FINAL REPORT

Strategies for the Management of *Botrytis* Gray Mold and Other Pathogens for Alaska's Peony Industry

TO:

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Project Title:
Strategies for the Management of *Botrytis* Gray Mold and Other Pathogens for Alaska's Peony Industry

Project Summary

The purpose of this project was to provide information on disease identification, mitigation, and management to peony growers in Alaska. *Botrytis* gray mold was identified by Alaska peony growers as one of the most important in-field and postharvest pathogens of peonies. Initial studies indicated there were multiple novel species of *Botrytis* infecting peonies in Alaska. The goals of this project included providing growers with the range of *Botrytis* species present in peony fields in Alaska, the prevalence of fungicide-resistant isolates, information on environmental conditions likely to influence disease development, the prevalence of *Botrytis* on different peony cultivars, and potential post-harvest cut flower treatments and their effect on *Botrytis*.

Additionally, this project served to identify the range of other peony pathogens in Alaska. Field surveys during this project helped to identify many other pathogens causing economic damage to Alaskan peony fields. Prior to this project, growers were unable to identify these diseases accurately, therefore limiting their ability to manage disease adequately. This project has led to the development of educational tools to enable peony growers in Alaska to diagnose and manage diseases affecting their crop.

- Successful management of *Botrytis* gray mold and other diseases is essential for the economic viability of the peony industry in Alaska. Alaska currently occupies a competitive niche in the world peony industry due to the ability to produce flowers at a time of year when world markets have few, if any, supplies of fresh cut peonies. Managing diseases is essential to the state's success in marketing high-quality flowers. Diseases build up over time. Therefore, rapid identification and effective management as new peony farms are established is key to maintaining the long-term health of the peony industry.

- This project built on a very limited 2013 survey and a 2014 SCBG project that was conducted to identify *Botrytis* species that were damaging peonies in Alaska. *Botrytis* gray mold is the single most important disease of Alaska field-grown peonies and cut stems in storage. *Botrytis* species tend to be aggressive, host specific pathogens that can reduce yields by 60% and have the potential to cause the complete pre- and post-harvest destruction of cut flowers. DNA sequencing of isolates from Alaska fields revealed five *Botrytis* species, not just the two, *B. cinerea* and *B. paeoniae,* that had been identified

previously on peonies. During our current project, a more extensive sampling of Alaska fields was conducted to verify species identification and to study the biology and pathogenicity of these *Botrytis* species.

- During previous surveys, growers also expressed an interest in regional differences in environmental conditions that favor *Botrytis* development, so an attempt was made to identify regional environmental triggers that result in disease manifestation. Previous interactions with growers also indicated that they were interested in using biopesticides to control gray mold on peonies. As a result, a number biopesticides were evaluated for their effectiveness in controlling *Botrytis* during our current project. Finally, our previous interactions with growers indicated there was a critical need for educational efforts to enable them to improve their disease management programs.

- Cultivars differ in the manifestation of *Botrytis* disease. Early in the season, the damage occurs on some plants at ground level where stems blackenand become limp very shortly after they emerge. Later in the season, often after flowering, circular lesions begin to appear on the foliage and enlarge until foliage is cut. Flower petals landing on the foliage can act as sugar sources for leaf infection. During the flowering season, spores can land directly onto the flower buds. In some instances, the infection is quite rapid, and the gray mold spores become visible on buds that are prevented from opening. Most often, however, spores germinate at the base of the bud near the sepals, often where nectar has dried. The infection does not become evident until stems are cut and placed into a high humidity cold storage room. Buds can show complete degradation in the cooler or become brown very shortly after returning to room temperature either in a shipping box or vase.

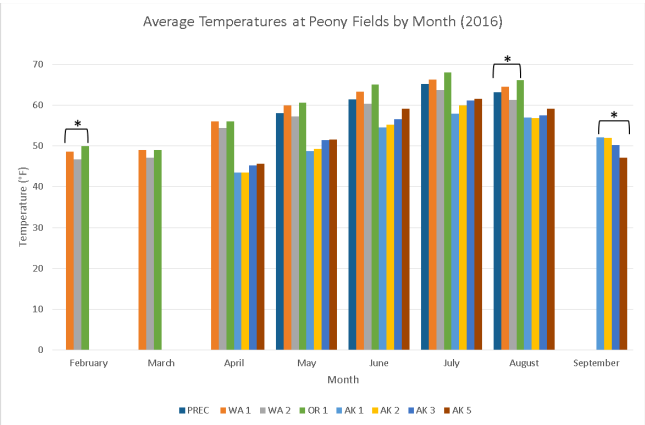
Project Approach

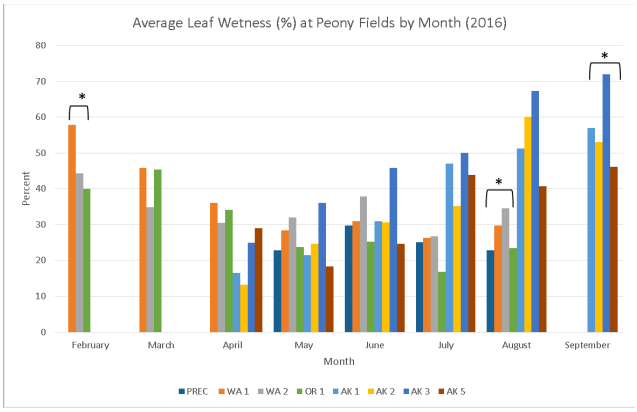
The following activities were performed and results, accomplishments, conclusions, and recommendations reached:

Activity 1: Travel to grower sites and install weather stations in Alaska—Weather stations were set up at 4 commercial peony farms during the 2015 and 2016 growing season in the 4 main peony production regions of Alaska: North Pole, Trapper Creek, Soldotna, and Homer. Data on temperature, leaf wetness, and rainfall were tracked for each location at 30-minute intervals from late April, prior to peony emergence, to mid-September, upon plant senescence. Monthly averages of each parameter were calculated for each farm location and the 2016 data are reported alongside environmental data collected in Washington and Oregon Figures 1, 2, & 3. One of the most significant findings from a disease development perspective is that leaf wetness, an essential component for fungal pathogen spore germination and infection, is low towards the

beginning of the season and increases throughout the season. This pattern is likely advantageous for Alaska peony growers as leaf wetness is low when plants are young and putatively more susceptible to fungal infections. The results also suggested that the ranges in leaf wetness among farms in Alaska likely indicates a range in the risk of disease development, depending on region, with the wetter regions more at risk.

Activity 2: Monitor progression of *Botrytis* infection on peonies — The progression of *Botrytis* infection on peonies was monitored at all four locations in Alaska where weather stations were installed and in September, samples were collected and final disease ratings were taken. Linear regression analyses were performed to determine any relationship of disease development to the environmental parameters measured. Temperature, rainfall, leaf wetness, individually and in combinations of parameters were plotted against final disease ratings for each location. For all individual parameters and combinations, no apparent correlation between environmental conditions and disease development were identified for the 2016 data due to lack of significant p-values.





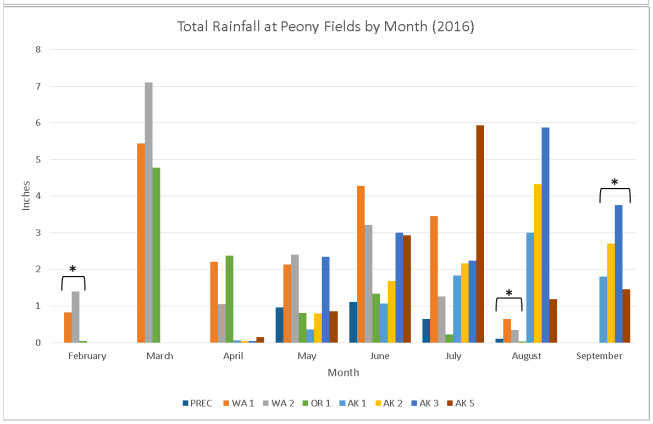


Figure 1. Monthly temperature, leaf wetness, and rainfall from late April, prior to peony emergence, to mid-September, upon plant senescence four farms in AK (AK1=Homer, AK2= Soldotna, AK3=Trapper Creek, and AK5=North Pole) alongside similar data from three sites in WA (PREC, WA 1, WA2) and one site in OR (OR1). An asterisk (*) indicates that data were collected for only part of the month. Where no bar is present, data were not collected.

