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Penthiopyrad applied in close proximity to *Rhizoctonia solani* provided effective disease control in sugar beet

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ABSTRACT

Rhizoctonia solani Kühn is an important pathogen of sugar beet (*Beta vulgaris* L.) that can cause damping-off and crown and root rot. Commercial cultivars which are highly resistant to the pathogen are not as high yielding as susceptible cultivars under low or absent disease pressure. These resistant cultivars often do not have resistance to other common pathogens such as *Aphanomyces cochlioides*, *Cercospora beticola*, and *Fusarium oxysporum*. Fungicides, such as azoxystrobin which belongs to the quinone outside inhibitors (QoI) class, are necessary for controlling *Rhizoctonia solani*, but there are concerns about the buildup of fungicide-resistant strains in the targeted pathogen population. There is a need to find effective fungicides from different chemical groups so they can be rotated with the current widely-used azoxystrobin to manage *R. solani*. The objective of this greenhouse study was to evaluate the efficacy of penthiopyrad, a succinate dehydrogenase inhibitor (SDHI), in managing *R. solani* on sugar beet using three different application methodologies. Penthiopyrad effectively controlled *R. solani* on sugar beet when applied at 210, 280, 420, or 550 g a.i./ha in-furrow at planting and as a soil drench at the 4-leaf stage. However, foliar application of penthiopyrad failed to provide disease control. These trials indicated that penthiopyrad needs to be in close proximity or direct contact with *R. solani* in the soil to provide effective control. Penthiopyrad has the potential to be used as an effective alternate partner with azoxystrobin for controlling *R. solani* and to help in mitigating the development of fungicide resistant isolates of *R. solani*.

1. Introduction

Sugar beet (*Beta vulgaris* L.) is grown in 50 countries as a primary source of sucrose and provides 20% of the world's sugar production (FAO, 2014). In 2013, the European Union was the world's largest sugar beet producer with a total production of 108.9 million metric tons, followed by the Russian Federation with 39.3 million metric tons, and the United States with 29.7 million metric tons (FAO, 2013). The United States has ten major sugar beet producing states including California, Colorado, Idaho, Michigan, Minnesota, Montana, Nebraska, North Dakota, Oregon, and Wyoming. Minnesota and North Dakota contributed 56% of the nation's sugar beet production (USDA-ERS, 2015), which resulted in $4 billion worth of total economic activities (Bangsund et al., 2012).

Rhizoctonia solani Kühn [Teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk], is considered to be one of the most devastating pathogens in sugar beet production in the USA including Minnesota and North Dakota (Whitney and Duffus, 1986). This fungus is comprised of different genetically isolated populations recognized as anastomosis groups (AGs) (Ogoshi, 1987). Several AGs were reported to cause root rot of sugar beet including AG-2-2 and AG-4 (Stojsin et al., 2011; Windels and Nabben, 1989).The subgroups of *Rhizoctonia solani* AG 2-2 IIIB and IV are the major causal agents of damping-off of and crown and root rot of sugar beet and are widely distributed in the Red River Valley of North Dakota and Minnesota (Brantner and Windels, 2007). Entire sugar beet fields can be destroyed if the diseases caused by *R. solani* are not managed (Khan et al., 2010; Windels and Brantner, 2005).

Crop rotation with wheat (*Triticum aestivum* L.) and barley (*Hordeum Vulgare* L.) is recommended to reduce the pathogen inoculum in the field rather than susceptible hosts such as soybean (*Glycine max* (L.) Merr.) and corn (*Zea mays* L.) susceptible to AG 2-2 IIIB (Engelkes and Windels, 1996; Ithurrart et al., 2004). For many

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years, Rhizoctonia-resistant cultivars were not widely used because of their lower potential yield compared to susceptible commercial cultivars (Panella and Ruppel, 1996). Growers typically used high yielding cultivars which were more susceptible to *R. solani* and relied on fungicides to protect sugar beet fields with a history of the disease. Azoxystrobin (Quadris®, Sygenta; Greensboro, NC, USA), a quinone outside inhibitor (QoI), was labeled for use on sugar beet in 1999 (Secor et al., 2010). Azoxystrobin effectively controls *R. solani* on sugar beet when it is applied in a timely manner, that is, before infection takes place (Khan et al., 2010; Kiewnick et al., 2001; Windels and Brantner, 2005). Azoxystrobin was one of the most widely used fungicides in Minnesota and North Dakota for controlling *R. solani* on sugar beet (Carlson et al., 2012). Another QoI fungicide, pyraclostrobin (Headline®, BASF; Research Triangle Park, NC, USA), was registered for use on sugar beet and used to control *Cercospora beticola* Sacc. in 2003 (http://www.epa.gov/pesticide-registration/conditional-registration-status-2000-2014). In 2009, growers also started using pyraclostrobin to control *R. solani* (https://www3.epa.gov/pesticides/chem_search/ppls/007969-00186-20091007.pdf).

