Biocontrol of Botrytis in Cut Flowers

This report is funded by the ASCFG Research Foundation.

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The pathogen: *Botrytis cinerea*

Like many other industries, the cut flower industry struggles with disease caused by *Botrytis cinerea*. This pathogen is the causal agent of gray mold (also known as botrytis blight) and infects many different crops across the world. Not only does it have a wide host range, but it also causes damage to crops throughout the production chain. Infection can lead to reduction in crop quality or crop loss. Symptoms or signs of a *Botrytis* infection in cut flowers can be found on petals, leaves, or stems. Symptoms include tan lesions on the petals, and eventually the growth of gray fuzzy spores that give gray mold its name (Smith, 2019). *Botrytis* thrives in low temperature and high humidity environments. Preventative measures include controlling temperature and humidity levels, and reducing time of standing moisture on petals. Fungicides are used to control gray mold in cut flowers, but resistance to several different fungicides has been reported (Muñoz, Faust, & Schnabel, 2019).

The alternative method of control: Beneficial bacteria

Biological control, or biocontrol, is an alternative method of controlling plant pathogens that is gaining the interest of researchers and growers alike (Meister Media Worldwide, 2014). Beneficial bacteria is one type of biocontrol agent that can be used to suppress pathogens in a plant system. *Pseudomonas* bacteria have shown great potential for the biocontrol of *Botrytis* in petunia (Gould, Kobayashi, & Bergen, 1996; South, Peduto Hand, & Jones, 2020).

The objectives of the current study were 1) To develop a pipeline to evaluate bacterial strains for biocontrol in cut flowers, and 2) To evaluate select beneficial bacteria for the ability to suppress gray mold in cut flowers.

Bacterial application and *Botrytis* inoculation details

Bacteria used in this study were previously selected for the biocontrol of *Botrytis* in petunia (South et al., 2020). Along with the application of the bacterial treatments, two groups of flowers received no bacterial application to serve as the controls. Control group 1 was not inoculated with *Botrytis* to account for any *Botrytis* already present on the flowers, while control group 2 was inoculated with *Botrytis* (Table 1).

Table 1

Treatment	Botrytis Inoculation (Y/N)
Control group 1 (no bacteria)	N
Control group 2 (no bacteria)	Y
Pseudomonas chlororaphis 14B11	Y
P. protegens AP54	Y
P. fluorescens 89F1	Y
P. frederiksbergensis 94G2	Y

'ABC 2 Blue' lisianthus (*Eustoma grandiflora*) were obtained from a local Ohio grower, Sunny Meadows Flower Farm (Columbus, OH). The flowers were separated into groups to be treated with the appropriate bacterial treatment. The bacteria culture was adjusted to the desired concentration (108 cell/mL) and applied through spray application (Fig 2A). Approximately 24 hours after the bacterial application, *Botrytis* spores (104 spore/mL) were sprayed onto the flowers. Flower stems were cut to 30 inches and placed in individual vases (Fig 2B). The individual flowers and vases were then covered in a plastic sleeve to create a high humidity environment. The flowers were kept in an interior room under fluorescent lighting at an average temperature of 22.7˚C (72.8˚F) (Fig 2C). Forty–eight hours after being inoculated with *Botrytis,* the flowers were taken out of the plastic sleeves and disease severity rating began (Fig 2D). Each flower was rated daily for disease severity on a scale of zero (no disease symptoms) to seven (collapsed flower due to disease) for seven days (Fig 3).

Figure 2: Bacterial treatment application and *Botrytis* inoculation of lisianthus. A) Stems were separated into groups for spray application of bacterial treatments; B) Flowers were inoculated with *Botrytis* spores and placed in individual vases; C) Each flower and vase were covered in a plastic sleeve to create high humidity conditions; and D) Plastic sleeves were removed and disease severity ratings began.

Figure 3: Flowers were rated daily using a disease severity scale 0 (no disease symptoms) to 7 (flower collapsed due to disease).

Bacterial strain identified for the biocontrol of Botrytis

After the rating period finished, the daily severity ratings were used to calculate the area under the disease progress curve, which gives a summary of the disease intensity over time. It considers the severity ratings over the 7 rating days. The flowers treated with the no bacteria/no *Botrytis* (Control 1) had the lowest AUDPC [area under the disease progress curve] (10.25) meaning it had the least amount of disease. The control flowers that were treated with no bacteria and inoculated with *Botrytis* (Control 2) had one of the highest average AUDPC (25.29). This shows that the *Botrytis* inoculation was successful. Considering the bacterial applications, one bacterial treatment, *Pseudomonas chlororaphis* 14B11, resulted in flowers with less disease compared to the no bacteria control flowers with an AUDPC of 16.50 (Fig 4).

Conclusions

The need for additional pathogen management options continues to grow as the issue of resistance to chemical pesticides and concern of environmental and human health continue to rise. Biocontrol provides growers another tool for controlling problematic diseases to allow the production and maintenance of high-quality crops through postharvest. The results from this study with lisianthus show that there is potential for the use of bacteria as biocontrol agents against *Botrytis*. *Pseudomonas chlororaphis* 14B11 is a promising candidate for the biocontrol of *Botrytis* and can potentially be formulated into biocontrol products to give growers additional options for the control of *Botrytis* in cut flowers.

References

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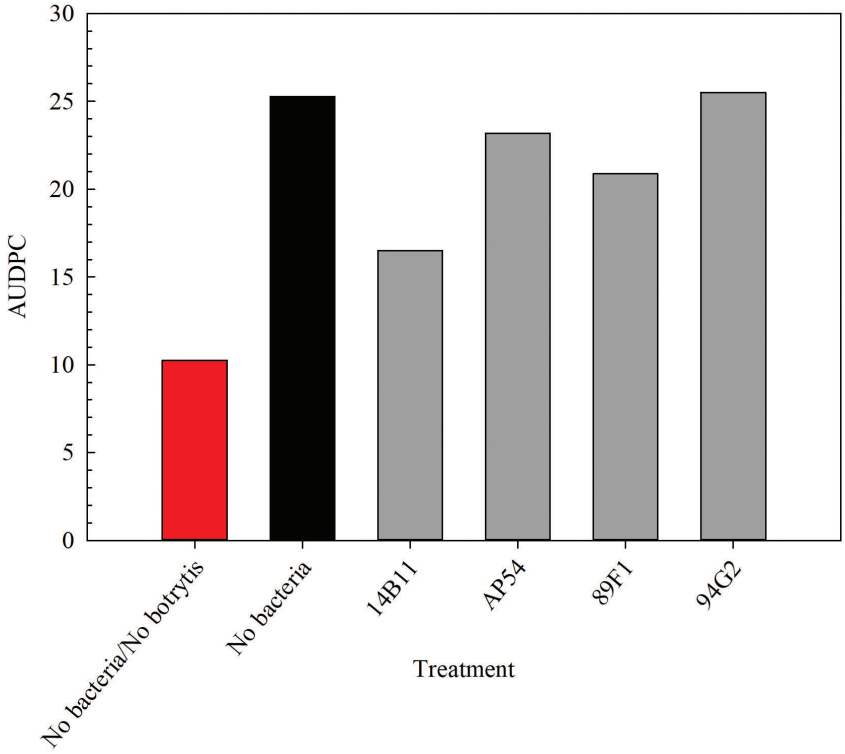
Figure 4: Area under the disease progress curve was used to summarize the daily severity ratings. The No bacteria/No *Botrytis* control is represented by the red bar. The four bacterial treatments were compared to the no bacteria control (black bar).



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