American Floral Endowment | Research Internships Scholarships Education

Funding the Future of Floriculture

Special Research Report #142: *Optimizing the efficacy of beneficial bacteria against botrytis blight in greenhouse crops, Part 2*

Category: Disease Management

Michelle Jones, Laura Chapin, and Sachin Naik
Horticulture and Crop Science, The Ohio State University, Wooster, OH

BACKGROUND

Botrytis cinerea is the causal agent of botrytis blight or gray mold, which is the most common and economically devasting disease for greenhouse crops. Fungicides are routinely used to control Botrytis; however, some Botrytis are developing resistance to those chemicals. Beneficial bacteria have been used successfully as biological control agents for disease control. Our laboratory has screened a collection of bacteria for their ability to control botrytis blight in petunia. We have identified some of those bacteria as effective microbial biocontrol agents (MBCAs). Further investigations with these bacteria isolates are required to characterize the bacteria and evaluate the efficacy of Botrytis disease control. Two of the three objectives of this project are reported in this final report (part 2) and obj 1 can be found in part 1.

These research objectives include:

- Objective 2 – To determine the best method of applying the biocontrol bacteria to plants to maximize Botrytis control.
- Objective 3 – To evaluate the effect of various combinations of bacteria against Botrytis.

MATERIALS AND METHODS

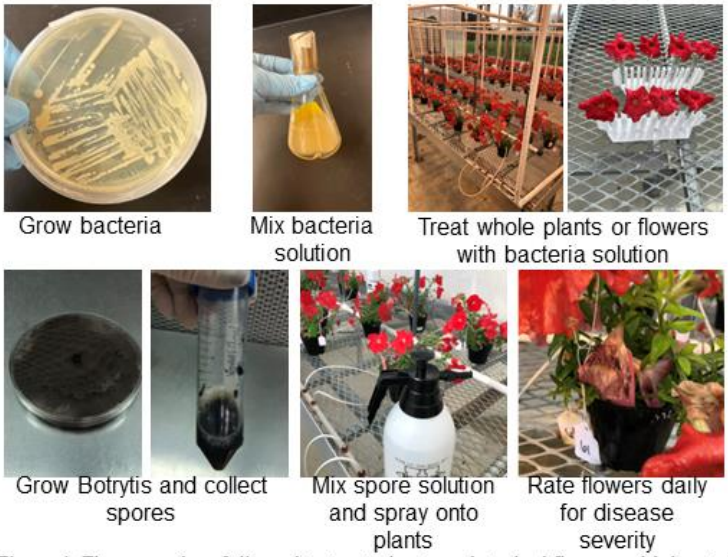

Figure 1. The procedure followed to treat plants or detached flowers with bacteria, to inoculate the plants or flowers with Botrytis, and to rate the disease severity of the flowers.

Experiment 2 – Bacteria application method and conditions to optimize Botrytis control.

Petunia × *hybrida* 'Carpet Red' seeds were germinated in plug trays. Bacterial treatments began at transplant. The bacteria were selected from the top-performing bacteria found to control Botrytis disease severity in previous experiments (South et al., 2020). Bacterial strains in this experiment were 14B11 and 89F1. Experimental treatments included three different application methods: (1) potting media drench, (2) foliage spray, or (3) both drench and spray (sprench). These treatments were repeated every other week until the plants were flowering. Flowers were tagged as they opened to track flower age. After the final bacteria treatment, plants were inoculated with Botrytis spores and placed in a high humidity and low light greenhouse (optimal conditions for Botrytis disease to develop). Each tagged flower was evaluated for disease severity every day after inoculation. Figure 1 illustrates the procedures followed.

Experiment 3 – Identifying bacteria mixtures (consortia) that control Botrytis blight in petunia.