In an attempt to give the test more power, 2016 data were combined with 2015 data from Alaska, Washington, and Oregon and the final disease ratings were compared to leaf wetness and temperature parameters conditions that are favorable for *Botrytis* spore germination and infection. The environmental parameters assessed were as follows: the number of leaf wetness periods greater than or equal to 4 hours; the average temperature during leaf wetness periods greater than or equal to 4 hours; and the number of instances of leaf wetness that occurred when temperatures were 53.6-86°F. The results of those linear regression analyses are shown in Figure 2 with R^2 and p-values, none of which are significant. Due to lack of statistically significant data in 2015 and 2016, the decision was made to abandon weather monitoring in 2017 as it would allow us to focus on research that is more likely to be valuable for growers. A list of potential reasons for lack of significant data are presented in the outcomes section below.

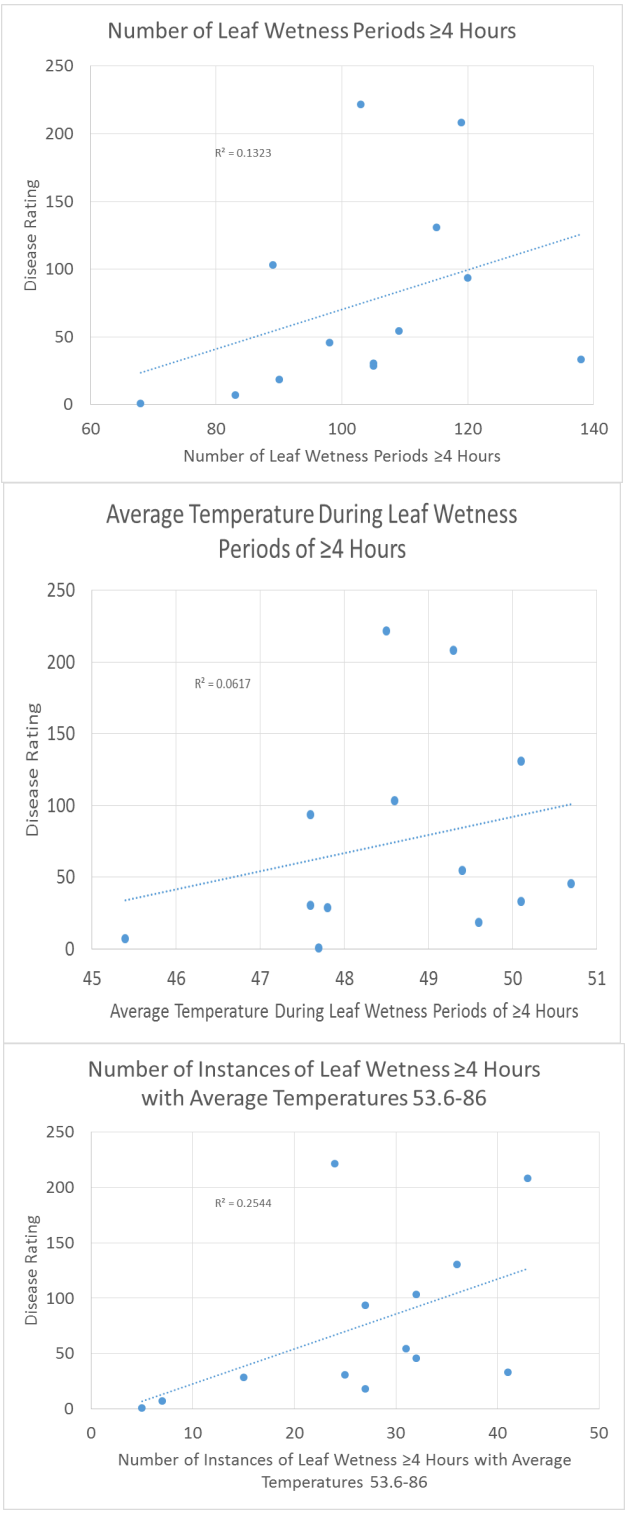


Figure 2. Relationship of leaf wetness and temperature to final disease ratings. P values were 0.22, 0.41 and 0.08 for the top, middle, and bottom regression, respectively.

Activity 3: Identify biopesticides and conventional fungicides that are effective in controlling Botrytis gray mold. - In 2016 and 2017 trials were conducted to evaluate the effectiveness of conventional fungicides and new biopesticides in controlling *Botrytis* species. on outdoor, container-grown 'Sarah Bernhardt' peonies. In 2016, a total of 18 products were evaluated. Disease pressure in this trial was low to moderate and both *Botrytis cinerea* and *Graphiopsis chlorocephala* were isolated from symptomatic plants. *Graphiopsis chlorocephala* was formerly known as *Cladosporium paeoniae,* and causes the disease called measles on peonies. In addition to the treatments applied to the 'Sarah Bernhardt' peonies, these same products were also simultaneously tested on a set of container-grown, mixed varieties of peonies which had been previously identified in 2015 to have high levels of *G. chlorocephala.*

For both sets of peonies, disease incidence was rated on a scale of 0 to 10 scale, where 0 = none, 1 = 1-10%, 2 = 11-20%, and 10 = 91-100% of the foliage were diseased. Visible fungicide residue was rated on a scale of 0-3, where 0 = none, 1 = slight, 2 = moderate, and 3 = severe fungicide reside on foliage. Basal rot stem decay due to *Botrytis* was assessed by counting the total number of stems and the number of decayed stems. An overall plant quality assessment was taken on July 6, 2016. Plant quality was rated on a scale of 1-9 where 9 = perfect plant, 6 = commercially acceptable (I would be that), 1 = dead. Residue was rated only on the 'Sarah Bernhardt' peonies on a scale of 1-3 where 1 = slight, 2 = moderate, and 3 = severe residue present. (Note: in Figures 4-11, columns with the same letter are not significantly different, P=0.05, Tukey's Studentized Range Test.)

Disease and plant quality on the 'Sarah Bernhardt' peonies were highly variable in the *Botrytis* trial (Data not shown). *Botrytis* disease incidence ratings on the foliage ranged from 1.2 to 4.8 and stem dieback severity ranged from 0.0 to 1.4 diseased stems per plant. The incidence of *G. chlorocephala* ranged from 0.0 to 3.8 and overall plant quality ranged from 3.4 to 8.0. None of the treatments had a statistically significant effect on disease ratings.

Disease and plant quality on the mixed varieties of peonies were also highly variable (data not shown). The severity of *G. chlorocephala* ranged from 0.0 to 6.3 and *Botrytis* severity ranged from 0.0 to 2.5. Overall plant quality ranged from 3.4 to 8.0. None of the treatments has a statistically significant effect on *Botrytis* disease ratings. However, applications of Pageant, BAS 703 06 (Orkestra) and both rates of SS00 had significantly less *G. chlorocephala* than the non-treated check.

In 2017, a total of 20 products were evaluated for their effectiveness in controlling *Botrytis* species. on container grown 'Sarah Bernhardt' peonies (Table 1). Foliar applications were applied with a CO2 sprayer equipped with an 8002LP Tee-Jet nozzle at 15 psi in the equivalent of 100 gallons of water and sprayed to wet. The initial applications occurred on April 4th and treatments were applied at 7 or 14-day intervals until the flower stems were harvested in mid-

May. Each treatment was applied to a single plant in each of five blocks. Disease development and visible residue levels were monitored as described for the 2016 trials.

