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Azoxystrobin is Needed before Infection for Control of *Rhizoctonia solani* in Sugarbeet

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Abstract

Rhizoctonia solani is pathogenic to sugar beet, causing seedling damping-off, and crown and root rot in mature sugar beet plants. Rhizoctonia root rot was reported as the most important problem faced by sugarbeet producers in Minnesota and North Dakota. Azoxystrobin, a strobilurin fungicide, was recommended for controlling *R. solani,* but growers needed to know when to apply the fungicide relative to when infection takes place for effective disease control. The objective of this greenhouse study was to determine the best time to apply azoxystrobin relative to the time of inoculation for controlling root rot caused by *R. solani* AG 2-2 IIIB. Four-leaf stage sugar beet plants received fungicide application at 0, 3, 10, 14 and 21 days after inoculation and at 0, 7, 14, 21 and 28 days before inoculation. Treatments included a non-inoculated control and an inoculated control. Azoxystrobin was applied as a hypocotyl drench, and inoculation was done by placing *R. solani* grown on barley grain 2 cm below the soil and from 2 cm from the roots. Fourteen days after treatments were completed, plants were evaluated for root rot disease severity. Azoxystrobin applied before inoculation resulted in significantly lower disease severity compared to when it was applied after inoculation, except when inoculation was followed two hours later by the fungicide treatment. This research demonstrated that azoxystrobin needs to be applied before infection takes place to provide effective control and the fungicide provided protection for up to 28 days.

Keywords: *Beta vulgaris*, Fungicide timing, Strobilurin, Root rot

Introduction

Rhizoctonia solani is a serious soil-borne pathogen of sugarbeet worldwide [1,2]. This pathogen was made of several anastomosis groups (AG), with AG 2-1, 2-2, 3, 4 and 5 known to be pathogenic on sugarbeet and causing seedling damping off, and crown and root rot in mature sugarbeet plants [3]. *R. solani* AG 2-2 is the most virulent group that was subdivided into intra specific groups AG 2-2 IIIB (the more aggressive) and AG 2-2 IV [4]. Both anastomosis groups are widely distributed in the Red River Valley [5] and are the most damaging to sugarbeet.

In the United States (U.S.), *R. solani* affects 24% of planted sugarbeet acreage [1]. However, yield losses can reach up to 50% in some sugarbeet fields where the pathogen population is high and in conditions favorable for disease development. Most of the losses incurred by *Rhizoctonia* in this region is a result of root rot. Typically, most plants infected with *R. solani* are killed and decomposed by October when the full harvest takes place. Surviving beets with lower levels of infection become infected with other microorganisms that increase the decay of stored beets, and thus reduce crop yield and white sugar recovery [6].

In Minnesota and North Dakota, Rhizoctonia crown and root rot is managed by using a combination of tolerant cultivars, agronomic practices, and fungicide application [7]. Since most of the cultivars available lack complete resistance to *R. solani,* growers plant tolerant cultivars, plant as early as possible in cool conditions so that the most susceptible early growth stages avoid the pathogen, and use fungicides to help prevent infection in fields with a known history of the disease. In North Dakota and Minnesota, Quadris (azoxystrobin, active ingredient [a.i.], 22.9%; Syngenta, Greensboro, NC) is the most widely used fungicide for controlling Rhizoctonia root rot [7]. This strobilurin fungicide has protectant, curative, eradicant, translaminar and systemic properties. It prevents spore germination, mycelial growth, penetration of the fungus and has anti-sporulant properties [8].

In most field trials done to determine the efficacy of azoxystrobin for controlling *R. solani*, the fungicide is applied first followed by inoculation in the crown [9]. These treatments usually result in excellent control of *R. solani* because the pathogen gets killed from direct contact with the fungicide.

Prior to 2008, conventional sugarbeet was used that needed conventional herbicides and mechanical cultivation to manage weeds. As a result, infections by *R. solani* were believed to take place through the root or the upper part of the hypocotyl when infested soil was deposited within sugarbeet crown during weed control. By 2010, growers had rapidly adopted herbicide tolerant sugarbeet that was present on 95% of sugarbeet acreage in the US [10] which then became 100% after the technology was approved for use in California. Since glyphosate provides excellent control of weeds in herbicide tolerant sugarbeet, growers did not need to cultivate to assist in weed control resulting in no cultivation or a significant reduction in the number of cultivations. However, since 2009, growers in North Dakota and Minnesota listed Rhizoctonia as their worst production problem [11], even though fields were not cultivated. Most of the fields with Rhizoctonia were infected with root rot (*Personal observation by the corresponding author*).

