance to the SDHI and DMI classes of fungicides has not been reported for *R. solani*, the Fungicide Resistance Action Committee (2016) reports a medium to high resistance risk for SDHI and a medium resistance risk for DMI. Therefore, to guard against resistance development in the populations of *R. solani* treated with the currently used SDHI and DMI seed treatments, it is pertinent that the sensitivities of historical isolates be compared with sensitivities of recently collected isolates. This would establish acceptable levels of fungicide sensitivities which can then be used in subsequent monitoring programs to determine possible shifts in the sensitivity of *R. solani* populations. The objectives of our research were to (i) establish premarket introduction profiles of two different mode-of-action chemistries in *R. solani* populations; (ii) identify possible shifts in sensitivity to each fungicide class; and (iii) determine, using *in vivo* assays, the effectiveness of the selected fungicides in managing isolates showing a range of *in vitro* sensitivities.

Materials and Methods

Collection, isolation, and storage of *R. solani* isolates. Historical isolates of *R. solani* evaluated were recovered from diseased soybean and sugar beet plants between 1986 and 2000. Two isolates from Illinois (Liu and Sinclair 1991) were included as part of the historical group. Other isolates included those recovered from Ohio (Dorrance et al. 2003), which were obtained from Dr. Anne Dorrance at the Ohio State University (Wooster), and those recovered from the Red River Valley in Minnesota (MN) and North Dakota (ND), which were obtained from Dr. Carol Windels at the University of Minnesota (Crookston) (Liu and Sinclair 1992). Because of the soybean-sugar beet (*Beta vulgaris*) rotation system in the Red River Valley of Minnesota and North Dakota, and the observation that sugar beet is a host to the AG and subgroups of *R. solani* that also infect soybean, isolates recovered from sugar beet were included for evaluation in this study. This group of historical isolates represented two important AG (Table 1) previously reported to be highly aggressive on soybean (Liu and Sinclair 1991; Muyolo et al. 1993; Nelson et al. 1996).

To assess the current sensitivities of *R. solani* populations to fungicides, isolates recovered after 2011, when most of the active ingredients had not been introduced to the market, were evaluated. The isolates were collected from Illinois, Arkansas, and Ontario, Canada (Table 1). Isolates from Arkansas and Ontario were originally collected by Dr. John Rupe (University of Arkansas, Fayetteville) and Mr. Albert Tenuta (Ontario Ministry of Agriculture and Food), respectively. These isolates are curated in Dr. Ahmad Fakhoury's laboratory (Southern Illinois University, Carbondale). Isolates from Illinois were collected from soybean seedlings with sunken reddish-brown lesions on hypocotyls, root rot, or damping-off symptoms. These isolates were recovered from eight counties between 2012 and 2014. Roots of all seedlings were washed under running tap water to remove soil particles, disinfected with 1% NaOCl, and rinsed in sterile

distilled water. Cut sections from lesions of symptomatic plants were placed on Ko and Hora medium (Ko and Hora 1971) and incubated at room temperature for 24 to 48 h. Hyphal tips from colonies resembling *Rhizoctonia* were transferred to potato dextrose agar (PDA) amended with rifampicin to minimize bacterial contamination. All isolates were identified based on cellular nuclear number using a safranin O staining technique (Matsumoto et al. 1932), cultural and morphological characteristics, hyphal anastomosis reactions (Kronland and Stanghellini 1988), and sequence comparison of the ribosomal DNA internal transcribed spacer (ITS) regions with the ITS4 and ITS5 primer (White et al. 1990). Only isolates determined to be multinucleate and identified as *R. solani* were selected for assessments. All isolates collected after 2011 were identified to five AG (Table 1).

Plugs (5 mm in diameter) of selected isolates on PDA were placed in 1.5-ml microcentrifuge tubes containing 850 μ l of 15% glycerol and stored at -80°C for long-term storage. Hyphal tips from cultures were transferred to new antibiotic-amended PDA plates containing sterile table beet seed and incubated 10 days at 25°C . Colonized beet seed were dried under a sterile flow hood for 48 h, placed in 2.0-ml Nalgene cryogenic vials (Sigma-Aldrich, St. Louis), and stored at 4°C . New cultures from 4°C storage were used for all experiments to avoid using genetically unstable isolates that might have arisen due to repeated subculturing (Sneh and Adams 1996).

***In vitro* sensitivity assay.** In total, 40 pre-2001 isolates and 80 isolates collected after 2011 were evaluated for their sensitivity to four active ingredients belonging to two different fungicide classes: technical-grade formulations of penflufen (98.72% active ingredient [a.i.]) and sedaxane (95% a.i.) for the SDHI, and technical-grade formulations of prothioconazole (97.7% a.i.) and ipconazole (40.7% a.i.) for the DMI. The effective concentration that resulted in 50% mycelial growth inhibition (EC_{50}) values were determined using a modified version of the mycelial growth assays described by Martin et al. (1984b) and Carling et al. (1990). A pilot study, using 10 randomly selected isolates from both groups of isolates, was initially conducted to determine the fungicide concentrations required for each fungicide class (data not shown). Appropriate volumes, based on percent active ingredients, of each fungicide were dissolved in acetone to obtain stock solutions, and serial dilutions were carried out to obtain the following concentrations for the two chemistry classes: 0.001, 0.01, 0.1, and 1 $\mu\text{g/ml}$ for the SDHI and 0.01, 0.1, 1, and 10 $\mu\text{g/ml}$ for the DMI.

