Identification and Management of *Botrytis* Gray Mold Species for Alaska's Peony Growers

Final Report

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Project Summary:

Botrytis gray mold is the single most important disease of Alaskan 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 postharvest destruction of cut flowers *Botrytis* gray mold has been tentatively identified in nearly every commercial peony operation in Alaska from the Interior to the Kenai Peninsula. Preliminary DNA sampling of Alaska grower fields in 2013 revealed *Botrytis* species grouping into 5 distinct genetic clades, not two as had been identified previously on peonies. We found a greater diversity of *Botrytis* species impacting peonies in Alaska than in Washington, Oregon, and The Netherlands. One of our selections is a new species not found in other regions.

Our sampling of roots for Botrytis was not successful, but our tests indicated that *Botrytis* was able to infect root tissue and cause chocolate brown lesions on cut root surfaces. Disease development was low during the 2015 growing season as indicated by the low disease values. Disease development within Alaska was variable and ranged from 0.69 (very little to disease) to 78.57 (0-500 rating scale). Disease ratings were higher in Trapper Creek and Delta Junction than in Soldotna and Homer. Although data were not statistically significant after one year, there was a slight trend toward increasing periods of leaf wetness and increasing temperatures during periods of leaf wetness as contributing to higher disease.

Because *Botrytis* levels were very low in 2015, fungicide treatments were inconclusive. They did show that at recommended commercial rates, no fungicide showed phytotoxic effects on peonies (Pageant®, Dithane®, Champ® and Zerotol®). From 0 to 64% of petals that dropped and stuck to leaf surfaces showed evidence of *Botrytis* infection on the leaves. Petals are a significant food source for *Botrytis* infection and should be removed from the field before petal fall.

Approach

Twenty-six Alaska peony farms in three major production regions (Interior, Mat-Su Valley, Soldotna area, and the Kenai Peninsula) were surveyed during the 2014 growing season. Samples were collected for molecular analysis of the G3PDH gene to identify the species and races of Botrytis of concern to peony growers. A phylogenetic tree was created to show the relationship among species and to identify new species unique to Alaska. We sampled roots to attempt to recover *Botrytis* from rootstocks and root material to determine if roots sold to growers are carriers for *Botrytis*. Clean roots were also inoculated to determine if *Botrytis* had the ability to colonize root tissues.

Weather stations were set up at four peony farms in Alaska in four different peony production regions: Delta Junction, Trapper Creek, Soldotna, and Homer. Four additional weather stations were deployed in Washington and Oregon. The weather stations gathered season-long data on temperature, rainfall, and leaf wetness to ascertain possible environmental triggers for *Botrytis* growth. The incidence of disease related to these environmental parameters were analyzed using linear regression. Disease progression was monitored either in-person or remotely using photographs supplied by growers. Final disease data were taken in person at the end of the growing season. We developed a disease rating system to evaluate disease development which resulted in a possible range of 0 to 500, with 0 being no disease and 500 being total dieback due to disease.

The effectiveness of four fungicides (commercially sold as Pageant®, Dithane®, Champ® and Zerotol®) was evaluated for Botrytis control and phytotoxicity. Each was applied six times during the early season through cutting stage.

Flower petals that drop naturally after bloom. We speculated that petals that land on a leaf or stem might be a conduit for Botrytis infection. The UAF staff selected up to 50 leaves at random from 20 peony selections and cultivars or selections, removed the brownish petal from their surface, and recorded the incidence of *Botrytis* on the leaf surface beneath the petal.

Goals and Outcomes Achieved:

- Goal 1: Expanded DNA analysis of peonies on grower cooperator farms to explore the species of *Botrytis* occurring in Alaska.