Table 1. Products included in the 2017 peony fungicide test.

Trade name and formulation	% active ingredient and common name	FRAC Code[1]
Badge	24.6% copper oxychloride, 22.9% copper hydroxide	M01
BAS 703 01F (Orkestra)	21.3% pyraclostrobin +21.3 % fluxapyroxad	11 + 7
Botector	1.06 x 10^9 cfu/g *Aureobasidium pullulans*	NC
BW165N	8 x 10^7 cfu/g *Ulocladium oudemansii* U3 strain	NC
Chipco 26019 N/G	50% iprodione	2
Daconil Weather Stik SC	54% chlorothalonil	M5
Decree 50WDG	50% fenhexamid	17
F9110 WG	20% extract of *Lupinus*	NC
Fore 80 WP	80% mancozeb	M3
Kenja 400 SC	36.0 isofetamid	C2
MBI110 AF5	1 x 10^8 cfu/mL *Bacillus amyloliquifaciens* strain F727	NC
Medallion 50WP	50% fludioxonil	12
NUP 09092 50L	40.3 % fludioxonil	12
Pageant 38 WG	12.8% pyraclostrobin + 25.2% boscalid	11 + 7
Palladium 62.5WG	37.5% cyprodinil + 25% fludioxonil	9 + 12
Prophytex EC	*Bacillus subtilis* strain B1111	44
Prophytex WP	*Bacillus subtilis* strain B1111	44
Proud 3	5.6% thyme oil	NC
S2200 4SC	42-45% mandestrobin	11
Zerotol	27.1% Hydrogen dioxide + 2.0 perozyacetic acid	NC

[1]FRAC Code List 2017. http://www.frac.inf accessed 15 May 2017

(Note: Some of these pesticides were tested under an experimental use permit granted by WSDA. Application of a pesticide to a crop or site that is not on the label is a violation of pesticide law and may subject the applicator to civil penalties. In addition, such an application may also result in illegal residues that could subject crops to seizure or embargo action by WSDA and/or the U.S. Food and Drug Administration. It is your responsibility to check the label before using products to ensure lawful use and obtain all necessary permits in advance.)

Treatments of Daconil and Fore resulted in significantly higher residue levels on the foliage than the non-treated check and all of the other fungicides except Badge X2, which had intermediate residue ratings (Data not shown). Disease ratings on the peonies were low. *Botrytis* disease incidence ratings on foliage during the period between emergence and flower harvest ranged from 0.0 to 2.4 and the percent of stems with basal decay ranged from 7.5% to 41.8%. The incidence of measles, caused by *G. chlorocephala,* ranged from 0.0 to 3.6. None of the treatments significantly reduced the incidence of basal stem decay or the incidence of foliar symptoms often associated with *Botrytis* infection. However, applications of Orkestra (8 fl oz),

the high rate of S2200 (15 fl oz), and Palladium (6 oz) had significantly less measles than the non-treated check (Data not shown).

Given the limited disease development on the plants, leaves were harvested after the last treatment application and inoculated with mycelial plugs of *B. cinerea* and *B. paeoniae* to assess the residual activity of the fungicide treatments. Checks consisted of non-sprayed leaves that were inoculated with mycelial plugs of *B. cinerea, B. paeoniae,* or plugs of uncolonized media. Lesion development on the treated leaves was compared to the size of lesions that developed on inoculated checks. No lesions developed on the non-inoculated checks (Figure 3).

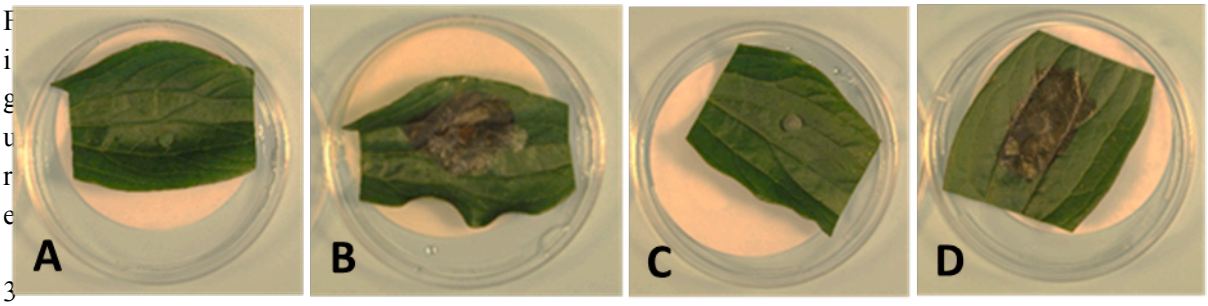
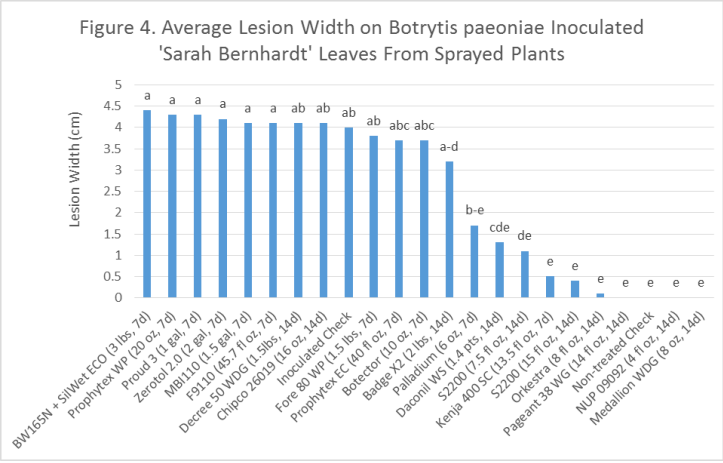
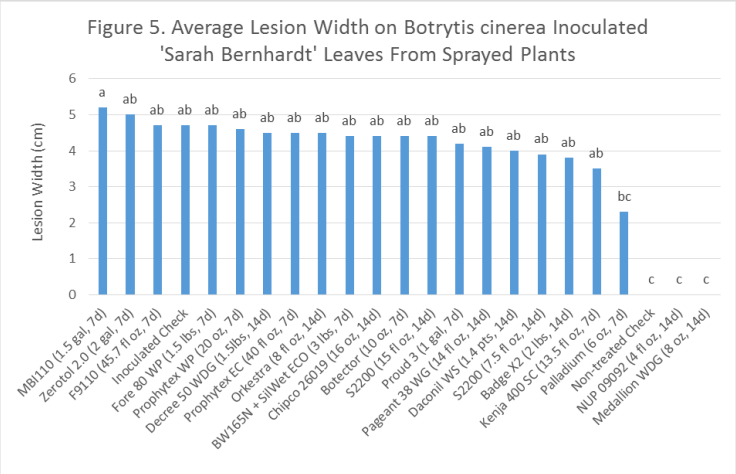


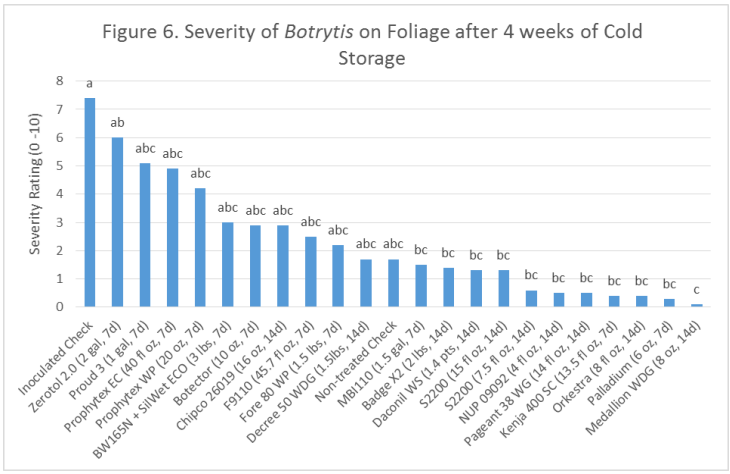
Figure 3. Lesion development on peony leaves inoculated with *Botrytis paeoniae* (B) and *B. cinerea* (D) on May 22, 2017. No lesions developed on the non-inoculated checks (A & C).