Exp 3.1 Whole plant assay. A consortium or community of bacteria may work better than a single bacteria strain. Bacteria of interest identified in the above experiments were tested on whole plants using the best application methods as identified in Exp 2. Namely, the bacteria treatments were applied as a foliar spray and a potting media drench and only flowers treated with Botrytis at 4-5 days after anthesis were evaluated. We identified strains and combinations of strains through the in-lab analysis (Part 1 Exp 1.1-1.5) that should be included in these greenhouse experiments. The treatments for this experiment were:

Bacteria strain 14B11
Bacteria strain 15H3
Bacteria strain AP54
Bacteria strains 14B11+15H3
Bacteria strains 15H3+AP54
Bacteria strains 14B11+15H3+AP54
Cease (a commercial MBCA)
Untreated (negative control)

Exp 3.2 Detached flower assay. The same consortia of bacteria and single bacteria strains were evaluated on flowers that were detached from petunia plants. The flowers selected for this experiment were 4 days after anthesis. The flowers were cut from the plant and put into a test tube filled with reverse osmosis (RO) water. Each flower was sprayed with a bacteria solution. After the bacteria solution dried on the detached flowers for two hours, the flowers were sprayed to run off with a solution of Botrytis spores. The flowers were closed inside Plexi-glass chambers sealed with weather stripping to create a high-humidity environment, and the chambers were covered with

cloth to decrease light and placed at room temperature for optimal disease development. Each flower was rated for disease severity 48 hours after inoculation.

RESULTS

Exp 2. Application technique did not significantly affect Botrytis disease control by the bacteria.

Spray plus drench application of 14B11 and 89F1 tended to have lower disease severity ratings than the negative control. However, there were no significant differences in disease using the different application techniques. Without a clear indication of one application technique resulting in less disease than another technique, it is best to apply the beneficial bacteria as both a potting media drench and foliar spray (data not shown).

This experiment did provide us with valuable information about the importance of flower age when selecting flowers to rate for Botrytis disease. Flowers that were 3-5 days after anthesis resulted in more consistent disease ratings than older or younger flowers. All future experiments will utilize petunia flowers in this age range.

Exp 3.1 Bacteria mixtures trialed on whole petunia plants decreased Botrytis disease. We trialed bacteria and mixtures of bacteria for their efficacy in decreasing Botrytis disease severity. In this experiment, the whole petunia plant was treated with the bacteria and then inoculated with Botrytis. After five days, disease severity ratings for each flower were recorded. We found that the combination of 14B11+15H3+AP54 did not decrease disease. All other single bacteria strains (14B11, 15H3, AP54) and mixtures of bacteria (14B11+15H3, 14B11+AP54, 15H3+AP54) decreased disease severity compared to the negative control (Figure 2). We confirmed that these single bacteria can act as MBCAs and determined that mixtures of the bacteria are also efficient MBCAs. The commercial MBCA treatment (Cease) did not decrease disease severity in this experiment. These greenhouse-based experiments by nature have a lot of variability and that may have contributed to the variation in the data we collected.

Exp 3.2 Bacteria mixtures trialed on detached petunia flowers decreased Botrytis disease. The disease symptoms on detached flowers were more consistent compared to whole plant experiments. We found that 14B11, 15H3, AP54, 14B11+AP54, and 15H3+AP54 significantly decreased the disease severity caused by Botrytis (Figure 3). The combinations of 14B11+15H3 and 14B11+15H3+AP54 did not decrease disease severity. This aligns well with results found in Report Part 1, which indicate that 15H3 has negative effects on the growth and function of 14B11 (see Part 1).

CONCLUSIONS

Objective 2 - Determine the best method of applying bacteria to plants to maximize Botrytis control.

Applying the bacteria as a spray, a drench, or a spray and drench showed no differences in disease severity. Through these experiments, we did find that the age of

the flower analyzed should be opened for 3-5 days before Botrytis inoculation. This has helped us obtain more consistent results in greenhouse experiments.

