Evaluation of Fungicide Strategies for Controlling Leaf Spot Complex in Manitoba Potato Fields

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Abstract

Leaf Spot Lesion Complex (LSC) is a significant foliar disease affecting potato production, necessitating effective fungicide management strategies. This study evaluated the efficacy of twelve fungicide treatments in controlling LSC in *Ranger Russet* potatoes under field conditions in Manitoba. A randomized complete block design was implemented, with three replicated plots per treatment. Disease severity was assessed using image analysis to quantify lesion coverage on sampled leaves. Tuber yield and quality parameters, including specific gravity and internal defects, were also evaluated. Results indicated that Treatment 3 (Scala and Sercadis) applied at row closure and early bulking stages yielded the highest tuber production, followed by Treatment 10, which utilized systemic fungicides at different growth stages. No significant differences were observed in tuber quality across treatments, suggesting that fungicide applications did not negatively impact potato quality. Disease severity varied among treatments, with lesion percentages ranging from 8.59% to 22.54%, where Treatment 7 exhibited the highest disease incidence. Pathogen isolation confirmed the presence of *Alternaria solani*, *Alternaria alternata*, and *Botrytis cinerea*, highlighting the complexity of LSC and the necessity for multi-fungicide strategies. In vitro assays demonstrated the varying efficacy of fungicides against isolated pathogens. Veltyma, Proline Gold, Luna T, and Scala significantly reduced mycelial growth of *A. alternata* and *B. cinerea*, while Luna T and Miravis Duo achieved complete inhibition of *A. solani* and *A. alternata*. The findings suggest that integrated fungicide strategies combining contact, systemic, and specialized fungicides are essential for effective LSC management. Additionally, fungicide selection and rotation play a critical role in minimizing disease severity while preventing resistance development. This study underscores the importance of targeted fungicide applications in sustaining potato health and optimizing yield potential.

Keywords

Leaf Spot Lesion Complex (LSC), Tuber Yield and Quality, Fungicide Efficacy, *Alternaria solani*, *Alternaria alternata*, *Botrytis cinerea*, Biological control, Systemic and Contact Fungicides, contact fungicides.

Introduction

Alternaria is the most abundant plant pathogen, and as temperatures rise, Alternaria becomes more common and may become more concerning under climate change conditions (Delgado-Baquerizo et al., 2020). Alternaria species can live in different ways, but they mostly survive as saprophytes in soil and decaying plant matter (Thomma, 2003). Early blight, caused by the fungus *Alternaria solani*, and brown leaf spot, caused by *Alternaria alternata*, are serious diseases that affect potato crops worldwide. These diseases cause leaf loss later in the growing season, leading to lower potato yields (Shah et al. 2004). Since there are no resistant plant varieties available, farmers must use fungicides to manage the disease (Jones and Perez, 2023). Applying fungicides at the right time can help reduce these yield losses (Douglas and Groskopp, 1974).

Botrytis cinerea is a type of fungus that infects plants and causes a disease called gray mold. This disease affects many plant species and leads to major financial losses, especially in fruit production during storage and transport. Some of these plants produce defense compounds called phytoalexins. Because *B. cinerea* infects so many plant species, it must be able to tolerate a wide variety of phytoalexins. Previous research has shown that *B. cinerea* can break down the sesquiterpenoid phytoalexin capsidiol, a key defense compound in Nicotiana and Capsicum plants, by converting it into a harmless substance called capsenone using the enzyme BcCPDH (Kuroyanagi et al. 2022). It can also break down rishitin, a similar phytoalexin found in potatoes and tomatoes (Bulasag et al. 2024). To manage *B. cinerea*, different strategies are used, such as reducing moisture in plant canopies, removing infected plants (Jacometti et al. 2010), and applying synthetic fungicides (Rosslenbroich and Stuebler, 2000). Fungicides are the main method for controlling this fungus, but *B. cinerea* has developed resistance to these chemicals over time (Esterio et al. 2017).

