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Evaluation of Biorational Products for Botrytis Blight Management in Floriculture Crops

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Abstract. **The use of biorational products offers an alternative to the conventional chemical fungicide approach to manage botrytis blight caused by *Botrytis cinerea*. Biorationals are a broad category of products that include biological control agents (BCAs), biologically derived products, compounds that induce natural plant disease resistance mechanisms, and mineral elements. We evaluated 15 biorational products and two chemical fungicides on detached petunia (*Petunia ×hybrida*) and rose (*Rosa ×hybrida*) flowers inoculated with *B. cinerea* spores following treatments. The two chemical fungicides, Miravis Prime and Captan, were evaluated as commercial control. In the first experiment, five products showed a reduction in disease severity in petunia flowers (reduction percentages shown in parentheses are relative to the inoculated control): Zivion, a formulation of natamycin, a natural fermentation product of *Streptomyces natalensis* (66%); ON-Gard Calcium, a calcium chloride product delivered in soy protein (79%); Howler, a formulation of *Pseudomonas chlororaphis* strain AFS009 (51%); Affirm, a polyoxin D zinc salt (36%); and Regalia, an extract from giant knotweed (*Reynoutria sachalinensis*) (36%). In the second experiment, the five effective products were applied to petunia flowers individually and in combinations. The combination of Zivion + Howler and ON-Gard Calcium + Howler reduced disease severity by 77% and 79%, respectively, compared with the inoculated control, whereas ON-Gard Calcium + Zivion showed a 91% reduction in disease severity. In the third experiment, the same 15 biorational products from the first experiment were applied as a dip application on rose flowers. Applications were made 1 or 8 days before inoculation with *B. cinerea* spores. When biorational products were applied 1 day before inoculation, Affirm, ON-Gard Calcium, Actigard (acibenzolar-S-methyl, an inducer of systemic-acquired resistance, and Zivion showed a reduction in disease severity of 63%, 33%, 23%, and 18%, respectively. When the biorational products were applied 8 days before inoculation, Actigard, Affirm, and ON-Gard Calcium showed a reduction in botrytis severity by 16%, 54%, and 31%, respectively. In the fourth experiment, the four most effective products were evaluated as single and combination applications on rose flowers. The combinations of Actigard + ON-Gard Calcium, ON-Gard Calcium + Zivion, and Actigard + Zivion reduced botrytis blight by 66%, 62%, and 53%, respectively, whereas Actigard + Affirm, ON-Gard Calcium + Affirm, and Zivion + Affirm reduced disease severity by 85%, 77%, and 76%, respectively. This work demonstrates that tank mixes of biorational products, which provide different modes of action, can have comparable efficacy to chemical fungicides for controlling botrytis blight in petunia and rose flowers.**

Botrytis cinerea is the causal agent of botrytis blight (gray mold) of ornamental, nursery, vegetable, field, and fruit crops (Dik and Wubben 2007). The pathogen is ubiquitous and can be spread in greenhouses through the air. *B. cinerea* can remain as conidia, mycelia, or sclerotia for long periods of time on living or decaying tissue (Williamson et al. 2007). It can infect plant tissue during the early stages of crop development and remain latent until the environment is conducive for fungal growth. The optimal conditions for fungal development are temperatures between 15 and 25 °C and relative humidity >93%. The disease can affect the whole plant, but flower petals are often the most susceptible tissue (Muñoz et al. 2019). Symptoms start as necrotic spots that, provided the proper environment (Pikovskyi et al. 2018), expand over time and often render plants unsellable.

Growers employ integrated management approaches involving cultural practices, biorational products, and chemical management to control disease. The use of chemical fungicides is one of the primary and often most effective strategies for the management of botrytis blight in greenhouse production and postharvest of the crops; however, the persistent use of active ingredients with similar modes of action, especially single-site fungicides, can lead to pathogen resistance (Fillinger and Elad 2016; Hahn 2014). In addition, concerns about pesticide applicator health, environmental safety, and chemical pesticide residues remaining on the crops make this management option less desirable, attracting attention to an increased use of biorational pesticides as part of an integrated management approach (Damalas and Eleftherohorinos 2011).

Biorationals, also called "third-generation pesticides" (Kapoor 2020), are believed to have minimal to no effects on the environment and encompass biological control agents (BCAs), botanical extracts, microorganism-derived compounds, plant nutrients, and systemic-acquired resistance (SAR) inducers (Copping and Menn 2000; Paulitz and Belanger 2001). BCAs consist of living organisms such as bacteria, fungi, or nematodes that can kill or suppress plant pathogens (Adriaens et al. 2007; Usta 2013). Botanical extracts consist of plant by-products that possess antimicrobial activity with different modes of action that include reduction of cell growth, inhibition of biofilm, and disruption of the cell wall of the pathogen (Nazzaro et al. 2017). Several studies have shown that essential oils such as thyme (*Thymus vulgaris*) may trigger host defense when applied against *B. cinerea* in apple (*Malus ×domestica* 'Red Fuji') fruit (Banani et al. 2018), whereas the extract of giant knotweed exhibited an antifungal effect on *B. cinerea* when used on tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) (Schmitt et al. 1996; Wurms et al. 2021). Microorganism-derived compounds are biofungicides obtained as a natural product of the fermentation of microorganisms such as *Streptomyces* (Copping and Menn 2000). For example, polyoxin D is a bacteria-derived product with documented effectiveness against botrytis blight in flowering plants such as cutflower rose (Elad 1988) and geranium (*Pelargonium ×hortorum*) (Webster 2005), and natamycin is a bacteria-derived compound commonly used as a food preservative that has been effective against botrytis blight on blueberries (*Vaccinium corymbosum*) (Saito et al. 2022) and strawberries (*Fragaria ×ananassa*) (Zhang et al. 2025).

Plant nutrients, such as calcium, help by improving cell wall strength, making the plant more resistant to fungal penetration (Gislerød 1997). The cell wall acts as a first layer of defense against pathogen attack (Shi et al. 2019). SAR inducers consist of plant metabolites, chemical compounds, or pathogens that trigger a defense response in the plant (Achuo et al. 2004; Kessmann et al. 1994), such as salicylic acid or acibenzolar-S-methyl, a compound that mimics the salicylic acid metabolic pathway (Oostendorp et al. 2001). The diversity in the modes of action of the different biorational products highlights their potential to be incorporated as part of an integrated disease management

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program; specific host-pathogen-biorational interactions are needed.

B. cinerea plays an important role in the floriculture industry as a major threat from propagation to the postharvest environment. Fungicide applications have been the standard approach for growers to manage this disease but concerns about pesticide risk and an increase in fungicide resistance create the necessity to explore alternative products for the management of botrytis blight. Biorational products have shown potential in controlling fungal pathogens; therefore, the first objective of this study was to individually evaluate 15 biorational products to manage *B. cinerea* in petunia and rose flowers, and the second objective was to evaluate the most effective products applied in combination.