Azoxystrobin was the fungicide most recommended to be applied to sugarbeet in an 18 cm foliar band at 167 g a.i./ha to 280 g a.i./ha for controlling *R. solani* [12,13]. Most growers use ground rig equipment to apply fungicides [7]. This means that wet field conditions, common in the spring, may adversely impact timing of fungicide application because the soil conditions do not allow for operation of a tractor and spray equipment. Most growers start spraying for foliar fungal diseases at first symptoms and have good to excellent disease control [7]. However, when symptoms are observed for Rhizoctonia root rot, it is too late to apply fungicides for effective control [14]. Growers need to be educated on the importance of timing of azoxystrobin application relative to the time of infection for effective disease control. There is

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currently no published research that illustrates or addresses this issue. As such, the objective of this study was to determine the best time to apply azoxystrobin relative to the time of inoculation for controlling root rot caused by *R. solani* AG 2-2 IIIB.

Materials and Methods

Trials were conducted at the North Dakoda State University greenhouse facility located in Fargo, North Dakoda (ND), USA. Three sugarbeet seeds of a susceptible cultivar (Proprietary material, Crystal Beet Seed, Moorhead, MN, USA) were sown in sunshine mix # 1 peat soil (Sun Gro Horticulture Canada Ltd., Canada) in 9.29 x 7.49 x 7.89 cm size pots. Plants were thinned at the two-leaf stage to allow one plant per pot. Plants were grown to the 4-leaf stage before treatment applications. Greenhouse conditions were set at 12 h photoperiod, and temperature was maintained at 27 ± 2°C. Sugarbeet plants were watered regularly to maintain the soil moisture essential for plant growth and pathogen development.

Inoculations were done using two barley grains colonized with *R. solani* AG 2-2 IIIB. Treatments included a non-inoculated control where no inoculum was applied to plants and an inoculated control where two grains of barley inoculum were placed in close proximity with plant roots at 2 cm below the soil surface, but no fungicide was applied. Other treatments were fungicide application as a hypocotyl drench at 0, 3, 10, 14 and 21 days after inoculation and inoculation application at 0, 7, 14, 21 and 28 days after fungicide application as a hypocotyl drench. The fungicide used was azoxystrobin, applied at the recommended rate of 0.67 L/ha. Approximately ~96 μl of fungicide solution was applied to each plant.

The experiment layout was a complete randomized design (CRD) with twelve treatments. There were four replicates with one plant per replicate. The experiment was repeated three times. Fourteen days after final fungicide application, plants were removed from pots, washed and roots were rated for root rot disease severity using a modified 0-7 rating scale [15]. The scale indicates: 0= healthy roots with no lesions; 1=crown area slightly scurfy; 2=<5% infection; 3=6-25% infection; 4=26-50% infection; 5 = 51-75% infection; 6=>75% infection; and 7=the root completely deteriorated or dead plant.

Root rot ratings were analyzed and transformed into relative effects using the non-parametric PROC RANK and MIXED procedures of SAS (version 9.3, SAS Institute Inc., Cary, NC).

Results

For all non-inoculated control plants, their roots were healthy with the lowest relative effect (0.2396) whereas the inoculated control had the most severe disease with the highest effect (0.8264), indicating that the *R. solani* inoculum was effective at causing infection (Table 1).

Generally, sugarbeet plants treated first with azoxystrobin followed by inoculation had lower root rot severity than those plants inoculated then treated with azoxystrobin. All plants treated with azoxystrobin had significantly lower root rot severity than the inoculated control except two treatments where azoxystrobin was applied 14 and 21 days after inoculation ($p<0.05$). Plants treated with azoxystrobin followed by inoculation at 7, 14, 21 and 28 days had similar relative effects of disease severity as the non-inoculated control. No significant differences in root rot severity were observed between plants receiving azoxystrobin and plants being inoculated with *R. solani* on the same day (within 2 hours between fungicide application and inoculation). Among the plants that were inoculated then treated with azoxystrobin at different timings, the plants receiving azoxystrobin at 0 day had significantly lower root rot severity than those plants treated with the fungicide at 3 and 10 days. Plants treated with the fungicide at 3 and 10 days had significantly smaller root rot severity than plants treated with the fungicide at 14 and 21 days. In contrast, the root rot severity among plants first treated with azoxystrobin and then inoculated was not significantly different from each other (Table 1).