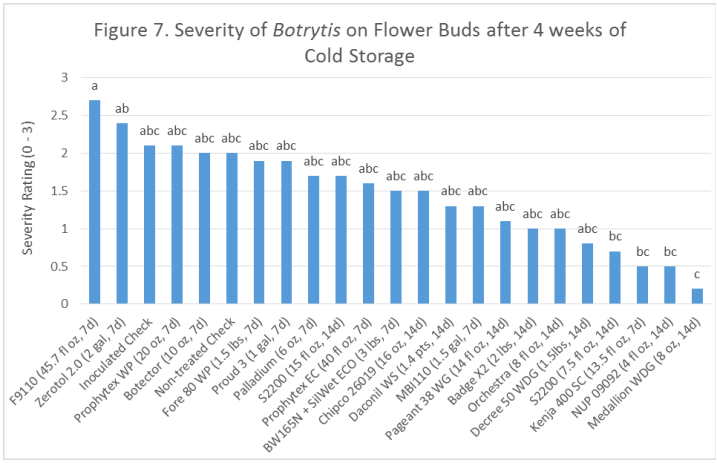
After 96 hours of incubation at 18C, lesion width on the *B. paeoniae*-inoculated leaves ranged from 0.0 to 4.37 cm and from 0.0 to 5.15 cm on the *B. cinerea*-inoculated leaves (Figures 4 & 5). Several fungicides either reduced or eliminated the growth of lesions compared to the inoculated checks in the *B. paeoniae*-inoculated leaves. The most effective treatments were Daconil WS, S2200, Kenja 400 SC, Orkestra, Pageant 38 WG, NUP 09092, and Medallion WDG. Fewer fungicides were effective against *B. cinerea* than *B. paeoniae*. Treatments of Medallion and NUP09092 were the only ones that had lesions that were significantly smaller than the inoculated checks in the *B. cinerea* test.



To assess the effect of the preharvest applications of fungicides during the growing season on the postharvest development of gray mold on the foliage and flower buds on cut stems during cold storage, three stems were harvested from each plant and held in cold storage for 4 weeks at 1 to 5C. Just prior to storing, the bundles of flowers were sprayed with *Botrytis cinerea* spores and then wrapped in paper to encourage disease development. The foliage was rated for disease severity on a scale of 0 to 10 scale, where 0 = no foliar decay and 10 = 91 to 100% of the foliage is dead. Disease development on the flowers was rated on a scale of 0-3 where 0 = none, 1 = slight infection (< 25% of flower infected), 2 = moderate infection (25-50%), 3 = severe (>50% of flower infected). Flowers that were held in cold storage for 4 weeks had high levels of disease on both the foliage and flowers (Figures 6 & 7). Disease ratings on the foliage ranged from 0.1 to 7.4 and treatments with MBI110, Badge X2, Daconil WS, S2200, NUP 09092, Pageant 38 WG, Kenja 400 SC, Orkestra, Palladium, and Medallion WDG had significantly lower disease ratings on the foliage than the inoculated check. However, compared to the inoculated check, none of the fungicides significantly lowered disease ratings on the flower buds.



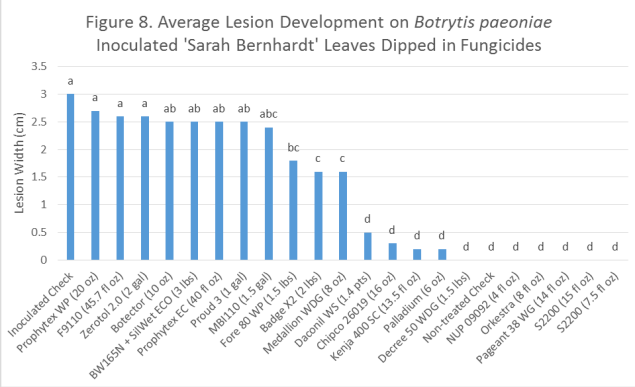


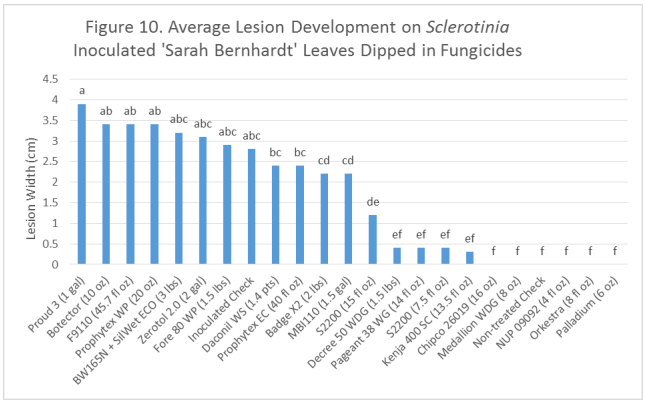


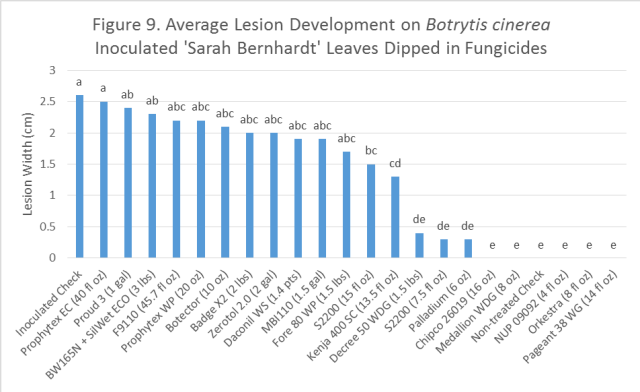
To determine if the limited effectiveness of some of the fungicides in the spray trials was due to inadequate fungicide coverage on the leaves, leaves were collected from field-grown 'Sarah

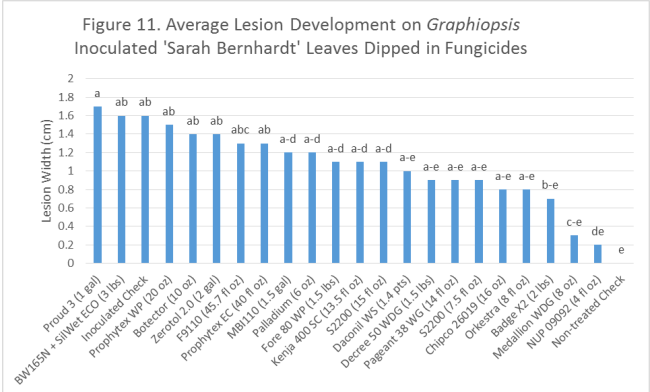
Bernhardt' peonies that had not been treated previously fungicides. The leaves were then dipped in fungicide solutions at the same concentrations used in the spray trial. The surface of the leaves were allowed to dry before placing mycelial plugs of *B. cinerea*, *B. paeoniae, Sclerotinia sclerotiorum,* and *Graphiopsis chlorocelphala* on the upper surfaces of the leaf sections. The inoculated leaves were incubated at 20C for 4 days with the exception of the *Graphiopsis* leaves which were incubated for 15 days. Inoculated and non-inoculated checks consisting of leaves that had not been treated with a fungicide were included in this test.

