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Efficacy of Azoxystrobin at Controlling *Rhizoctonia solani* at Early Growth Stages of Sugar Beet

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Abstract

Sugar beet (*Beta vulgaris L.*) is one of the most profitable crops grown in Minnesota and North Dakota and makes a significant economic contribution to these States. *Rhizoctonia* damping-off and crown and root rot caused by *Rhizoctonia* solani Kühn, AG 2-2 are important soil borne diseases of sugar beet. Control strategy against this disease is achieved through timely application of effective fungicides before infection takes place. For many years, azoxystrobin was applied at or just prior to cultivation when sugar beets were at the 4- to 10-leaf stage to protect against crown infection and subsequent *Rhizoctonia* crown and root rot. Since 2009, warmer conditions in spring resulted in earlier planting and reports of seedling mortality and root rot caused by R. solani. The research objective was to determine whether azoxystrobin protects sugar beet seeds and seedlings to the 4-leaf growth stages against R. solani infection in a favorable environment. The experiment was conducted in the greenhouse with a temperature of 20±2 ˚C using sugar beet cultivar Crystal 539, susceptible to R. solani. Azoxystrobin was applied directly over the seed in-furrow at planting, in an 18 cm band to soil covered seeds, and in an 18 cm band to cotyledonary, 2-leaf, and 4-leaf stages sugar beet. Fungicide application was followed by inoculation with R. solani AG 2-2 IIIB. Inoculated and non-inoculated (using sterile barley grains) controls were included for all the growth stages evaluated. There was no significant difference between the percentages of survivals in the non-inoculated controls compared to the sugar beet seed and different growth stages that were inoculated and treated with azoxystrobin indicating that the fungicide provided effective control of R. solani, irrespective of the sugar beet growth stages evaluated. Results indicated that sugar beet seeds and seedlings to the 4-leaf stages were susceptible to infection by R. solani in favorable environmental conditions. As such, necessary precautions should be taken to protect seeds and young plants especially when sugar beet is grown in fields with a history of the pathogen.

Keywords: *Rhizoctonia* solani; Pathogens; Hypocotyl; Soil-root; Crop rotation; Azoxystrobin

Abbreviations: PDA: Potato Dextrose Agar; PDB: Potato Dextrose Broth

Introduction

Rhizoctonia solani Kühn (teleomorph *Thanatephorus cucumeris* (AB Frank) Donk), is a pathogen of numerous crops worldwide [1,2]. There are many isolates of *R. solani* which are placed into anastomosis groups based on hyphal fusion [1-3]. Currently, 14 anastomosis groups of *R. solani* have been described [4,5]. *Rhizoctonia* solani AG 2-2 and AG 4 cause sugar beet damping-off and AG 2-2 (IIIB and IV) causes crown and root rot of older sugar beet (*Beta vulgaris L.*) plants [6-8] worldwide including North Dakota and Minnesota in the United States [9-13]. *Rhizoctonia* solani may cause pre-emergence damping-off but post-emergence damping-off or stunting of plants is more common [14] and occurs when planting into warm soils with a history of the disease. Infection begins below the soil surface and typical symptoms of damping-off include brown discoloration of the hypocotyl progressing upwards from the soil surface resulting in wilting and severely infected plants die. *Rhizoctonia* crown and root rot infections may begin in the crown or on the root surface at or below the soil-root interface [15]. *Rhizoctonia* crown and root rot may result in significant yield reduction and adversely impact sucrose extraction [16,17].

Management of *Rhizoctonia* damping-off, crown and root rot may be achieved by planting into cool soils since infection does not occur in soil temperatures below 15 °C [18]. Rotations of three to five years with crops that are non-hosts of *R. solani* AG 2-2 may reduce disease severity [19], but since the soil borne pathogen is present in all sugar beet production areas, the use of crop rotation alone may be inadequate for effective control [3]. There is no commercial sugar beet variety available which is completely resistant to *R. solani* and varieties with partial resistance typically have lower yields compared to susceptible varieties in the absence of disease [20]. Growers typically use an integrated system including early planting, crop rotation, resistant varieties as well as fungicide applications to manage *R. solani*. Among the fungicides, azoxystrobin (Quadris 2.08 SC; Syngenta, USA) provides effective

control of *R. solani*, but it must be applied before infection takes place [2,16,21-23].

Prior to the adoption of herbicide tolerant sugar beet in 2008, mechanical cultivation was widely used on 4-leaf and older beets to complement herbicides for acceptable weed control in North Dakota and Minnesota [24]. The deposition of *Rhizoctonia* infested soil into the crown of sugar beet plants during cultivation resulted in infection and crown and root rot [12]. Growers in North Dakota and Minnesota were advised to apply fungicides to sugar beet that were 8-12 leaf sugar beet to control crown and root rot [25]. In 2009 and 2010, growers reported *Rhizoctonia* crown and root rot as their worst production problem which was confirmed by the diagnostic laboratory at the University of Minnesota from field samples submitted by growers [26,27]. However, most of the fields affected had plants with symptoms of *Rhizoctonia* damping-off or root rot rather than crown rot (Observation of Khan).

