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Optimizing fungicide delivery methods for management of *Rhizoctonia solani* on sugar beet

^{1,2}Sushmita Kalika-Singh, ¹Sunil Bhandari, ¹Mayowa Aderoju, ¹Luis E del Río Mendoza and ¹Mohamed Khan

¹Department of Plant Pathology, North Dakota State University, Fargo, North Dakota, United State

²Department of Biology, University of Guyana, Berbice Campus, Corentyne, Guyana, South America

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Corresponding Author: Sushmita Kalika-Singh

Abstract

Root rot caused by *Rhizoctonia solani* is a major constraint to sugar beet (*Beta vulgaris* L.) production, leading to stand losses and significant yield reductions in regions such as the Red River Valley of North Dakota and Minnesota. Fungicides remain an important tool within integrated management programs; however, the effect of application method on their efficacy is not well documented. This study evaluated four fungicides: azoxystrobin (Quadris), prothioconazole (Proline), penthiopyrad (Vertisan), and fluxapyroxad + pyraclostrobin (Priaxor), applied using three methods: foliar spray (with soil protected), tray application beneath pots, and soil drench. Greenhouse experiments were conducted using a split-plot design with a susceptible sugar beet cultivar inoculated at the four-leaf stage. Disease severity was rated 28 days after inoculation using a 0–7 categorical root rot scale. Data was analyzed using non-parametric statistical methods. A significant interaction between fungicide and application method ($P < 0.05$) was detected. Quadris applied as a soil drench consistently reduced disease severity (mean = 3.9), whereas foliar and tray applications were less effective. These findings highlight the importance of application method in fungicide performance and support soil drench delivery, particularly with azoxystrobin, as an effective strategy for managing *R. solani* in sugar beet.

Keywords: Management, azoxystrobin, proline, priaxor, vertisan

Introduction

Sugar beet (*Beta vulgaris* L.) is an economically important crop in the United States where production is concentrated in northern states. Minnesota and North Dakota consistently lead national output, producing about 54% of the annual crop ^[1,2]. Despite its value, sugar beet production faces numerous challenges from soilborne and foliar diseases, among which *R. solani* is particularly destructive in the Red River Valley region ^[3].

R. solani causes seedling damping-off and crown and root rot, often resulting in severe stand and yield losses under favorable conditions ^[4,5]. The pathogen is genetically diverse, with anastomosis groups (AGs) differing in virulence and host range. In sugar beet, AG 2-2 IIIB and AG 2-2 IV predominate, with AG 2-2 IIIB being more aggressive and infecting additional crops, including dry beans and corn ^[6].

Disease management relies on an integrated approach combining cultural practices, partially resistant cultivars, and fungicides ^[7,8]. Fungicides are particularly important under high disease pressure, but their effectiveness can vary by chemistry and application method. Azoxystrobin (Quadris), a quinone outside inhibitor (QoI; FRAC 11), is widely used because of its strong preventive activity ^[4,5]. However, repeated QoI use increases the risk of resistance,

as seen in *R. solani* AG 1-IA populations in rice ^[9].

To delay resistance and improve control, fungicides with different modes of action are incorporated into management programs. These include penthiopyrad (Vertisan; SDHI, FRAC 7), prothioconazole (Proline; DMI, FRAC 3), and fluxapyroxad + pyraclostrobin (Priaxor; SDHI + QoI, FRAC 7 + 11), which target distinct biochemical pathways and can be rotated or tank-mixed to reduce selection pressure ^[10]. While their efficacy against *R. solani* has been demonstrated, comparative studies of application techniques remain limited.

Fungicides in sugar beet are applied as seed treatments, as in-furrow or banded soil applications, and occasionally as foliar sprays. Each method offers distinct advantages and limitations: seed treatments provide early protection but limited short residual activity; in-furrow placement targets the root zone but requires precise application; banded sprays protect later growth stages but may miss early infections ^[4]. While in-furrow azoxystrobin and seed-applied SDHIs have shown efficacy ^[11,12], less is known about the performance of foliar or post-emergence applications and their potential to suppress soilborne inoculum, particularly under variable environmental conditions.