Using a sterile cork borer, 5-mm-diameter plugs were excised from the edge of 4-day-old cultures on rifampicin-amended PDA, inverted, and transferred to the center of 100-by-15-mm petri dishes containing fungicide-amended media. There were two replicate plates for each isolate-fungicide concentration combination, and inoculated fungicide-amended plates were incubated at 25°C in continuous light. Nonamended PDA plates served as controls. At 48 h after inoculation, mycelial diameter at opposite axes was measured, and the average diameter was calculated. Diameter of the agar plug was subtracted from the average mycelial diameter, and the percent growth inhibition for each isolate-fungicide combination was determined using the formula $[(\text{diameter of control} - \text{diameter of fungicide-amended plate}) / (\text{diameter of control})] \times 100\%$ (Amaradasa et al. 2014). The EC_{50} for each isolate was calculated using linear interpolation (Wise et al. 2008).

Due to the large size of the experiment and space constraints, sensitivity of all isolates to all four fungicides could not be tested in a single trial; therefore, for each individual fungicide assayed, isolates were evaluated in batches or trials, and each batch comprised 10 isolates. To minimize variability across batches, an internal check, isolate 65L-2, was assayed for its sensitivity to each of the four active ingredients in 10 separate pilot trials, and the mean EC_{50} value and 95% confidence interval was determined for all 10 trials (Wong and Wilcox 2002). Only main experimental trials in which the EC_{50} value of the internal check fell within the 95% confidence interval obtained from the pilot trials were considered valid and were included for statistical analysis. The experiment was arranged as a randomized complete block design and repeated in a second run, with each run serving as a block. Each experiment comprised all isolates,

which were evaluated in a total of 12 batches. EC₅₀ data were log-transformed to ensure normal distribution and homogeneity of residuals before analysis in SAS (version 9.4; SAS institute Inc., Cary, NC). Data were analyzed using the mixed-model procedure (PROC

MIXED), with trial and AG type as fixed factors and isolate nested within AG as a random factor. Mean differences among AG were determined using the PDMIX800 macro (Saxton 1998) in SAS (version 9.4). Using the Pearson correlation procedure (PROC CORR) in SAS

Table 1. Origin and number of isolates within each anastomosis group (AG) of *Rhizoctonia solani* evaluated for sensitivity to penflufen, sedaxane, ipconazole, and prothioconazole

Isolate ID	Isolate group	Origin	AG
65L-2	Pre-2000	Illinois	AG-2-IIIB
RS 1018 ^z	Pre-2000	Illinois	AG-2-IIIB
PL_1 AD	Pre-2000	Ohio	AG-2-IIIB
NT_0 AD	Pre-2000	Ohio	AG-2-IIIB
NT_12 AD	Pre-2000	Ohio	AG-2-IIIB
NT_30 AD	Pre-2000	Ohio	AG-2-IIIB
NT_9 AD	Pre-2000	Ohio	AG-2-IIIB
NT_15	Pre-2000	Ohio	AG-2-IIIB
NT_11	Pre-2000	Ohio	AG-2-IIIB
NT_13 AD	Pre-2000	Ohio	AG-2-IIIB
NT_14 AD	Pre-2000	Ohio	AG-2-IIIB
NT_39 AD	Pre-2000	Ohio	AG-2-IIIB
NT_18 AD	Pre-2000	Ohio	AG-2-IIIB
91_1 AD	Pre-2000	Ohio	AG-2-IIIB
90_3_22	1990	Clay County, MN	AG-4
18_s_21	1988	Wilken County, MN	AG-2-2
95_16_2	1995	ND	AG-2-2
18_s_22	1988	MN	AG-2-2
95_3_6	1995	Polk County, MN	AG-4
87_4_70	1987	MN	AG-2-2
95_5_19	1995	Polk County, MN	AG-4
86_71_6	1986	Marshall County, MN	AG-2-2
88_11_3	1988	Pembina County, ND	AG-4
18_S_26B	1988	MN	AG-2-2
87_24_4A	1987	MN	AG-2-2
86_72_7	1986	Polk County, MN	AG-2-2
86_34_8	1986	Traill County, ND	AG-4
87_18_2	1987	Walsh County, ND	AG-4
95_16_6A	1995	Pembina County, ND	AG-4
95_8_12	1995	Pembina County, ND	AG-4
87_12_23	1987	Pembina County, ND	AG-4
88_12_2	1988	Polk County, MN	AG-4
7_S_44	1988	Wilken County, MN	AG-2-2
86_49_11	1986	MN	AG-2-2
18_T_9	1988	Polk County, MN	AG-2-2
93_29_6	1993	MN	AG-2-2
ST_10	1988	Polk County, MN	AG-4
87_36_4	1987	ND	AG-2-2
E_O_20	1987	Roseau County, MN	AG-4
95_4_19	1995	Polk County, MN	AG-4
K_ARSO2_1_20	2013	Arkansas	AG-11
K_ARSO2_2_5	2013	Arkansas	AG-7
K_ARSO2_1_7	2013	Arkansas	AG-7
K_ARSO2_1_6	2013	Arkansas	AG-7
K_ARSO2_1_9	2013	Arkansas	AG-7
K_ARSO2_1_8	2013	Arkansas	AG-7
BVT_18	2013	St. Clair County, IL	AG-11
S_P_19b	2013	Pike County, IL	AG-11
S_P_19a	2013	Pike County, IL	AG-11
K_4_18b	2013	St. Clair County, IL	AG-11
X12SDSa	2013	Illinois	AG-11
12RS52	2012	Champaign County, IL	AG-11
ER_4	2013	Whiteside County, IL	AG-2-IIIB
ER_19b	2013	Whiteside County, IL	AG-2-IIIB
ER_15	2013	Whiteside County, IL	AG-2-IIIB
ER_19a	2013	Whiteside County, IL	AG-2-IIIB
DK_8	2013	DeKalb County, IL	AG-2-IIIB

(continued in next column)

^z Isolate was collected by Dr. Sally Miller (Ohio State University) but curated in Dr. Darin Eastburn's laboratory (University of Illinois, Urbana-Champaign).