  26 Alaskan peony farms in 4 major production regions of Alaska (the Interior, the Mat-Su Valley, Soldatna area, and the Kenai Peninsula) were surveyed during the 2014 growing season. A total of 234 isolates of *Botrytis* were collected from these farms and are archived at the Washington State University (WSU) Puyallup Research and Extension Center (PREC). Due to difficulties in molecular techniques and availability of funding to analyze all 234 samples, 115 isolates were selected for further analysis. Molecular analysis of the G3PDH gene of these *Botrytis* isolates indicated that Alaskan *Botrytis* isolates grouped into at least 6 genetic clades (see supplemental PDF attachment "Alaskan peony Botrytis isolates G3PDH" for a phylogenetic tree illustrating the genetic clades), and potentially more than 6 species. The species found include *B. cinerea* (n=43) and *B. paeoniae* (n=22), which are commonly described on peony, and *B. pseudocinerea* (n=1), a cryptic *Botrytis* species that has only been described on peonies in Chile. The remaining isolates do not appear to be genetically identical to any other named species of *Botrytis*, however, further genetic analysis is currently underway to confirm exact placement of these isolates.

  These results indicate that there is a great diversity of *Botrytis* species impacting peonies in Alaska, much greater than in Washington, Oregon, or The Netherlands, as indicated by other surveys that we are currently performing. Fig. 1 shows pie graphs of the relative diversity of *Botrytis* species on peony in each of these major peony production regions. In WA and OR, 81% of isolates were either *B. paeoniae or B. cinerea* and in The Netherlands 100% of the isolates were these two species. However, in Alaska, only 58% of the total isolates were either *B. paeoniae* or *B. cinerea* with 41% of the Alaskan isolates collected categorized as species other than *B. cinerea*, *B. paeoniae*, or *B. pseudocinerea*.

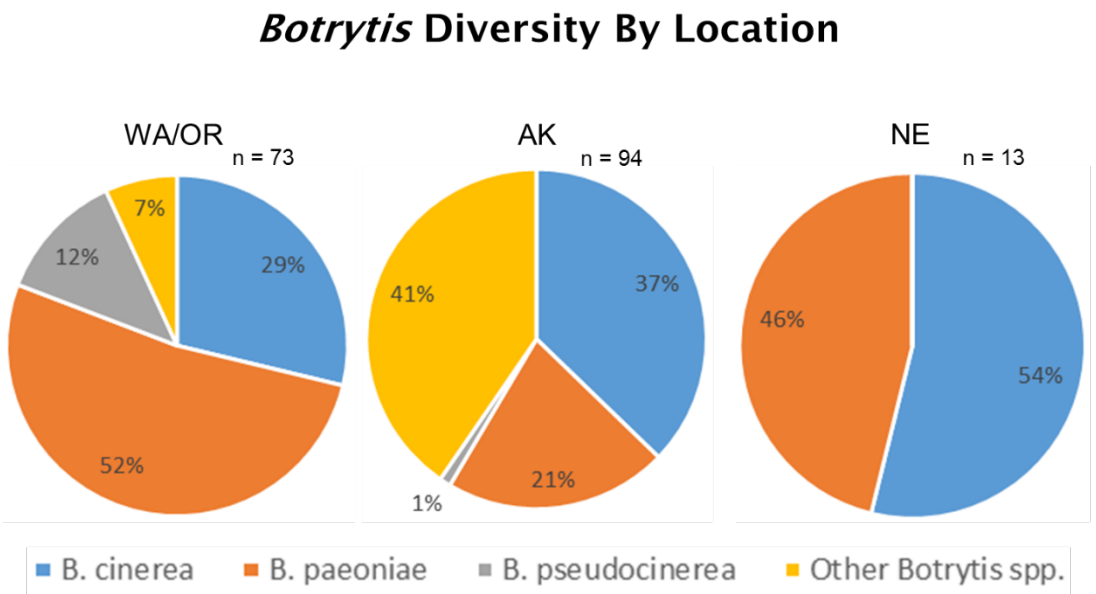

Figure 1. Diversity of *Botrytis* species found on peony in three major commercial peony production regions. WA/OR = Washington and Oregon, AK = Alaska, NE = The Netherlands.

One of the most frequently isolated species of *Botrytis* from peony in Alaska is currently under further analysis for formal description of a new species. WSU is collaborating with a team in Italy who found one isolate that is genetically identical to some of the isolates found in Alaska. We are currently performing additional genetic analysis, pathogenicity testing, and morphological analyses which we anticipate to be presented later this year.