Materials and Methods

Four replicated experiments were conducted to evaluate the effect of biorational products on *B. cinerea* infection using detached petunia (*Petunia ×hybrida*) and rose (*Rosa ×hybrida*) flowers as model crops. In Expt. 1, the effect of spray application of 15 biorational products was evaluated on detached petunia flowers that were inoculated with *B. cinerea* 1 d after treatment. In Expt. 2, combinations of the best performing products from Expt. 1 were evaluated on detached petunia flowers. In Expt. 3, the effect of dip application of 15 biorational products was evaluated on rose flowers where the application occurred 1 d (Expt. 3A) or 8 d (Expt. 3B) before inoculation with *B. cinerea* spores. In Expt. 4, combinations of the best performing products from Expt. 3A were evaluated on rose flowers.

B. cinerea culture maintenance and preparation of spore suspension. Two *B. cinerea* isolates were used for the experiments: PDRK3 (Bennett et al. 2020) was used for petunia inoculation, and S4GBR4O (Muñoz et al. 2019) was used for rose inoculation. For each isolate, one 5-mm-diameter potato dextrose agar (PDA; Difco Laboratories, Sparks, MD, USA) plug with actively growing mycelium was transferred to a petri dish with PDA medium and incubated in the dark for 8 to 10 d until sporulation occurred. Spores were harvested with sterile deionized water and adjusted to final concentrations of 1×10^4 spores/mL for petunia and 1×10^5 spores/mL for rose using a hemocytometer (Bright-Line 3110; Hausser Scientific, Horsham, PA, USA).

Biorational products and fungicide controls. Fifteen biorational products were evaluated using the middle of the label-recommended concentration range (Table 1). Two chemical fungicide products provided controls. Noninoculated and inoculated untreated controls were also included with each experiment.

Expt. 1. Evaluation of biorational products applied to detached petunia flowers. Petunia 'Dreams Burgundy Picotee' plugs were received from a commercial supplier (Ball Tagawa Growers, Arroyo Grande, CA, USA) and transplanted into 1.4 L pots (one plug per container) filled with a peat-based growing medium (Fafard 3B; Sun Gro, Anderson, SC, USA). Eighty petunia plants were grown in a glass greenhouse (Clemson University, Clemson, SC, USA) with a computer-controlled environment system (Argus Control Environmental Systems, White Rock, Canada). Plants were fertigated with a 250 ppm N solution using 15N–5P_2O_5–15K_2O (Peters Excel Cal-Mag Special, Dublin, OH, USA). All open flowers were removed the day before the experiment began to allow the harvest of newly opened flowers for the experiment.

A total of 180 flowers were harvested and immediately placed into 35-cm length × 20-cm width × 0.22-cm height trays. Thirty flowers were placed per tray with 1 L of water to keep the flowers hydrated. Sets of 10 flowers were taken from the trays, and the biorational products were sprayed to surface saturation without runoff with ∼1 mL/flower on the open, upper side of the petals with using a fine-mist sprayer (118.3 mL clear polyethylene terephthalate plastic bottle; The Cary Company, Addison, IL, USA) and allowed to dry. After 24 h, the flowers were inoculated with a spore suspension (1×10^4 spores/mL) by spraying 1 mL using a 118.3 mL clear polyethylene terephthalate plastic bottle. Flowers were placed in 30.4 cm long × 20.7 cm wide × 16.8 cm tall plastic storage containers (HMS Mfg. Co., Troy, MI, USA). A total of 32 plastic storage containers were used for this experiment. Each container possessed six flowers each having received a different spray treatment. A polystyrene sheet was placed inside each container and possessed six 10-mm diameter holes in which one flower was placed per hole. Water (600 mL) was placed at the bottom of each container to hydrate the flowers and to humidify the boxes once the lids were closed. Flowers were arranged in the containers using a completely randomized design to ensure equal conditions between the treatments. Flowers were incubated in the containers for 72 h. After inoculation, plants remained at 96% to 100% relative humidity until disease assessment. Humidity was measured with a psychrometer (RH300; Extech Instruments, Nashua, NH, USA). Ten flowers were used per treatment.

Disease progression data were collected at 72 h after inoculation. Each flower was rated from 0 to 8 based on a botrytis severity scale where 0 = no infection, 1 = 1% to 2%; 2 = 3% to 5%; 3 = 6% to 10%; 4 = 11% to 25%; 5 = 26% to 50%; 6 = 51% to 75%; 7 = 76% to 99% of the flower petal was affected with necrotic spots, and 8 = entire flower petal is infected with botrytis blight. This experiment was performed three times.

Expt. 2. Evaluation of biorational product combinations applied to detached petunia flowers. Seventy petunia 'Dreams Burgundy Picotee' plugs (PanAmerican Seed Co., Chicago, IL, USA) were transplanted into 1.4 L pots (one plug per container) using the same procedures as Expt. 1. The three most effective biorational products from Expt. 1. Howler, ON-Gard Calcium, Zivion (Fig. 1), and their combinations were evaluated and consider for accomplish this experiment. Two chemical fungicides were used as control groups: Miravis Prime and Captan 50 WP. Noninoculated and inoculated untreated controls were also used. Ten petunia flowers per treatment were evaluated and a total of 100 flowers were harvested, and the pedicel was immediately placed into one of the 17 plastic storage containers containing water. Treatment application, inoculation, incubation, and

Table 1. Products evaluated in this study, active ingredients, and application rates.

Product	Active ingredient	Application rate	Manufacturer
Actigard 50WG	Acibenzolar-S-methyl	0.04 g/L	Syngenta Crop Protection, Greensboro, NC, USA
Affirm WDG	Polyoxin D zinc salts	0.5 g/L	Cleary Chemicals, Alsip, IL, USA
Botector	*Aureobasidium pullulans*	0.75 g/L	Westbridge Agricultural Products, Vista, CA, USA
BotryStop WP	*Ulocladium oudemansii*	3.6 g/L	Bioworks Inc. Victor, NY, USA
Cease	*Bacillus subtilis* strain QST 713	10 mL/L	Bioworks Inc., Victor, NY, USA
Howler	*Pseudomonas chlororaphis* strain AFS009	6.23 g/L	Agbiome Inc., Research Triangle Park, NC, USA
ON-Gard Calcium	Calcium	20 mL/L	Bioworks Inc., Victor, NY, USA
Potassium silicate	Silicon	394 µL/L	Pfaltz & Bauer Inc., Waterbury, CT, USA
PureCrop 1	Soybean oil and corn oil	15.6 mL/L	West Coast AG Products, Ukiah, CA, USA
Regalia CG	*Reynoutria sachalinensis*	6.34 mL/L	Marrone Bio Innovations, Inc., Davis, CA, USA
Revitalize	*Bacillus amyloliquefaciens* strain D747	1.3 mL/L	Bonide Products Inc., Oriskany, NY, USA
RootShield WP	*Trichoderma harzianum*	7.39 g/L	Bioworks Inc., Victor, NY, USA
Triathlon BA	*Bacillus amyloliquefaciens* strain D747	25 mL/L	OHP Inc., Bluffton, SC, USA
Trichoderma asperellum	Experimental isolate	1×10^7 spores/L	
Zivion	Natamycin	4.84 mL/L	DSM, Heerlen, the Netherlands
Captan 50 WP	Captan	2.4 g/L	Southern Agricultural Insecticides, Boone, NC, USA
Miravis Prime	Fludioxonil + pydiflumetofen	700 µL/L	Syngenta, Greensboro, NC, USA

