Combining Penthiopyrad with Azoxystrobin is an Effective Alternative to Control Seedling Damping-off Caused by *Rhizoctonia solani* on Sugar Beet

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Abstract:

The soil-borne fungus *Rhizoctonia solani* causes damping-off on sugar beet seedlings. Growers rely on fungicides to protect sugar beet in fields affected by *R. solani*. Quinone outside inhibitor (QoI) fungicides, such as azoxystrobin, have been applied as in-furrow and foliar sprays to manage *R. solani,* but repeated use of QoI fungicides pose risks in fungicide resistance. Penthiopyrad is a novel fungicide with the succinate dehydrogenase inhibitor (SDHI) mode of action. The objectives of this study were to compare the efficacy of penthiopyrad used as a sole seed treatment versus azoxystrobin as an in-furrow or a post-planting application for controlling *R. solani*; to determine if a penthiopyrad seed treatment combined with azoxystrobin as a post-planting application can improve control of *R. solani* over sole penthiopyrad seed treatment, azoxystrobin in-furrow or post-planting spray application. Seedling survival rate and area under disease progress curve (AUDPC) for seedling loss rate were used to measure the efficacy of each treatment. A sole penthiopyrad seed treatment at 14 g a.i. kg^{-1} of seeds, and penthiopyrad seed treatments at 7 and 14 g a.i. kg^{-1} of seeds combined with one azoxystrobin in-furrow application 14 days after planting resulted in similar seedling survival rate and AUDPC as achieved with the standard azoxystrobin in-furrow application. However, post-planting foliar spray of azoxystrobin alone failed to control seedling damping-off. Our research suggests that penthiopyrad can be used as a seed treatment to provide early protection to vulnerable seedlings while azoxystrobin can be used as a post-planting application to protect the ensuing adult plants.

Keywords: *Beta vulgaris*, Fungicides, Rhizoctonia damping-off, Sugar beet

1. Introduction

Sugar beet (*Beta vulgaris* L.) is mainly grown in temperate regions and provides 14% of the world's sucrose production (Draycott, 2006; FAOSTAT, 2017). The United States (U.S.) is the world's fourth largest producer of sugar beet, preceded by the Russian Federation, France, and Germany (FAOSTAT, 2017). In 2019, 33 million tonnes of sugar beets were harvested from approximately 450,000 ha in eleven U.S. states - Michigan, Minnesota, North Dakota, Colorado, Montana, Nebraska, Wyoming, California, Idaho, Oregon, and Washington (USDA-ERS, 2019). Minnesota and North Dakota accounted for 50.9% of the total U.S. sugar beet production (USDA-ERS, 2019), where the sugar beet industry generates around $4.9 billion in total economic activities (Bangsund et al., 2012).

One of the most damaging pathogens to both sugar beet production and industry is a soil-borne basidiomycete *Rhizoctonia solani* Kühn [Teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk]. This fungus is a significant plant pathogen for many economic crops including sugar beet (Anderson, 1982; Ogoshi, 1987). Anastomosis groups (AGs) are used to classify various *R. solani* species based on the hyphal compatibility reaction, and some AGs can be divided into subgroups with their distinct host ranges and biochemical properties (Arakawa and Inagaki, 2014; Carling et al., 2002). *R. solani* AG 2-2 IIIB, AG 2-2 IV, and AG 4 cause severe damping-off of sugar beet seedlings, reducing plant stands in the early growing season, while AG 2-2 IIIB and AG 2-2 IV are also the most virulent isolates responsible for crown and root rot on mature sugar beet plants (Rush et al., 1994; Windels and Nabben, 1989). *R. solani* AG 2-2 IIIB was reported to be more aggressive than AG 2-2 IV on sugar beet seedlings and mature roots (Bolton et al., 2010; Engelkes and Windels, 1996) and considered a threat to sugar beet production in the U.S. and Europe (Garcia et al., 2001; Rush et al., 1994; Strausbaugh et al., 2011). The *R. solani*-caused

damage varies from field to field, but a significant yield loss (above 50%) can occur when warm and humid weather conditions favor disease development in the absence of effective management strategies (Khan et al., 2010; Windels and Brantner, 2005). Severely affected sugar beet roots result in increased respiration rates, sucrose losses, and inverted sugar accumulation during storage (Campbell et al., 2014).