Newer fungicides are more targeted and have less impact on the environment. However, their use is limited because fungi can develop resistance through point mutations in the gene encoding the target protein (Gudmestad et al., 2013). There are many fungicides available to control early blight, but strobilurin or quinone outside inhibitor (QoI) fungicides are often preferred. These fungicides provide broad protection against many fungal and oomycete diseases, have less impact on the environment, and are less toxic to mammals and bees compared to traditional fungicides like chlorothalonil, mancozeb, and mefenoxam (Rosenzweig et al., 2008). The overuse and misuse of chemical fungicides have led to the development of resistant plant pathogens, which can pose risks to food safety and human health. Because of this, there is growing interest in finding alternative antifungal methods for controlling plant diseases. *Bacillus* species are known for producing various antifungal compounds that can help suppress or kill fungal pathogens (Chaurasia et al., 2005). This study aims to (1) evaluate different fungicide and biological combinations for managing LSC, (2) identify the pathogens associated with LSC in Manitoba, and (3) assess fungicide susceptibility against key pathogens, particularly Alternaria spp.

Materials and Methods

Experimental Design

A field trial was conducted to evaluate the efficacy of twelve different fungicide treatments in managing the Leaf Spot Lesion Complex in potatoes. Each treatment was applied in three replicated plots following a randomized complete block design. The study aimed to assess the extent of foliar disease severity and its potential impact on potato yield and quality. The Ranger Russet potato variety was planted with a seed spacing of 13 inches and a row spacing of 36 inches. Pre-plant fertilization was applied on May 13, followed by seeding on May 17. The hilling process was completed on June 5. Fungicide treatments can be categorized into three main groups (Table 1). Contact fungicides, such as Manzate and Bravo, provide broad-spectrum, preventive protection by remaining on the plant surface and preventing fungal infection. Systemic fungicides, including Veltyma, Proline Gold, Miravis Duo, and Luna T, offer curative and residual activity by being absorbed into the plant and providing extended disease control. Specialized fungicides, such as Scala and Sercadis, are designed for targeted control of specific fungal diseases.

Plant Material and Sampling

Leaf samples showing leaf lesions symptoms were collected from the field trial conducted by MHPEC in Carberry. Potato leaves were collected at the late bulking stage from each treatment plot. The sampled leaves were carefully detached and placed on a standardized blue background to ensure consistency in image analysis. Each set of leaves corresponded to a specific fungicide treatment, labeled numerically from 1 to 12.

Isolation and Identification of Fungal Isolates

To isolate pathogens from infected leaves, small leaf pieces (5 × 5 mm) were cut from the center of diseased areas using a sterile scalpel. These samples were placed on tap water agar plates and spread using sterile needles. The plates were then incubated at 25°C to allow fungal spores (conidia) to germinate. Once germinated, individual conidia were carefully transferred to potato dextrose agar (PDA) using a dissecting microscope (Fairchild et al. 2013). The PDA contained 100 mg of streptomycin sulfate and was incubated in the dark at 25°C. The common fungal isolates were identified based on their colony and conidial morphology. *Alternaria solani* has large, beaked conidia with both transverse and longitudinal septa, often produced singly or in short chains. *Alternaria alternata* forms long chains of smaller conidia that lack the long beak seen in *A. solani*. *Botrytis cinerea* produces round to oval conidia arranged in branched, grape-like clusters, distinguishing it from the chain-forming Alternaria species. To confirm species identity, molecular analysis was performed by sequencing the internal transcribed spacer (ITS) regions (ITS1 and ITS2) (Table 2) (Sambrook et al., 1989).

Image Analysis

Leaf images were processed using OpenCV and NumPy in Python to quantify disease severity. The following steps were performed:

I. Image Preprocessing: Conversion to HSV color space to enhance differentiation between healthy (green) and diseased (brown) tissue.
II. Segmentation: Threshold-based color segmentation to classify pixels into green (healthy) and brown (lesion) areas.