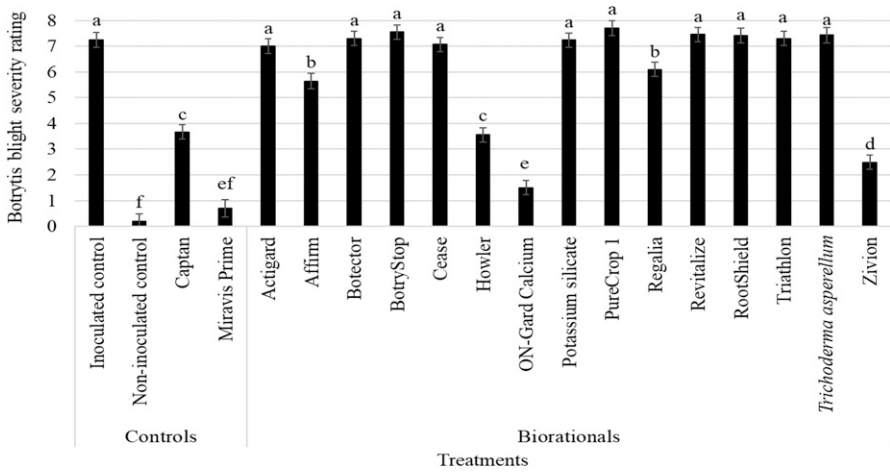

Fig. 1. Botrytis blight severity on detached petunia flowers treated with spray application of 15 biorational products applied 24 h before inoculation with a *Botrytis cinerea* spore suspension (Expt. 1). Captan and Miravis Prime were used as chemical fungicide controls. Inoculated and noninoculated controls were included. Data show results 72 h after inoculation. Lettering indicates significant differences between the treatments using Fisher's least significant difference test ($\alpha = 0.05$). Error bars represent ±1 standard error. Data were averaged over three replications (n = 30).

data collection were identical to Expt. 1. This experiment was performed three times.

Expt. 3A. Evaluation of the biorational products applied to rose flowers 1 d before inoculation. One-hundred fifty-two 'Orange Crush' rose cut flower stems were obtained from an Ecuadoran grower through a wholesale distributor (Carolina Florist Supply, Anderson, SC, USA). The flowering stems were placed in cardboard boxes in a 5 °C cooler for 24 h before the start of the experiment. Then the roses were removed from the cooler and 10 cm was cut from the base of the stem to improve hydration. Any visibly damaged leaves were removed. Roses were randomly selected and placed in groups of eight roses per treatment. The 15 biorational products were applied at the rates indicated in Table 1. Each flower bud was dipped for 15 s into the treatment solution, moving the head of the rose gently to improve solution contact with the multiple layers of flower petals. The roses were then placed in a 0.55-m length × 0.12-m width × 0.12-m height humid chamber covered with clear heavy duty, polyethylene plastic film. Ten trays were placed in the bottom of each humid chamber. Each tray contained 1 L water to hydrate the roses and to humidify the chamber. Each tray held a 35-cm length × 20-cm width × 0.22-cm height PVC structure with plastic mesh netting to hold the stems upright. Experiments were performed using a completely randomized design. For this, roses were randomized within the open humid chamber for 24 h to allow treatment solutions to dry. Then the roses were inoculated with a 1×10^5 spores/mL suspension using a 118 mL clear polyethylene terephthalate plastic bottle with a mist sprayer.

Visual ratings were performed 3, 5, and 7 d after inoculation based on a total flower area affected by botrytis using a 0 to 8 botrytis severity scale where 0 = no symptoms, 1 = necrotic spots covering 1% to 5% of the petal tissue, 2 = necrotic spots covering 6% to 15% of the petal tissue, 3 = necrotic spots covering 16% to 29% of the petal tissue, 4 = necrotic lesions covering 30% to 50% of the petal tissue, 5 = Necrotic lesions covering 51% to 60% of the tissue, 6 = necrotic lesions covering 61% to 70% of the petal tissue, 7 = necrotic spots covering 71% to 95% of the petal tissue, 8 = necrotic spots covering 96% to 100% of the petal tissue (adapted from Muñoz et al. 2025). This experiment was performed three times.

Expt. 3B. Evaluation of the biorational products applied to rose flowers 8 d before inoculation. In this experiment the dip applications of the products were performed at two commercial greenhouses in Cundinamarca, Colombia (lat. 4°59′16.9″N, lat. 4°48′03.3″N). Individual 'Orange Crush' rose cut flower buds were covered with clear plastic bags for the 8 weeks of growth before harvest to avoid contact with chemical applications. A total of 150 flowers were used for this experiment, and a completely randomized design was implemented. The following 12 biorational treatments were tested with the rates shown in Table 1: Actigard, Affirm, BotryStop, Cease, Howler, ON-Gard Calcium, PureCrop, Regalia, Revitalize, RootShield, Triathlon, and Zivion. After harvest, the stems were randomized and organized in a set of 10 roses before being dipped in the treatments as described in Expt. 3A. Immediately after the treatment applications, roses were grouped into their respective treatments in bunches and packed in plastic bouquets. The bouquets were labeled and placed in a cardboard box and cooled to 2 °C in a forced-air cooler, then the boxes were stored at 5 °C for 24 h before being shipped by airfreight to Clemson University, Clemson, SC, USA. Boxes arrived after 7 d. Upon arrival, the flowers were placed in the cooler at 5 °C for 24 h. The stems were taken out of the boxes, and 15 cm were cut from the base of the stems to improve water uptake. Flowers were inoculated 8 d after treatments as described in Expt. 3A, labeled, randomized, and placed in the humid chambers. Visual ratings were performed 3, 5, and 7 d after inoculation as per Expt. 3A. The experiment was performed twice.

Expt. 4. Evaluation of biorational product combinations applied to rose flowers 1 d before inoculation. Seventy-eight 'Orange Crush' roses were obtained from an Ecuadoran grower through a wholesale distributor (Carolina Florist Supply). After receiving the flowers, the same procedures from Expt. 3A were applied before starting the treatment application. Experiments were performed following a completely randomize design. For this, roses were randomly selected and placed in groups of six roses per treatment. The roses were then treated with the various biorational products. For roses, the four most effective biorational products from Expt. 3A were considered, including Actigard, Affirm, ON-Gard Calcium, Zivion, and their combinations were applied as described in Expt. 3A. Visual ratings were performed 3, 5, and 7 d after inoculation as per Expt. 3A. This experiment was performed three times.