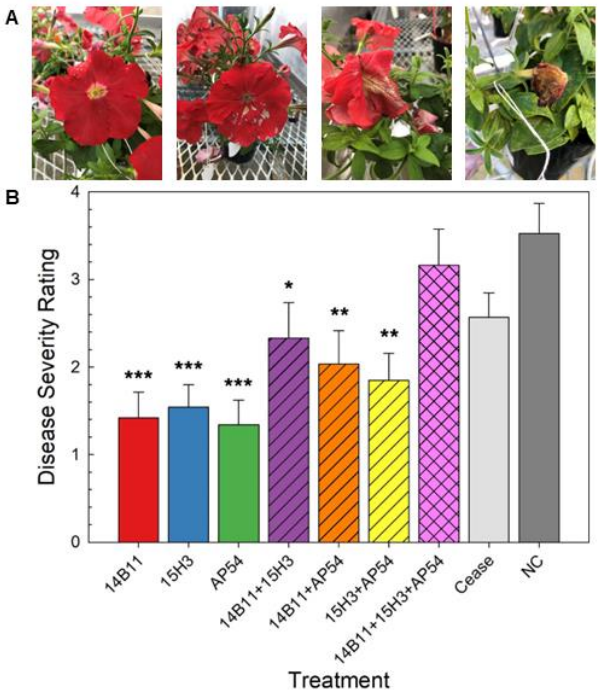

Figure 2. Control of Botrytis disease severity when whole plants were treated with single bacteria or mixtures of bacteria. (A) Example flowers with varying degrees of disease severity. (B) Average disease severity rating of flowers after treatment with single bacteria (solid bars), a mixture of two bacteria (dashed bars), or a mixture of three bacteria (cross-hatched bars). Bars with asterisks indicate treatments that significantly lowered disease severity when compared to the negative control (gray bar).

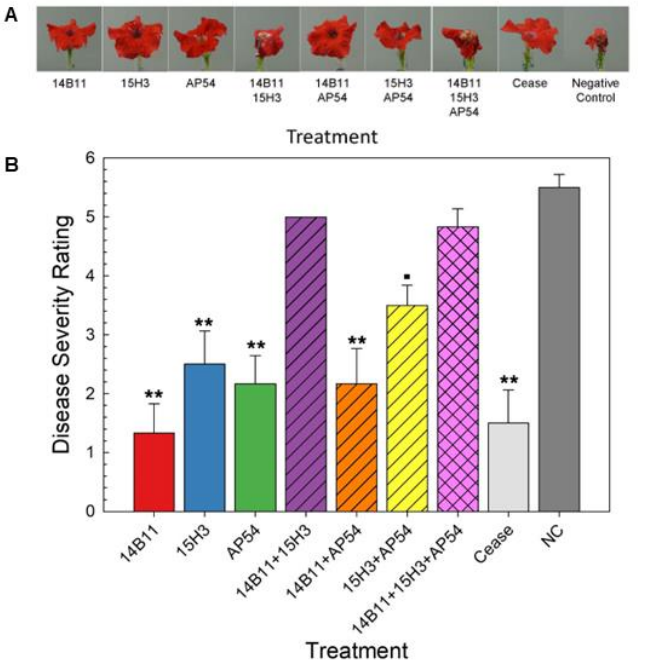

Figure 3. Control of Botrytis disease with single bacteria strains and consortia on detached flowers. (A) Flowers treated with bacteria that represent the average disease severity rating. (B) Average disease severity rating of flowers after treatment with single bacteria (solid bars), a mixture of two bacteria (dashed bars), or a mixture of three bacteria (cross-hatched bars). Bars with asterisks and a dot indicate treatments that significantly lowered disease severity when compared to the negative control (gray bar).

Objective 3 - Evaluate the effect of various combinations of bacteria against Botrytis.

We used information gathered in Objective 2 to determine which bacteria strains to trial with whole plants or detached flowers, and determined which strains worked together to improve Botrytis control and which were antagonistic.

IMPACT OF RESEARCH TO THE INDUSTRY

Combining bacteria strains together can be a powerful tool in creating an MBCA with efficacy in a diverse variety of floriculture crops. We have identified key lab assays that should be conducted to evaluate bacteria so informed decisions can be made on what bacteria to include in a consortium for future product formulation. Through these lab assays and plant trials, we identified bacteria consortia that act as an MBCA for Botrytis control in petunia.

American Floral Endowment
Phone: 703.838.5211 afe@endowment.org / www.endowment.org