Compared to the inoculated checks, 12 products significantly reduced lesion sizes of *B. paeoniae*, and 9 significantly reduced lesion sizes of *B. cinerea* and *G. chlorocephala* (Figures 8, 9, & 10). Eight fungicides (Orkestra, NUP 09092, Pageant, Chipco 26019, Medallion, Palladium, Decree, and Kenja) significantly reduced lesion development of all three pathogens. With respect to *B. paeoniae*, three additional fungicides had significantly lower lesion size development. These were as follows: Daconil WeatherStik, Badge X2, and Fore. Far fewer fungicides controlled lesion development on the leaves inoculated with *Graphiopsis*. Only treatments of NUP 09092 and Medallion had lesions that were significantly smaller than the inoculated checks (Figure 11). The increased number of fungicides that were effective in controlling the *Botrytis* lesions in this dip test illustrates the importance of having good coverage on plants when applying fungicide sprays.









Activity 4: Travel to the APGA Conference and disseminate information to growers about research trials— Updates on this project were provided to growers at the 2016 Alaska Peony Grower's Association (APGA) Conference in Homer and the 2017 APGA Conference in Fairbanks, AK. At the 2016 conference the PhD student presented the environmental and disease development data from the 2015 field season. Growers were especially interested to see the data on the differences in climate patterns between regions of Alaska and Washington and Oregon.

At the 2017 conference, Dr. Chastagner and PhD student, Andrea Garfinkel held a pre-conference workshop on disease management of peonies that included information on: basic plant pathology and disease management, proper use of fungicides including how to properly use fungicides to reduce the risk of fungicide resistance, information on how to read a pesticide label, and how to identify common peony diseases and their management strategies. During the regular program, the PhD student, Andrea Garfinkel, presented information on the diseases observed in surveys of peonies across the United States, indicating those which were most common in Alaska, and Dr. Chastagner presented information on the efficacy of reduced-risk and biocontrol fungicides in controlling diseases on bulb crops and the potential to reduce the number of fungicide applications in *Botrytis* disease management programs by using a crop phenology-based, integrated disease management program. In 2017, Dr. Holloway presented the results of her research on post-harvest handling of peony cut flowers

Activity 5: Travel to Alaska to collect material from peony farms to identify range of peony pathogens—Three trips were made to the peony fields during 2016 to survey for pathogens: in April, July, and September. In July 2016, a 10-day survey of fields ranging from the Interior to the Kenai Peninsula was conducted. During this time, the PhD student, Andrea Garfinkel, and Dr. Chastagner visited 35 fields and collected samples with a variety of disease symptoms.

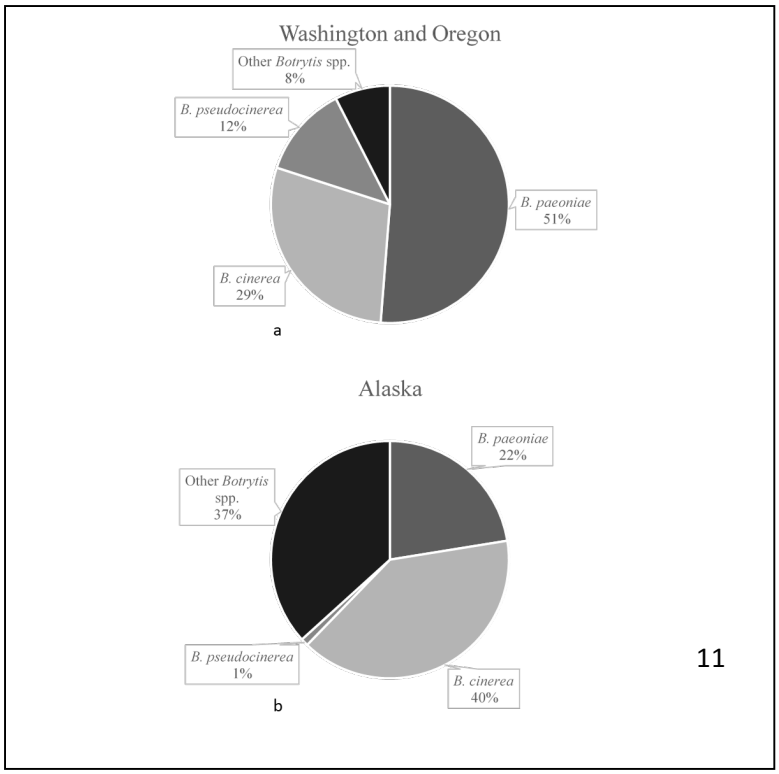


Figure 12. Percentage of isolates collected from peony in a) Washington and Oregon (n=80) and b) Alaska (n=98) that were identified as belonging to the genus *Botrytis*.

Activity 6: Isolate and identify pathogens obtained from peony samples—The PhD student, Andrea Garfinkel, isolated from 126 peony tissue samples. Multiple fungal plant pathogens were identified including several *Botrytis* spp, *Mycocentrospora acerina*, and *Phoma* spp. The *M. acerina* and *Phoma* spp. represent the first reports of these pathogens on peony in the state. Pathogenicity trials were conducted to confirm their ability to cause disease on peony.

A total of 179 isolates of *Botrytis* from peony were identified from Alaska, Washington, and Oregon. The breakdown of the identity of these isolates are described in Figure 12. In short, the majority of isolates from Washington and Oregon were identified as being either *B. cinerea* or *B. paeoniae*, whereas 35% of the isolates from Alaska were species other than *B. cinerea*, *B. paeoniae*, or *B. pseudocinerea*. Many of the isolates from Alaska represented new species, including the one that has been described as *B. euroamericana* as a result of this project. We also identified a number of fields that had peonies with symptoms of *Tobacco rattle virus* (TRV). Some fields appeared to have the disease in high frequencies. While at these farms, growers were advised on how to manage the disease appropriately and/or contact suppliers regarding the quality of planting material. These findings were reported to growers in the state at the 2017 APGA conference, at a workshop, during several grower field tours (see Activity 4) and via various publications (see Activity 11). This included providing growers with images of the disease symptoms caused by the pathogens found on peonies in Alaska (Figure 13).

Figure 13. Disease symptoms observed on peonies in Alaska. Botrytis gray mold on leaves (A), flower buds (B), and stems (C); *Mycocentrospora acerina* stem lesions (D); *Phoma* stem lesion (E); and ringspot symptoms on leaves caused by *Tobacco rattle virus* (F).

Activity 7: Conduct pathogenicity and rootstock infection studies on peonies— Pathogenicity trials were conducted for the *M. acerina* and *Phoma* spp. isolated from peony (see Activity 6).

Roots were inoculated with *Botrytis paeoniae* in the fall of 2015 to determine the potential for commercial rootstocks to become infected by *Botrytis*. Rootstocks were inoculated in three locations using agar plugs that had been colonized by *B. paeoniae*. The inoculated locations included: a cut root surface, an area below the next year's developing bud, and on a basal stem piece that remained intact on the rootstock. Inoculated material was incubated in a greenhouse. Only the cut root surface became infected with *B. paeoniae,* as confirmed by isolations. Rootstocks were then potted and left outside to vernalize over the winter. In the spring of 2016, plants were routinely observed for above-ground *Botrytis* disease development. None was observed in any of the treatments. At the end of the season, rootstocks were washed clean of soil and observed for lesion development. Lesions were not observed on any of the tissue and *B. paeoniae* could not be reisolated from any root tissue. Furthermore, there was no increase in disease development in above-ground tissues on inoculated plants versus control plants. Our

results suggest that our method of inoculation to test the potential for movement of *B. paeoniae* within rootstock is either ineffective or the pathogen is not very aggressive on peony root tissue. Due to our lack of success with this method, we chose not to repeat this test in 2016, as indicated in our previous annual report.