A number of additional isolates comprising other clades have been subject to further genetic analysis by the RPB2 and HSP60 genes and the results are currently being analyzed. Two additional isolates comprising unique clades have also been confirmed as pathogenic on peony.

- Goal 2: Sampling common root stock sources to determine if *Botrytis* is being imported on or within root tissues.

Surveys were conducted to attempt to recover *Botrytis* from rootstocks and root material, however, these attempts were unsuccessful. The chance of finding *Botrytis* in rootstock was likely low (maybe like finding a "needle in a haystack," so-to-speak).

Rootstocks were inoculated in the fall of 2015 to determine if *Botrytis* had the ability to colonize root tissues. Our tests indicated that *Botrytis* was able to infect root tissue and cause chocolate brown lesions on cut root surfaces (Fig. 2). The ability of the fungus to colonize root tissues increases our understanding of the biology of this pathogen and the possible epidemiological importance as it relates to movement of rootstock. Plants with inoculated roots are currently growing at the PREC and will be observed over the summer of 2016. In these experiments, only plugs of fungal mycelia were used (rather than conidia as indicated in the proposal) as we determined this the best way to maximize chance of infection and only one method to simulate infection during root processing and harvesting (rather than also infest soil with sclerotia) was used because we believed that this scenario was the most likely in the movement of this pathogen.

Figure 2. Chocolate brown lesion caused by *Botrytis paeoniae* on the surface of a cut peony root (indicated by red arrow).

An initial set of 8 molecular markers have been developed to test population structure of *B. paeoniae* in the major rootstock production areas and Alaska. These 8 markers, focusing on

repetitive regions of the genome called “microsatellites,” were developed from a draft genome of a *B. paeoniae* isolate collected during this study. 64 isolates of *B. paeoniae* have been identified out of the total collected in all survey locations (WA, OR, AK, and The Netherlands) and subject to analysis using the 8 molecular markers. Initial results indicated that these 8 microsatellite markers are insufficient to fully characterize the population structure, therefore, we are in the process of developing 8 additional microsatellite markers to use to answer the question of pathogen movement. Furthermore, as more *B. paeoniae* isolates are identified, additional isolates will be subject to analysis.

- Goal 3: Identifying environmental triggers (air temperature, leaf moisture, etc.) that might control regional and seasonal differences in disease manifestation.

Weather stations were set up at 4 peony farms in Alaska during the 2015 growing season. The weather stations were deployed at farms in four different peony production regions in Delta Junction, Trapper Creek, Soldatna, and Homer. Four additional weather stations were deployed in Washington and Oregon. The weather stations gathered season-long data on temperature, rainfall, and leaf wetness. A comparison of these data with the data collected in Washington and Oregon can be seen in Figs. 3-5. Temperatures during the growing season varied 5-7°F among the Alaskan farms during the 2015 growing season, with Fairbanks (gray bar, Fig. 3) having the highest temperatures in June and July. Total precipitation ranged from under 1 inch to almost 4.5 inches per month (Fig. 4). Leaf wetness values were consistently highest in Trapper Creek (green bar, Fig. 5).