The pathogen can be managed by rotating sugar beet fields at least every third year with non-host crops such as wheat (Buhre et al., 2009). However, this management option will largely limit crop choice for farmers since the AG 2-2IIIB strain of *R. solani* has a wide host range, including corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) (Engelkes and Windels, 1996; Ithurrart et al., 2004). Commercial sugar beet cultivars are typically resistant against crown and root rot (Panella et al, 2015; Ruppel and Heck, 1994), while a few germplasms are resistant against both seedling damping-off and crown and root rot caused by *R. solani* (Mcgrath et al., 2015; Nagendran et al., 2009). Panella and Ruppel (1996) reported that resistant cultivars had lower potential yields than susceptible commercial cultivars. Growers typically apply fungicides to protect sugar beet in fields affected previously by *R. solani*. Azoxystrobin (Quadris, Syngenta; Greensboro, NC, USA) is a quinone outside inhibitor (QoI) fungicide registered for use on sugar beet crops since 1999 (Secor et al., 2010). It has been used as an in-furrow application fungicide at planting and as a foliar spray during the growing season. Researchers have shown in field and greenhouse studies that the fungicide should be applied preventively and in a timely manner to consistently provide effective control of *R. solani* (Khan et al., 2017; Khan et al., 2010; Windels and Brantner, 2005). Azoxystrobin, now considered an industry standard for controlling *R. solani*, has been the most widely used fungicide in sugar beet producing states, including Michigan,

77 Montana, Minnesota and North Dakota (Carlson et al., 2012; Harveson et al., 2004; Kirk et al.,
78 2008; Hakk et al., 2015).

79 However, QoI fungicides typically have a high risk for the buildup of a fungicide-resistant
80 pathogen subpopulation due to their site-specific mode of action, particularly when used in fields
81 with consecutive or repeated applications (Vincelli, 2002). This is because QoI fungicides act at a
82 specific point to bind the Qo site of the cytochrome bc1 enzyme complex to block electron transfer
83 in the mitochondrial respiration pathway, halting ATP synthesis in the fungal organism (Balba,
84 2007). *R. solani* has been considered at low risk of development of resistance against fungicides
85 (FRAC, 2019), since it is typically a soil-borne pathogen with only one disease cycle per year with
86 limited sexual reproduction (Brent and Hollomon, 2007). However, this fungus has been reported
87 with the QoI fungicide resistance, as reported for *R. solani* AG 3 in potatoes (Djébali et al., 2014),
88 AG 2-2 IIIB in turf grass (Blazier and Conway, 2004), and 1-IA in rice fields (Olaya et al., 2012).
89 Furthermore, Arabiat and Khan (2016) reported that *R. solani* isolates from sugar beet fields
90 showed reduced sensitivity to QoI fungicides.

91 Penthiopyrad is the second generation of succinate dehydrogenase inhibitor (SDHI)
92 fungicides with broad fungicidal activity against both basidiomycetes and ascomycetes (Yanase et
93 al., 2013). Our previous research (Liu and Khan, 2016) showed that penthiopyrad applied as an in-
94 furrow application or soil drench in the proximity of sugar beet seeds provided effective reduction
95 of *R. solani* damping-off when in close proximity to the pathogen. In North Dakota, it is currently
96 registered for sugar beet as Kabina ST (Mitsui Chemicals; Minato-ku, Tokyo, Japan) for seed
97 treatment and as Vertisan (Dupont; Wilmington, DE, USA) for a soil application. Using
98 penthiopyrad as a seed treatment is convenient for growers to protect sugar beet seedlings from
99 Rhizoctonia seedling damping-off, because azoxystrobin used as an in-furrow application has been

limited in field practice (Quadris® Label, Syngeta; https://www.syngenta-us.com/current-label/quadris). Therefore, further research is needed to know how penthiopyrad seed treatment can be integrated with azoxystrobin used as a post-planting application to effectively control *R. solani* during the whole growing season and to maximize fungicide longevity by mitigating potential fungicide-resistant issues. The objectives of this study were: to compare the efficacy of penthiopyrad used as a sole seed treatment versus azoxystrobin as an in-furrow or a post-planting application for controlling *R. solani*; to determine if a penthiopyrad seed treatment combined with azoxystrobin as a post-planting application can improve control of *R. solani* over sole penthiopyrad seed treatment, azoxystrobin in-furrow or post-planting spray application.