Given the economic impact of *R. solani* and the need for sustainable management, optimizing fungicide placement is

critical. This study assessed the performance of four fungicides: azoxystrobin, penthiopyrad, prothioconazole, and fluxapyroxad + pyraclostrobin, applied by different methods under controlled greenhouse conditions. The objective was to determine which fungicide–application combinations most effectively suppress *R. solani* in sugar beet while reducing the risk of resistance development.

Materials and Methods

Greenhouse Evaluation and Experimental Design

Greenhouse experiments were conducted at the Jack Dalrymple Agricultural Research Complex, North Dakota State University, to evaluate fungicide application strategies for managing *R. solani*. Conditions were maintained at $22 \pm 2^\circ\text{C}$. A susceptible sugar beet cultivar (Hilleshog) was used, with three seeds sown per pot ($10 \times 10 \times 12$ cm) in a peat-based medium (Sunshine Mix 1, Sun Gro Horticulture, Alberta, Canada). Pots were watered as needed, and seedlings were thinned to one healthy plant per pot at emergence. Plants were grown to the four-leaf stage prior to inoculation and treatment. The study followed a split-plot design with nine replications, and the entire experiment was conducted twice.

Inoculation and Fungicide Application

Barley-based inoculum of *R. solani* AG 2-2 IIIB was prepared using actively growing isolates following the method of [11]. At the four-leaf stage, each sugar beet plant was inoculated by placing a single colonized barley grain 1 cm from the root and 2 cm below the soil surface.

Fungicide efficacy was evaluated using three application methods (whole plots) applied prior to inoculation: (1) foliar spray with soil covered by aluminum foil to prevent soil contact, (2) tray application with fungicide solution delivered to trays beneath the pots, and (3) soil drench near the soil-hypocotyl interface. All fungicides were applied at recommended post-emergence field rates, dissolved in 1 L of water.

Four fungicides (split plots) representing different FRAC groups were tested: azoxystrobin (22.9% a.i.; Quadris, QoI) at 1133 mL ha^{-1} , prothioconazole (41% a.i.; Proline, DMI) at 417 mL ha^{-1} , penthiopyrad (20.6% a.i.; Vertisan, SDHI) at 1023 mL ha^{-1} , and a premix of fluxapyroxad + pyraclostrobin (28.6% a.i. each; Priaxor, SDHI + QoI) at 584 mL ha^{-1} (8 fl oz a⁻¹).

Foliar sprays were delivered to leaves using a calibrated spraying system (De Vries Manufacturing, Hollandale, MN, USA) at 138 kPa with a TeeJet 8001E nozzle. For tray applications, 1 L of solution was divided equally between two trays (30×24 cm) beneath the pots, allowing root uptake; excess solution was discarded after soil saturation. For drench applications, 1 mL of solution was applied per plant at 1 cm soil depth using a syringe, corresponding to 98.8 L ha^{-1} based on a population of 98,800 plants ha⁻¹. Plants were watered 24 h after treatment and inoculation, then irrigated daily.

Disease Assessment

Rhizoctonia crown and root rot severity was evaluated 28 days post-inoculation. Plants were carefully uprooted, and tap roots were gently washed under running water to remove soil. Disease severity was scored using a 0–7 scale, where 0 = no visible symptoms, 1 = slightly scurfy crown, 2 = <5% tissue infection, 3 = 6–25% infection, 4 = 26–50% infection, 5 = 51–75% infection, 6 = >75% infection, and 7 = completely deteriorated root or dead plant [13].