Table 1. (continued from preceding column)

Isolate ID	Isolate group	Origin	AG
DK_19	2013	DeKalb County, IL	AG-2-IIIB
DK_15a	2013	DeKalb County, IL	AG-2-IIIB
DK_13	2013	DeKalb County, IL	AG-2-IIIB
K_ILSO2_3_25c	2013	Warren County, IL	AG-2-IIIB
X42210_b	2013	Illinois	AG-2-IIIB
DK_6a	2013	DeKalb County, IL	AG-2-IIIB
DK_4a	2013	DeKalb County, IL	AG-2-IIIB
DK_16	2013	DeKalb County, IL	AG-2-IIIB
DK_3b	2013	DeKalb County, IL	AG-2-IIIB
42304h	2013	Illinois	AG-2-IIIB
DK_14	2013	DeKalb County, IL	AG-2-IIIB
DK_10	2013	DeKalb County, IL	AG-2-IIIB
DK_7	2013	DeKalb County, IL	AG-2-IIIB
DK_11	2013	DeKalb County, IL	AG-2-IIIB
DK_3a	2013	DeKalb County, IL	AG-2-IIIB
DK_15b	2013	DeKalb County, IL	AG-2-IIIB
DK_4b	2013	DeKalb County, IL	AG-2-IIIB
DK_6b	2013	DeKalb County, IL	AG-2-IIIB
K_ILSO2_3_25a	2013	Warren County, IL	AG-2-IIIB
X42210_c	2013	Illinois	AG-2-IIIB
K_ILSO2_2_13c	2013	Warren County, IL	AG-2-IIIB
211_1 PDA	2012	Champaign County, IL	AG-2-IIIB
12RS41	2012	Williamson County, IL	AG-2-IIIB
211_1a KH	2012	Champaign County, IL	AG-2-IIIB
211_1b KH	2012	Champaign County, IL	AG-2-IIIB
IL 2014a	2014	Champaign County, IL	AG-2-IIIB
IL 2014b	2014	Champaign County, IL	AG-2-IIIB
X248_3b KH	2012	Jackson County, IL	AG-3
C_far_500_3	2013	Champaign County, IL	AG-3
C_far_500_10a	2013	Champaign County, IL	AG-3
C_far_500_6	2013	Champaign County, IL	AG-3
EV_3	2013	Jackson County, IL	AG-4
BVT_28	2013	St. Clair County, IL	AG-4
EV_6	2013	Jackson County, IL	AG-4
BVT_3	2013	St. Clair County, IL	AG-4
BVT_11	2013	St. Clair County, IL	AG-4
BVT_16	2013	St. Clair County, IL	AG-7
EV_19	2013	Jackson County, IL	AG-7
EV_7	2013	Jackson County, IL	AG-7
BVT_20	2013	St. Clair County, IL	AG-7
WONS 13_8_7	2013	Ontario	AG-2-IIIB
WONS 13_8_8	2013	Ontario	AG-2-IIIB
WONS 13_8_6	2013	Ontario	AG-2-IIIB
PDONS 13_8_6	2013	Ontario	AG-2-IIIB
PDONS 13_8_3	2013	Ontario	AG-2-IIIB
WONS 13_8_5	2013	Ontario	AG-2-IIIB
PDONS 13_12_6	2013	Ontario	AG-2-IIIB
PDONS 13_8_5	2013	Ontario	AG-2-IIIB
WONS 13_12_3	2013	Ontario	AG-2-IIIB
PDONS 13_8_4	2013	Ontario	AG-2-IIIB
WONS 13_8_3	2013	Ontario	AG-2-IIIB
PDONS 13_8_8	2013	Ontario	AG-2-IIIB
WONS 13_8_1	2013	Ontario	AG-2-IIIB
PDONS 13_8_7	2013	Ontario	AG-2-IIIB
WONS 13_12_1	2013	Ontario	AG-2-IIIB
WONS 13_8_2	2013	Ontario	AG-2-IIIB
WONS 13_8_4	2013	Ontario	AG-2-IIIB
ONSO2_14	2012	Ontario	AG-2-IIIB
ONSO2_18	2012	Ontario	AG-2-IIIB
ONSO2_15	2012	Ontario	AG-2-IIIB
ONSO2_13	2012	Ontario	AG-2-IIIB
ONSO2_16	2012	Ontario	AG-2-IIIB
ONSO2_17	2012	Ontario	AG-2-IIIB

(version 9.4), associations between EC_{50} values of active ingredients belonging to the same chemistry class were determined. For the AG-2-2 and AG-4 isolates, combined EC_{50} values obtained from the pre-2001 isolates and isolates collected after 2011 were further analyzed to determine significant differences in mean EC_{50} values within each AG. To compare degree of sensitivity among isolates, the following in vitro sensitivity (Martin et al. 1984b) scale was adopted: extremely sensitive ($EC_{50} < 1 \mu\text{g/ml}$), moderately sensitive ($1 \leq EC_{50} \leq 10 \mu\text{g/ml}$), and tolerant ($EC_{50} > 10 \mu\text{g/ml}$).