III. Quantification: Pixel areas of both healthy and diseased regions were measured, and the percentage of lesion coverage was calculated relative to the total leaf area.

Data Collection

Data collection focused on two key parameters: yield and tuber quality. Yield measurements included total yield and the effects of different treatments on yield performance. Tuber quality was assessed based on specific gravity and the presence of internal defects, such as rot, greening, and hollow heart.

Statistical Analysis

Statistical comparisons were performed using IBM SPSS Statistics Version 30.0.0.0 (172) to determine significant differences between treatment zones.

Table 1. Fungicide application schedule for managing leaf spot complex in potatoes. The table outlines in-furrow and foliar fungicide applications applied throughout the growing season. The in-furrow application was conducted on May 17, 2024, using Velum Rise and Minuet (*Bacillus subtilis*) in treatments 2–12. Foliar applications were initiated on June 27, 2024, and continued at weekly intervals until September 5, 2024. Different fungicide treatments, including single and combined applications of Manzate, Scala, Bravo, Proline Gold, Miravis Duo, Luna T, and Sercadis, were applied.

Tr. #	In Furrow	Foliar Applications										
		1	2	3	4	5	6	7	8	9	10	11
	17-May-24	*27-Jun*	*4-Jul*	*11-Jul*	*18-Jul*	*25-Jul*	*1-Aug*	*8-Aug*	*15-Aug*	*22-Aug*	*29-Aug*	*5-Sep*
1	0	Manzate	Manzate	Bravo	Manzate	Bravo	Manzate	Manzate	Bravo	Manzate	Manzate	Manzate
2	Velum Rise+Minuet (7)	Veltyma+Manzate (3,11)	Manzate	Bravo	Manzate	Bravo	Manzate	Manzate	Bravo	Manzate	Manzate	Manzate
3	Velum Rise+Minuet (7)	Veltyma+Manzate (3,11)	Manzate	Bravo	Scala + Bravo (9)	Bravo	Manzate	Sercadis + Manzate (7)	Bravo	Manzate	Manzate	Manzate
4	Velum Rise+Minuet (7)	Veltyma+Manzate (3,11)	Manzate	Bravo	Proline Gold + Manzate (7, 3)	Bravo	Manzate	Manzate	Bravo	Manzate	Manzate	Manzate
5	Velum Rise+Minuet (7)	Veltyma+Manzate (3,11)	Manzate	Bravo	Miravis Duo + Manzate (7, 3)	Bravo	Manzate	Manzate	Bravo	Manzate	Manzate	Manzate
6	Velum Rise+Minuet (7)	Veltyma+Manzate (3,11)	Manzate	Bravo	Proline Gold + Manzate (7, 3)	Bravo	Manzate	Scala + Bravo (9)	Bravo	Manzate	Manzate	Manzate
7	Velum Rise+Minuet (7)	Veltyma+Manzate (3,11)	Manzate	Bravo	Miravis Duo + Manzate (7, 3)	Bravo	Manzate	Scala + Bravo (9)	Bravo	Manzate	Manzate	Manzate
8	Velum Rise+Minuet (7)	Veltyma+Manzate (3,11)	Manzate	Bravo	Proline Gold + Manzate (7, 3)	Bravo	Manzate	Sercadis + Manzate (7)	Bravo	Luna T + Manzate (7, 9)	Manzate	Manzate
9	Velum Rise+Minuet (7)	Veltyma+Manzate (3,11)	Manzate	Bravo	Miravis Duo + Manzate (7, 3)	Bravo	Manzate	Sercadis + Manzate (7)	Bravo	Luna T + Manzate (7, 9)	Manzate	Manzate
10	Velum Rise+Minuet (7)	Veltyma+Manzate (3,11)	Manzate	Bravo	Proline Gold + Manzate (7, 3)	Bravo	Manzate	Miravis Duo + Manzate (7, 3)	Bravo	Luna T + Manzate (7, 9)	Manzate	Manzate
11	Velum Rise+Minuet (7)	Veltyma+Manzate (3,11)	Manzate	Bravo	Miravis Duo + Manzate (7, 3)	Bravo	Manzate	Miravis Duo + Manzate (7, 3)	Bravo	Manzate	Manzate	Manzate
12	Velum Rise+Minuet (7)	Veltyma+Manzate (3,11)	Manzate	Bravo	Proline Gold + Manzate (7, 3)	Bravo	Manzate	Miravis Duo + Manzate (7, 3)	Bravo	Manzate	Manzate	Manzate

