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# Special Research Report #139: Use of Beneficial Microbes to Decrease Disease Severity in Ornamentals.

Disease Management

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## BACKGROUND

The commercial greenhouse industry relies heavily on chemical pesticides when producing ornamental plants. Growing concerns about environmental contamination and worker and consumer safety have led to increased interest in effective alternatives. Biopesticides, which are derived from natural materials, provide viable alternatives to the use of synthetic chemicals. Bacteria from a diverse number of genera have been found to colonize plant roots and benefit plants by stimulating growth or by suppressing plant diseases and pests. Some of these bacteria act as biocontrol agents by eliciting an induced systemic resistance (ISR) that is effective against foliar and root pathogens as well as insect herbivores. Biocontrol by beneficial microbes may result from the production of antibiotics or fungal cell wall lysing enzymes or it may be due to competition with pathogenic microorganisms. *Pseudomonas* species have been widely studied for their ability to promote growth by both stimulating the plant and by directly suppressing pathogens. Many pseudomonads produce antibiotics like 2,4-DAPG (2,4-diacetylphloroglucinol), which control a variety of root and seedling diseases by suppressing pathogenic fungi.

Gray mold or botrytis blight is a disease caused by a fungal pathogen, *Botrytis cinerea*. This pathogen causes disease is most major greenhouse crops, including containerized ornamentals. *Botrytis* infection can lead to reduced crop quality or plant death. *Botrytis* is particularly problematic in the greenhouse in early spring due to the cool temperatures, high humidity, and wet foliage and flowers. Fungicides are available to control gray mold, but *Botrytis* is resistant to several active ingredients. Preventative measures against *Botrytis* include environmental and cultural practices such as increased air movement and keeping a clean space, but these practices are not always feasible or reliable. Alternative management methods of *Botrytis* are needed for the greenhouse industry. The ***goal*** of this research was to investigate the efficacy of selected beneficial bacteria strains for the biocontrol of *Botrytis* in containerized ornamentals.

## MATERIALS & METHODS

### The beneficial bacteria collection

This collection includes 44 strains of bacteria in the genus *Pseudomonas from an OSU collection*. The strains represent nine different species, and the bacteria were isolated from various soil, water and plant samples. An additional 15 strains were obtained from Dr. Joseph Kloepper (Auburn University). Identities were determined by sequencing (Subedi et al., 2019). All of the bacteria were evaluated in greenhouse trials to identify the strains that could reduce *Botrytis cinerea* disease symptoms in *Petunia x hybrida* "Carpet Red Bright" (Fig. 1).