A total of 16 microsatellite markers were developed for *B. paeoniae*, 15 of which are polymorphic in the isolates that have been tested from our collections. Development of these markers was aided by two draft genome sequences of *B. paeoniae*, one developed during this project and one that was provided by a Dutch university. The results of this project's marker development has been submitted for publication with the primer sequences and allele sizes. The microsatellite markers have been applied to 73 *B. paeoniae* isolates that this project's leaders collected throughout the United States and The Netherlands. Although there are 15 polymorphic loci, there are relatively few alleles per locus and few genotypes. Statistical tests to determine the number of populations represented in these 73 samples are inconclusive, suggesting either they represent one population or more information is needed to elucidate differences. The 73 isolates tested represents a small increase in the number of samples we tested and reported in our previous annual report, however, the results were still inconclusive. Further information about this objective and potential reasons for non-significant results is discussed below in the outcomes section.

Activity 8: Conduct fungicide resistance studies—A total of 50 isolates identified as *B. paeoniae* and 50 isolates identified as *B. cinerea* were tested in-vitro for their resistance to 7 fungicides (Table 2). Each isolate was grown on potato dextrose agar (PDA) amended with three rates of each fungicide (0.1, 1.0 and 10 ppm ai) to determine the concentration required to inhibit the growth of each isolate on PDA alone by 50% (EC50). The 50 isolates of each species represented those collected from Alaska, Washington and Oregon (Table 3).

Table 2. Fungicides included in the fungicide resistance tests.

Trade Name and formulation	% active ingredient and common name	FRAC Code[1]
Chipco 26019 N/G	50% iprodione	2
Cleary's 3336F	41.25% thiophanate-methyl	1
Decree 50WDG	50% fenhexamid	17
Emerald	70% boscalid	7
Empress	23.3% pyraclostrobin	11
Medallion 50WP	50% fludioxonil	12
Vangard WG	75% cyprodinil	9

[1]FRAC Code List 2017. http://www.frac.inf accessed 15 May 2017

Table 3. Geographical sources of isolates included in fungicide resistance tests.

	State			
Organism	AK	OR	WA	Total
B. cinerea	29	7	14	50
B. paeoniae	20	6	24	50

There was very little difference in the sensitivity of the isolates from the different states. Overall, all of the *B. cinerea* and *B. paeoniae* isolates were very sensitive to fenhexamid with EC50 values of <0.1 ppm. About 2% of the *B. cinerea* and *B. paeoniae* isolates had EC50s >10 ppm of iprodione. The addition of thiophanate-methyl, even at 10 ppm had very little effect on the growth of any of the isolates included in our tests. It is unclear if the lack of sensitivity is due to resistance or a problem with the testing method. The percentage of *B. cinerea* isolates with EC50s >10 ppm for boscalid, pyraclostrobin, and cyprodinil was 67.3, 49.0, and 98.0%, respectively. For *B. paeoniae* isolates the percentages with EC50's >10 ppm for the same fungicides were 31.9, 2.1, and 78.7%, respectively. These data suggest that strains of *B. cinerea* and *B. paeoniae* from peony fields in Alaska, Oregon, and Washington are resistant to a number of commonly used *Botrytis* fungicides. This indicates that grower disease management programs need to include practices such as fungicide rotations to manage fungicide resistance problems.

Activity 9: Post-harvest analysis of *Botrytis* by cultivar and incidence of *Botrytis* following treatment with chemicals that promote cut flower longevity. Cultivars showed varying levels of *Botrytis* infection in the field and as cut flowers. The incidence of the disease was low in 2017, Table 4 shows the vase life of individual cultivars growing at the Georgeson Botanical Garden with a notation if they showed *Botrytis* anywhere on the plant. Some cultivars exhibited *Botrytis* as stem blackening shortly after emergence. A second category showed leaf lesions usually late in the season. Finally, some *Botrytis* showed up in the buds during post-harvest storage (34$\pm$ 3^{o}F; 90% RH) for one week followed by vase life studies (68^{o}F UAF horticulture lab, 24-hr fluorescent light, tap water)

Table 4. Vase life and presence of *Botrytis* on peony cultivars growing at the UAF Georgeson Botanical Garden, 2017*.

Cultivar	Flower class	Days to full bloom	Days from full bloom to petal fall/wilt	Total vase life	Botrytis presence
Alexander Fleming (Dr. Alexander Fleming)	Double	2.0	5.0	7.0	
Bowl of Cream	Double	1.8	8.0	9.8	Leaf lesions, flower buds
Bridal Icing	Bomb	1.5	4.5	6.0	Flower buds
Corinne Wersan	Double	1.8	5.4	7.2	
Festiva Maxima	Double	1.3	4.0	5.3	Flower buds, leaf lesions
Festiva Powder Puff	Double	1.0	5.4	6.4	
Gay Paree	Anemone	1.2	4.6	5.8	
George W. Peyton	Double	2.0	5.4	7.4	
Heidi	Japanese	1.2	6.4	7.6	
Joker	Double	3.0	6.3	9.3	Flower buds
Kansas	Double	2.0	5.4	7.4	
Ken Shan		3.0	6.0	9.0	
Lady Alexandra Duff	Double	2.7	5.7	8.5	Flower buds
Lady Kate	Double	2.2	6.0	8.2	Flower buds
La Lorraine	Double	3.0	5.3	8.3	
Largo	Japanese	2.0	4.0	5.5	
Lauren	Japanese	1.2	6.2	7.4	
Leslie Peck	Japanese	1.4	4.2	5.6	

Lora Dexheimer	Double	3.0	3.8	6.8	
Love's Touch	Semi-double to double	1.8	5.4	7.2	Flower buds
Lowell Thomas	Semi-double	2.6	3.2	5.8	
Mme Claude Tain	Double	2.0	7.0	9.0	
Mme Emile Debatene	Double	2.8	4.0	6.8	
Mary Jo LeGare	Double	3.0	5.8	8.8	
Mons. Martin Cahuzac	Double	2.0	6.6	8.6	
Nippon Beauty	Japanese	2.0	4.4	6.4	
Paul M. Wild	Double	2.3	4.7	6.2	
Petite Renee	Japanese	1.6	3.4	5.0	
President Roosevelt	Double	2.0	6.0	8.0	
President Taft	Double	2.2	5.6	7.8	
Sadie Fisher	Double	2.0	2.4	5.4	
Sarah Bernhardt 1	Double	2.5	5.8	8.2	Emerging shoots, leaf lesions, flower buds
Sarah Bernhardt 2	Double	2.0	6.1	8.1	Flower buds
Shirley Temple	Double	3.2	5.6	8.8	Flower buds
Sitka	Japanese	2.2	5.6	7.8	Flower buds
Victorian Blush	Double	1.8	6.4	8.2	Flower buds

*All cultivars grown at the Georgeson Botanical Garden except Mme Claude Tain, Sarah Bernhardt 2, and Bowl of Cream grown at Far North Peonies

A second experiment examined 10 post-harvest treatments with chemicals routinely used in the floral industry to prolong vase life. Three replicates of 10 stems each of 'Sarah Bernhardt peonies were harvested and stored in a cooler ((34± 3°F; 90% RH), for one week. One treatment consisted of spraying the foliage and flower buds with Floralife Clear Crowning Glory Hydration and Protection Solution® Spray according to manufacturer's directions prior to storage. All other treatments occurred after storage as a pre-box-and-ship treatment. The treatments prior to placing in the vase were:

1. Floralife Clear Crowning Glory Hydration and Protection Solution® – post storage, foliage and flower buds sprayed to drip, then dried
2. Floralife Crystal Clear 200® plant food- food packets dissolved in water, 1 hr stem soak prior
3. Hyaluronic acid- Jarrow formula, hydration liquid, 1 hr stem soak
4. Floralife Quick Dip 100® Instant hydration pretreatment, 1 second dip
5. Trehalose powder Swanson Brand – 1 hour stem soak
6. Chrysal Professional Glory Flower and Foliage Shield®- spray
7. Tap Water- 1 hour hydration
8. No treatment- dry stems from cold storage into box
9. Direct to vase- no treatment, no storage

Following treatments, flowers were inserted into a standard peony shipping box (Polar Peonies) to simulate air transport for 24 hours. Boxes were packed with cotton batting and two frozen gel packs wrapped in newsprint. Boxes were held at 68°F. After 24 hours, stems were cut 2 inches and placed in jars of tap water under the same laboratory conditions.