2. Materials and Methods

2.1 Greenhouse Environments, Plant Material, and Inoculum Production

The research was conducted in the Agricultural Experiment Station greenhouse at North Dakota State University in Fargo, ND, USA. Greenhouse conditions were set to allow for a 16-h photoperiod, and temperature was maintained at 22 ± 2°C (Argus Control Systems Ltd.; British Columbia, Canada). Plants were watered daily to maintain adequate moisture for plant growth and disease development.

Two sugar beet commercial cultivars BTS 89RR10 and BTS 89RR50 used in this research were resistant and susceptible to *R. solani*, respectively (Niehaus, 2013). Sugar beet seeds were sown and grown in plastic trays measuring 27 x 13 x 13 cm (T. O. Plastics Inc.; Clearwater, MN, USA) filled with peat mix (Sunshine mix 1, Sun Gro Horticulture Ltd.; Alberta, Canada). *R. solani* AG 2-2 IIIB was used in this study, because few *R. solani* AG-4 isolates have been recently found in sugar beet fields in the U.S. (M.F.R. Khan, personal communication, October 2019). A single *R. solani* AG 2-2 IIIB isolate was obtained from Dr. Carol Windels (University of Minnesota,

Crookston, MN). It was maintained and pure cultures were grown on potato dextrose agar (PDA) and then used to produce the inoculum of *R. solani*-infested barley grains as described by Noor and Khan (2015).

2.2 Inoculation and Fungicide Treatments

A 2.5-cm depth furrow was made in the middle of each tray into which ten evenly spaced sugar beet seeds were planted. Azoxystrobin (Quadris, 22.9%, Syngenta) at 167 g a.i. ha^{-1} was applied as in-furrow directly over the sugar beet seeds in the in-furrow treatment, followed by *R. solani* inoculation. Sugar beet seeds of BTS 89RR10 and BTS 89RR50 were treated with penthiopyrad (Kabina ST, 40%, Mitsui, Japan) as a seed treatment at 5, 7, and 14 g a.i. kg^{-1} (one kg = 100,000 seeds) by Betaseed, Inc. (Tangent, OR, USA). Inoculation was done to place one *R. solani*-infested barley grain 1 cm to the side of each sugar beet seed (Noor and Khan, 2015). Sugar beet seeds in all treatments except the negative control were inoculated with the *R. solani*-infested barley grains at planting. After inoculation, seeds and inocula were covered with the peat soil. Each of these penthiopyrad seed treatments was also combined with azoxystrobin at 167 g a.i. ha^{-1} used as an 18-cm band application treatment on 2-leaf stage sugar beet seedlings at 14 days after planting. The fungicide azoxystrobin was delivered in a spraying booth (De Vries Manufacturing; Hollandaise, MN, USA) calibrated to deliver 47 L ha^{-1} at 138 kPa through a single flat fan nozzle (4001E).

In total, there were ten treatments as seen in Table 1 and below:

treatments 1, 2, 3: sole penthiopyrad-treated seeds at 5, 7, and 14 g a.i. kg^{-1} of seeds;

treatments 4, 5, 6: penthiopyrad-treated seeds at 5, 7, and 14 g a.i. kg^{-1} of seeds combined respectively with an 18-cm band application of azoxystrobin at 167 g a.i. ha^{-1} 14 days after planting;

