CROP ROTATION EFFECTS ON RHIZOCTONIA SOLANI AG 2-2

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Rhizoctonia solani (= *R. solani*) is a common, soilborne fungal pathogen of crops grown throughout the world. The fungus does not produce spores but is composed of genetically isolated populations called anastomosis groups or AGs (4). In the last decade, *R. solani* AG-2-2 has been increasing in prevalence and severity on sugarbeet in the Red River Valley. This trend has been caused by various factors including wet summers that favor infection and disease development and by increased production of bean crops (also susceptible to stem and root rot caused by *R. solani* AG 2-2). Close rotation of these susceptible crops gradually results in a build up of inoculum in soil and increases in disease. *Rhizoctonia solani* AG 2-2 is further divided into intraspecific groups (ISGs) known as IIIB and IV; AG 2-2 IIIB tends to attack soybean and other bean crops and AG 2-2 IV tends to attack sugarbeet (4), although both ISGs can attack both crops (1, 5).

Cereal crops (e.g., wheat, barley, corn) are recommended for rotation with broadleaf crops (e.g., sugarbeet, soybean, sunflower) because they are not known to be susceptible to attack by *R. solani* AG 2-2 in the upper Midwest. In Europe (2) and the southeastern United States (6, 7), however, *R. solani* AG 2-2 IIIB is reported as causing crown and brace root rot on corn. In fact, *R. solani* AG 2-2 IIIB causes stalk rot of corn in Germany and also is reported as primarily responsible for Rhizoctonia root and crown rot on sugarbeet in Europe (2). Recently, corn production has been increasing in northwest Minnesota and eastern North Dakota, so there are concerns if this crop is another host for *R. solani* AG 2-2 IIIB and AG 2-2 IV when grown in close rotation with sugarbeet or bean crops.

Scant data are available to document prevalence of *R. solani* AG 2-2 IIIB and AG 2-2 IV in Minnesota and North Dakota. Preliminary identification of a few cultures from sugarbeet in our laboratory has revealed both ISGs but AG 2-2 IV predominated. In a 2003 to 2004 field experiment, we found that inoculation of soil with *R. solani* AG 2-2 IIIB resulted in no obvious disease on wheat, soybean, sunflower or corn but when sugarbeet was grown the following season, Rhizoctonia root and crown rot was most severe following corn (9). Since distribution and occurrence of *R. solani* AG 2-2 IIIB and AG 2-2 IV in sugarbeet growing regions of Minnesota and North Dakota are unknown, a large-scale survey was initiated in the summer of 2005 and will continue in 2006. Also, it is unknown if *R. solani* AG 2-2 IIIB and AG 2-2 IV are equally aggressive on crops commonly grown in Minnesota and North Dakota or how rotation of these crops in *R. solani*-infested fields affects sugarbeet production. The following report summarizes the first year of a field trial to answer these questions.

OBJECTIVES

Our objectives are to conduct field trials to determine relative pathogenicity and survival of *R. solani* AG-2-2 IV and AG-2-2 IIIB on 1) rotation crops (corn, wheat, soybean) and 2) a subsequent sugarbeet crop.

MATERIALS AND METHODS

Crops grown in 2005. A field trial was established at the University of Minnesota, Northwest Research and Outreach Center (NWROC), Crookston in a split-plot trial of four replicates. Main plots were inoculated with *R. solani* AG 2-2 IIIB, *R. solani* AG 2-2 IV, and not inoculated. Inoculum of *R. solani* was grown on sterile barley grain for 3 weeks and then air-dried. On May 17, 2005, barley grain inoculum (11.3 ounces) was sprinkled over each main plot (33 x 30 ft) and incorporated with a Melroe multiweeder to about a 2-inch depth. Rotation crops were sown as subplots of main plots the following day. Seed of spring wheat 'Knudson', soybean 'GoldCountry 923RR', and corn 'Pioneer 39D81' were sown at rates of 90 lb, 60 lb, and 30,000 seed per acre, respectively. Each crop was fertilized, treated with pesticides, and maintained following recommended practices.

Disease ratings. On August 16, 25 wheat plants per plot were assessed for root rot by rating the subcrown internode on a 0 to 3 scale where 0 = clean and 3 = more than 50% surface with lesions and discoloration (8). Subcrown internodes are easier to rate than crown roots, are an indicator of general root health, and can be infected by several soilborne pathogens. On August 23, 25 soybean plants were removed per plot and washed to assess basal stems and roots for disease on a 1 to 5 scale where 1 = no symptoms and 5 = shoot dead and more than 75% of stem girdled (1). Corn roots (25 per plot) were dug from plots on September 4 and 7. Roots were thoroughly washed to remove adhering soil and then rated for disease on a 1 to 5 scale where 1 = less than 2% root surface with lesions and 5 = plant dead (7). Basal stem diameters of each corn plant also were measured with a caliper positioned at the middle of the first internode above brace roots.