Statistical analysis. Data analysis was performed using JMP pro version 16.0.0 (SAS Institute Inc., Cary, NC, USA). Each one of the experiments was independently run for the statistical analysis, considering the average response for the three replications. The effect of the treatments was assessed using analysis of variance and Fisher's least significant difference test was used to compare means between significant treatments at $P < 0.05$. For rose, the cumulative effect of the products over time was analyzed using the area under the disease progression curve calculated with data collected at 3, 5, and 7 d after inoculation using the calculation previously described (Bennett et al. 2020). For petunia, the effect of treatments on the botrytis severity response was evaluated 72 h after inoculation.

Synergistic or antagonistic effects of the combinations were calculated using the following equation: E = If + IN – IfIN/100 from Colby (1967), where If is the observed percentage of control provided by one of the single products, and IN is the observed percentage of control provided by the other single products used for the combination. E is the expected percentage control by the combination of the two products. When the observed control is higher than the expected, the combination presents a synergistic effect; when the observed control presents a lower result than the expected, control is referred to the antagonistic effect described by Peng et al. (2014).

Results

In petunia flowers, ON-Gard Calcium reduced disease severity by 79% compared with the inoculated control, which was not significantly different from Miravis Prime, but it showed a significant difference from the noninoculated control (Fig. 1). Zivion and Howler reduced disease severity by 66% and 51%, respectively, which was better than

Captan. Overall, Regalia and Affirm showed a reduction of 17% and 22%, respectively, in botrytis blight severity in comparison with the inoculated control; however, the results varied between experimental replications. No significant differences were observed between the other 10 treatments and the inoculated control.

From the first experiment, the three best performing biorational products were chosen for further evaluation as individual applications and in combination with one another (Fig. 2). ON-Gard Calcium, Zivion, and Howler reduced botrytis blight by 79%, 66%, and 51%, respectively, compared with the inoculated control and were equivalent to the noninoculated control. ON-Gard Calcium, Zivion, and Howler performed better than Captan, but they did not perform as well as Miravis Prime. All combinations performed better than the individual products, except for Howler + Zivion, which was the same as Howler applied alone. The combination of Howler + Zivion, ON-Gard Calcium + Howler, and ON-Gard Calcium + Zivion reduced botrytis blight by 77%, 79%, and 91%, respectively, compared with the inoculated control. The ON-Gard Calcium + Zivion combination was equivalent to Miravis Prime. The combinations of Howler + Zivion and ON-Gard Calcium + Howler showed antagonistic effects, whereas the combination of ON-Gard Calcium + Zivion showed a synergistic interaction. Synergistic effects of the three-active-ingredient combination could not be calculated.

In rose flowers treated 1 d before inoculation, Zivion showed a 18% reduction in botrytis blight severity compared with the inoculated control (Fig. 3). Cease also exhibited a disease severity reduction of 13% in comparison with the inoculated control. The application of Actigard and ON-Gard Calcium showed a disease severity reduction of 23% and 33%, respectively. Affirm reduced botrytis blight severity by 63%, similar to the Miravis Prime fungicide control. The other biorational treatments had no significant effect on disease severity.

In rose flowers treated 8 d before inoculation, Affirm reduced disease severity by 54% (Fig. 4). The use of ON-Gard Calcium showed a 31% reduction in disease severity. Both Actigard and Zivion reduced disease severity by 13% in comparison with the inoculated control. Cease, Howler and RootShield showed a reduction of 11%.

In rose flowers, all biorational combinations reduced disease severity compared with the inoculated control (Fig. 5). Actigard, Affirm, ON-Gard Calcium, and Zivion performed better than the inoculated control and showed a reduction of botrytis blight of 49%, 61%, 33%, and 40%, respectively. The combination of Actigard + Affirm and Affirm + ON-Gard Calcium performed similar to Miravis Prime. The combinations of Actigard + Affirm, Actigard + ON-Gard Calcium, Affirm + ON-Gard Calcium, Affirm + Zivion, and ON-Gard Calcium + Zivion showed synergistic effects, whereas the Actigard + Zivion combination showed an antagonistic effect.

Discussion

Spray applications of ON-Gard Calcium (1000 mg/L Ca) on detached petunia flowers and dip applications on rose flowers consistently reduced botrytis blight symptoms comparable to the fungicide Miravis Prime. This is consistent with the results from previous studies where the use of calcium at concentrations of 800 and 1200 mg/L had a positive effect in the reduction of *B. cinerea* on petunia flowers (Bennett et al. 2020), whereas concentrations of 1000 and 2000 mg/L Ca using calcium chloride resulted in decreased botrytis blight severity of cut roses (Muñoz et al. 2025). This effect is potentially a combined result of the structural role of calcium-forming bonds with the pectic material located in the middle lamella, which strengthen the cell wall and effectively increase the resistance of the tissue to fungal penetration (Gislerød 1997) and the role of calcium as a signaling molecule responsible for modulating a series of physiological and molecular downstream responses (Liu et al. 2024).

Rose flowers receiving a dip application of RootShield exhibited reduced botrytis blight severity only when applied 8 d before inoculation. The active ingredient in RootShield is *Trichoderma harzianum*, a fungus related to a strong lytic and antibiotic effect (Gams and Meyer 1998). It is possible that RootShield was only effective when applied 8 d before inoculation because time is required for the fungus to colonize the petal tissue and induce resistance in the host plant. The biorationals Actigard, Affirm, Cease, ON-Gard Calcium, and Zivion are not living organisms and reduced disease severity on rose flowers when they were applied 1 d and 8 d before inoculation, indicating a faster effect.

Howler was a more promising treatment for petunia than rose. The active ingredient listed on the Howler label is a bacterium (*Pseudomonas chlororaphis*); however, Wesche et al. (2025) reported that no living bacteria was found in commercial lots, and the