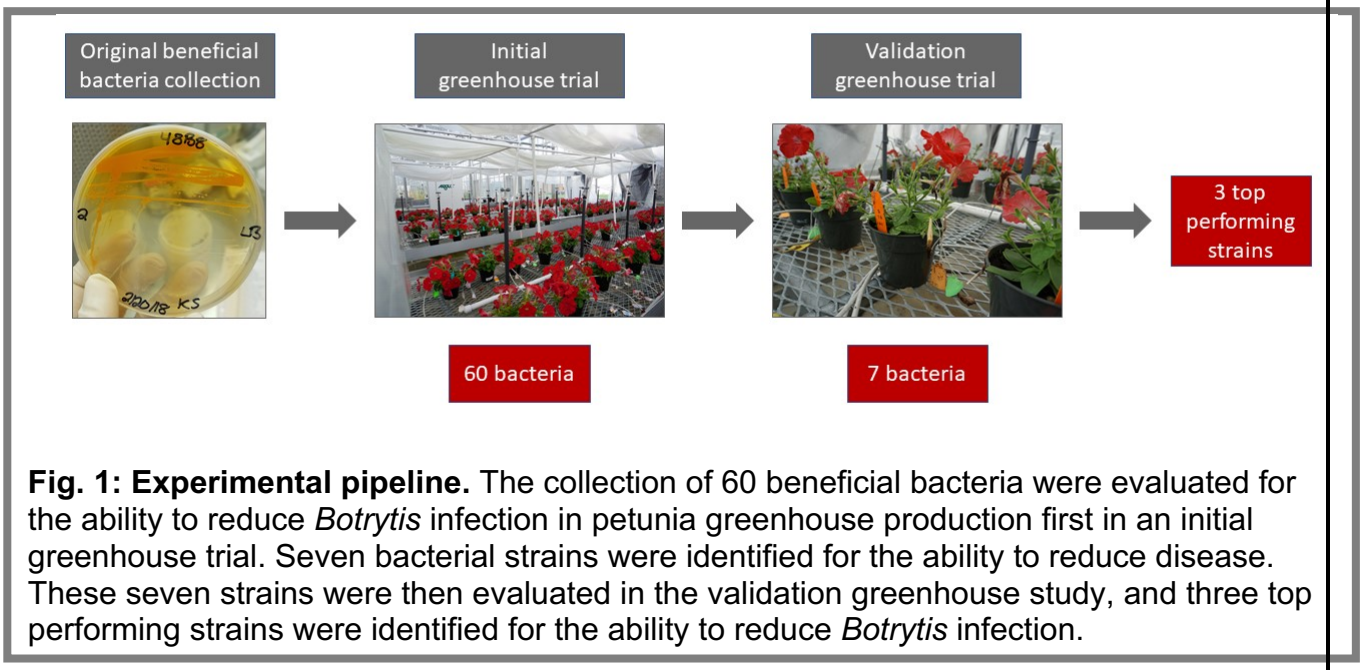



**Fig. 1: Experimental pipeline.** The collection of 60 beneficial bacteria were evaluated for the ability to reduce *Botrytis* infection in petunia greenhouse production first in an initial greenhouse trial. Seven bacterial strains were identified for the ability to reduce disease. These seven strains were then evaluated in the validation greenhouse study, and three top performing strains were identified for the ability to reduce *Botrytis* infection.

**Initial greenhouse trial.** Identify strains with the potential to reduce disease in a greenhouse production setting.

- **Plant material:** *Petunia x hydrida* "Carpet Red Bright" in 4.5" pots of Pro-Mix PGX media with 50 ppm N 15-5-15 Ca Mg fertilizer.
- **Treatments:** 60 bacterial strains + positive control (commercial biopesticide) + negative control (no bacteria).
- **Experimental Design:** Augmented design: 3 replicates of each bacterial treatment and 12 replicates of each control treatment.
- **Methods:**

**1.**

**2.**

1. First bacterial application at transplant and then every two weeks after that

for a six-week period. Applications were made through spray to the flowers and leaves and drench to the media.

2. All open flowers were tagged after the last bacterial application, and plants were then moved to high humidity/low temperature environment.

3. Plants were then inoculated with *Botrytis* through spray application of spores.

3  

4. Tagged flowers were evaluated daily until 12 days after the *Botrytis* inoculation using a disease severity rating scale (0: no disease to 7: flower senesced because of *Botrytis*). The daily ratings were used to calculate disease severity index.

4. 


**Validation greenhouse trial.** Validate the ability of the seven top performing strains to reduce severity of *Botrytis* infection.

- The same methods described in the initial greenhouse trial were used except for the experimental design.
- **Experimental Design:** Randomized complete block design: 12 replicates of each bacterial treatment and of each control treatment, replicated in time.

**Validation Trial: 7 strains evaluated:**
*Pseudomonas chlororaphis* 14B11
*P. frederiksbergensis* 94G2
*P. fluorescens* 89F1
*P. protegens* 15H3
*P. lini* 48C10
*Pantoea agglomerans* AP47
*Comamonas acidovorans* AP54

**RESULTS**

**Initial greenhouse trial.** Collection of 60 bacterial strains evaluated in petunia for ability to reduce disease.

- Seven strains were selected from the original bacterial collection for the ability to reduce disease severity in petunia.
- The disease severity index was calculated for each bacterial treatment each rating day based on daily ratings of the flowers (Fig. 2).
- The top seven strains reduce severity of *Botrytis* infection compared to the negative control.