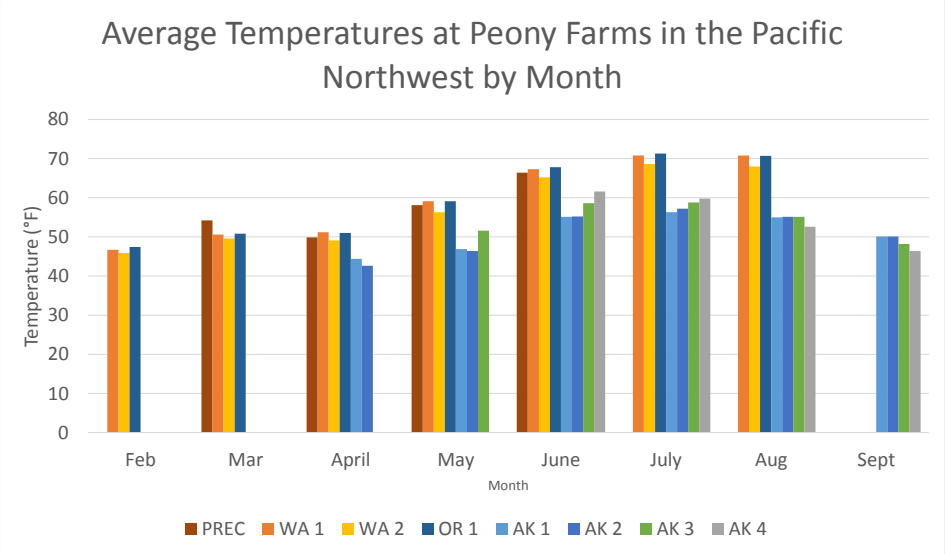

Figure 3. Average temperatures at peony farms in the Pacific Northwest during the 2015 growing season by month. PREC= Puyallup Research and Extension Center in Puyallup, WA, WA 1= Washington Farm 1, WA 2 = Washington Farm 2, OR 1 = Oregon Farm 1, AK 1 = Homer Farm, AK 2 = Soldatna Farm, AK 3 = Trapper Creek Farm, AK 4 = Delta Junction Farm.

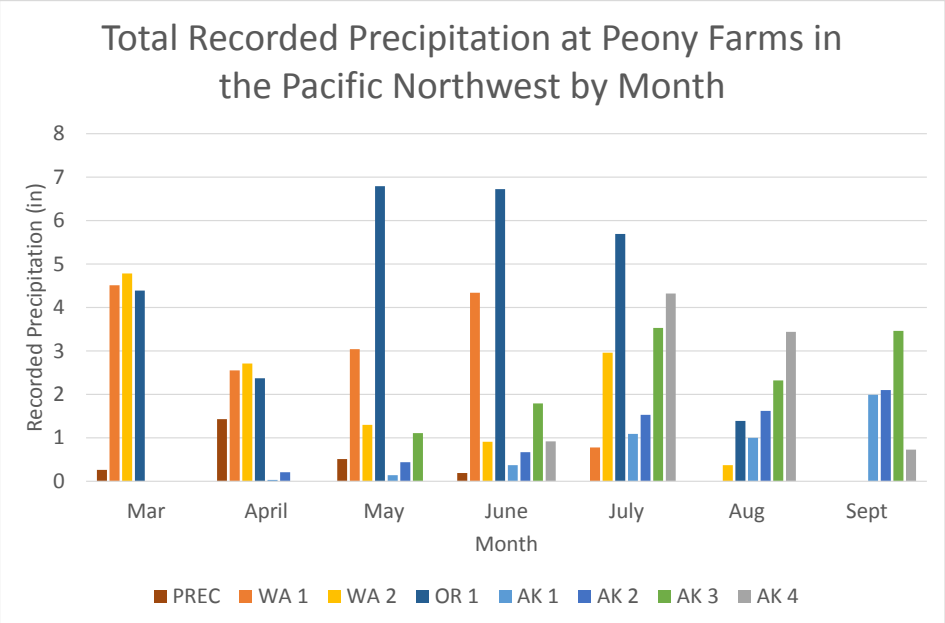

Figure 4. Total rainfall at peony farms in the Pacific Northwest during the 2015 growing season by month. PREC= Puyallup Research and Extension Center in Puyallup, WA, WA 1= Washington Farm 1, WA 2 = Washington Farm 2, OR 1 = Oregon Farm 1, AK 1 = Homer Farm, AK 2 = Soldatna Farm, AK 3 = Trapper Creek Farm, AK 4 = Delta Junction Farm.