After roots were assessed for disease, attempts were made to isolate *R. solani* (a total of 100 plants of each crop per soil treatment). For wheat, subcrown internodes were surface-disinfested in bleach, rinsed twice in sterile distilled water, and placed on potato-dextrose agar (PDA). For soybean, a 1-inch piece of each basal stem was treated and cultured in the same fashion. For corn, a 1-inch segment of root with lesions or discoloration was excised from each plant, or if no discoloration occurred, was randomly removed from an apparently healthy root. Corn root pieces were cultured as previously described. After 14 days, PDA was examined for growth of *R. solani* and other fungi. If *R. solani* was present, transfers were made to fresh PDA so cultures could be purified and further identified.

Harvest. Wheat plots were not harvested because of severe lodging. Soybeans were harvested on September 29 with a small plot combine (two middle rows per plot) and analyzed for percent oil and protein with an Infratec 1229 grain analyzer. Corn was hand-harvested on October 19 by removing ears from all plants in the two middle rows of each plot, which then were dried at 105 ^{0}F for 48 hours. Kernels were removed with a corn sheller (Hocking Valley Improved AU170) and percent moisture was determined with an Infratec 1229 grain analyzer.

Data analysis. Data were subjected to analysis of variance and if significant (P = 0.05), means were separated by Least Significant Difference.

RESULTS

Disease ratings. No aboveground symptoms of *Rhizoctonia* diseases (obvious stunting, yellowing or necrosis of foliage, necrosis at the soil line) were observed on crops in the *Rhizoctonia*-inoculated and non-inoculated control plots.

Root rot ratings of wheat subcrown internodes were the same in *R. solani*-inoculated and non-inoculated plots and averaged 1.86 (Table 1). Isolation of *R. solani* from subcrown internodes was infrequent and not significantly different among *R. solani*-inoculated and non-inoculated plots (average = 1.2%), although recovery from wheat tended to be somewhat higher in plots inoculated with *R. solani* AG 2-2 IIIB (Table 1). The "common root rot" pathogens F*usarium graminearum* and *Bipolaris sorokiniana* were isolated from 39 and 36 % of subcrown internodes, respectively (data not shown). Isolation of these fungi was not related to plot treatment.

Soybeans were not rated for root rot because all plants had some typical discoloration and no girdling of basal stems, regardless of soil treatment. Isolation of *R. solani* from basal stem root pieces was infrequent and not significantly different among *R. solani*-inoculated and non-inoculate plots (average = 1.9%), although recovery from soybean tended to be highest in plots inoculated with *R. solani* AG 2-2 IIIB (Table 1).

Root rot ratings of corn were significantly higher in plots inoculated with *R. solani* AG 2-2 IIIB compared to plots inoculated with AG 2-2 IV and the non-inoculated control, which both had negligible disease (Table 1, Figure 1). Corn from the AG 2-2 IIIB plots had obvious lesions, discoloration, and smaller root systems (Fig. 1A) than in the AG 2-2 IV-inoculated (Fig. 1B) and non-inoculated plots (Fig. 1C). Recovery of *R. solani* from roots also was significantly higher in plots inoculated with AG 2-2 IIIB (9.3%) compared to plots inoculated with AG 2-2 IV and the non-inoculated control, which both averaged recoveries of less than 1% (Table 1). Basal stem diameters of corn in the *R. solani* AG 2-2 IIIB plots were somewhat smaller than plants in the AG 2-2 IV and non-inoculated plots, but were not significantly different.

Table 1. Root rot ratings, isolation of *Rhizoctonia solani,* yield, and quality of wheat, soybean, and corn sown in plots inoculated with *R. solani* AG 2-2 IIIB or AG 2-2 IV compared to non-inoculated soil in a field trial at the University of Minnesota, Northwest Research and Outreach Center, Crookston in 2005.

Crop and variables measured [U, Z]	Soil Treatment: Inoculated with *R. solani*[T] AG 2-2 IIIB	Soil Treatment: Inoculated with *R. solani*[T] AG 2-2 IV	Soil Treatment: Not inoculated
Wheat 'Knudson'			
RRR (0-3 scale) [V]	1.9 a	1.8 a	1.9 a
Root pieces with *R. solani* (%) [W]	2.3 a	0.8 a	0.5 a
Yield [Y]	-	-	-
Soybean 'Gold Country 923RR'			
RRR (1-5 scale) [V]	NR	NR	NR
Root pieces with *R. solani* (%) [W]	2.3 a	2.0 a	1.5 a
Yield (bu/A) [Y]	30.0 a	33.0 a	32.0 a
Oil (%)	19.0 a	18.8 a	19.0 a
Protein (%)	32.0 a	32.6 a	32.4 a
Corn 'Pioneer 39D81'			
RRR (1-5 scale) [V]	2.8 a	1.1 b	1.1 b
Root pieces with *R. solani* (%) [W]	9.3 a	0.5 b	0.8 b
Basal stalk diameter (cm) [X]	12.5 a	12.9 a	12.9 a
Yield (bu/A) [Y]	131.0 a	118.0 a	132.0 a
Moisture content (%)	17.1 a	16.0 a	15.9 a