In vivo sensitivity assay. Four and six isolates, selected on the basis of their in vitro sensitivity levels to the SDHI and DMI fungicides, respectively (Table 2), were evaluated in the greenhouse to determine the effectiveness of these fungicides in protecting soybean stands and controlling root and hypocotyl rot of soybean. Inocula for all isolates were prepared as described by Paulitz and Schroeder (2005), with slight modifications. Briefly, 250 ml of sorghum seed were soaked in 250 ml of distilled water in 500-ml flasks and autoclaved for 30 min at 137.9 kPa and 120°C. After 24 h, a second autoclaving was carried out, after which flasks were allowed to cool. Beet seed colonized with the isolates were recovered from storage at 4°C and maintained on PDA for 4 days. Inoculation of autoclaved sorghum seed was carried out by transferring five 15-mm-diameter agar plugs from the 4-day-old PDA culture of each isolate into separate flasks containing autoclaved sorghum seed. Inoculated sorghum seed were incubated at room temperature and shaken every other day to ensure uniform colonization of seed. After 2 weeks of incubation, inoculated sorghum seed were dried in a laminar flow hood for 3 days, after which they were stored in paper bags and maintained at 4°C before use.

Seed of 'Williams 82' soybean were treated with slurries of four fungicide seed treatments: penflufen at 2.13 g a.i. per 100 kg of seed (Evergol Prime; Bayer CropScience, Research Triangle Park, NC), sedaxane at 1.09 g a.i. per 100 kg of seed (Vibrance; Syngenta Crop Protection, Greensboro, NC), ipconazole at 1.14 g a.i. per 100 kg of seed (Vortex; Bayer CropScience), and prothioconazole (Proline; Bayer CropScience). Plastic pots (12.7 cm in diameter) were half filled with a layer of steam-pasteurized 2:1 sand/silt loam soil, covered with 2 g of inoculum, and then covered with an approximately 5-cm layer of soil before planting; each pot received 10 seeds. Two separate experiments were conducted for the SDHI and DMI fungicides. The experiment was set up as a randomized complete block in a split-plot arrangement with four replications. Isolate served as the whole plot and fungicide seed treatment as the subplot. Fungicide seed treatment consisted of two active ingredients and a nontreated but inoculated control for both SDHI and DMI fungicide class. The experiment, which was conducted in the greenhouse under a 14-h photoperiod and $24 \pm 3^\circ\text{C}$, was repeated in a second trial. At 18 days after planting, plants were evaluated for stand, root, and hypocotyl disease severity using a 0-to-5 scale modified from Nelson et al.

(1996), as follows: 0 = no lesion on root or hypocotyl; 1 = lesions < 2.5 mm on hypocotyl and $\leq 20\%$ of roots with lesions or rot symptoms; 2 = lesions 2.5 to 5 mm on hypocotyl and 20 to 40% of roots with lesions or rot symptoms; 3 = lesions > 5 mm on hypocotyl and 40 to 60% of roots with lesions or rot symptoms; 4 = lesions girdling entire hypocotyl and 60 to 80% of roots with lesions or rot symptoms; and 5 = plant dead, or no roots, or $> 80\%$ of roots with lesions or rot symptoms. The disease severity rating for each subplot unit was converted to a disease severity index (DSI) using the formula $DSI = \frac{\sum_{i=1}^s s_i p_i}{5 \times N}$, where s is the disease score for each plant, p is the number of plants with disease score, and N is the total number of plants in each subplot unit. Because we observed that, for both fungicide classes, all isolates could not be grouped into the three sensitivity categories adopted for the in vitro assay, we selected isolates that fell on the low and high spectrum of the sensitivity range obtained for each fungicide class (Table 2). For statistical analysis, tests for normality and homogeneity of residuals were conducted, and box cox transformations (Box and Cox 1964) were carried out as follows: DSI was transformed to $DSI' = DSI^{-1}$ and stand count (SC) data were transformed to $SC' = SC^3$ for both SDHI and DMI data were transformed to $SC' = SC^2$ for the DMIs and $SC' = SC^3$ for the SDHIs. Statistical analysis was carried using PROC MIXED in SAS (version 9.4). To determine whether trials could be pooled, a test of homogeneity of variance of both trials was conducted, and when heterogeneity was not significant, data were pooled. Comparison of least-square means was conducted with Fisher's protected least significant difference test at $\alpha = 0.05$, using the PDMIX800 macro (Saxton 1998) in SAS (version 9.4). Mean separations and analysis of variance (ANOVA) presented are from the analysis of transformed data.

Results

In vitro sensitivity of pre-2001 isolates. The EC_{50} values obtained with the DMI were found to be consistently higher than those of the SDHI. For example, the respective minimum and maximum EC_{50} values obtained for the SDHI fungicides were 0.007 and 0.155 $\mu\text{g/ml}$ for penflufen, and 0.026 and 0.215 $\mu\text{g/ml}$ for sedaxane (Fig. 1). In contrast, for the DMI, 0.349 and 3.162 $\mu\text{g/ml}$ were the minimum and maximum mean EC_{50} values for ipconazole, respectively, while prothioconazole had minimum and maximum values of 0.200 and 3.665 $\mu\text{g/ml}$, respectively (Fig. 2).