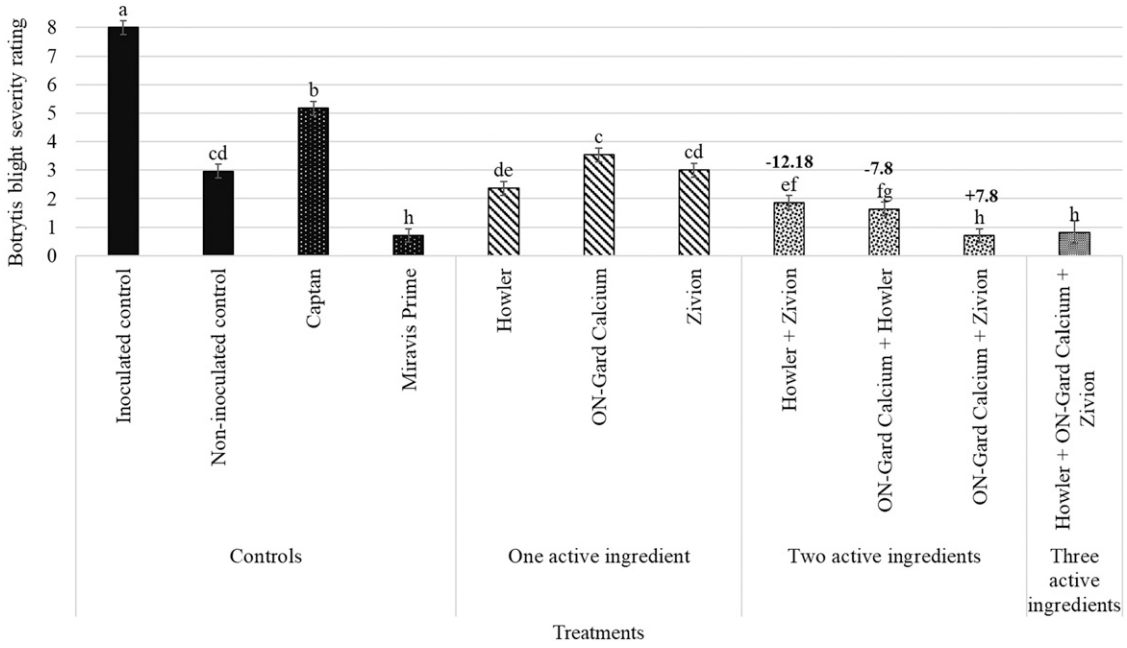

Fig. 2. Botrytis blight severity on detached petunia flowers treated with three biorational products and their four combinations 24 h before inoculation with a *Botrytis cinerea* spore suspension (Expt. 2). Captan and Miravis Prime were used as chemical fungicide controls. Inoculated and noninoculated controls were included. Data show results 72 h after inoculation. Lettering indicates significant differences between the treatments using Fisher's least significant difference test ($\alpha = 0.05$). Error bars represent ±1 standard error. Data were averaged over three replications (n = 30). Numbers above the bars indicate antagonistic (–) or synergistic (+) effects (Colby 1967).

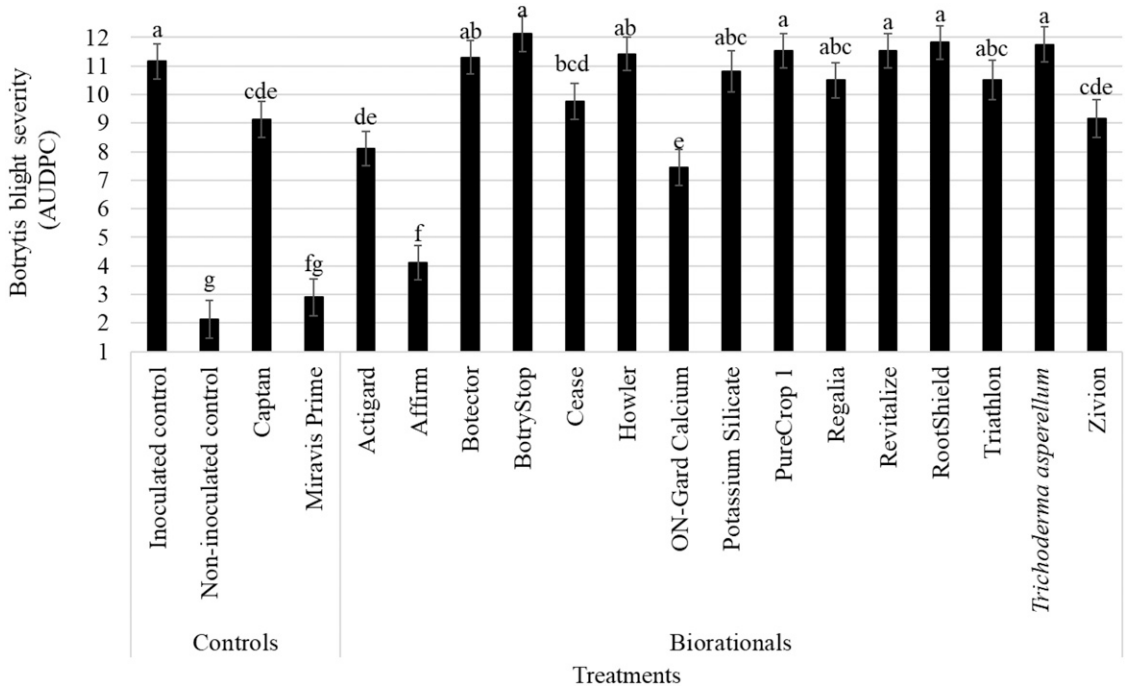

Fig. 3. Botrytis blight severity on rose flowers treated with a dip application of 15 biorational products 1 d before inoculation (Expt. 3A). Captan and Miravis Prime were used as chemical fungicide controls. Inoculated and noninoculated controls were included. Botrytis blight severity is expressed as the area under the disease progression curve (AUDPC) including the severity ratings from days 3, 5, and 7 after inoculation with a *Botrytis cinerea* spore suspension (10^5 spores/mL). Lettering indicates significant differences between the treatments using Fisher's least significant difference test ($\alpha = 0.05$). Error bars represent ±1 standard error. Data were averaged over three replications (n = 24).

effectiveness of Howler was attributable to a fermentation product of *P. chlororaphis*, that is, Howler should be considered a biofungicide, not a BCA.

Our results showed that Actigard showed a reduction of botrytis blight on roses when it was applied 8 and 1 d before inoculation but did not show any effect on petunia flowers. The active ingredient in Actigard, acibenzolar-S-methyl, induces plant defense mechanisms against diseases (Lawton et al. 1996). When it was used as postharvest treatment in strawberries, Actigard reduced botrytis severity (Terry and Joyce 2000). In grapes, botrytis was reduced when acibenzolar-S-methyl was applied as a spray; however, the efficacy increased when the fruits were immersed in the product in comparison to