Results and Discussion

Tuber Yield and Quality

Treatment 3, which involved the application of Scala and Sercadis at the row closure and early bulking stages, resulted in the highest total tuber yield. This was followed by Treatment 10, which consisted of a combination of systemic fungicides applied at different growth stages (Fig. 1A–D). These results indicate that targeted fungicide applications can effectively mitigate leaf spot complex (LSC) and contribute to increased potato yield. No statistically significant differences were observed in tuber specific gravity (Fig. 1E) or internal defects (Fig. 1F) across treatments, suggesting that fungicide applications did not adversely affect tuber quality suggesting that these fungicide strategies do not compromise potato quality.

Disease Severity Assessment

The lesion severity in potato leaves varied among the 12 different treatments, as assessed through image analysis (Fig. 2). Each treatment included 15 leaves collected from 3 plots, and the percentage of brown lesions relative to the total leaf area was calculated. Lesions percentages of 12.04%, 8.59%, 11.31%, 10.91%, 12.31%, 11.72%, 12.89%, 14.29%, 10.18%, 9.41%, 1466% for treatments 1, 2, 3, 4, 5, 6, 8, 9, 10, 11 and 12, respectively. Treatment 7 (Image 7) had the highest lesion percentage at 22.54%, indicating the least effective disease control, where it exhibited the highest degree of foliar damage, with extensive brown lesions covering a significant portion of the leaves.

Pathogen Isolation and Identification

Pathogens isolated from leaves at the late bulking stage across all plots included *Alternaria solani*, *Alternaria alternata*, and *Botrytis cinerea* (Table 2). These results highlight the complexity of LSC and the need for multi-fungicide strategies to manage diverse pathogen populations and mitigate disease impacts in Manitoba potato fields.

Dose-Response of the Tested Fungicides on the In Vitro Growth of Selected Isolated Pathogens

Fungal growth was quantified as the mean colony diameter (mm) across different fungicide concentrations, including the recommended field dose (RD), against three fungal

pathogens: *Alternaria solani* (As), *Alternaria alternata* (Aa), and *Botrytis cinerea* (Bc). Control plates of As, Aa, and Bc exhibited the largest colony diameters, indicating uninhibited fungal growth. The 4000 ppm and 2000 ppm (Recommended Dose; RD) concentrations of Veltyma significantly reduced mycelial growth of As compared to the control, whereas the 1000 ppm and 500 ppm concentrations displayed intermediate effects when compared to 4000 ppm and 2000 ppm (Fig. 3A). However, 1000 ppm and 500 ppm still significantly reduced As growth compared to the control (Fig. 3A). All tested concentrations of Proline Gold and Scala significantly reduced mycelial growth of As compared to the control (Fig. 3B, 3E). Complete inhibition was observed for As at all selected concentrations of Luna T (Fig. 3C) and Miravis Duo (Fig. 3D), confirming its higher sensitivity to both fungicides. The 2000 ppm concentration of Sercadis significantly reduced mycelial growth of As and Aa compared to the control, whereas the 1000 ppm (RD) and 500 ppm concentrations displayed intermediate effects when compared to 2000 ppm and significant effects when compared to the control (Fig. 3F). All tested concentrations of Veltyma (Fig. 3A), Proline Gold (Fig. 3B), Luna T (Fig. 3C), and Scala (Fig. 3E) significantly reduced mycelial growth of Aa compared to the control. Complete inhibition was observed for Aa at all selected concentrations of Miravis Duo (Fig. 3D), confirming its higher sensitivity to this fungicide. Complete inhibition was also observed for Bc at all selected concentrations of Veltyma (Fig. 3A), Proline Gold (Fig. 3B), Luna T (Fig. 3C), and Miravis Duo (Fig. 3D). All tested concentrations of Scala (Fig. 3E) significantly reduced mycelial growth of Bc compared to the control.