SDHI. For penflufen and sedaxane, the ANOVA for the EC_{50} produced a significant effect of AG ($P < 0.0001$). However, trial and the interaction between trial and AG were not significant (Table 3). For penflufen, the 27 AG-2-2 isolates had a significantly higher mean EC_{50} of 0.056 $\mu\text{g/ml}$, with a range of 0.016 to 0.155 $\mu\text{g/ml}$. EC_{50} values for the 13 AG-4 isolates were 0.007 to 0.035 $\mu\text{g/ml}$, with a mean of 0.017 $\mu\text{g/ml}$. For sedaxane, the EC_{50} values of the AG-2-2 isolates were 0.029 to 0.215 $\mu\text{g/ml}$, with a mean of 0.089 $\mu\text{g/ml}$, while the

Table 2. List of isolates evaluated in the greenhouse assay for sensitivity to the succinate dehydrogenase inhibitor (SDHI) and demethylation inhibitor (DMI) fungicides

Isolate number	Isolate ID	EC_{50} ($\mu\text{g/ml}$) ^a	Active ingredient
SDHI			
1	BVT-3	0.014	Penflufen
2	ER-15	0.081/0.184	Penflufen/sedaxane
3	K_ILSO2_2_13c	0.244/0.251	Penflufen/sedaxane
4	BVT-18	0.035	Sedaxane
DMI			
5	EV-6	0.078	Ipconazole
6	X42210_b	0.990	Ipconazole
7	DK-6	2.414	Ipconazole
8	BVT-28	0.239	Prothioconazole
9	DK-15a	1.514	Prothioconazole
10	DK-10	4.390	Prothioconazole

^a EC_{50} = effective concentration that reduced mycelial growth by 50%. Because isolates could not be grouped into all four in vitro sensitivity categories, isolates in the low and high spectrum of sensitivity for each fungicide class were selected for the in vivo study.

Fig. 1. Overall distribution of effective concentration that reduced mycelial growth by 50% (EC_{50}) values of the pre-2001 and post-2011 *Rhizoctonia solani* isolates for the succinate dehydrogenase inhibitor fungicides. Dashed lines represent mean EC_{50} value for each fungicide and solid lines represent median of distribution.

AG-4 isolates had a significantly lower mean EC₅₀ of 0.051 µg/ml, with EC₅₀ values of 0.026 to 0.119 µg/ml (Table 4). Based on the range of EC₅₀ values obtained, all isolates were extremely sensitive (EC₅₀ < 1 µg/ml) to penflufen and sedaxane. A significant ($P < 0.0001$) positive correlation ($r = 0.38$) was observed between the sensitivity to penflufen and sedaxane.

DMI. For ipconazole, there were significant ($P < 0.0001$) differences between the mean EC₅₀ values obtained for the AG-2-2 and AG-4 isolates (Table 4). Isolates of AG-2-2 resulted in a significantly lower mean EC₅₀ value of 1.338 µg/ml compared with the AG-4 isolates, which had a mean EC₅₀ value of 1.894 µg/ml (Table 4). For

prothioconazole, main effects of AG, trial, and trial–isolate interactions were not significant (Table 3). The mean EC₅₀ value for the AG-2-2 isolates was 1.497 µg/ml, while that of the AG-4 isolates was 1.809 µg/ml (Table 4). A significant ($P < 0.0001$) positive correlation ($r = 0.50$) was observed between the sensitivity to ipconazole and prothioconazole.

In vitro sensitivity of the post-2011 isolates. The EC₅₀ values obtained with the SDHI were consistently lower than those of the DMI. For the SDHI, the minimum and maximum EC₅₀ values for penflufen were 0.014 and 0.244 µg/ml, respectively, while those for Sedaxane were 0.022 and 0.282 µg/ml (Fig. 1). For the DMI, the minimum and maximum EC₅₀ values for ipconazole were 0.078 and 3.434 µg/ml, respectively, while those for prothioconazole were 0.239 and 6.802 µg/ml (Fig. 2).

SDHI. For both penflufen and sedaxane, there were significant differences among AG; however, the effect of trial and the interactions between trial and AG were not significant (Table 3). For penflufen, the AG-2-2 isolates not only had the widest range of EC₅₀ values but also had the highest mean EC₅₀ value of 0.054 µg/ml, although this was not significantly different from the means of the AG-3 (0.041 µg/ml), AG-7 (0.047 µg/ml), and AG-11 (0.040 µg/ml) isolates (Table 4). For sedaxane, the AG-7 isolates had the highest mean EC₅₀ value of 0.162 µg/ml, which was significantly higher than those of the other four AG. A wide range of EC₅₀ values were observed for the AG-2-2, AG-7, and AG-11 isolates but not for the AG-3 or AG-4 isolates (Table 4). A significant ($P < 0.0001$) positive correlation ($r = 0.40$) between the sensitivities to penflufen and sedaxane was observed.

DMI. For the pre-2001 isolates, a significant AG effect and a non-significant effect of trial and trial–AG interaction was observed for ipconazole and prothioconazole (Table 3). For ipconazole, the mean EC₅₀ value obtained for the AG-11 isolates (1.797 µg/ml) was the highest, followed by isolates of AG-2-2 (1.143 µg/ml), AG-7 (0.495 µg/ml), AG-4 (0.298 µg/ml), and AG-3 (0.199 µg/ml). The EC₅₀ values of the AG-2-2 and AG-11 isolates were highly variable, as follows: 0.148 to 3.434 µg/ml for the AG-2-2 isolates and 0.913 to

Fig. 2. Overall distribution of effective concentration that reduced mycelial growth by 50% (EC₅₀) values of the pre-2001 and post-2011 *Rhizoctonia solani* isolates for the demethylation inhibitor (DMI) fungicides. Dashed lines represent mean EC₅₀ value for each fungicide and solid lines represent median of distribution.