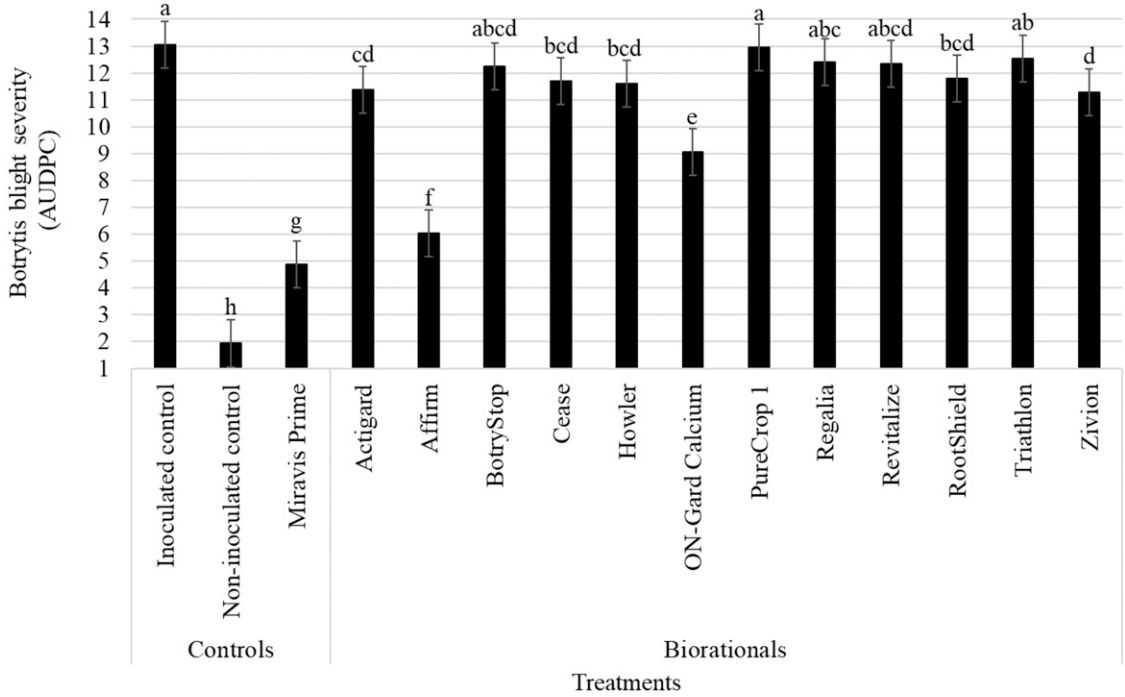

Fig. 4. Botrytis blight severity on rose flowers treated 8 d before inoculation with a dip application of 12 biorational products (Expt. 3B). Miravis Prime was used as a chemical fungicide controls. Inoculated and noninoculated controls were included. Botrytis blight severity is expressed as the area under the disease progression curve (AUDPC) including the severity ratings from days 3, 5, and 7 after inoculation with a *Botrytis cinerea* spore suspension (10^5 spores/mL). Lettering indicates significant differences between the treatments using Fisher's least significant difference test ($\alpha = 0.05$). Error bars represent ±1 standard error. Data were averaged over three replications (n = 30).

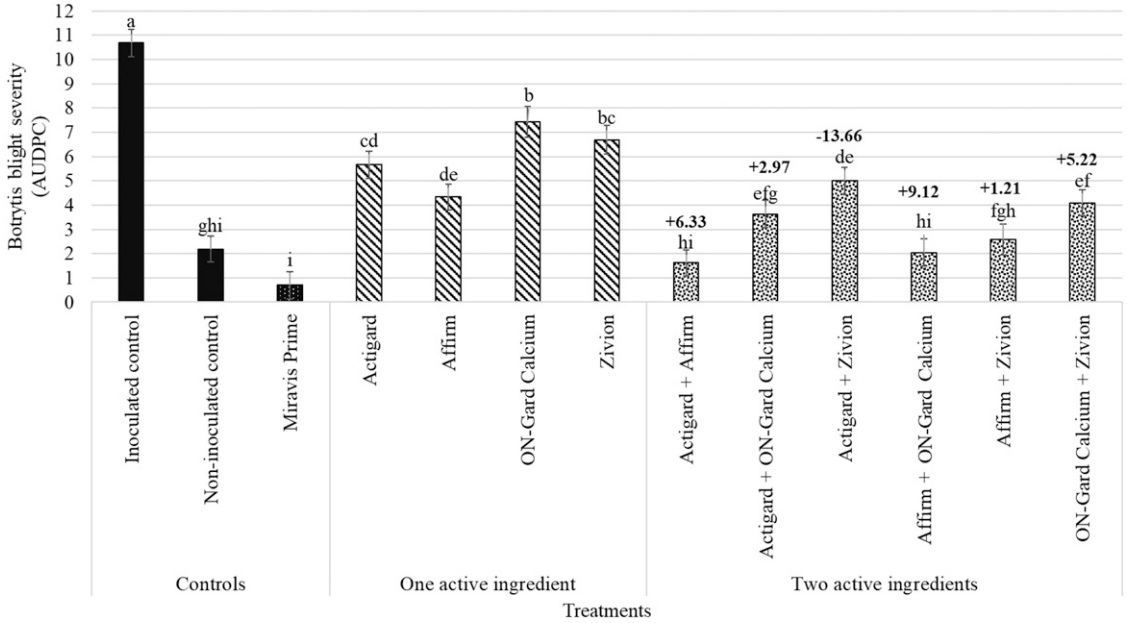

Fig. 5. Botrytis blight severity rating on rose flowers dipped with four biorationals and their six combinations (Expt. 4). Miravis Prime served as a chemical fungicide controls. Botrytis blight severity was expressed as the area under the disease progression curve (AUDPC), which included the severity ratings at days 3, 5, and 7 after inoculation with a *Botrytis cinerea* spore suspension. Lettering indicates significant differences between the treatments using Fisher's least significant difference test ($\alpha = 0.05$). Error bars represent ±1 standard error. Data were averaged over three replications (n = 18). Numbers above the bars indicate antagonistic (–) or synergistic (+) effects (Colby 1967).

when the product was applied as a spray (Youssef et al. 2019).

Zivion showed a reduction of the disease on petunia and rose flowers. The active ingredient in Zivion is natamycin, which is a product obtained from the fermentation of *Streptomyces natalensis* (Oostendorp 1981) and has antifungal properties. Natamycin binds the ergosterol of fungi, limiting the growth of the pathogen (Aparicio et al. 2016) and appears to be a promising tool for botrytis blight management; however, considering the ultraviolet sensitivity of this compound best results are most likely to be obtained from postharvest treatments.

Botector, BotryStop, Revitalize, RootShield, Triathlon, and *T. asperellum* had no effect on disease severity for rose or petunia. Studies have reported that these BCAs yield variable results because they require the proper environmental conditions (temperature, light, humidity) for growth and to achieve their antagonistic effect against the pathogen (Tatagiba et al. 1998). For example, *B. cinerea* control has been effective when the BCA is capable of surviving and adapting to the environment. In cyclamen (*Cyclamen persicum*), a reduction of the botrytis infection was achieved when BotryStop was applied 48 h before inoculation, whereas no effect was observed when BotryStop was applied at the same time as the pathogen (Kessel et al. 2002). Studies have reported that combining different microorganisms can improve their efficacy due to their different modes of action (Sylla et al. 2015); for example, disease severity was reduced by 80% to 99% when *Pichia guilermondii*, a yeast, and *Bacillus mycoides*, a bacterium, were used in combination (Guetsky et al. 2001). Further investigation is needed to understand why the living BCA products used in this study did not provide control of botrytis blight.