Data Analyses

Data were analyzed in SAS 9.4 (Cary, NC, USA). Disease severity was evaluated on a 0–7 scale [13]. To assess the effects of fungicides, application methods, and their interaction on *R. solani* severity, both parametric and non-parametric approaches were used due to the categorical nature of the rating scale. In both approaches, severity scores were ranked in ascending order, with the lowest disease severity receiving the lowest rank. For the parametric analysis, Levene's test was first conducted to assess the homogeneity of variances of ranks. A combined analysis of variance (ANOVA) was then performed using the GLIMMIX procedure in SAS, with fungicide, application method, and their interaction as fixed effects, and trial and replication as random effects. Treatment mean ranks were compared using the Tukey-Kramer post hoc test at $P = 0.05$.

For the non-parametric analysis, ranks were used to calculate treatment relative effects following Shaw and Madden (2004). Ranks were assigned across all treatment-replication combinations, and the F1_LD_F1 and LD_CI SAS macros were employed. The F1_LD_F1 macro tested the significance of main effects (whole-plot and split-plot) and their interaction, while the LD_CI macro was used to compute 95% confidence intervals for the split-plot main effects and the whole-plot $\times$ split-plot interaction [14].

Results

Levene's test confirmed homogeneity of variances ($P = 0.9235$), allowing a combined analysis of variance (ANOVA). The ANOVA indicated that fungicide application method, fungicide type, and their interaction were all significant ($P = 0.0172$, <0.0001 , and <0.0001 , respectively; Table 1). The significant interaction was driven primarily by the drench application. Foliar and tray applications of all fungicides were largely ineffective and did not differ from the inoculated, non-protected control ($P > 0.05$). In contrast, drench applications significantly reduced disease severity, with Quadris showing the lowest mean rank ($P = 0.05$) compared to Priaxor, Proline, Vertisan, and the control. Post-hoc tests confirmed that the drench method had significantly ($P > 0.05$) lower disease severity than both foliar and tray applications, and among fungicides, Quadris was consistently the most effective (Table 1).

Table 1: Treatment relative effect and median disease severity for the main effect of fungicide application methods and fungicides used in greenhouse trials to manage *Rhizoctonia* root rot of sugar beet caused by *Rhizoctonia solani*.

Main Effect	Levels	Parametric combined analysis of variance	Treatment relative effect Mean (95% confidence interval)		Median Severity	
		Least square mean ranks	Trial 1	Trial 2	Trial 1	Trial 2
Method	Foliar	9.02 A	0.57 (0.55 – 0.59)	0.54 (0.51 – 0.58)	7	7
	Tray	8.86 A	0.57 (0.55 – 0.59)	0.56 (0.53 – 0.59)	7	7
	Drench	6.12 B	0.36 (0.31 – 0.41)	0.39 (0.35 – 0.44)	5.5	7
Fungicide	Quadris	7.12 C	0.48 (0.42 – 0.53)	0.41 (0.34 – 0.48)	7	7
	Proline	7.85 BC	0.48 (0.43 – 0.54)	0.50 (0.41 – 0.55)	7	7
	Vertisan	7.80 BC	0.51 (0.48 – 0.55)	0.47 (0.41 – 0.52)	7	7
	Priaxor	8.15 B	0.46 (0.42 – 0.51)	0.55 (0.51 – 0.59)	7	7
	Check	9.08 A	0.57 (0.53 – 0.61)	0.57 (0.54 – 0.61)	7	7

Means within a column followed by the same letter are not significantly different (Tukey-Kramer, $P = 0.05$). Treatment relative effects were calculated using ranked disease severity scores as described by Shaw and Madden (2004). Disease severity was rated on a 0–7 scale, where 0 = no disease and 7 = completely deteriorated root or dead plant (Windels and Nabben-Schindler, 1996). Median severity represents the central tendency of disease ratings for each treatment in each trial.

Non-parametric analyses conducted separately for each trial were consistent with ANOVA results. The method of application (whole-plot factor) was highly significant ($P < 0.0001$), and fungicide type (split-plot factor) was significant in both trials ($P = 0.0399$ and 0.00079 for trials 1 and 2, respectively). The interaction between application method and fungicide type was also significant ($P = 0.04$ and 0.01), indicating that fungicide efficacy depended on the application technique (Figure 1). Priaxor, Proline, and Vertisan provided inconsistent protection, with significant effects in only one trial, while tray and foliar applications consistently resulted in higher disease severity.