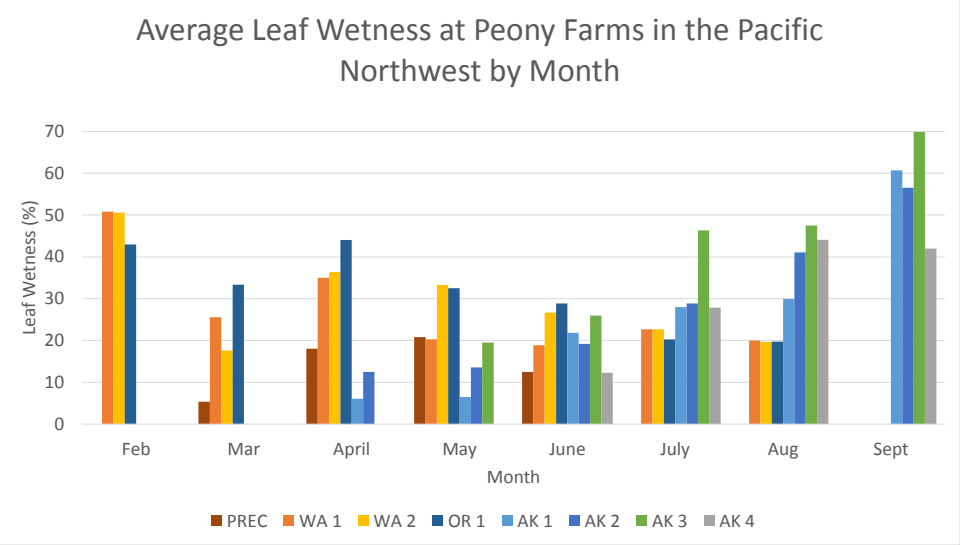

Figure 5. Average leaf wetness at peony farms in the Pacific Northwest during the 2015 growing season by month. PREC= Puyallup Research and Extension Center in Puyallup, WA, WA 1= Washington Farm 1, WA 2 = Washington Farm 2, OR 1 = Oregon Farm 1, AK 1 = Homer Farm, AK 2 = Soldatna Farm, AK 3 = Trapper Creek Farm, AK 4 = Delta Junction Farm.

Disease progression at all of the sites was monitored either in-person or remotely using photographs supplied by growers. Final disease data were taken in person at the end of the growing season. A disease rating scheme was devised to rate disease development which resulted in a possible range of 0 to 500, with 0 being no disease and 500 being total dieback due to disease. Final disease ratings are shown below:

PREC – 12.89
WA 1 – 30.62
WA 2 – 93.40
OR 1 – 33.30
AK 1 – 0.69
AK 2 – 7.00
AK 3 – 28.78
AK 4 – 78.57

PREC= Puyallup Research and Extension Center in Puyallup, WA, WA 1= Washington Farm 1, WA 2 = Washington Farm 2, OR 1 = Oregon Farm 1, AK 1 = Homer Farm, AK 2 = Soldatna Farm, AK 3 = Trapper Creek Farm, AK 4 = Delta Junction Farm.

Disease development was low during the 2015 growing season as indicated by the low disease values. Disease development within Alaska was variable and ranged from 0.69 (very little to disease) to 78.57 (a low to moderate amount of disease). Disease ratings were higher in Trapper Creek and Delta Junction than in Soldatna and Homer.

In order to determine if there was any relationship between disease development and the environment, linear regression analyses were performed (Figs. 6-8). Due to various factors, AK 4 and the PREC were excluded from the analysis. Linear regressions were inconclusive as p-values were not significant, however, this is likely due to the low number of datapoints in the linear regression and the corresponding lack of statistical power. We are currently deploying weather stations for the 2016 growing season and will add the data collected in 2016 to the data collected in 2015 to see if any trend emerges. Regardless of lack of statistical significance, it appears that a trend towards increasing periods of leaf wetness and increasing temperatures during periods of leaf wetness are contributing to higher disease (Figs. 6-8).