All treatments receiving azoxystrobin followed by inoculations had zero median root rot severities (i.e. with <1% of the root area with visible lesions) (Table 1). Sugarbeet plants treated with azoxystrobin before inoculations also showed no above ground symptoms such as yellowing or wilting of leaves.

Discussion

Timing of fungicide application is crucial for the control of

Table 1: Median disease severity, relative effect, and its confidence interval for the disease severity caused by *R. solani* 2-2 IIIB on sugar beet treated with azoxystrobin (0.67 L/ha) at pre- and post-inoculations.

Treatment	Median disease rating[b]	Estimated relative effect (P)*	CI(95%) for p[a]	
Inoculation followed by azoxystrobin at:			**Lower limit**	**Upper limit**
0 days (2 hours)	7	0.4731	0.3591	0.5905
3 days	7	0.6855	0.5522	0.7895
10 days	7	0.6800	0.5455	0.7856
14 days	7	0.7963	0.7534	0.8318
21 days	7	0.7934	0.7478	0.8309
azoxystrobin followed by inoculation at:				
0 days (2 hours)	0	0.3452	0.2692	0.4322
7 days	0	0.2642	0.2181	0.3178
14 days	0	0.3134	0.2480	0.3893
21 days	0	0.3189	0.2491	0.4002
28 days	0	0.2642	0.2181	0.3178
Non-inoculated check	0	0.2396	0.2132	0.2688
Inoculated check	7	0.8264	0.8019	0.8476

[a]CI: Confidence interval. Relative treatment effects were calculated by performing non-parametric one-way analysis.
[b]Median disease rating: Disease severity ratings were determined using a scale of 0 to 7.

Rhizoctonia root rot of sugarbeet under conditions favorable for infection by *R. solani*. The *R. solani* isolate used in the study is known to be aggressive on sugarbeet [4]. This study demonstrated that it is difficult to control *R. solani* after infection takes place. Azoxystrobin prevented the fungus from quickly causing complete root damage when it was applied 3 and 10 days after inoculation. This was possibly due to the fungicide reducing mycelial growth [16]. Similar results were obtained in field trials conducted in Nebraska and Wyoming when foliar application of azoxystrobin was made at the time of crown inoculation and one week after inoculation [17]. Azoxystrobin applied 2 to 3 weeks after inoculation did not protect sugarbeet plants because infection by *R. solani* AG 2-2 IIIB had probably already occurred. This suggests that azoxystrobin does not have curative effects for *R. solani* infections which is consistent with research reported by Windels and Brantner [18].

Azoxystrobin application prior to inoculation was more efficient in controlling root rot disease severity in this study. Azoxystrobin provided effective protection even when applied 28 days before inoculation. Strobilurin fungicides have systemic properties once taken up by plants with a residual period of 7-21 days [13]. Protection lasting beyond 21 days could be due to the fungicide stimulating the plants defense mechanism to provide protection against the pathogen. Sugarbeet plants produce large amounts of pectin lyase inhibitor protein that inhibits pectin lyase produced by *R. solani* [19]. The pectin lyase produced by *R. solani* was reported to be responsible for pathogenicity in sugarbeet cultivars [20]. Windels and Brantner [18] postulated that sugarbeet plant defense responses to *R. solani* may have been triggered when plants are exposed to azoxystrobin and *R. solani* inoculum under favorable conditions suitable for infection.

In this research, azoxystrobin was applied as a hypocotyl drench to control Rhizoctonia root rot. This targeted method of fungicide application may have contributed to better protection of the vulnerable root and hypocotyl to the fungus. *R. solani* is known to cause infection when the soil temperature at the 10 cm depth goes above 16^{o}C [21]. In areas where *R. solani* is endemic and fungicides are necessary for control, our study suggests that azoxystrobin will provide protection against *R. solani* for 28 days when applied before conditions are favorable for infection.

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