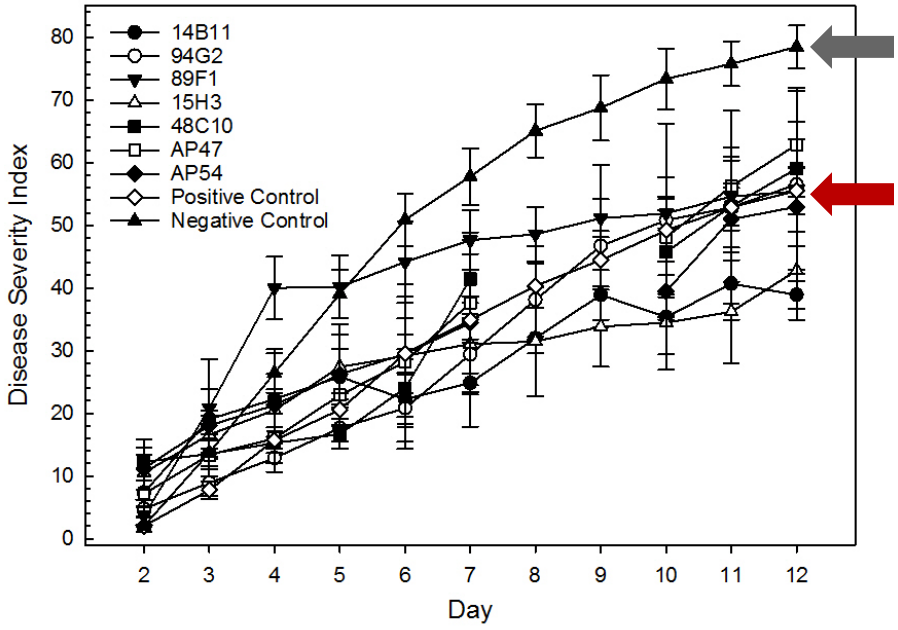



**Fig 2.** Disease severity index of the two controls and top 7 performing strains identified from the initial greenhouse trial. The gray arrow indicates the negative control, while the red arrow indicates the positive control.

**7 strains moved on to the Validation Greenhouse Trials.**

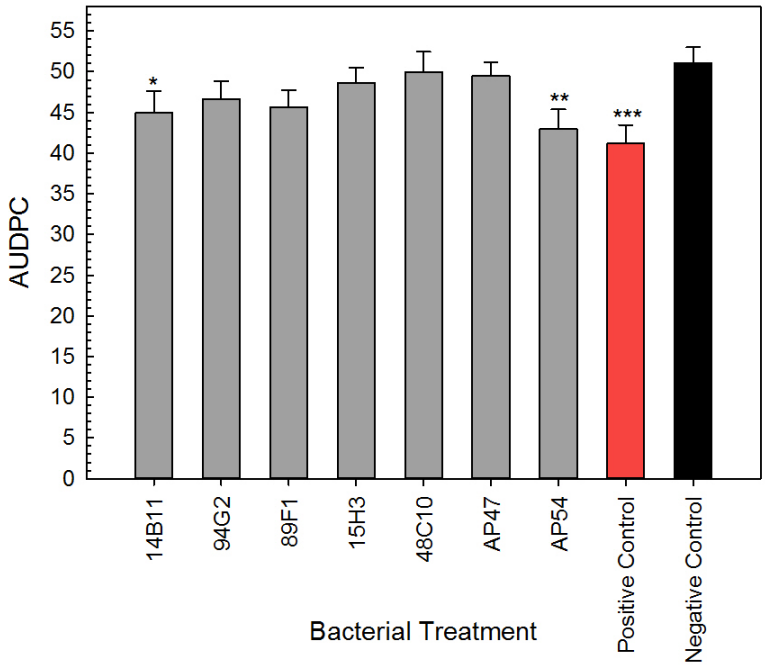
**Validation greenhouse trial.** Seven bacterial strains evaluated for the ability to reduce disease severity in petunia.

Area under the disease progress curve (AUDPC)

- AUDPC was calculated based on the daily ratings of the flowers.
- AUDPC summarizes the disease severity over the rating period.

**Fig 3.** AUDPC for the seven strains evaluated in the validation greenhouse trial. The black bar is the negative control and the red bar is the positive control.

The positive control, 14B11, and AP54 had a decrease in AUDPC value compared to the negative control.



## Disease Severity Index

- Disease severity index is calculated from the daily flower ratings.
- It shows the disease severity of plants receiving a certain treatment each rating day.