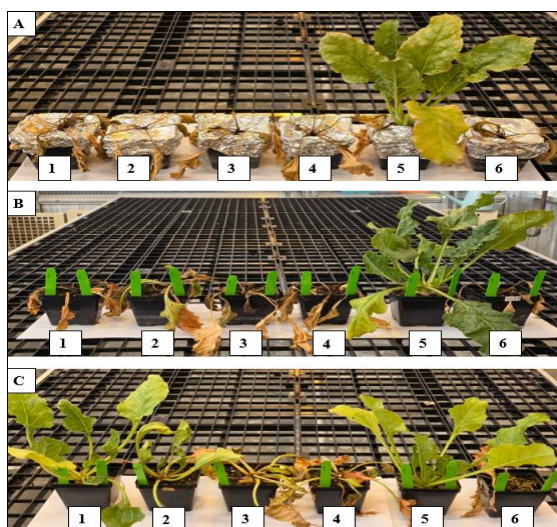


Fig 1: Impact of different fungicide treatments on the severity of *Rhizoctonia solani* in sugar beet. Treatments are arranged from left to right as follows: 1 – Quadris, 2 – Proline, 3 – Vertisan, 4 – Priaxor, 5 – Bon-Inoculated Control, and 6 – Inoculated Control. (A) Foliar applications with soil covered to limit fungicide penetration; (B) fungicides applied in trays; and (C) fungicides delivered as a soil drench.

Analysis of relative effects showed that the drench method was most effective ($P = 0.05$), with median and mean

disease severity values of 5.5 and 3.9 in trial 1 and 7 and 4.2 in trial 2 (Figure 2). Foliar and tray methods exhibited median severities of 7 and mean severities of 5.7–5.8, indicating lower efficacy. The inoculated, non-protected control had the highest disease severity (mean and median = 7). Among fungicides, Quadris had the lowest relative effect (0.48) and was significantly more effective than the control in both trials and more effective than Priaxor in trial 2.

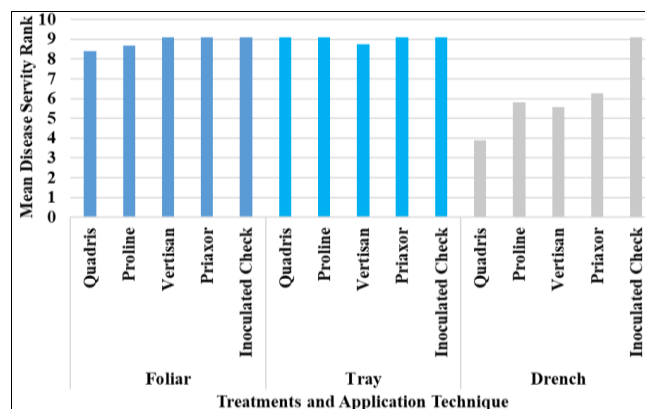


Fig 2: Disease severity of *Rhizoctonia solani* under varying fungicide treatments and application methods.

Across analyses, soil drench application was the most effective method for reducing *R. solani* severity, with Quadris providing the most consistent suppression (Figure 1). Foliar and tray applications were largely ineffective, and the performance of other fungicides varied by trial and application methods.

Discussion

Sugar beet production is challenged by both foliar and soilborne pathogens, including *R. solani*, which causes seedling damping-off and crown and root rot in mature plants, particularly in the Red River Valley region [3]. Surveys indicate that approximately one-third of growers rely solely on foliar sprays, foregoing in-furrow fungicide applications, which may limit their ability to effectively manage soilborne pathogens such as *R. solani*. Resistance to QoI fungicides like azoxystrobin that target the cytochrome b protein to inhibit electron transport and mitochondrial respiration [15], can emerge through a single amino acid substitution [16]. Although *R. solani* is generally considered low-risk for resistance development, resistant isolates have been reported in other crops, including rice, potato, and bentgrass [17,9,18], highlighting the importance of using

diversified chemistries and proper deployment strategies to mitigate potential resistance.