146 treatment 7: azoxystrobin at 167 g a.i. ha^{-1} applied in-furrow directly over seeds without
147 penthiopyrad seed treatment at planting;
148 treatment 8: azoxystrobin at 167 g a.i. ha^{-1} sprayed on leaves in an 18-cm band 14 days
149 after planting seeds without penthiopyrad seed treatment;
150 treatment 9: positive control in which seeds were inoculated with *R. solani*-infested barley
151 grains without any fungicide use;
152 treatment 10: negative control in which seeds were inoculated with sterilized barley grains
153 without any fungicide use.
154 **2.3 Data Collection and Data Analysis**
155 Experimentation was conducted twice as a complete randomized design (CRD) with four
156 replicates. We observed and recorded total emerged seedlings, seedlings lost to damping off by *R.*
157 *solani* and heathy seedlings in each replicate at 10, 20, 30, and 45 days after planting. We used
158 seedling survival rate and seedling loss rate as indicators to measure seedling damping off
159 incidences. Seedling survival rate (%) was calculated as:
160 Seedling survival rate (%) = (Total healthy seedlings)/(Total emerged seedlings)*100
161 The seedling loss rate was calculated as:
162 Seedling loss rate (%) = (Total emerged seedlings – Total healthy seedlings)/(Total emerged
163 seedlings)*100
164 Finally, the areas under the disease progress curve (AUDPC) was calculated treating the seedling
165 loss rate as a measurement for seedling damping off incidence following Madden et al. (2007):
166 $$\text{AUDPC}= \sum_{i=1}^{n-1}[(y_i + y_{i+1})/2](t_{i+1} - t_i)$$
167 where y_i= seedling loss rate at the i*th* observation, t_i= time (days) at the i*th* observation, y_{i+1} =
168 seedling loss rate at the (i+1*th*) observation and t_{i+1} = time (days) at the (i+1*th*) observation, and n

= total points of observations. So, the calculated AUDPC for seedling loss rate acted as an indicator to measure the accumulative effects of damping off caused by *R. solani* AG 2-2 IIIB. Dead seedlings and infected plants were collected and re-isolated to confirm the presence of *R. solani* AG 2-2 IIIB to cause the death or infection.

The homogeneity for variances between the two experiments was tested by Levene's test before the data from the two experiments were combined. Analysis of variance (ANONA) was performed for the seedling survival rate and AUDPC. Means between treatments were separated using the post hoc test of Fisher's Least Significant Difference (LSD) at a statistically significant level $p= 0.05$. All the statistical analyses were conducted using the general linear model procedure (Proc GLM) in SAS (Version 9.3, SAS Institute Inc.; Cary, NC, USA).

3. Results

Data from two experiments of seedling survival rates and AUDPC were combined since their homogeneity test for variance was not significantly different (p=0.86 and p=0.89, respectively). No significant differences were found between the resistant and susceptible cultivars across all ten treatments on seedling survival rate (p=0.53) and AUDPC (p=0.77).

Figure 1 shows the seedling survival rate of each treatment at 30 days after inoculation. In the negative control, 94% of total seedlings emerged without the presence of seedling damping-off symptoms. As expected, seedling survival rate was significantly reduced by *R. solani* AG 2-2 IIIB and was the lowest in the positive control which indicated that the *R. solani*-infested barley grains worked effectively. Clearly, a band spray of azoxystrobin at 167 g a.i. ha^{-1} on leaves at 14 days after planting did not prevent the seedling damping off (p>0.05) and resulted in as low seedling survival rate as in the positive control (Figure 1). Compared with the positive control, all sole seed treatments at three levels of penthiopyrad and their respective

combination with azoxystrobin as post-planting application significantly increased seedling survival rate ($p<0.05$). However, when compared with the negative control, sole penthiopyrad seed treatment at 14 g a.i. kg^{-1} of seeds at planting, sole azoxystrobin in-furrow application at 167 g a.i. ha^{-1} at planting and combinations of seed penthiopyrad treatment at 7 and 14 g a.i. kg^{-1} with azoxystrobin application at 167 g a.i. ha^{-1} 14 days after planting were equally effective in protecting seedlings from damping off and thus these four treatments did not differ significantly from the negative control in seedling survival rate ($p>0.05$).

Figure 2 shows progress in the seedling loss rate because of damping-off caused by *R. solani* for each treatment. For both the positive control and the sole azoxystrobin treatment as an 18-cm band spray on leaves at 14 days after inoculation (DAI), the seedling loss rate progressed rapidly and the seedling loss rates reached approximately 90% at 45 DAI. For sole penthiopyrad treatments at 5, 7, and 14 g a.i. kg^{-1}, there was an accelerated increase of seedlings loss rate after 30 DAI. While penthiopyrad seed-treatments with azoxystrobin application at 14 days after planting showed consistently a slow progress seedlings loss rate throughout the experiment.