Table 3. Analysis of variance for effective concentration that reduced mycelial growth by 50% for the pre-2001 and post-2011 isolates of *Rhizoctonia solani* to penflufen, sedaxane, ipconazole, and prothioconazole

Source ^z	<i>P</i> values ^y									
	Pre-2001 (<i>n</i> = 40)					Post-2011 (<i>n</i> = 80)				
	df	Pen	Sed	Ipcon	Proth	df	Pen	Sed	Ipcon	Proth
AG	1	<0.0001	<0.0001	<0.0001	0.4716	4	0.016	<0.0001	<0.0001	0.0001
Trial	1	0.9651	0.6669	0.3401	0.4587	1	0.265	0.242	0.103	0.192
Trial × AG	1	0.176	0.613	0.6011	0.611	4	0.862	0.181	0.639	0.072
Isolate (AG)	38	<0.0001	<0.0001	<0.0001	<0.0001	75	<0.0001	<0.0001	<0.0001	<0.0001
Residual	118	...	...	...	...	235	...	...	...	...

^y Abbreviations: penflufen (Pen), sedaxane (Sed), ipconazole (Ipcon), and prothioconazole (Proth).

^z AG = anastomosis groups.

Table 4. Mean and range of effective concentration that reduced mycelial growth by 50% of the pre-2001 and post-2011 *Rhizoctonia solani* isolates to penflufen, sedaxane, ipconazole, and prothioconazole^y

		Penflufen			Sedaxane			Ipconazole			Prothioconazole		
Isolates	N ^z	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
Pre-2001													
AG-2-2	27	0.056 a	0.016–0.155	0.032	0.089 a	0.029–0.215	0.043	1.338 b	0.349–2.559	0.636	1.497 a	0.242–3.554	0.904
AG-4	13	0.017 b	0.007–0.035	0.010	0.051 b	0.026–0.119	0.025	1.894 a	0.351–3.162	0.796	1.809 a	0.200–3.665	1.026
Post-2011													
AG-2-2	55	0.054 a	0.028–0.244	0.030	0.103 b	0.048–0.281	0.030	1.143 b	0.148–3.434	0.554	2.328 a	0.275–6.802	1.706
AG-3	4	0.041 ab	0.035–0.049	0.006	0.071 bc	0.070–0.073	0.006	0.199 c	0.132–0.365	0.101	0.435 b	0.338–0.523	0.110
AG-4	5	0.034 b	0.014–0.043	0.013	0.064 c	0.048–0.072	0.010	0.298 c	0.078–0.463	0.146	0.493 b	0.239–0.676	0.189
AG-7	9	0.047 ab	0.021–0.071	0.016	0.162 a	0.095–0.263	0.067	0.495 c	0.198–0.817	0.215	1.245 ab	0.334–2.733	0.943
AG-11	7	0.040 ab	0.021–0.071	0.015	0.058 c	0.022–0.129	0.015	1.797 a	0.913–2.490	0.911	0.759 b	0.283–1.791	0.538

^y Units in micrograms per milliliter; nontransformed values are reported. Means with the same letter are not significantly different using Fisher's protected least significant difference ($\alpha = 0.05$); SD = standard deviation of the mean.

^z Number of isolates.

2.490 µg/ml for the AG-11 isolates. All isolates of AG-3, AG-4, and AG-7 had EC₅₀ values below 1 µg/ml and, therefore, were considered extremely sensitive to ipconazole. Highly variable EC₅₀ values were similarly observed for prothioconazole, especially for the AG-2-2 (0.275 to 6.802 µg/ml), AG-7 (0.334 to 2.733 µg/ml), and AG-11 (0.283 to 1.791 µg/ml) isolates, permitting their classification into the extremely sensitive and moderately sensitive categories. All AG-3 and AG-4 isolates were extremely sensitive (EC₅₀ < 1 µg/ml) to prothioconazole. EC₅₀ values of ipconazole and prothioconazole were not significantly correlated ($P = 0.11$; $r = 0.09$).

Comparison of sensitivities of the pre-2001 and post-2011 isolates of AG-2-2 and AG-4 for the four fungicides. AG-2-2 isolates. For penflufen, the mean EC₅₀ values of the pre-2001 isolates were not significantly different from those of the post-2011 isolates; however, for sedaxane, the mean EC₅₀ of the post-2011 isolates was significantly greater than that of the pre-2001 isolates. The pre-2001 isolates had a higher mean EC₅₀ for ipconazole (Table 4), which was significantly different from that for the isolates recovered after 2011. For prothioconazole, compared with the pre-2001 isolates, a significantly higher mean EC₅₀ was observed for the isolates recovered after 2011.

AG-4 isolates. The mean EC₅₀ value of the post-2011 group was numerically higher but not significantly different from those of the pre-2001 group for penflufen and sedaxane. Ipconazole and prothioconazole treatments, however, showed contrasting results: the pre-2001 group had significantly higher mean EC₅₀ values than the post-2011 group.

In vivo sensitivity assay. SDHI fungicides. For both dependent variables (SC and DSI), the main effect of isolate was not significant (Table 5), whereas that of fungicide was highly significant ($P < 0.0001$). A significant isolate–fungicide interaction was observed for SC but not for DSI (Table 5). Isolates 1, 3, and 4 did not significantly decrease SC with the application of penflufen and sedaxane (Table 6). For isolate 2, SC observed with penflufen was not significantly different from the nontreated control (Table 6). All four isolates resulted in significantly lower DSI with the application of penflufen and sedaxane.