The combinations of ON-Gard Calcium + Howler and ON-Gard Calcium + Zivion performed better on petunia than the products by themselves. In rose flowers, all combinations performed significantly better than the products alone except for Actigard + Zivion, which performed as well as Actigard and Zivion alone. Actigard + Affirm and ON-Gard Calcium + Affirm performed similarly to Miravis Prime. The combination of Howler + Zivion and ON-Gard Calcium + Zivion showed antagonistic effects meaning the observable control was higher than the expected control. Howler EVO, a new formulation of Howler, contains antifungal metabolites including pyrrolnitrin, which has been shown to reduce the P450 14α-demethylase gene *CYP51* and to result in synergistic interactions with demethylation inhibitor (DMI) fungicides in plant pathogenic fungi such as *Monilinia fructicola* and *Colletotrichum siamense* (Wesche et al. 2024a, 2024b). It is not known whether Howler suppresses the *CYP51* gene in *B. cinerea* or if synergies exist between Howler and DMI fungicides against *B. cinerea*–induced diseases. Natamycin, the active ingredient of Zivion, is a polyene macrolide that, in contrast to DMIs, does not bind to enzymes involved in ergosterol synthesis. Instead, it binds to ergosterol itself, and thus its mode of action is different from that of azoles. This study shows that at least in *B. cinerea,* the potential suppression of *CYP51* expression combined with ergosterol-binding did not lead to synergistic interactions. Synergistic effects were observed for the combination of ON-Gard Calcium + Zivion. The mechanism of this synergy is unknown. The calcium in ON-Gard Calcium's $CaCl_2$ strengthens plant cell walls by binding to pectin. It also inhibits the polygalacturonase enzyme in plant pathogenic fungi at concentrations starting at 100 to 500 μg/mL of calcium (Bennett et al. 2020; Muñoz et al. 2025). Thus, it is possible that a combination of factors involved in strengthening the plant cell wall and direct effects on fungal metabolic processes and ergosterol function led to the phenomenon of synergy.

These results demonstrate that some biorational products and their combinations showed efficacy against botrytis blight on ornamental flowers. ON-Gard Calcium, Howler, and Zivion and their combinations showed promising results for managing botrytis blight in petunia flowers, whereas Actigard, Affirm, ON-Gard Calcium, and Zivion and their combinations showed promising results for managing botrytis blight on cut flower roses. To our knowledge, no past studies have reported on the modes of action of the combinations of biorational products evaluated in this study. As such, detailed mechanistic information is currently lacking in literature. Therefore, this knowledge gap invites further research to elucidate the mechanisms underlying the observed efficacy.

References Cited

Achuo EA, Audenaert K, Meziane H, Höfte M. 2004. The salicylic acid–dependent defense pathway is effective against different pathogens in tomato and tobacco. Plant Pathol. 53(1): 65–72. https://doi.org/10.1111/j.1365-3059.2004.00947.x.

Adriaens T, San Martin y Gomez G, Maes D. 2007. Invasion history, habitat preferences and phenology of the invasive ladybird *Harmonia axyridis* in Belgium, p 69–88. In: Roy HE,

Wajnberg E (eds). From biological control to invasion: The ladybird *Harmonia axyridis* as a model species. Springer, Dordrecht, The Netherlands. https://doi.org/10.1007/978-1-4020-6939-0_6.

Aparicio JF, Barreales EG, Payero TD, Vicente CM, de Pedro A, Santos-Aberturas J. 2016. Biotechnological production and application of the antibiotic pimaricin: Biosynthesis and its regulation. Appl Microbiol Biotechnol. 100(1): 61–78. https://doi.org/10.1007/s00253-015-7077-0.

Banani H, Olivieri L, Santoro K, Garibaldi A, Gullino ML, Spadaro D. 2018. Thyme and savory essential oil efficacy and induction of resistance against *Botrytis cinerea* through priming of defense responses in apple. Foods. 7(2):11. https://doi.org/10.3390/foods7020011.

Bennett K, Jent J, Samarakoon UC, Schnabel G, Faust JE. 2020. Reduction of *Botrytis cinerea* infection on petunia flowers following calcium spray applications. HortScience. 55(2):188–191. https://doi.org/10.21273/HORTSCI14208-19.

Colby SR. 1967. Calculating synergistic and antagonistic responses of herbicide combinations. Weeds. 15(1):20–22. https://doi.org/10.2307/4041058.

Copping LG, Menn JJ. 2000. Biopesticides: A review of their action, applications and efficacy. Pest Manag Sci. 56(8):651–676. https://doi.org/10.1002/1526-4998(200008)56:8<651::AID-PS201>3.0.CO;2-U.

Damalas CA, Eleftherohorinos IG. 2011. Pesticide exposure, safety issues, and risk assessment indicators. Int J Environ Res Public Health 8(5): 1402–1419. https://doi.org/10.3390/ijerph8051402.

Dik AJ, Wubben JP. 2007. Epidemiology of *Botrytis cinerea* diseases in greenhouses, p 319–333. In: Elad Y, Williamson B, Tudzynski P, Delen N (eds). Botrytis: Biology, pathology and control. Springer, Dordrecht, The Netherlands. https://doi.org/10.1007/978-1-4020-2626-3_17.

Elad Y. 1988. Latent infection of *Botrytis cinerea* in rose flowers and combined chemical and physiological control of the disease. Crop Prot. 7(6):361–366. https://doi.org/10.1016/0261-2194(88)90003-8.

Fillinger S, Elad Y. 2016. Botrytis—The fungus, the pathogen and its management in agricultural systems. In: Fillinger S, Elad Y (eds). Springer International Publishing, Basel, Switzerland. https://doi.org/10.1007/978-3-319-23371-0.

Gams W, Meyer W. 1998. What exactly is *Trichoderma harzianum*? Mycologia. 90(5):904–915. https://www.tandfonline.com/doi/abs/10.1080/00275514.1998.12026984.

Gislerød HR. 1997. The role of calcium on several aspects of plant and flower quality from a floricultural perspective. Acta Hortic. 481:345–352. https://doi.org/10.17660/actahortic.1999.481.4.

Guetsky R, Shtienberg D, Elad Y, Dinoor A. 2001. Combining biocontrol agents to reduce the variability of biological control. Phytopathol. 91:621–627. https://doi.org/10.1094/phyto.2001.91.7.621.

Hahn M. 2014. The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study. J Chem Biol. 7(4):133–141. https://doi.org/10.1007/s12154-014-0113-1.

Kapoor B. 2020. Biorational pesticides: An enviro-safe alternative to pest control. Indian Farmer. 7(8):90.

Kessel GJT, De Haas BH, Van Der Werf W, Köhl J. 2002. Competitive substrate colonization by *Botrytis cinerea* and *Ulocladium atrum* in relation to biological control of *B. cinerea* in cyclamen. Mycol Res. 106(6):716–728. https://doi.org/10.1017/S0953756202005956.

Kessmann H, Staub T, Hofmann C, Maetzke T, Herzog J, Ward E, Uknes S, Ryals J. 1994. Induction of systemic acquired disease resistance in plants by chemicals. Annu Rev Phytopathol. 32:439–459. https://doi.org/10.1146/annurev.py.32.090194.002255.

Lawton KA, Friedrich L, Hunt M, Weymann K, Delaney T, Kessmann H, Staub T, Ryals J. 1996. Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. Plant J. 10(1):71–82. https://doi.org/10.1046/j.1365-313x.1996.10010071.x.