Table 1 shows the integral effects of each treatment on AUDPC for seedling loss rate (i.e. a measurement of the seedling damping off incidences). As expected, all emerged seedlings in the negative control survived without damping-off incidences and therefore, the AUDPC was zero. The positive control had the highest AUDPC followed by the sole azoxystrobin treatment with an 18-cm band spray of azoxystrobin at 167 g a.i. ha^{-1} on leaves at 14 days after inoculation, but both these treatments did not differ significantly ($p>0.05$). The standard in-furrow application of azoxystrobin at 167 g a.i. ha^{-1} at planting had the lowest AUDPC, but did not differ significantly from sole penthiopyrad seed treatment at 14 g a.i. kg^{-1} of seeds and the combinations of penthiopyrad seed treatments at 7 and 14 g a.i. kg^{-1} of seeds with azoxystrobin

at 167 g a.i. ha^{-1} applied at 14 days after inoculation (p>0.05). Sole penthiopyrad seed treatments at 5 and 7 g a.i. kg^{-1} of seeds and the combination of penthiopyrad seed treatments at 5 g a.i. kg^{-1} of seeds with azoxystrobin application at 167 g a.i. ha^{-1} 14 days after inoculation did not differ in AUDPC (p>0.05), but they had significantly larger AUDPC than the standard in-furrow application of azoxystrobin at 167 g a.i. ha^{-1} at planting (p>0.05).

4. Discussion

The resistant cultivar (BTS 89RR10) was not superior to the susceptible one (BTS89RR50) in increasing the seedling survival rate (p=0.53) or in reducing the AUDPC (p=0.77) caused by *R. solani* in this study. Two types of resistance in sugar beet germplasm against *R. solani* are known and depend on the plant age when infection takes place: seedling damping-off resistance and root rot resistance. Sugar beet resistance usually refers to root and crown rot resistance determined via screening plants after inoculation on mature sugar beet root. Gaskill (1968) reported that none of the sugar beet cultivars tested were resistant to seedling damping-off caused by *R. solani* inoculation at sugar beet planting. Few sugar beet germplasms have been reported to have the resistance to *R. solani* damping-off (Mcgrath et al., 2015; Nagendran et al., 2009), but this damping-off resistance was screened by inoculation at two- to four- true leaf stage instead of following Gaskill (1968) who conducted the inoculation at planting. Furthermore, these germplasms are not commercially available to sugar beet growers. By inoculation at planting, Liu et al. (2019) demonstrated that sugar beet seedlings of commercial cultivars were highly susceptible to *R. solani* until at four-weeks-old when plants were at about six leaf stage, even for the most resistant cultivars. In the current study, barley grains infested with *R. solani* placed at the time of planting also induced severe seedling damping-off incidences (i.e. high seedling loss rate) in the resistant cultivar (BTS 89RR10) in the positive control.

Early chemical protection is required for vulnerable sugar beet seedlings, especially when conditions are ideal for infection by soil borne fungi . *R. solani* is a ubiquitous soil-borne fungus overwintering as sclerotia and on infected root debris, which serves as initial inoculum to cause seedling damping-off in the next crop when soil temperatures become warm enough. According to Khan et al. (2010), the soil temperature threshold at which *R. solani* becomes infective to sugar beets is 18°C. In North Dakota and Minnesota, the average soil temperature can be expected to reach 18°C by May during which sugar beet drilling takes place (NDAWN; http://ndawn.ndsu.nodak.edu/). This temperature threshold, which generally occurs in May in northern sugar beet production areas in the US, indicates that *R. solani* surviving from the previous year could be active in the early growing season to cause damping-off to vulnerable sugar beet seedlings. Our study demonstrated that azoxystrobin applied in-furrow at 167 g a.i. ha^{-1} at planting provided excellent control of *R. solani* and seedling establishment achieved was comparable to that in the negative control. However, the sole use of azoxystrobin at the same rate applied as a post spray on seedling leaves at 14 days after planting led to losses of most seedlings to *R. solani*, with a similar poor seedling survival rate to that in the positive control. It was too late to spray azoxystrobin at 14 days after planting because most of the sugar beet seedlings would have been infected if inocula of *R. solani* were present. Thus, effective fungicides must be applied before *R. solani* infection takes place (Windels and Brantner, 2005).