DMI fungicides. Results from the ANOVA revealed significant main effects of isolate and fungicide for SC and DSI and a significant isolate–fungicide interaction for DSI only (Table 5). Regardless of isolate, significantly higher SC were obtained with ipconazole and prothioconazole treatments (Table 6). Isolates 6, 7, 8, and 10 did not significantly decrease DSI with the application of ipconazole and prothioconazole. However, isolates 5 and 9 resulted in significantly higher DSI with ipconazole and prothioconazole, respectively (Table 6).

Discussion

The sensitivity of *R. solani* to several fungicide classes has been studied in various in vivo and in vitro assays (Amaradasa et al. 2014;

Barnes et al. 1990; Blazier and Conway 2004; Campion et al. 2003; Carling et al. 1990; Goll et al. 2014; Kataria et al. 1991a,b; Li et al. 2014; Martin et al. 1984a,b; Meyer et al. 2006; Zhang et al. 2009), with varied results obtained within and among the different AG tested. Unfortunately, the majority of the fungicides evaluated in these studies are currently not in use for managing diseases caused by this pathogen. Although our study focused on more recent active ingredients, similar variability within and among AG was observed for both the historical and the nonhistorical groups. The historical group represented isolates recovered prior to 2001, when all four active ingredients evaluated had

Table 6. Effects of fungicide seed treatments on disease severity index (DSI) and stand count (SC) of soybean seed treated with succinate dehydrogenase inhibitor (SDHI) and demethylation inhibitor (DMI) fungicides^z

Fungicide class, isolate	Treatment	DSI (0 to 1)	SC
SDHI			
1 (extremely sensitive)	Penflufen	0.28 b	9 a
	Sedaxane	0.27 b	10 a
	No treatment	0.63 a	8 b
2 (extremely sensitive)	Penflufen	0.32 b	8 b
	Sedaxane	0.28 b	10 a
	No treatment	0.75 a	8 b
3 (extremely sensitive)	Penflufen	0.29 b	10 a
	Sedaxane	0.35 b	9 a
	No treatment	0.59 a	9 a
4 (extremely sensitive)	Penflufen	0.34 b	10 a
	Sedaxane	0.30 b	9 a
	No treatment	0.54 a	7 b
DMI			
5 (extremely sensitive)	Ipconazole	0.73 a	6 a
	Prothioconazole	0.49 b	7 a
	No treatment	0.76 a	1 b
6 (extremely sensitive)	Ipconazole	0.48 b	8 a
	Prothioconazole	0.42 b	9 a
	No treatment	0.68 a	7 b
7 (moderately sensitive)	Ipconazole	0.52 b	9 a
	Prothioconazole	0.45 b	10 a
	No treatment	0.78 a	8 b
8 (extremely sensitive)	Ipconazole	0.42 b	9 a
	Prothioconazole	0.44 b	9 a
	No treatment	0.50 a	7 b
9 (moderately sensitive)	Ipconazole	0.36 b	9 a
	Prothioconazole	0.56 a	10 a
	No treatment	0.67 a	7 b
10 (moderately sensitive)	Ipconazole	0.52 b	10 a
	Prothioconazole	0.53 b	9 a
	No treatment	0.79 a	8 b

^z Means with the same letter are not significantly different using Fisher's protected least significant difference ($\alpha = 0.05$)

Table 5. Analysis of variance for the effects of fungicide seed treatment on plant stand and disease severity indices of soybean treated with succinate dehydrogenase inhibitor (SDHI) and demethylation inhibitor (DMI) fungicides

Dependent variable	SDHI			DMI		
	DF	MS	$P > F$	DF	MS	$P > F$
Stand count						
Isolate	3	99,900.0000	0.386	5	6,445.6757	0.0011
Fungicide	2	898,330.0000	<0.0001	2	12,889.0000	<0.0001
Isolate × fungicide	6	137,676.0000	0.037	10	297.7193	0.6188
Block	3	6,451.4722	0.973	3	170.1190	0.8987
Block × isolate	9	88,092.0000	0.156	15	881.3391	0.0051
Disease severity index						
Isolate	3	0.4146	0.1584	5	1.3517	<0.0001
Fungicide	2	32.7085	<0.0001	2	5.7827	<0.0001
Isolate × fungicide	6	0.8796	0.0639	10	0.8768	<0.0001
Block	3	0.3785	0.1841	3	0.0291	0.8462
Block × isolate	9	0.1889	0.9024	15	0.1073	0.8148

not been registered for use as a seed treatment on soybean or sugar beet. The reason for the observed variability within AG of this group is unknown but may be a reflection of the high level of genetic diversity that is a characteristic of the two AG of *R. solani* that represent this group.

Based on the sensitivities of the two isolate groups, our results showed that all isolates examined were more effectively controlled by the SDHI fungicides in vitro. From the range of EC₅₀ values obtained, the isolates were extremely sensitive (EC₅₀ < 1 µg/ml) to penflufen and sedaxane (Table 7). Although the mean and median EC₅₀ values of the post-2011 isolates were slightly higher than those of the historical isolates, the values did not exceed the extremely sensitive threshold. Contrary to what was observed with the SDHI, all isolates could be categorized as either extremely sensitive or moderately sensitive (1 ≤ EC₅₀ ≤ 10 µg/ml) to the DMI (Table 7). According to Hewitt (1998), sensitivity shifts are more often observed with fungicidal compounds that have been in use for many decades (Hewitt 1998). The DMI fungicides are reported to have been available for use since the mid-1970s (Mueller et al. 2013), although they were not used on soybean until their approval as a section 18 emergency product for the management of soybean rust in the mid-2000s. Given the potential for cross-resistance between members of this fungicide group (Fungicide Resistance Action Committee 2016), the likelihood of a previous exposure of our historical and post-2011 isolates to other DMI fungicides cannot be completely ruled out, and this might explain the observed reduced sensitivity of both groups of isolates to the DMI. Alternatively, differences in pathogen sensitivities could reflect the natural levels of variation within the populations of *R. solani* examined in this study.