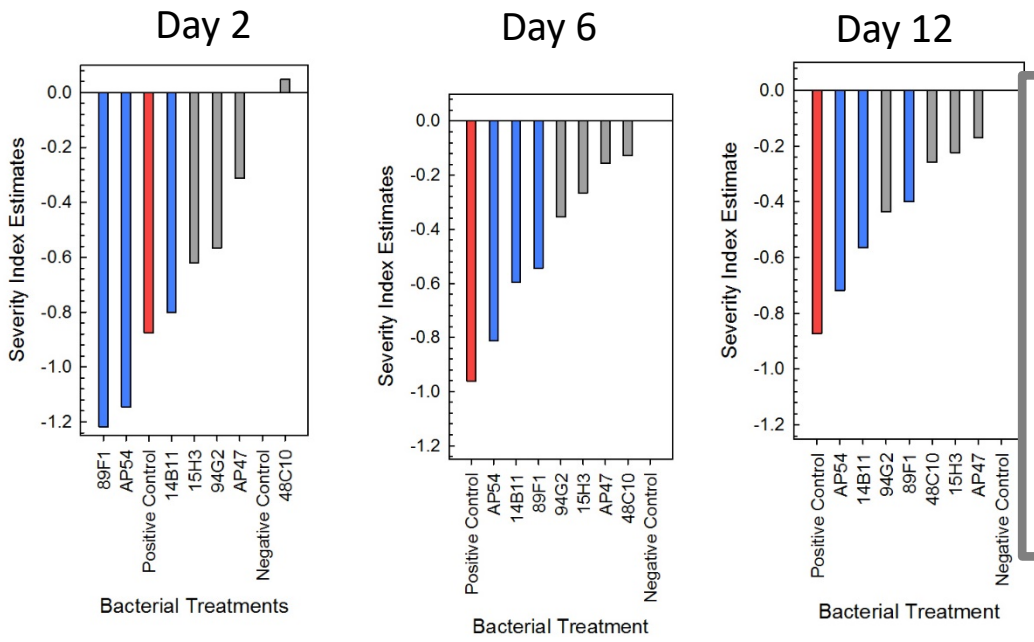



**Fig 4.** Disease severity index fixed effect estimates of treatment effect on rating day compared to the negative control. The more negative the value, the less disease. Red bars represent the positive control, and blue bars represent the top three strains.

The positive control, 14B11, AP54, and 89F1 have reduced a disease severity index compared to the negative control.

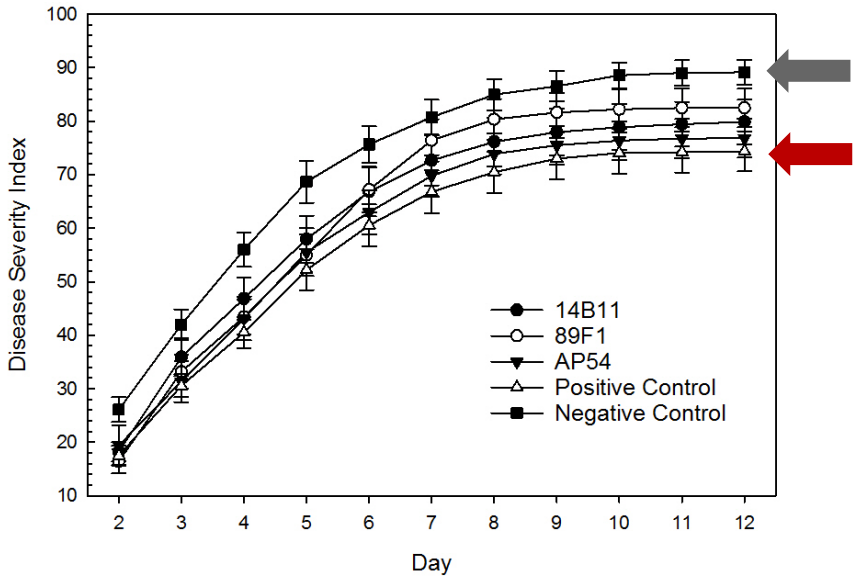



**Fig 5.** Disease severity index of the two controls and top three performing strains identified from the validation greenhouse trial. The gray arrow indicates the negative control, while the red arrow indicates the positive control.

AP54, 14B11, and 89F1 were selected as the top performing strains based on the ability to reduce severity of *Botrytis* infection in petunia.

## CONCLUSIONS

- The greenhouse trials were effective in identifying bacteria strains with the potential to reduce *Botrytis* infection in petunia.
- These trials lead to the identification of three top performing strains with the ability to reduce disease severity when applied to plants in a greenhouse production setting.

## INDUSTRY IMPACT

- The identified strains have the potential to be formulated into products for the biocontrol of *Botrytis* in floriculture crop production.
- Biocontrol products give growers additional management tools to produce high quality plants.
- Biocontrol product use can also lead to the reduction in fungicide applications.
- Biocontrol products can provide additional methods to continue to move greenhouse production to a more sustainable system.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