Together, these results suggest that fungicide selection plays a crucial role in disease suppression. Treatments with mixed-mode fungicides targeting multiple pathogen pathways were generally more effective. The findings underscore the importance of integrating fungicide rotation strategies to mitigate resistance development and enhance long-term disease control. Also, this study underscores the importance of selecting appropriate fungicide combinations to manage LSC effectively without affecting the yield.

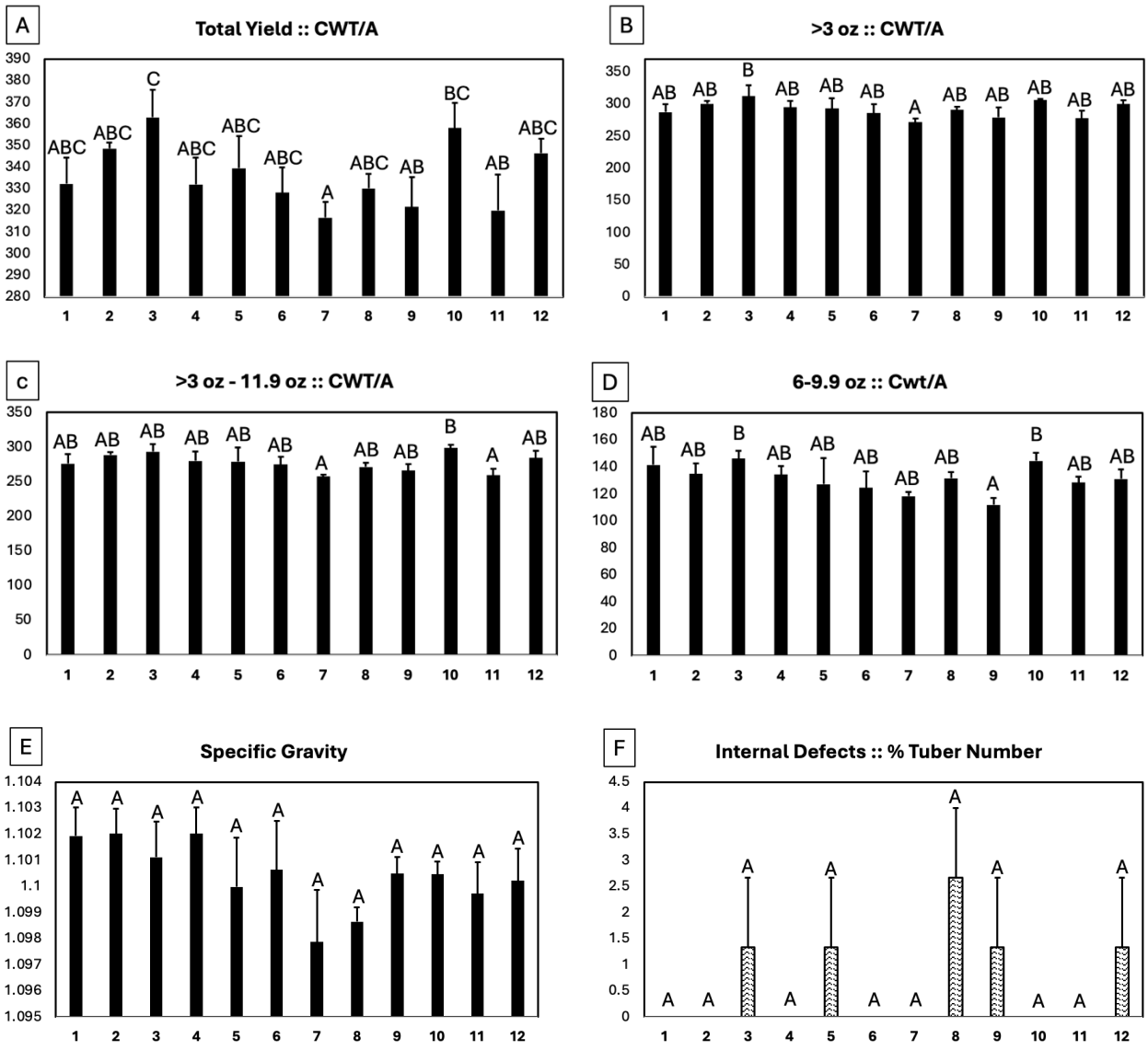

Figure 1. Effects of twelve fungicide treatments on potato yield, quality, and internal defects. (A) Total Yield (CWT/A), (B) Yield of tubers >3 oz (CWT/A), (C) Yield of tubers between 3–11.9 oz (CWT/A), (D) Yield of tubers between 6–9.9 oz (CWT/A), (E) Specific Gravity, **(F)** Internal Defects. Letters on bars indicate statistically significant differences; one-way ANOVA; post hoc least significant difference; $P < 0.05$. Bars sharing the same letter are not significantly different from each other.