Due to the genetic diversity of *R. solani*, an evaluation of the sensitivity of the different *R. solani* AG that cause seedling diseases of soybean to currently used seed-treatment fungicides will be important in making seed treatment recommendations, provided the predominant AG in any one field is known. We found that penflufen and sedaxane provided strong activity toward the AG-2-2 and AG-4 isolates of the historical and post-2011 groups. Although the mean EC₅₀ value of the pre-2001 AG-4 isolates for both active ingredients was numerically lower than that of the AG-4 isolates of the post-2011 group, the range of EC₅₀ values obtained for all AG-4 isolates in both groups fell within the extremely sensitive category. There were no isolates representative of AG-3, AG-7, and AG-11 in the pre-2001 group but the results obtained from the post-2011 group confirm the excellent activity of these two active ingredients toward these AG. Our results with both penflufen and sedaxane agree with those of other authors who evaluated the sensitivity of different *R. solani* AG to members of the SDHI fungicide chemistry class. For example, Goll et al. (2014) tested the sensitivity of *Rhizoctonia* spp. recovered from Europe to sedaxane and reported a low baseline sensitivity for isolates of AG-3PT (0.022 µg/ml), AG-3TB (0.043 µg/ml), AG-4 (0.028 µg/ml), and AG-11 (0.020 µg/ml). Carboxin, an older member of the SDHI class, has also been shown to possess strong activity toward

different AG (Kataria et al. 1991b; Martin et al. 1984b). Martin et al. (1984b) reported an EC₅₀ of 0.31 mg/liter for AG-2 isolates and 0.01 mg/liter for isolates of AG-4, while Kataria et al. (1991b) found that AG-7 isolates were highly sensitive, followed by AG-4 and AG-2-2 isolates.

A surprising finding from evaluating the DMI fungicides was that ipconazole and prothioconazole exhibited good activity toward the post-2011 AG-4 isolates but weak activity toward some of the historical AG-2-2 and AG-4 isolates and the post-2011 AG-2-2 isolates. The poor activity of the DMI toward the historical AG-4 isolates is in contrast to that reported by Barnes et al. (1990), who found good activity of diniconazole (mean EC₅₀ = 0.028 mg/liter), cyproconazole (mean EC₅₀ = 0.056 mg/liter), and tebuconazole (mean EC₅₀ = 0.166 mg/liter) toward AG-4 isolates that were previously unexposed to these fungicides. For both AG, the observed differences in fungicide activity may be due to a small sample size that may have been inadequate to allow for an unbiased comparison of both isolate groups, especially for the AG-4 isolates.

The goal of the in vivo fungicide evaluation conducted in this study was to determine whether reduced fungicide sensitivity in the in vitro assays translated into reduced fungicide efficacy when applied as seed treatments. For both fungicide classes, we found that, in most cases, isolates exhibiting high and moderate sensitivity to certain active ingredients in the in vitro assays were effectively controlled by the respective fungicides in the greenhouse. Therefore, a high EC₅₀ value in vitro should not be interpreted as resistance unless a correlation has been established in in vivo assays. Results from the in vivo assay with isolates 5 and 7 in response to the application of a DMI fungicide also highlight the inappropriateness of subjective assessments (such as disease severity ratings) alone for evaluating fungicide effectiveness in greenhouse or field studies. Isolates resulting in significantly higher DSI with the application fungicide may not always significantly reduce SC concomitantly.

In conclusion, our results indicate that *R. solani* isolates are more sensitive to the SDHI class of fungicides than to the DMI fungicides. Although considerable variation in fungicide sensitivity was observed within and among AG of this fungus, sensitivity shifts were not detected for both fungicide classes. Data from our in vitro assays can provide a benchmark for assessing future declines in sensitivity to these active ingredients by *R. solani* populations. Although our results from in vitro assays indicate reduced sensitivity of some isolates to the DMI fungicides, results from greenhouse assays conducted suggest that protection in the field will be acceptable. Although none of the fungicides evaluated provided complete disease control in the greenhouse assays, significantly lower DSI and higher SC of plants from treated seed compared with plants from nontreated seed further confirms the effectiveness of seed treatments in improving stand establishment and reducing the risk of total crop failure in the presence of disease risk factors. Finally, our results indicate that reduced in vitro fungicide sensitivity may not always correlate with disease control in the field.

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Table 7. Percentages of *Rhizoctonia solani* isolates of the pre-2001 and post-2011 groups within each in vitro sensitivity category

Fungicide	Category ^z	Percentage of isolates	
		pre-2001	post-2011
Penflufen	Extremely sensitive	100	100
	Moderately sensitive	...	...
	Tolerant	...	...
Sedaxane	Extremely sensitive	100	100
	Moderately sensitive	...	...
	Tolerant	...	...
Ipconazole	Extremely sensitive	22.50	52.50
	Moderately sensitive	77.50	47.50
	Tolerant	...	...
Prothioconazole	Extremely sensitive	30	43.75
	Moderately sensitive	70	56.25
	Tolerant	...	...

^z Extremely sensitive (EC₅₀ < 1 µg/ml), moderately sensitive (1 ≤ EC₅₀ ≤ 10 µg/ml), and tolerant (EC₅₀ > 10 µg/ml).

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