Growers wanted to know whether they should wait until plants are at the 4- to 10-leaf stages before applying azoxystrobin as they had done previously to prevent infection through crown inoculation at cultivation, or should they apply azoxystrobin earlier to younger plants since they were planting into soils that were warm and favorable for infection by *R. solani* [23]. The objective of this study was to determine whether azoxystrobin gives protection to sugar beet seeds and seedlings at the cotyledonary, 2- and 4-leaf stages against *R. solani* in an environment favorable for infection and disease development.

Greenhouse Trial

Greenhouse trial was conducted at North Dakota State University in Fargo, North Dakota. Sugar beet cultivar Crystal 539RR susceptible to *R. solani* [28], was used for this experiment. Trays measuring 25 x 14 x 13 cm (T.O. Plastics Inc., Clearwater, MN) were filled with Sunshine Mix 1 peat (Sun Gro Horticulture Ltd., Alberta, Canada). One 3cm deep furrow was made with a Hume seed finder (Hume Global Enterprises, Fresno, CA, USA) in the middle of each peat filled tray into which 10 evenly spaced seeds were planted. Sugar beet seeds and sugar beet at cotyledonary, 2-leaf and 4-leaf stages were treated with azoxystrobin (Quadris 2.08 SC, Syngenta, Greensboro, NC, USA) at 167 g a.i ha^{-1}, followed by inoculation. Azoxystrobin was applied in-furrow directly over sugar beet seeds followed by inoculation and covered with soil; azoxystrobin was applied in an 18 cm band directly over soil covered seeds, and cotyledonary, 2-leaf and 4-leaf sugar beet followed by inoculation. Treatments were applied using a spraying system (De Veries Manufacturing, Hollandale, MN) calibrated to deliver 145 liters ha^{-1} spray solution at 276kPal with a speed of 2.1kph through a single flat fan nozzle 4001E (Teejet Technologies, Springfield, IL). Inoculation was done by placing barley grains, one per seed or seedling, colonized with a pathogenic isolate of *R. solani* AG 2-2 IIIB at about 2cm below the soil surface and about 1cm away from the sugar beet seed or hypocotyl. Dr. Carol Windels, University of Minnesota, provided the *Rhizoctonia* solani AG 2-2 IIIB isolate which was recovered from sugar beet. R. solani AG 2-2 IIIB was grown on PDA (Potato Dextrose Agar) plate for 14 days. One fourth strength of PDB (Potato Dextrose Broth) was prepared using 18g PDB in 2,500-2,800 ml of de-ionized water per tray. The PDB solution was poured into an aluminum foil tray containing 4.5kg of untreated barley grain. The tray was tightly covered with aluminum foil and stored at 4-7 °C. After 72hr, trays were removed from cold storage, water was drained and the trays were sealed with paper tape and autoclaved for 2hr at 12 °C. Autoclaved barley grains were cooled for 2hr, then inoculated using the R. solani AG 2-2 IIIB isolate at one plate per tray under a laminar flow bench hood to avoid any potential contamination. Trays were resealed with paper tape and stored at room temperature for about 3 to 4 weeks. The inoculated barley grain was dried for 4 days in the greenhouse and then stored at -20 °C until required for use.

For each of the growth stages of sugar beet an inoculated and non-inoculated (treated with sterile barley grain) control was included. Trays were watered daily to provide adequate conditions for favorable plant and pathogen growth. Greenhouse condition was set to allow for a 12-hour photoperiod and temperature was maintained at 20±2 °C (Argus Control Systems, Ltd., British Columbia, Canada). Stand counts of surviving sugar beet plants were taken 21 days after treatments. The experimental design was a randomized complete block design with four replicates. The experiment was repeated twice.

Data Analysis

Each experiment was analyzed separately, and a folded F-test was used to determine homogeneity of the data. Experimental data were combined since no significant differences were observed at F=0.025 level of confidence. Analysis of variance was done using the SAS general linear models (Proc GLM) procedure (Version 9.3, SAS Institute Inc., Cary, NC). Fisher's protected least significant difference of means at $\alpha = 0.05$ was calculated to compare treatment means.

Results

The analysis of variance indicated that there were significant differences among the treatments at $P \leq .0001$ level of confidence. At 21 days after treatment, all azoxystrobin applications resulted in significantly greater number of survivals compared to the inoculated control where R. solani caused death of most or all the plants, irrespective of whether seeds, cotyledonary, 2-leaf or 4-leaf stages of sugar beet were used at the time of treatment. Sugar beet at the seed, cotyledonary, 2-leaf and 4-leaf stages were all equally susceptible to R. solani Ag 2-2. There were no significant differences in the percentage of survivals between the non-inoculated control and azoxystrobin treatments which indicated that the fungicide was effective in preventing R. solani from causing disease and mortality. There were no significant differences in survivals among the band application of azoxystrobin to soil covered seeds and cotyledonary, 2-leaf and 4-leaf stages sugar beet. Azoxystrobin provided control of R. solani and did not cause any significant reduction in plant stand when used as an in-furrow or band application (Table 1).