Penthiopyrad used as seed treatment allows the chemical to be used at a low rate on the seed to protect the seedlings from *R. solani* infection. In the current study, penthiopyrad used as seed treatment at 14 g a.i. kg^{-1} of seeds was observed to be safe to sugar beet plants and effective against *R. solani* being comparable to the standard azoxystrobin applied in-furrow at planting. These results agreed with those of Windels and Brantner (2009). They found that a penthiopyrad

seed treatment at as high as at 84 g a.i. kg^{-1} of seeds was still effective 28 days post-applications of azoxystrobin in managing *R. solani* AG 4, AG 2-2 IV, and AG 2-2 IIIB, with no observed phytotoxicity.

In the current study, the efficacy of penthiopyrad seed treatment declined gradually with the decreasing dose. This could be due to the penthiopyrad treatment around seeds being washed off by daily watering over time, while the soil-borne fungus *R. solani* still survived in the soil. Penthiopyrad seed treatment can also be washed off by rainfall in fields and is likely to be degraded by the soil microbiome. In the current study, penthiopyrad seed treatments at 7 and 14 g a.i. kg^{-1} of seeds combined with an 18-cm band application of azoxystrobin at 167 g a.i. ha^{-1} 14 days after planting still maintained effectiveness in controlling *R. solani*. The effect of the azoxystrobin in-furrow application combined with penthiopyrad seed treatment possibly depended on whether penthiopyrad seed treatment could still effectively prevent infection for 14 days when *R. solani* is present, since a sole azoxystrobin sprayed at 167 g a.i. ha^{-1} on leaves in an 18-cm band 14 days after planting without penthiopyrad seed treatment at planting was not effective after the infection occurred. Combined with the azoxystrobin treatment, penthiopyrad at 7 g a.i. kg^{-1} of seeds performed better than at 5 g a.i. kg^{-1} of seeds, indicating that 5 g a.i. kg^{-1} might be too low to be effective against *R. solani* infection after 14 days. In future studies, discerning the period of penthiopyrad seed treatment effectiveness in the field will help growers when to apply azoxystrobin after the penthiopyrad-treated seeds are sown to achieve a satisfactory control of *R. solani* throughout the growing season.

Penthiopyrad is a SDHI fungicide and was shown here to be a viable seed treatment. When it is combined with an azoxystrobin post application, penthiopyrad can be a useful option for fungicide resistance management of *R. solani*. In North Dakota and Minnesota, QoI class

fungicides, azoxystrobin and pyraclostrobin have been used intensively as both in-furrow and post application by sugar beet growers to manage *R. solani* (Carlson et al., 2012). Azoxystrobin is still effective in controlling the pathogen on sugar beet, but recent concerns with the reduced sensitivity of *R. solani* isolates from sugar beet fields to QoI fungicides have been raised by Arabiat and Khan (2016). Thus, the continued use of QoI fungicide in a repeated manner may result in a *R. solani* population that cannot be adequately reduced in sugar beet fields, as has occurred in rice fields in Louisiana (Olaya et al., 2012). In addition, most growers are not using azoxystrobin in-furrow since the current label (https://www.syngenta-us.com/current-label/quadris) does not recommend the use of azoxystrobin in-furrow with a starter fertilizer at planting, a common practice by many producers in North Dakota, Minnesota, Michigan, Nebraska, Wyoming and Montana (Noor and Khan, 2015; *personal communication* Mohamed Khan), and when cool soil conditions will prevail after planting. In conclusion, it would be useful for sugar beet growers to have a SDHI fungicide such as penthiopyrad used as a seed treatment to provide early protection to vulnerable seedlings and provide further protection to the succeeding adult plants with a post-planting QoI azoxystrobin fungicide application. This combination of fungicide chemistries would maximize the effective life of fungicides through better fungicide resistance management while providing a longer period of control of *R. solani* in sugar beet fields.

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437 Table 1. Effects of seed treatments with penthiopyrad and their combination with azoxystrobin as
438 post-planting application on area under disease progress curve (AUDPC) calculated with
439 seedling loss rate as an indicator of seedling damping-off incidence caused by *R. solani* applied
440 at planting.