Liu Y, Wu J, Li Y, Deng W, Cao K, Li Z, Wang L. 2024. Calcium chloride enhances phenylpropanoid metabolism, antioxidant ability and phytohormone signaling to effectively alleviate chilling injury in postharvest nectarines. Postharvest Biol Tech. 217:113122. https://doi.org/10.1016/j.postharvbio.2024.113122.

Muñoz M, Behnke LE, Bridges WC, Schnabel G, Faust JE. 2025. Postharvest calcium chloride dips. An effective strategy to reduce *Botrytis* blight severity and increase petal strength in cut roses. Postharvest Biol Tech. 219:113292. https://doi.org/10.1016/j.postharvbio.2024.113292.

Muñoz M, Faust J, Schnabel G. 2019. Characterization of *Botrytis cinerea* from commercial cut flower roses. Plant Dis. 103(7):1577–1583. https://doi.org/10.1094/PDIS-09-18-1623-RE.

Nazzaro F, Fratianni F, Coppola R, De Feo V. 2017. Essential oils and antifungal activity. Pharmaceuticals. 10:4–86. https://doi.org/10.3390/ph10040086.

Oostendorp JG. 1981. Natamycin. Antoine Van Leeuwenhoek. 47:170–171. https://doi.org/10.1007/bf02342201.

Oostendorp M, Kunz W, Dietrich B, Staub T. 2001. Induced disease resistance in plants by chemicals. Eur J Plant Pathol. 107:19–28. https://doi.org/10.1023/a:1008760518772.

Paulitz TC, Belanger RR. 2001. Biological control in greenhouse systems. Ann Rev Phythopathol. 39:103–133. https://doi.org/10.1146/annurev.phyto.39.1.103.

Peng D, Li S, Chen C, Zhou M. 2014. Combined application of Bacillus subtilis NJ-18 with fungicides for control of sharp eyespot of wheat. Biological Control. 70:28–34. https://doi.org/10.1016/j.biocontrol.2013.11.013.

Pikovskyi MY, Kolesnichenko OV, Melnyk VI, Serediuk OO. 2018. Flowering and ornamental plant-hosts of *Botrytis cinerea* Pers. Bioresour Nat Manage. 10:5–10. https://doi.org/10.31548/bio2018.05.001.

Saito S, Wang F, Xiao CL. 2022. Natamycin as a postharvest treatment to control gray mold on stored blueberry fruit caused by multi-fungicide resistant *Botrytis cinerea*. Postharvest Biol Tech. 187:111862. https://doi.org/10.1016/j.postharvbio.2022.111862.

Shi Z, Yang H, Jiao J, Wang F, Lu Y, Deng J. 2019. Effects of graft copolymer of chitosan and salicylic acid on reducing rot of postharvest fruit and retarding cell wall degradation in grapefruit during storage. Food Chem. 283:92–100. https://doi.org/10.1016/j.foodchem.2018.12.078.

Schmitt A, Eisemann S, Strathmann S, Emslie KA, Seddon B. 1996. The use of *Reynoutria sachalinensis* extracts for induced resistance in integrated disease control: Effects on *Botrytis cinerea*, p 46. Book of Abstracts of the XIth International Botrytis Symposium, Wageningen, The Netherlands.

Sylla J, Alsanius BW, Krüger E, Wohanka W. 2015. Control of *Botrytis cinerea* in strawberries by biological control agents applied as single or combined treatments. Eur J Plant Pathol. 143(3):461–471. https://doi.org/10.1007/s10658-015-0698-4.

Tatagiba S, Maffia LA, Barreto RW, Alfenas AC, Sutton JC. 1998. Biological control of *Botrytis cinerea* in residues and flowers of rose (*Rosa hybrida*). Phytoparasitica. 26(1):8–19. https://doi.org/10.1007/BF02981261.

Terry LA, Joyce DC. 2000. Suppression of grey mould on strawberry fruit with the chemical plant activator acibenzolar. Pest Manag Sci. 56(11):989–992. https://doi.org/10.1002/1526-4998(200011)56:11<989::AID-PS229>3.0.CO;2-A.

Usta C. 2013. Microorganisms in biological pest control—A review (bacterial toxin application and effect of environmental factors), p 287–317. In: Silva-Opps M (ed). Current progress in biological research. InTech. https://doi.org/10.5772/55786.

Webster BJ. 2005. Managing foliar blights on specialty crops (MS Thesis). Michigan State University, East Lansing, MI, USA. https://doi.org/10.25335/M53B5WK35.

Wesche J, Wu P, Luo C-X, Faust JE, Schnabel G. 2024a. Bioproducts of *Pseudomonas chlororaphis* suppress DMI fungicide-induced CsCYP51A and CsCYP51B gene expression in *Colletotrichum* siamense and generate synergistic effects with metconazole and propiconazole. Phytopathology. https://doi.org/10.1094/PHYTO-03-24-0090-R.

Wesche J, Zeng Z, Luo C-X, Schnabel G. 2024b. Pyrrolnitrin in *Pseudomonas chlororaphis* strain ASF009 metabolites reduces constitutive and DMI-induced *MfCYP51* gene expression in *Monilinia fructicola*. Plant Dis. 109:657–663. https://doi.org/10.1094/PDIS-07-24-1470-RE.

Wesche J, Repp J, Hu M, Faust J, Schnabel G. 2025. Cross-resistance between *Pseudomonas chlororaphis* strain AFS009 metabolites (Howler EVO) and fludioxonil in *Botrytis cinerea*. Plant Dis. 109(6):1366–1371. https://doi.org/10.1094/PDIS-10-24-2211-RE.

Williamson B, Duncan GH, Harrison JG, Harding LA, Elad Y, Zimand G. 1995. Effect of humidity on infection of rose petals by dry-inoculated conidia of *Botrytis cinerea*. Mycol Res. 99(11): 1303–1310. https://doi.org/10.1016/S0953-7562(09)81212-4.

Wurms KV, Ah Chee A, Wood PN, Taylor JT, Parry F, Agnew RH, Hedderley D, Elmer PAG. 2021. Lipid-based natural food extracts for effective control of botrytis bunch rot and powdery mildew on field-grown winegrapes in New Zealand. Plants (Basel). 10(3):423. https://doi.org/10.3390/plants10030423.

Youssef K, Roberto S, Colombo R, Canteri M, Elsalam K. 2019. Acibenzolar-S-methyl against *Botrytis* mold on table grapes in vitro and in vivo. ASB J. 5(1):52–52. https://doi.org/10.33158/ASB.2019v5i1p52.

Zhang X, Wang L, Chen Y-Y, Dai Y, Li M-Q, Zhang H-W. 2025. Natamycin and potassium sorbate syngeristically enhance resistance to *Botrytis cinerea* by activating the phenylpropanoid metabolism in harvested strawberry. Postharv Biol Tech. 222:113361. https://doi.org/10.1016/j.postharvbio.2024.113361.