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Table 1: Effect of azoxystrobin (167g a.i ha^{-1}) fungicide on controlling R. solani AG 2-2 IIIB on sugar beet seed, cotyledonary, 2-leaf and 4-leaf sugar beet in a soil-less potting mix watered daily in a greenhouse maintained at 20 ± 2 °C and a 12- hr day length. In-furrow application was made directly over seeds followed by inoculation and covering of seeds. Band applications, 18cm wide, were made over soil covered seeds, cotyledonary, 2-leaf and 4-leaf sugar beet followed by inoculation. Inoculation was done by placing one R. solani infected barley grain 1 cm away from seed or hypocotyl and 2cm below the soil surface. Stand counts were taken 21 days after treatments. There were four replicates per treatment and the experiment was repeated. The data shown is from the combined experiments.

Treatments	Method of Fungicide Application	Mean Number of Survivals (%)
Inoculated control for in-furrow treatment to seeds	No fungicide	0
Non-inoculated control for in-furrow treatment to seeds	No fungicide	88
Azoxystrobin 167g a.i ha^{-1} + Inoculated	In-furrow	86
Inoculated control for band application on soil covered seeds	No fungicide	0
Non-inoculated control for band application on soil covered seeds	No fungicide	90
Azoxystrobin 167g a.i ha^{-1} on soil covered seeds + Inoculated	18cm band	90
Inoculated control for cotyledonary stage	No fungicide	0
Non-inoculated control for cotyledonary stage	No fungicide	90
Azoxystrobin 167g a.i ha^{-1} at cotyledonary stage + Inoculated	18cm band	93
Inoculated control for 2-leaf stage	No fungicide	0
Non-inoculated control for 2-leaf stage	No fungicide	90
Azoxystrobin 167g a.i ha^{-1} on 2-leaf stage + Inoculated	18cm band	89
Inoculated control for 4-leaf stage	No fungicide	1
Non-inoculated control for 4-leaf stage	No fungicide	90
Azoxystrobin 167g a.i ha^{-1} on 4-leaf stage + Inoculated	18cm band	90
LSD (P=0.05)		6

Conclusion and Management Implications

In conventional sugar beet, cultivation was necessary to complement herbicide applications to provide acceptable levels of weed control and 99.7% of survey respondents indicated using this practice in North Dakota and Minnesota in 2005 [29]. Cultivation was typically done at the 4 to 10-leaf stages and the movement of *R. solani* infested soil into the crown of plants caused crown rot [12]. Typically, in research trials conducted to evaluate fungicide efficacy for *R. solani* in sugar beet, fungicides were applied to the plants just prior to artificial inoculation of the crown with *R. solani* [2]. As such, sugar cooperatives typically recommended that fungicides be applied to 4 to 10-leaf stages sugar beet for effective control of *Rhizoctonia* crown rot [30]. In 2008, Roundup Ready seeds became widely available and was rapidly adopted by growers with over 95% of sugar beet acreage planted using this technology by 2010 [23,31]. Roundup Ready sugar beet facilitated the use of glyphosate which provided excellent weed control and significantly reduced the need for mechanical cultivation which was costly and time consuming [31]. In 2010, only 10% of sugar beet acres were cultivated compared to 200% in 2000 [27], probably to help in drying out fields and make them less favorable for pathogens. This significant reduction in cultivation would have likely reduced the possibility of sugar beet fields becoming infected with *R. solani* through deposition of infested soils at cultivation into crowns of sugar beet plants. Although the production practice of cultivation had changed with the adoption of glyphosate tolerant sugar beet, the recommendation for *R. solani* control with azoxystrobin remained the same [25,30]. During 2009 through 2012, growers in North Dakota and Minnesota reported that *Rhizoctonia* root rot was their most important problem [32,33]. During this period, growers were planting sugar beet when average daily soil temperature at the 10 cm depth was already at 18 ˚C and moisture was adequate for seedling emergence [34,35]. These environmental conditions were also favorable for infection by *R. solani* AG 2-2IIIB [21,23]. If growers were to wait until plants were at the 4-leaf and older stages before applying azoxystrobin, protection from *R. solani* would not be effective since infection would have already taken place and azoxystrobin does not provide curative control [17]. Our research was consistent with other research [17,21,23], which showed that sugar beet seeds and seedlings were susceptible to infection by *R. solani* in favorable conditions. We also demonstrated, under greenhouse conditions, that seeds and seedlings can be effectively protected from *R. solani* infection with azoxystrobin preventatively. This would suggest that sugar beet seeds and seedlings planted in fields with a history of *R. solani* should be protected with an effective fungicide before

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environmental conditions including temperature and moisture [21,23], become favorable for infection and development of *R. solani*.

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