[T] Inoculum of *R. solani* AG 2-2 IIIB and *R. solani* AG 2-2 IV was grown on sterile barley grain for 3 weeks, air-dried, sprinkled onto field plots. Main plots were inoculated with each population of *R. solani* (11.3 ounces per 990 ft^2) and the control was not inoculated. All plots then were disked with a Melroe multiweeder. Treatments arranged in a split-plot design with four replicates.

[U] Rotation crops were sown on May 17, 2005 as subplots of main plots inoculated the previous day with *R. solani* AG 2-2 IIIB or AG 2-2 IV or not inoculated. Plots were arranged in a randomized block design with four replicates.

[V] RRR = root rot rating (total of 100 plants for each crop and soil treatment). **Wheat** subcrown internodes were assessed for root rot on August 16 with a 0 – 3 scale where 0 = clean and 3 = more than 50% surface with lesions and discoloration (8). **Soybeans** were removed and cleaned August 23 for rating of basal stems and roots with a 1 – 5 scale where 1 = no symptoms and 5 = shoot dead and more than 75% of stem girdled (1) but all plants had similar amounts of slight discoloration and no root rot, so they were not rated (NR). **Corn** roots and brace roots were removed on September 4 and 7, washed, and rated with a 1 – 5 scale where 1 = less than 2% root surface with lesions and 5 = plant dead (7).

[W] One section of root (~ 1-inch long) of each plant was removed after disease assessment (total of 100 plants per crop and soil treatment), surface-sterilized, and cultured on potato-dextrose agar for isolation of *R. solani*.

[X] Basal stem diameters of corn were measured in the middle of the first internode above the brace roots (total of 100 per soil treatment).

[Y] Yield data was not collected for wheat because of lodging; soybeans were harvested on September 29 and corn on October 19, 2005

[Z] For each row, values followed by the same letter are not significantly different, $P = 0.05$.

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Crop yields. For soybean and corn, there were no statistical differences in yield when these crops were grown in plots inoculated with *R. solani* AG 2-2 IIIB, *R. solani* AG 2-2 IV, or not inoculated (Table 1). Nor were there differences among main plot treatments for percent oil and protein of soybean or moisture content of corn (Table 1).

DISCUSSION

The first year of this field trial indicated that *R. solani* AG 2-2 IV was not pathogenic on wheat, soybean, or corn and infected roots of these crops at very low frequencies. Since *R. solani* AG 2-2 IV is considered the major cause of Rhizoctonia root and crown rot of sugarbeet (4), except in Europe where AG 2-2 IIIB predominates (2), these results are viewed with cautious optimism. In plots inoculated with *R. solani* AG 2-2 IIIB, wheat and soybean were