The purpose was to determine if the incidence of *Botrytis* was changed with the individual treatments. Although vase life was affected by the treatments (not published here), the incidence of *Botrytis* was impossible to study because of confounding physiological disorders caused by the treatments. *Botrytis* causes browning of the petals and receptacle and may or may not be visible when the stems are placed into the cooler. The result after one week can be a single small patch of brown, or the entire bud can be engulfed in brown. Some of the post-harvest treatments also caused significant browning mostly of the guard petals, in some cases amounting to 100% of the treated stems (treatments showing damage: hyaluronic acid, no water, Floralife Plant Food, Chrysal). Although we tried to ascertain if the browning was caused by disease or the treatments, it was impossible, at times, to separate the two. Therefore, no conclusions were drawn from this experiment on *Botrytis* incidence.

Activity 10. Analyze data, prepare quarterly and annual reports—Data have been analyzed and quarterly and annual reports have been submitted throughout the duration of the project.

Activity 11: Develop, organize, and execute educational programs for Alaskan peony growers—Educational programming was provided to peony growers in multiple forms and at various times during the project. Presentations and workshops were given at the APGA grower's conference during January 2017 (see Activity 4). The PIs, Dr. Patricia Holloway and Gary Chastagner, and PhD student, Andrea Garfinkel, attended the Mat-Su Peony Farm Tour and the Arctic Alaska Peonies Farm Tours (Interior) in July of 2016 and 2017 and gave field presentations on how to identify and manage diseases, the range of *Botrytis* species discovered during surveys in Alaska, identification and management of TRV, and post harvest issues with peonies.

During the 2017 tours, a new Fact Sheet on TRV management in peonies was provided to growers. Growers' guides, including the TRV Fact Sheet and manuscripts are described below in Activity 11. We also conducted a photo quiz of diseases, physiological disorders, weather-related traumas, and insect pest damage to growers. Participants were asked to guess what they were viewing in a series of photographs. The quiz was well received, and more than 30 participants at each farm tour tried their luck. The PIs were pleasantly surprised to see the level of retention and identification exhibited by participants. Average response was 70% correct answers. Of course, in most instances, the individuals who felt they would not be embarrassed by low scores were the ones who chose to participate.

Activity 12: Prepare final report, grower disease management guides, and manuscripts for publication—This report is satisfying our objective of submitting a final report for this project. A general disease management guide is in preparation for future publication through WSU extension. An extension Fact Sheet has been published on TRV in peonies and can be found as open-access at this URL: http://extension.wsu.edu/publications/pubs/fs284e/. A journal publication describing the range of pathogens found on peonies in the United States, including Alaska, has been prepared for submission to the journal Plant Disease. A manuscript describing one of the new species of *Botrytis, B. euroamericana*, was published in Mycologia (http://www.tandfonline.com/eprint/MSxd2r4FbC9i2x3ptq67/full).

Dr. Chastagner and Andrea Garfinkel also coauthored a chapter on the management of diseases on peonies for the new Springer "Plant Disease Management. Handbook of Florists' Crops Diseases" book, and provided more in-depth information to growers about TRV on peonies in the Fall 2016 issue of the Association of Specialty Cut Flower Growers (ASCFG) Cut Flower Quarterly.

This project did not benefit any other commodity groups outside of specialty crops.

This project would not have been possible without the knowledge, leadership and collaboration of Washington State University professor and graduate student, Dr. Gary

Chastagner and Andrea Garfinkel and the Alaska Peony Growers Association. Alaska does not have a full-time expert at the University of Alaska or State in the dynamics of *Botrytis* and other fungal diseases. This partnership was critical to the success of this project and to the future of the peony industry in Alaska and worldwide. Andrea Garfinkel, WSU Ph.D., student, organized surveys to collect disease samples, identified the diversity of *Botrytis* species and other pathogens on peonies, developed molecular markers to detect *B. paeoniae*, conducted pathogenicity studies, helped organize educational activities, and helped prepare publications, updates and necessary reports

Dr. Gary Chastagner directed all plant pathology research and worked with a dynamic team at Washington State University, Puyallup to complete these studies.

Dr. Patricia S. Holloway was the APGA Industry Professional and Collaborator on this project. She provided input an assistance relating to the environmental monitoring and fungicide trials, was a liaison with growers collaborators, and helped prepare updates and necessary reports.

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Katie Coats, WSU Molecular Biology Research Assistant, assisted Andrea Garfinkel with the molecular studies to identify pathogens and the development of the molecular markers for *B. paeoniae*.

Annie DeBauw, WSU Agriculture Research Tech. III, conducted disease control trials and assisted with the preparation of reports.
WSU hourly part-time help provided assistance with the maintenance of plant material and isolate collection, isolations from disease samples, pathogenicity studies, and fungicide-resistance tests.

Todd Steinlage- Plant Pathologist, Alaska Division of Agriculture Plant Materials Center worked in partnership with the WSU researchers to clarify and identify Tobacco rattle virus in Alaska peonies.

Janice Chumley, Alaska Cooperative Extension Service, Kenai, assisted in field collection of Tobacco rattle virus and Botrytis samples.

Growers - This project would not have been possible without the cooperation of a number of growers in Alaska who provided access to their fields, helped collect environmental data, and provided plant material needed for this project. The PIs and PhD student would specifically like to thank the following farms for their assistance with the environmental monitoring studies: Alaska Perfect Peony, Arctic Sun Peonies, Boreal Peonies, DeGoede

Bulb Farm, Echo Lake Peonies, Hoffman Acres Farms, Oregon Perennial Company, and Our American Roots.

Goals & Outcomes Achieved

The performance objectives and a description of their completion are described below:

Objective 1: Correlate *Botrytis* disease development with environmental conditions by tracking temperature, leaf wetness, and rainfall at peony fields in the four major peony production areas in Alaska—We were unable to complete this objective due to lack of statistical significance in the data we collected (see Activities 1 & 2). The failure to identify a correlation likely is not due to the irrelevance of the environmental data collected in disease development, but rather the prevalence of confounding and uncontrolled factors in the systems observed such as: differences in patterns of fungicide use, the prevalence of fungicide resistance, the presence of a diversity of *Botrytis* species present among fields, initial inoculum loads present in fields; differences in phenological development in periods conducive to disease development, planting density, and irrigation practices. In-vitro tests to assess variability among *Botrytis* species to infect peonies under various environmental conditions could lead to better understanding of conditions favorable to disease development.

Objective 2: Identify biopesticides and conventional fungicides that are effective in controlling *Botrytis* gray mold. – Extensive trials were conducted in 2016 and 2017 (see Activity 3) to identify conventional fungicides and biopesticides. Although none of the biopesticides provided effective control in any of the trials, a number of the conventional fungicides were effective in reducing disease development. The effectiveness of the specific fungicides varied by pathogen and the type of trial that was conducted. With respect to management of Botrytis gray mold, it is clear that there are fewer fungicides that are effective in controlling *B. cinerea* than *B. paeoniae*. It is unclear why this difference occurs, but additional studies are needed to obtain a better understanding of the fungicide sensitivity of Alaska's diverse *Botrytis* pathogens on peonies. The postharvest storage tests also suggest that while preharvest applications of fungicides have a significant effect on the development of gray mold on foliage in cold storage, they appear to have minimal effect on disease development on the flower buds. Additional studies are needed to confirm these results and potentially identify postharvest treatments that are effective in limiting disease development on flower buds. Although a number of new biopesticides were included in our trials, none of them proved to be effective under our test conditions. Additional work is needed to identify ways to potentially increase the efficacy of these types of products under field conditions.