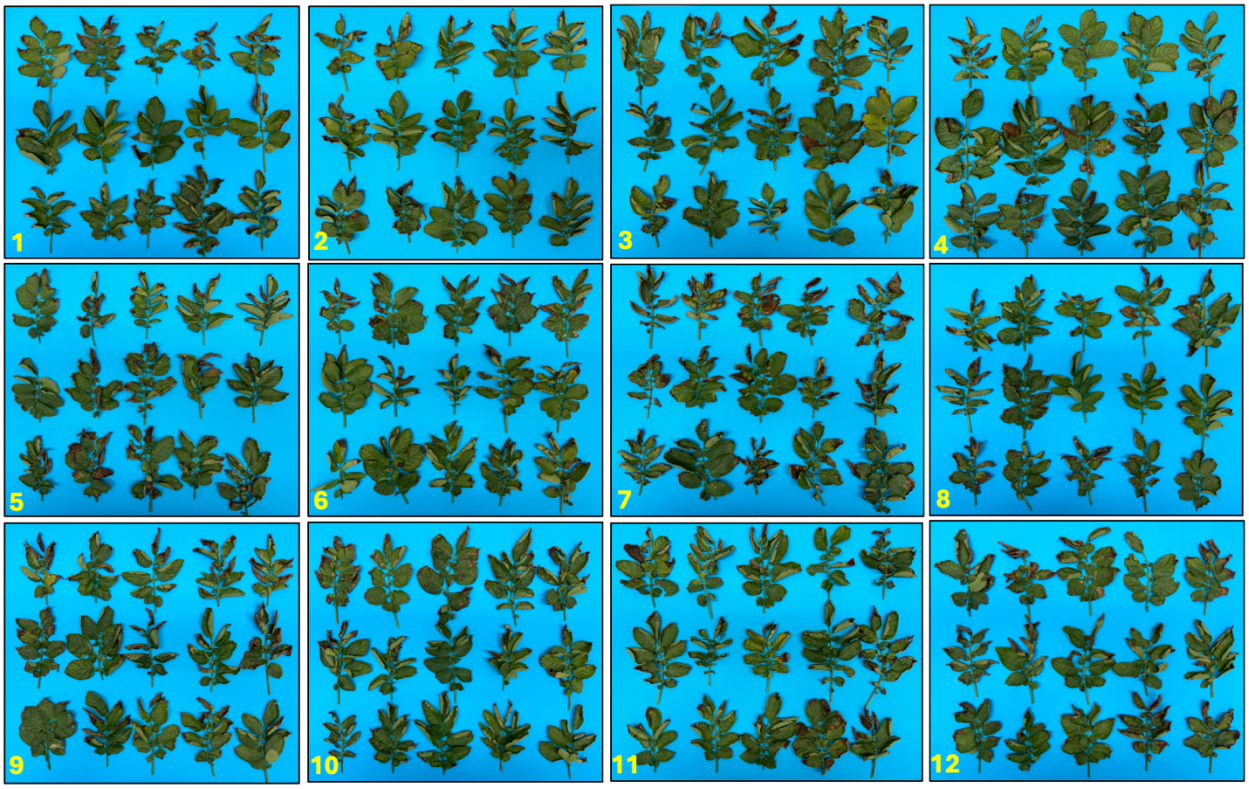

Figure 2. Lesion severity in potato leaves from twelve different treatments in a fungicide trial targeting the Leaf Spot Spot Complex. Each panel (1–12) represents a different fungicide treatment, with detached leaves arranged on a uniform blue background for clear visual assessment. Visible brown lesions indicate disease progression, while green leaf areas represent healthy tissue. The severity of the lesions varies among treatments, demonstrating differences in disease control efficacy. Quantitative analysis of lesion area percentages was performed using image processing techniques with OpenCV and NumPy in Python.

Table 2. Isolation of fungal pathogens from potato leaf spot lesions across different plots. The table lists the fungal species isolated from each plot, identified based on morphological and molecular characteristics.

Plot	**Isolated Pathogens**	***Plot***	**Isolated Pathogens**
1	*Alternaria alternata*	*19*	*Alternaria alternata*
2	*Alternaria alternata*	*20*	*Alternaria alternata*
3	*Alternaria alternata*	*21*	*Stemphylium vesicarium*