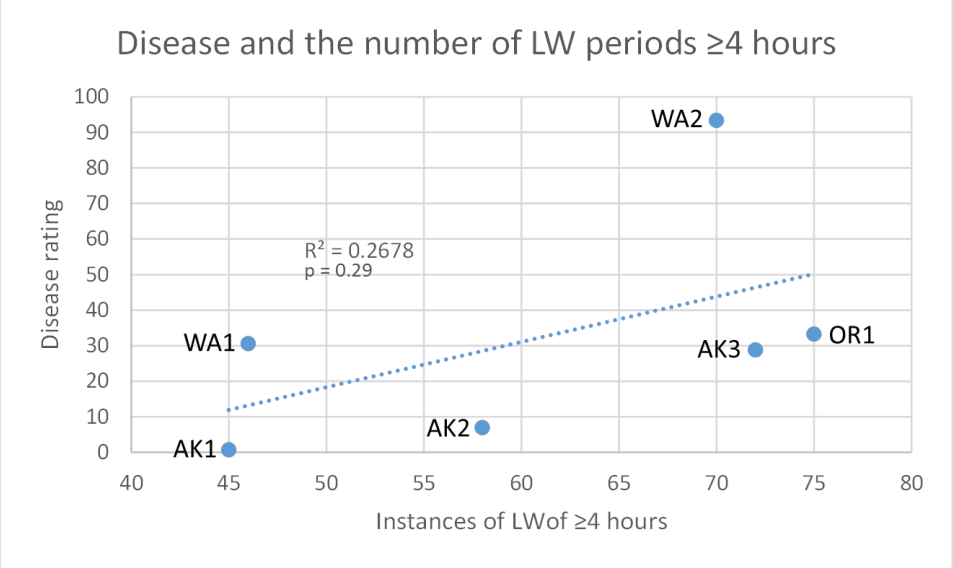

Figure 6. Linear regression describing the relationship between the number of leaf wetness periods that were greater than or equal to 4 hours at peony farms during the 2015 growing season.

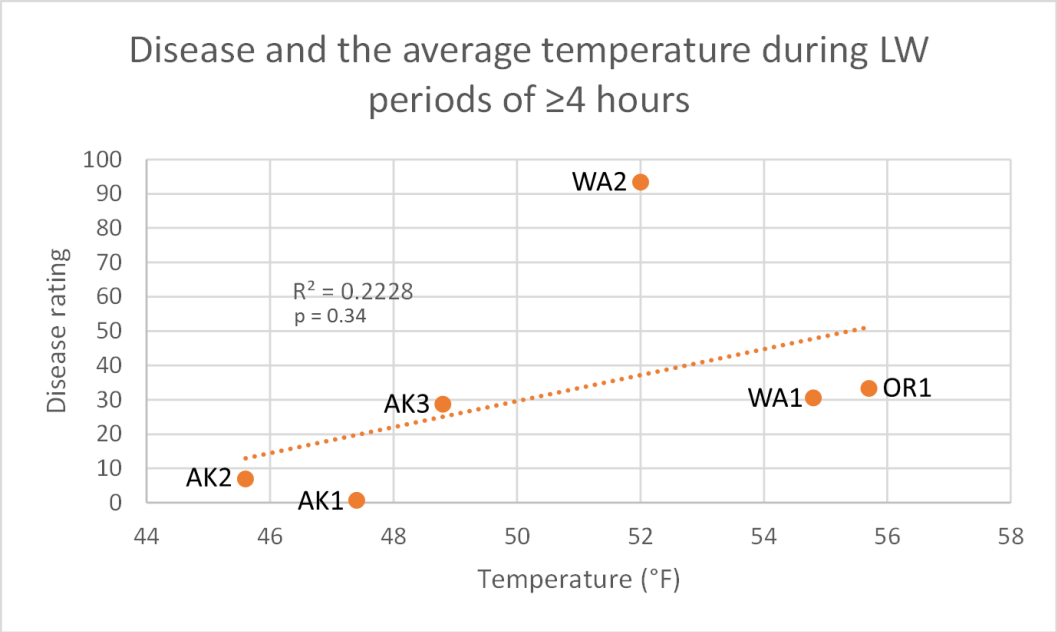

Figure 7. Linear regression describing the relationship between the temperature during periods of leaf wetness that were greater than or equal to 4 hours at peony farms during the 2015 growing season.