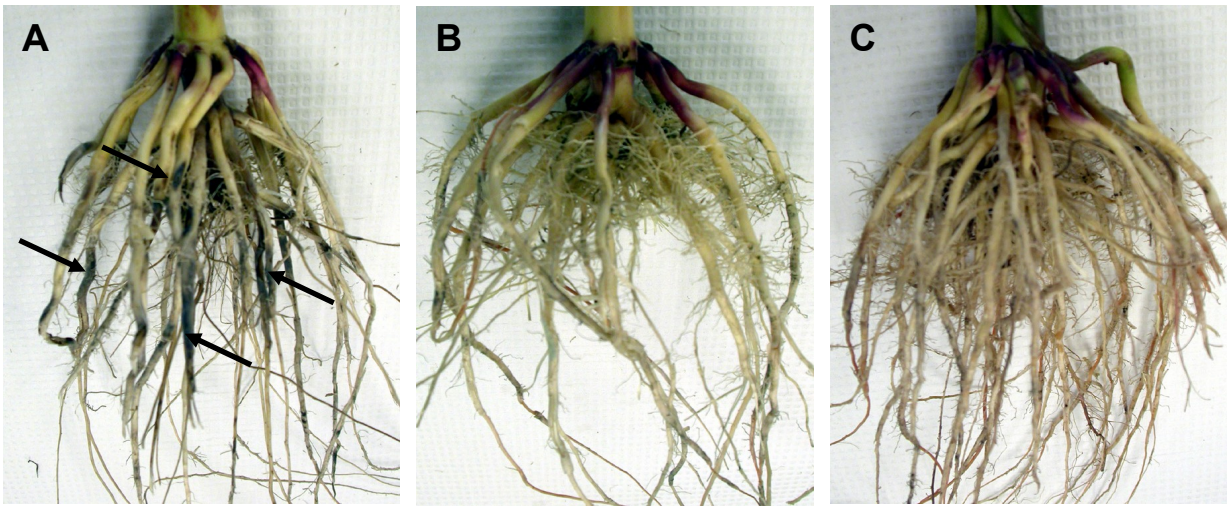

Figure 1. Corn roots of 'Pioneer 39D81' removed from plots in early September that were inoculated in mid May, 2005 with **A)** *Rhizoctonia solani* AG 2-2 IIIB (arrows note lesions), **B**) *R. solani* AG 2-2 IV or **C**) not inoculated. Dark color on crown of plants shown in **A-C** is a dark red pigment (not crown and brace root rot).

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not obviously affected, although this intraspecific group (ISG) is a common pathogen on soybean (4, 5). On the other hand, lesions and rotting of corn roots in plots inoculated with AG 2-2 IIIB were unexpected because no aboveground symptoms (stunting, yellowing, stalk breakage) were observed, nor were yields reduced. In a related trial in 2003, Windels and Brantner (9) also did not observe aboveground symptoms of Rhizoctonia diseases on corn, soybean, or wheat in plots inoculated with *R. solani* AG 2-2 IIIB, but the subsequent sugarbeet crop was most severely affected by Rhizoctonia following corn, least affected when following wheat, and intermediate after soybean. In 1996, Nelson et al. (3) reported that cultures of *R. solani* AG 2-2 from soybean in the Red River Valley caused some lesions on corn seedlings in greenhouse experiments, but they did not identify cultures to intraspecific group. Our results suggest that if *R. solani* AG 2-2 IIIB is present in fields, corn is a host contributing to the build-up of inoculum in soil, perhaps more so than cultivation of soybean and edible bean.

Recovery of *R. solani* from wheat, soybean, and corn at very low frequencies in plots inoculated with AG 2-2 IV suggests the fungus was not actively increasing on these crops when they were assessed for disease. In fact, recovery of *R. solani* in these plots was equal to non-inoculated plots, which apparently, were naturally infested or contaminated with *R. solani*. The tendency to recover *R. solani* somewhat more frequently from wheat and soybean, and significantly more often from corn, in plots inoculated with AG 2-2 IIIB compared to plots inoculated with AG 2-2 IV may be attributed to AG 2-2 IIIB being highly aggressive (1). Lack of isolation of *R. solani* from every lesion on corn roots is not unusual because other microorganisms colonize roots infected by pathogens, and thereby, obscure and/or inhibit isolation of the initial pathogen. Laboratory tests will be done to verify identity of the intraspecific groups of *R. solani* cultured from wheat, soybean and corn in the 2005 field trials.

R. solani is an excellent competitive saprophyte so results reported here for re-isolation from various crops may not predict the build-up of inoculum or survival for the 2006 growing season. The pathogen may increase inoculum in several ways: by causing root rot of rotation crops; by infecting roots, which remain symptomless; or by saprophytically colonizing crop residue after harvest. Perhaps residues of certain crops or varieties are colonized by one or both intraspecific groups of *R. solani* AG 2-2.

To determine the presence and distribution of ISGs of *R. solani* AG 2-2 in the RRV and southern Minnesota, surveys are being conducted throughout the region in 2005 and 2006 to collect cultures of *R. solani* from sugarbeet with crown and root rot. In 2005, over 160 cultures of *R. solani* were obtained from diseased sugarbeet plants. About 130 cultures were from samples provided by agriculturists for the disease survey and additional cultures were

from samples submitted to the NWROC or Plant Disease Clinics at the University of Minnesota or North Dakota State University for diagnosis. Field histories reveal the previous crops typically were wheat, soybean, or corn. Each culture will be purified and microscopically identified to anastomosis group by pairing with known AG tester cultures (5). Then, each culture of *R. solani* AG 2-2 will be separated into ISGs IIIB or IV by a temperature differential test (IIIB grows at 95 ^{0}F but IV does not). Although this initial separation is useful (5), results are not always clear cut, so further characterization requires molecular techniques. Discussions are underway with potential collaborators to accomplish this type of analysis.

Crop rotation is a common practice for managing plant diseases and improving crop production. Benefits of crop rotation are complicated, vary from region to region, and are affected by a multitude of factors. Our research suggests corn increases inoculum of *R. solani* AG 2-2 IIIB in infested fields but it is unknown if all corn varieties are susceptible. Also, the distribution and prevalence of AG 2-2 IIIB and AG 2-2 IV in the RRV and southern Minnesota are unknown. This information is critical in adopting crop rotation practices that avoid or delay build-up of inoculum and to manage disease in fields where the pathogens are established.

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