4	-	*21*	*Alternaria alternata*
4	*Alternaria alternata*	*22*	*Alternaria alternata*
5	*Alternaria alternata*	*23*	*Alternaria alternata*
6	*Alternaria tenuissima*	*24*	*Alternaria alstroemeriae*
7	*Alternaria alternata*	*25*	*Alternaria alternata*
7	*Alternaria alstroemeriae*	*25*	*Alternaria sp. RS-4*
8	*Alternaria alstroemeriae*	*26*	*Alternaria tenuissima*
9	*Alternaria alternata*	*27*	*Alternaria alstroemeriae*
10	*Alternaria compacta*	*28*	*Alternaria alternata*
11	*Botrytis cinerea*	*29*	*Alternaria alternata*
12	*Alternaria solani*	*30*	*Alternaria alternata*
13	*Alternaria tenuissima*	*30*	*Chaetomium elatum*
14	*Alternaria alternata*	*31*	*Alternaria alternata*
14	*Alternaria tenuissima*	*32*	*Alternaria alternata*
15	*Alternaria alternata*	*32*	*Alternaria alternata*
15	*Alternaria tenuissima*	*33*	*Alternaria tenuissima*
16	*Alternaria alternata*	*34*	*Alternaria alternata*
16	*Alternaria alternata*	*35*	*Alternaria alternata*
17	*Alternaria alternata*	*35*	*Alternaria alternata*
18	*Alternaria tenuissima*	*36*	*Alternaria alstroemeriae*