QoI fungicides inhibit mitochondrial respiration in fungi by binding to the quinol site of the cytochrome bc1 complex, blocking electron transfer and halting ATP synthesis (Balba, 2007). Due to the specific single site mode of action, the widespread and continuous use of QoI fungicides without rotation with other modes of action is not recommended since this can result in the development of fungicide resistant fungal populations and fungicide failures (Brent and Hollomon, 2000). Isolates of *Cercospora beticola* from sugar beet fields have been reported to be less sensitive to QoI fungicides due to G143A mutation (Kirk et al., 2012; Bolton et al., 2013). Compared to the polycyclic air-dispersed pathogen *C. beticola*, *R. solani* was considered as having a low risk of development of resistance against fungicides (FRAC, 2014). Azoxystrobin-resistant isolates of *R. solani* AG 1-IA on rice were reported from a field failure in Louisiana in 2011 (Olaya et al., 2012) and AG-2-2 IIIB from turfgrass (Blazier and Conway, 2004). It would be useful to have fungicides with different modes of action that can effectively manage *R. solani* on sugar beet and be used in rotation with QoI fungicides to prolong the usefulness of fungicides.

The succinate dehydrogenase inhibitor (SDHI) fungicides were reported to provide effective control of rice sheath blight caused by *R. solani* AG 1-IA (Chen et al., 2014). It would be useful to determine whether a chemical with a different mode of action, such as penthiopyrad (Vertisan®, Dupont; Crop protection, Wilmington, DE, USA), is able to manage *R. solani* without being phytotoxic to sugar beet, so that it can be considered as a rotating chemical for QoI fungicides. The objective of this greenhouse study was to evaluate the efficacy of penthiopyrad using different application methodologies for management of *R. solani* on sugar beet.

2. Materials and methods

2.1. Greenhouse conditions

Research was conducted in the Agricultural Experiment Station at North Dakota State University, Fargo, ND, USA. Crystal 539RR, a sugar beet cultivar susceptible to *R. solani* (Niehaus, 2011) was used in this research. Sugar beet was grown in plastic trays measuring 27 × 13 × 13 cm and plastic pots (T. O. Plastics Inc.; Clearwater, MN, USA) measuring 10 × 10 × 12 cm, which were filled with peat mix (Sunshine mix 1, Sun Gro Horticulture Ltd.; Alberta, Canada). *R. solani* AG 2-2 IIIB (obtained from Dr. Carol Windels, University of Minnesota, Northwest Research and Outreach Center, Crookston, MN, USA) was grown on sterilized barley grains for inoculum production as described by Gaskill (1968) and modified by Noor and Khan (2015). Three experiments were conducted to evaluate the efficacy of penthiopyrad (Vertisan, 20.6 EC), compared to azoxystrobin (Quadris, 22.9 F) which is considered as the industry's standard, for controlling *R. solani* on sugar beet using in-furrow (experiment 1), band (experiment 2), or soil drench (experiment 3) applications. In all experiments, penthiopyrad was used at 550, 420, 280, and 210 g a.i. ha^{-1} and azoxystrobin was applied at 167 g a.i. ha^{-1}. The greenhouse conditions were set to allow for a 12-h photoperiod and temperature was maintained at 22 ± 2 °C (Argus Control Systems Ltd.; British Columbia, Canada). Sugar beet plants were watered daily to maintain adequate moisture favorable for plant growth and disease development.

2.2. In-furrow application

In experiment 1, a 2.5 cm deep furrow was made in the center of each tray (27 × 13 × 13) into which 10 seeds were spaced evenly. Fungicides were applied directly over the seeds using a spraying system (De Vries Manufacturing; Hollandaise, MN, USA) calibrated to deliver 47 L ha^{-1} solution at 138 kPa through a single flat fan nozzle (4001E). After fungicide application, inoculation was done by using a tweezer (VWR; Chicago, IL, USA) to place one *R. solani*-infested barley grain 1 cm away from each seed (Noor and Khan, 2015). The positive control was inoculated with *R. solani*-infested barley grains while the negative control was inoculated with sterilized barley grains without *R. solani*; the controls had no fungicide treatment. The seeds and inoculum were then covered with peat mix. This experiment was repeated three times as a randomized complete block design (RCBD) with four replicates. Sugar beet survivors (not completely dead) were counted at 28 days after inoculation (DAI), and their roots were carefully removed from trays, washed under running tap water, and evaluated for root rot symptoms present on the tap root. Root rot symptoms (Harveson et al., 2009) were evaluated using a 0 to 7 scale: 0 (no disease), 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) (Hecker and Ruppel, 1977; Scholten et al., 2001).

2.3. Band application

In experiment 2, three seeds were planted 2 cm deep in each pot and thinned at the 2-leaf growth stage to allow one vigorous seedling per pot. When plants were at the 4-leaf growth stage, fungicides were applied in an 18-cm band using the spraying system as described above, followed by inoculation using a single *R. solani* colonized barley grain which was placed 1 cm away and at a depth of 2 cm from each plant and covered with peat mix. The positive and negative controls were set up by inoculating with infested or sterilized barley grains without the fungicide treatment, respectively. This experiment was repeated twice in a completely randomized design (CRD) with six replicates. At 21 DAI, plants were carefully removed from the pots, and their roots were washed and evaluated according to the disease scale as describe in experiment 1.

2.4. Soil drench application

In experiment 3, 4-leaf stage sugar beet plants grown as for the band-application experiment were used. The treatments (1 ml of fungicide solution) were injected with a syringe (HSW Norm-Ject; Dudley, MA, USA) into the soil-hypocotyl interface at 1 cm depth and 0.5 cm away from the sugar beet plant. The amount of fungicide solution for each plant was determined based on the active ingredient per hectare divided by the total number of sugar beet plants