Objective 3: Use molecular markers to determine if *B. paeoniae* is being introduced into Alaska via infested rootstock—Molecular markers were developed (see Activity 7) and tests were run to assess movement using the *B. paeoniae* isolates collected throughout the course of studies

conducted in Alaska. Results of these tests were statistically inconclusive, potentially due to the small number of *B. paeoniae* isolates collected in Alaska. Nonetheless, inoculation trials showed that *B. paeoniae* could infect roots, although perhaps infection does not spread after planting. *B. paeoniae* was identified in 2017 on roots from a rootstock producer in Oregon. Therefore it is likely that the potential for movement of this pathogen exist. Although the movement of *B. paeoniae* could not be confirmed, the markers developed indicated other surprising results. These included the likelihood that there is no sexual recombination occurring in *B. paeoniae* based on the distribution of mating types among the isolates sampled. This is a major contribution to the knowledge of *B. paeoniae* because never before have the frequencies of mating types been described for this pathogen, nor have the sequences of the mating type idomorphs (alleles) been described as was done as a result of this project. Furthermore, this project's technique that was used to identify microsatellite markers in *B. paeoniae* is a novel method never used before with fungi. These contributions to science, including the primers for the microsatellite loci, will be published and can therefore be used in the future to answer additional questions about *B. paeoniae* biology.

Objective 4: Conduct surveys at a minimum of 3-4 peony farms in each of the four major production regions of Alaska to identify the range of all pathogens that infect peonies in Alaska—35 farms were surveyed to identify the range of pathogens found in peonies in Alaska, for a total of more than 8 average per region. These surveys confirmed a greater diversity of *Botrytis* in peonies than has been seen in any agroecosystem. This includes up to 10 unnamed new *Botrytis* species. As a result of this study, one of the new species found in Alaska was formally named *B. euroamericana,* as published in the journal Mycologia. Additional species will be described in a future publication. Two new fungal pathogens, *Mycocentrospora acerina* and a *Phoma* spp. were identified on peonies in Alaska, with pathogenicity trials confirming their ability to cause disease. These results will be published in the journal Plant Disease and a diagnostic guide will be developed to help Alaskan growers identify these diseases in the field. Furthermore, our surveys helped to identify a widespread problem with TRV in peonies in Alaska. Due to the prevalence of this pathogen, we developed a grower's guide (see Activity 11 and Objective 5 below) to help growers identify and manage this disease.

Objective 5: Develop and provide educational programs and materials for peony growers regarding *Botrytis* and other peony disease identification and management—One extension Fact Sheet on TRV in peonies has been published, with Alaska-specific information on the virus' vector, and more in-depth information about TRV on peonies was reported in the Fall 2016 issue of the ASCFG Cut Flower Quarterly. The PI and PhD student also coauthored a chapter on the management of diseases on peonies for the new Springer "Plant Disease Management. Handbook of Florists' Crops Diseases" book. Research updates and ways to improve disease management were provided to growers who attended the 2016 and 2017 APGA Annual Conferences, a 2017 workshop, and four regional farm tours in 2016 and 2017.

The measurable outcomes and a description of their status are described below:

Outcome 1: Determine if *B. paeoniae* is being introduced into Alaska via infested rootstock (GOAL) by applying molecular markers developed with funding from a 2014 Alaska SCBG and 2014 Washington SCBG (TARGET) to a minimum of 20 Alaskan *B. paeoniae* isolates (BENCHMARK) by the end of this project (PERFORMANCE MEASURE).—The goal of determining if *B. paeoniae* is being introduced on infected rootstock was not achieved. However, a number of additional benefits were gained as a result of pursuing this goal. See Objective 3 above.

Outcome 2: Disseminate information on the diagnosis and management of diseases on peonies (GOAL) by developing a Peony Diagnostic Guide (TARGET) that is provided to a minimum of 100 growers (BENCHMARK) that are attending the annual APGA conference in 2017 (PERFORMANCE MEASURE).—An extension Fact Sheet on TRV on peonies was produced and disseminate it to approximately 120 growers in attendance at two summer field tours during 2017 in Alaska. This publication is also available for free online to Alaska peony growers. A full diagnostic guide on all diseases of peonies will be ready for submission to WSU extension prior to the end of 2017. All results of this project were also posted on the blog, HortAlaska Peonies: https://alaskapeony.wordpress.com/.

Beneficiaries

Information on disease diagnosis and management was given to the approx. 80 people who attended the APGA conference in 2016 and 250 people in 2017 as well as the 18 people who attended our day-long workshop in 2017. This study also directly benefitted the approx. 240 people who attended the farm tours in Fairbanks, Willow and Kenai Peninsula in 2017. There are currently 113 peony farms in the state of Alaska, all of which will benefit from the information developed as a result of this study. A link was send to all 113 farms with the fact sheet on Tobacco rattle virus, and all will receive a copy of this report.

Lessons Learned

There were a number of positive and negative lessons from this project. Examples of lessons learned that were outcomes of this project include:

- the realization that *B. paeoniae* exists at low frequencies in Alaska, complicating the issue of collecting enough isolates sufficient for microsatellite analysis;
- too many confounding factors likely exist to adequately correlate environmental data with disease development in the systems studied, and more controlled in-vitro studies of *Botrytis* on peonies would likely be an important first step in determining environmental conditions conducive to disease development;

- important regional differences in environmental conditions exist among Alaskan peony production regions in terms of temperature, rainfall, and leaf wetness. Understanding the seasonal changes in these parameters could help understand the risk of disease development throughout the state;
- the realization that not all diseases can be easily diagnosable in the field and there are still questions about the cause of some symptoms seen on peonies in Alaska;
- that the great amount of diversity in pathogens infecting peonies in Alaska increases the need for accurate diagnosis. Additional research into the biology and epidemiology of these pathogens is warranted;
- research into post-harvest environment and potential treatments for *Botrytis can* be difficult and confounding. Without an on-site, trained plant pathologist in Alaska, many of these studies will not be possible in the future.
- that growers are eager for information on peony diseases and that there is an ongoing need for education, given the number of new growers and the likelihood that diseases will continue to increase as plantings mature;
- one-on-one interactions with growers are extremely valuable and on-site tours of peony farms are essential for identifying novel diseases, their prevalence in the field, and possible management solutions;
- none of the tested biopesticide products appeared to be effective at managing Botrytis gray mold, but a number of conventional fungicides were efficacious and could be used to improve disease management in peonies in Alaska.

Examples of unexpected findings include:

- Analysis of the markers revealed an important aspect of *B. paeoniae*, biology, namely that it is likely not undergoing sexual recombination.
- Even though at least five new species of *Botrytis* were expected, many more species were found than anticipated. As a result of collecting *Botrytis* isolates for this study, up to 10 new species of this fungus may have been identified.
- There are a number of pathogens present in Alaska on peonies that have never been reported before in the literature on peonies in the United States.

Outcome 1 was not achieved due to a small sample size of *B. paeoniae* isolates for which a population genetics analysis can be performed. Although we collected hundreds of isolates, our sampling revealed that *B. paeoniae* exits at a relatively low frequency in Alaska peony farms, therefore, successful completion of this objective would likely require collection of many more isolates than was accomplished during the course of this study.

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Additional Information

- N/A