Experiment 2. Evaluating *Botrytis* biocontrol efficacy when beneficial bacteria are applied with and without additional calcium.

Approach: Calcium (Ca) applications have been shown to reduce *Botrytis* disease severity in petunia and other crops. This experiment was designed to compare Ca and beneficial bacteria biocontrol effects and to determine if applying Ca + beneficial bacteria would provide better biocontrol of *Botrytis*. Petunias 'Carpet Red Bright' were started from seed and grown in the greenhouse. Beneficial bacteria and On-Gard Ca
(BioWorks) treatments began when seedlings were transplanted to 4.5-inch pots and were applied every two weeks. Treatments included five bacteria previously shown to control *Botrytis* disease severity. These bacteria included *Pseudomonas fluorescens* 89F1, *P.chlororaphis* 14B11, *P. protegens* AP54, *P. frederiksbergensis* 94G2, and *P. protegens* 15H3.Bacteria were applied individually and as bacteria + On-Gard Ca applications. Controls included no bacteria (LB growing media only), LB + On-Gard Ca, and the biopesticide Cease. Bacteria treatments were applied as a spray + drench application, and the On-Gard Ca was sprayed on the plants at the recommended rate (16 fl. oz./100gal) after the bacteria treatment had completely dried on the leaves (at least 5 hours after the bacteria treatment). When petunias were flowering, *Botrytis*inoculations and disease evaluations were as described for experiment 1.

Summary of results: Treating plants with On-Gard Ca kept the disease symptoms slightly lower than the control. In the early days of disease development (3 and 4 days after *Botrytis* inoculation), plants treated with bacteria+On-Gard Ca tended to have less disease than bacteria alone for strains 14B11and 89F1. AP54 alone had lower disease ratings than AP54+On-Gard Ca.
Take home: The addition of On-Gard Ca to bacteria treatments did not always have an additive effect on *Botrytis* disease control. For some bacteria strains, the addition of On-Gard Ca improved disease control. For others, there was no improvement in disease control when On-Gard Ca was applied with the bacteria strain.

Objective 2. to evaluate the effect of various combinations of bacteria against *Botrytis*.

Experiment 1. Comparing the biocontrol effects of individual bacteria to consortia (groups) of bacteria.

Approach: Petunia plants were grown as described above, and bacteria treatments began at transplant. Based on previous experiments, the application method for the beneficial bacteria was a spray + drench biweekly. Five beneficial bacteria (obj 1, exp 2) were applied individually. Consortia treatment 1 included bacteria strains 89F1+14B11+AP54 and consortia treatment 2 included 94G2 +15H3+14B11. When petunias were flowering, *Botrytis* inoculations and disease evaluations were as described for objective 1.

Summary of results: Of the two consortia treatments, disease severity ratings were consistently lower, with the first consortia containing 89F1, 14B11, and AP54 compared to the second consortia of bacteria that contained 94G2, 15H3, and 14B1. While disease severity was reduced by both consortia compared to the control (no bacteria), the treatments that had the lowest disease severity from 3 to 7 days after inoculation with *Botrytis* were the individual bacteria AP54 and Cease. Disease control from AP54 was similar to or better than Cease at all time points. The area under the disease progress curve (AUDPC) was lower with the AP54 treatment than either of the consortia treatments or any of the other individual bacteria treatments. AUDPC was lower with consortia 1 (that contained AP54) than with consortia 2.

Take home: This experiment confirmed that we have identified multiple bacteria that can reduce *Botrytis* disease severity, but the specific combinations we applied in this experiment with Petunia did not improve efficacy. It is possible that bacteria like AP54 already control *Botrytis* using multiple modes of action or that the three bacteria combined in this experiment may inhibit other bacterial growth and action rather than acting synergistically. This will be further evaluated in yr 2.

Year 2 plans

Objective 3: to identify potential modes of action (mechanisms) for *Botrytis* control. Most biocontrol agents exert control of pathogens through one or more modes of action or mechanisms. They may indirectly affect disease severity by competing with the pathogen for nutrients or space or by inducing resistance in the plant. Some beneficial bacteria may directly interfere with the pathogen through parasitism or by producing antimicrobial compounds. Knowing the modes of action for specific biocontrol bacteria will help optimize application methods and improve efficacy. To characterize the modes of action that the OSU bacteria use to control *Botrytis,* we will conduct a series of assays that look at enzyme activities like proteases, chitinases, and cellulases that the bacteria can use to inhibit the fungal pathogen. We will also conduct diffusion plate assays to determine if the bacteria produce chemicals like antibiotics that can diffuse through the media and directly inhibit *Botrytis*. An inverted plate assay where the bacteria and *Botrytis* are in the same air space but not physically on the same plate will tell us if the bacteria produce volatile compounds that can inhibit the pathogen. The genomic DNA of all the OSU bacteria has been completely sequenced, so we can complement the microbiology assays with analyses that identify genes within the genomes of bacteria that could be involved in inducing resistance or other modes of action.

Objective 4. to evaluate the efficacy of the best bacterial strains in multiple *Botrytis* susceptible plant species. It is important that any MBCA has a broad host range so that it is effective at controlling *Botrytis* in the diverse species of plants grown in greenhouses. This objective will evaluate the most effective bacterial strains in three additional plant species. Plants that are very susceptible to *Botrytis*, such as geraniums, will be included.

Objective 5. to evaluate the use of OSU biocontrol bacteria as a cut flower dip to control Botrytis during vase life. *Botrytis* spores are ubiquitous and gray mold can be a problem during the shipping and retailing of cut flowers. Flower heads can be dipped in biocontrol agents to prevent the onset of *Botrytis* blight in cut flowers. In these experiments, three to five cut flower species will be sourced from cut flower growers. Bacteria treatments will include the top two bacteria from the OSU group (mentioned previously) and Cease. Cease contains Bacillus subtilis QST713 and has been labeled for postharvest dip on cut flowers. Flower heads will be immersed in Cease or the individual bacteria culture and then let dry. Cut stems will be placed in vases of water and kept for three days in a humid environment to allow for the development of *Botrytis* symptoms. If natural inoculum pressure does not cause disease, we will inoculate the flowers with *Botrytis cinerea* as described previously for whole-plant greenhouse studies. Flower vase life will be evaluated in an interior evaluation room with a set temperature of 21° C and fluorescent lighting provided for 12 hrs. *Botrytis* disease ratings will be as previously developed.

Why is this important? Knowing more about the conditions that affect the efficacy of beneficial biocontrol bacteria will allow us to create consortia or combinations of bacteria that are even more effective in numerous plant species and under different greenhouse environments. These beneficial bacterial strains have the potential to be formulated into MBCAs for the ornamental greenhouse industry. Biocontrol products added into an integrated pest management plan can help the ornamental greenhouse industry move toward a more sustainable production system.