Alternative fungicides, such as Vertisan (penthiopyrad; SDHI), have been suggested as replacements for azoxystrobin. In this study, Vertisan was applied at 1023 ml/ha (205 g a.i./ha), slightly below the 210–550 g a.i./ha range evaluated by Liu and Khan (2016). Despite the lower rate, Vertisan applied as a drench provided protection statistically similar to Quadris in both trials (non-parametric analysis) and remained superior to the inoculated, non-protected control (parametric analysis), although its efficacy differed from azoxystrobin. These findings confirm Liu and Khan's results, demonstrating that soil-directed applications of penthiopyrad are effective, whereas foliar sprays are generally less reliable, highlighting the critical role of application method in managing soilborne pathogens. These observations highlight the critical importance of application method in achieving effective disease control, as soil-directed applications often outperform foliar sprays for soilborne pathogens. Chemical fungicides remain essential for sugar beet disease management, particularly prior to the six- to eight-leaf stage to prevent infection and yield loss [19,20].

Results from this study demonstrate that selection of fungicide type and application method are key determinants for disease suppression. Data were analyzed using two approaches involving ranked severity scores. The non-parametric approach provided a robust theoretical framework for analyzing categorical data but lacked the capacity to conduct combined analyses across trials. In contrast, the parametric approach allowed a combined analysis, yielding a more streamlined interpretation. Both approaches, however, produced consistent results, reinforcing the validity of the conclusions.

The drench application of Quadris was the most effective treatment, directly targeting the root system to ensure optimal fungicide absorption and systemic protection against *R. solani*, which infects plants through the roots [21]. Drench applications provide direct contact with the hypocotyl and root, a critical factor in managing soilborne diseases [12], although they require higher solution volumes to achieve penetration into the root zone. While drench applications are not commonly used in large-scale commercial sugar beet production in North Dakota and Minnesota [12], this study used soil-directed applications to demonstrate fungicide behavior at the infection site. The purpose was to show that these fungicides are primarily xylem-restricted and do not translocate effectively from leaves to roots. This information helps growers understand the limitations of foliar applications and supports informed decisions regarding the timing and placement of fungicides early in the season to optimize disease control.

In contrast, foliar and tray applications were less effective, as fungicide contact with the root zone was limited. *R. solani* infection occurs externally through roots, and the fungicides used in this study are not phloem-mobile, limiting downward translocation from leaves [22]. Tray applications may have been ineffective due to rapid absorption by root hairs without maintaining sufficient fungicide concentrations in the surrounding soil to prevent infection. Similarly, foliar or tray applications of Quadris showed diminished systemic activity, further highlighting

the importance of both fungicide chemistry and placement. Although the drench application of Quadris showed the highest efficacy under greenhouse conditions, its field application requires consideration of environmental factors such as rainfall and irrigation, which may affect fungicide movement and penetration. Additionally, the relatively short duration of greenhouse experiments may not fully reflect the persistence required under field conditions, and repeated applications may be necessary to maintain protection. Nevertheless, growers should consider applying drench treatments before conditions favoring *R. solani* infection, particularly when average daily soil temperatures at the four-inch soil depth reach 18°C (65°F) or higher and soil moisture is abundant [23]. Timing fungicide applications prior to rainfall can further enhance effectiveness [24].

Conclusion

This study demonstrates that fungicide efficacy against *R. solani* in sugar beet depends strongly on application method. Azoxystrobin (Quadris) provided the most consistent suppression but only when delivered as a soil drench, emphasizing the importance of targeting the root zone. Foliar and tray applications were largely ineffective. These findings highlight the need to integrate fungicide selection with appropriate application strategies to manage soilborne pathogens. Adoption of soil-directed applications, combined with careful timing and rotation with fungicides of different modes of action, can reduce disease severity and contribute to sustainable management of *R. solani* in sugar beet production.

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