Treatment	Inoculation	Seed treatment (Penthiopyrad)	Azoxystrobin Applied at 167 g a.i. ha^{-1}	AUDPC*
1	Yes	5 g a.i. kg^{-1}		66bc
2	Yes	7 g a.i. kg^{-1}		63c
3	Yes	14 g a.i. kg^{-1}		31e
4	Yes	5 g a.i. kg^{-1}	As 18-cm band 14 days after planting	53cd
5	Yes	7 g a.i. kg^{-1}	As 18-cm band 14 days after planting	24e
6	Yes	14 g a.i. kg^{-1}	As 18-cm band 14 days after planting	35de
7	Yes		As In-furrow at planting	21ef
8	Yes		As 18-cm band 14 days after planting	87ab
9	Yes			97a
10	No			0f

441

442 *Means of AUDPC that contain a common letter were not significantly different using the post hoc
443 test of Fisher's Least Significant Difference (LSD) which was calculated with a statistically
444 significant level at p=0.05.

445

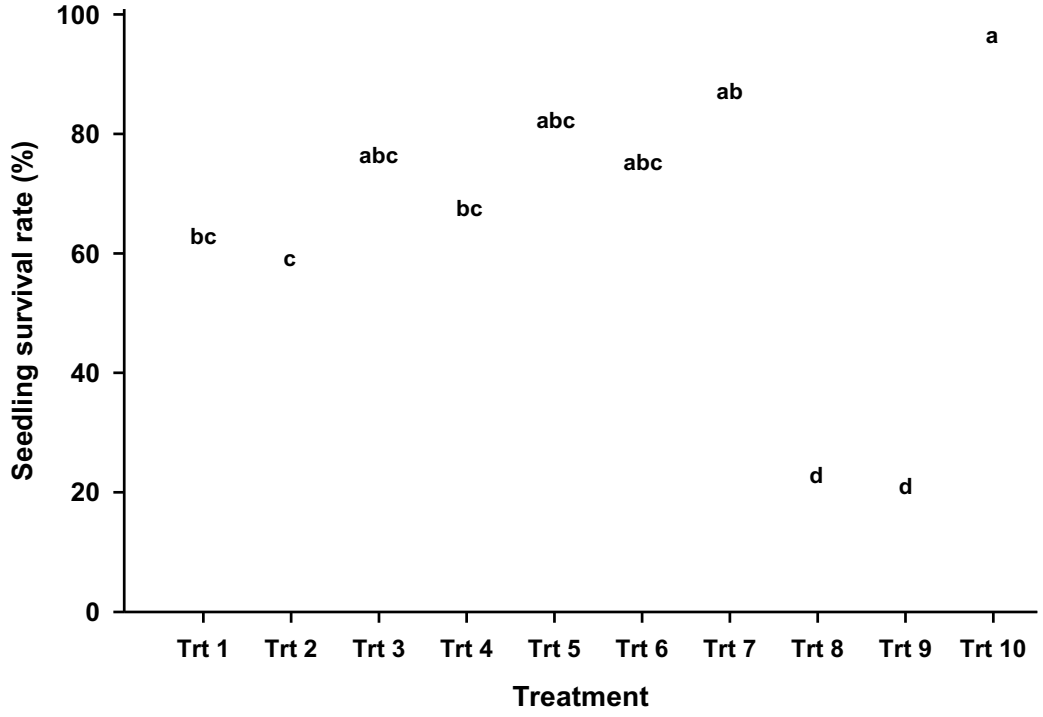

Figure 1 Effects of seed treatments with penthiopyrad and their combination with azoxystrobin as post-planting application on seedling survival rate (%) in response to *R. solani* 30 days after inoculation. Mean values of seedling survival rates on the bar chart that contain a common letter were not significantly different using the post hoc test of Fisher's Least Significant Difference (LSD) which was calculated with a statistically significant level at p=0.05.