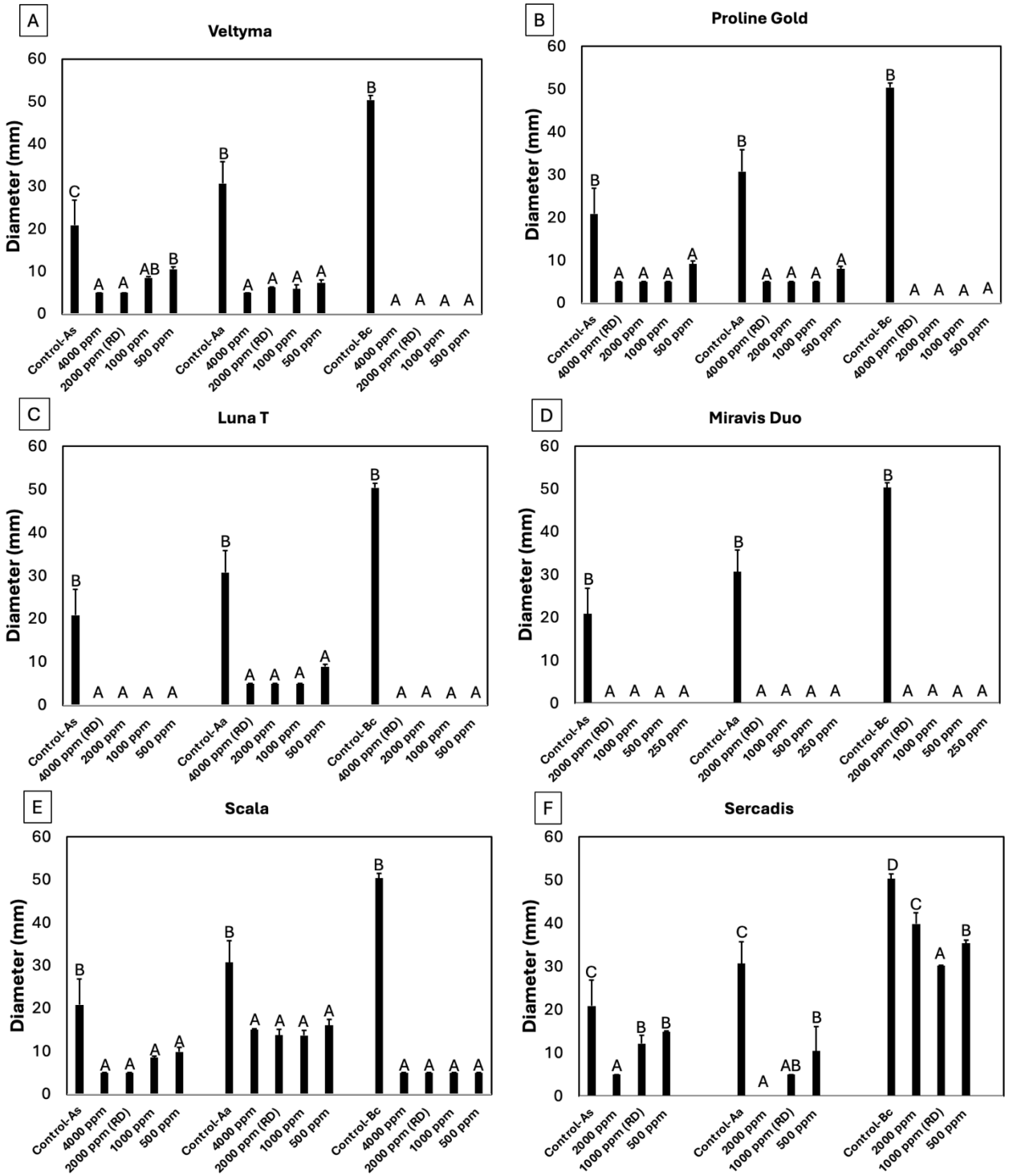

Figure 3. Dose-response of six fungicides on the in vitro growth of *Alternaria solani* (As), *Alternaria alternata* (Aa), and *Botrytis cinerea* (Bc). (A) Veltyma, (B) Proline Gold, (C) Luna T, (D) Miravis Duo, (E) Scala, and (F) Sercadis. Fungal growth was measured as mean colony diameter (mm) under different fungicide concentrations (ppm), including the recommended field dose (RD). Letters on bars indicate statistically significant differences; one-way ANOVA; post hoc least significant difference; $P < 0.05$. Bars sharing the same letter are not significantly different from each other.

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