Treatment 1, 2, 3: sole penthiopyrad-treated seeds respectively at 5, 7, and 14 g a.i. kg^{-1} of seeds; treatment 4, 5, 6: penthiopyrad-treated seeds at 5, 7, and 14 g a.i. kg^{-1} of seeds respectively combined with an 18-cm band application of azoxystrobin at 167 g a.i. ha^{-1} 14 days after planting; treatment 7: azoxystrobin applied in-furrow at 167 g a.i. ha^{-1} directly over seeds without penthiopyrad seed treatment at planting; treatment 8: azoxystrobin sprayed at 167 g a.i. ha^{-1} on leaves in an 18-cm band 14 days after planting without penthiopyrad seed treatment at

planting; treatment 9: positive control in which seeds were inoculated with *R. solani*-infested barley grains without fungicide use; and treatment 10: negative control in which seeds were inoculated with sterilized barley grains without fungicide use.

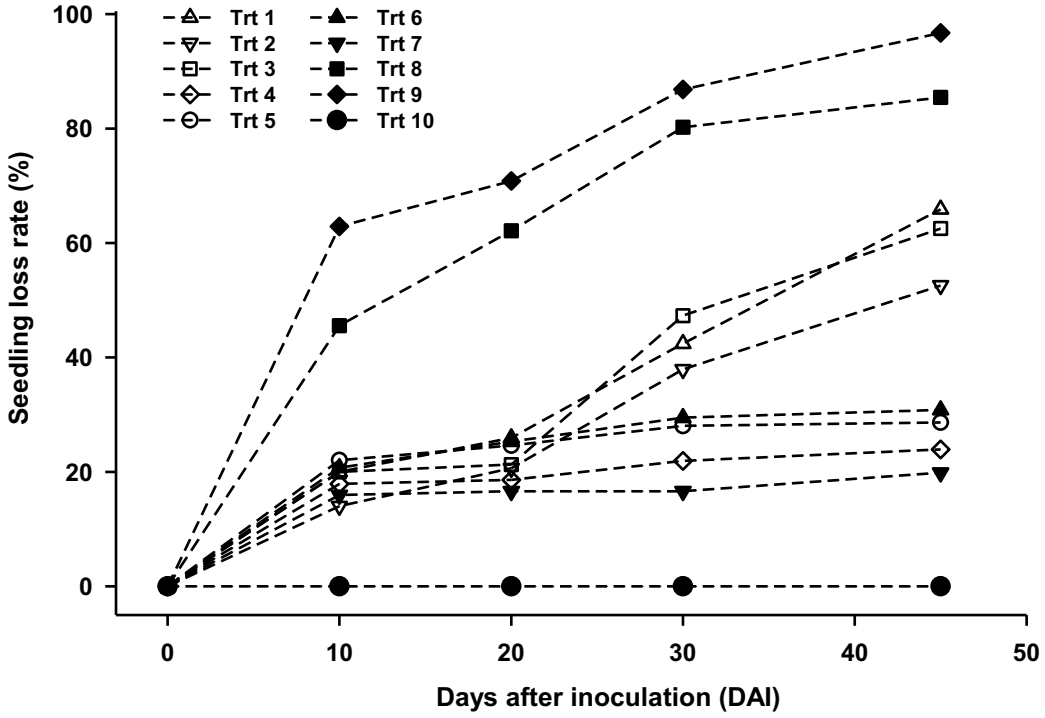

Figure 2 Rate of progress with time in seedling loss caused by *R. solani* damping-off.

Treatment 1, 2, 3: sole penthiopyrad-treated seeds respectively at 5, 7, and 14 g a.i. kg^{-1} of seeds; treatment 4, 5, 6: penthiopyrad-treated seeds at 5, 7, and 14 g a.i. kg^{-1} of seeds respectively combined with an 18-cm band application of azoxystrobin at 167 g a.i. ha^{-1} 14 days after planting; treatment 7: azoxystrobin applied in-furrow at 167 g a.i. ha^{-1} directly over seeds without penthiopyrad seed treatment at planting; treatment 8: azoxystrobin sprayed at 167 g a.i. ha^{-1} on leaves in an 18-cm band 14 days after planting without penthiopyrad seed treatment at planting; treatment 9: positive control in which seeds were inoculated with *R. solani*-infested

barley grains without fungicide use; and treatment 10: negative control in which seeds were inoculated with sterilized barley grains without fungicide use.