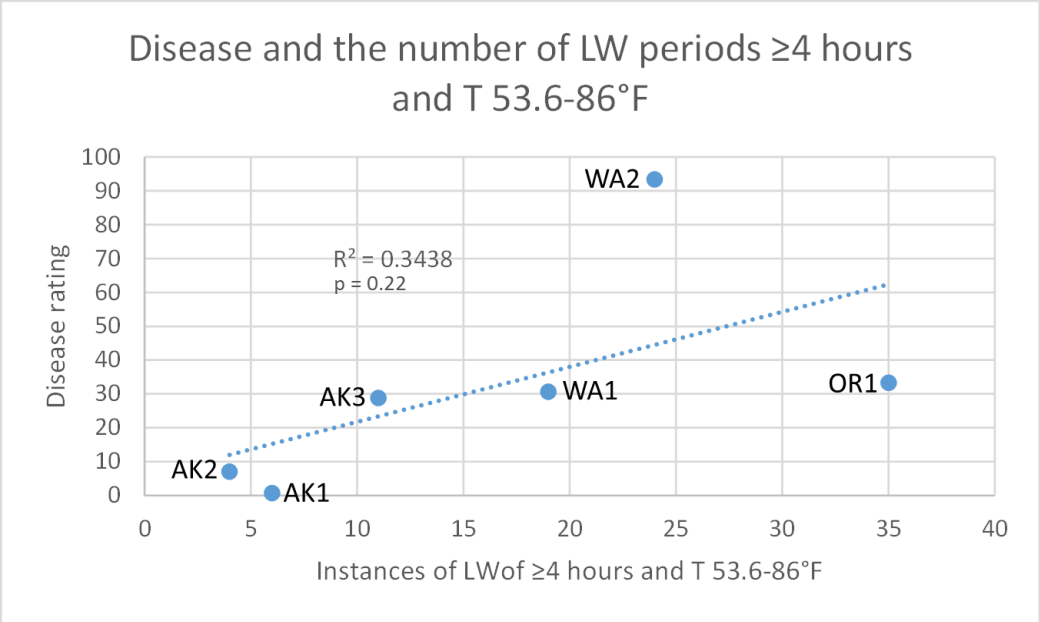

Figure 8. Linear regression describing the relationship between the number of leaf wetness periods that were greater than or equal to 4 hours that occurred when average temperatures were between 53.6 and 86°F (ideal temperature for *B. cinerea* conidia germination) at peony farms during the 2015 growing season.

We are currently in the process of determining any influence of seasonal variation on *Botrytis* infection.

- Goal 4: Examining the efficacy of different fungicides including potential biopesticide controls that might be used by Alaska growers to manage this disease complex in commercial fields.

  Based on information from growers, a disease management trial was set up in a peony planting at the UAF peony research plots. The trial compared the effectiveness of four fungicides applied 6 times during the early season through cutting stage in managing *Botrytis* infections. The fungicides are sold commercially as Pageant®, Dithane®, Champ® and Zerotol®. Pageant® is a well-known fungicide used throughout Alaska with fairly good Botrytis control. By the end of this trial, *Botrytis* was scattered throughout the entire plot as tiny spots on leaves or at the base of spent flowers. The incidence was so low, however, that no differences were recorded among treatments and the control. At commercial rates, however, no fungicide shows any phytotoxicity. Additional trials that are being supported by the IR-4 program will be conducted during the 2016 growing season at WSU Puyallup.

  In a separate trial, we demonstrated that flower petals that fall onto leaves resulted in increased *Botrytis* infections. These observations can be used in future experiments to enhance disease development in future disease control trials

Cultivar (n= number of petals examined)	*Botrytis* detected beneath petals on leaves (%)
Eskimo Pie (n=2)	50
Double Red (n=47)	38
Corinne Wersan (n=48)	23
Avalanche (n=50)	32
President Taft (n=50)	52
Lowell Thomas (n=50)	64
Love's Touch (n=50)	40
Double White (n=50)	40
Sadie Fisher (n=50)	50
Helen Hayes (n=28)	7
Festiva Powder Puff (n=50)	28
Lora Dexheimer (n=50)	50
Petite Renee (n=50)	38
Joker (n=22)	36

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Mary Jo Legare (n=12)	17
Leslie Peck (n=31)	0
Gay Paree (n=5)	20
Festiva Maxima (n=33)	15
Heidi (n=22)	0
Sarah Bernhardt (n=50)	48

Beneficiaries:

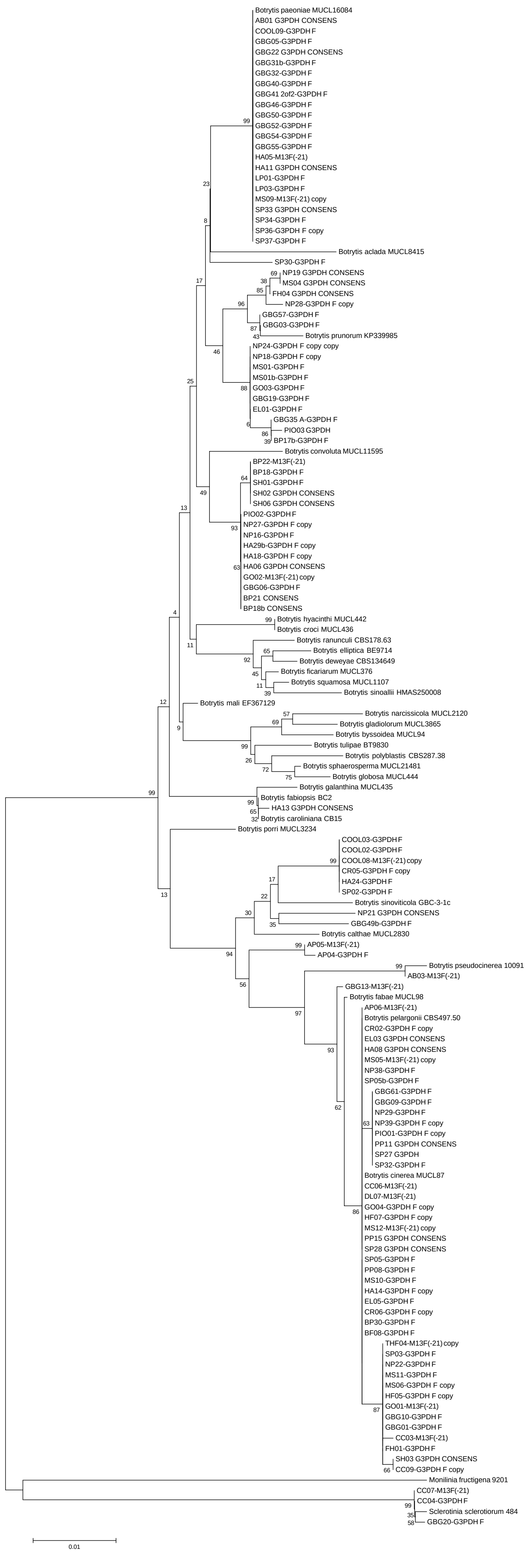
The project has benefited all peony growers in Alaska by providing baseline knowledge of a disease that will most definitely show up in their fields and storage facilities. It will also provide critical research for future *Botrytis* studies that emphasize management systems for growers. Results from this project have been shared with growers via presentations at the APGA annual conferences in 2015 and 2016 and by direct grower education via farm visits.

Lessons Learned:

The large diversity of *Botrytis* species on peonies in Alaska was unexpected. As a result of our surveys, we also learned that that there are a number other pathogens causing diseases on peonies. Additional work on *Botrytis* and some of these other pathogens will be completed on during a newly funded project. We showed that four fungicides commonly used on peonies showed no phytotoxic effects on peonies, but their efficacy on *Botrytis* control was not clear because of warm, dry weather and low incidence of Botrytis. Our study hints that leaf wetness measurements and air temperature might lead to a useful predictive model for Botrytis severity in grower fields. Sanitation in fields is critical to managing *Botrytis*. Petals from old flowers can be a significant food source for the entry of *Botrytis* into leaves.

Additional Information:

A phylogenetic tree showing the genetic diversity of 112 Botrytis species and 2 *Sclerotinia* spp. that were obtained from diseased samples can be found in the attached file.


Botrytis paeoniae MUCL16084
AB01 G3PDH CONSENS
COOL09-G3PDH F
GBG05-G3PDH F
GBG22 G3PDH CONSENS
GBG31b-G3PDH F
GBG32-G3PDH F
GBG40-G3PDH F
GBG41 2of2-G3PDH F
GBG46-G3PDH F
GBG50-G3PDH F
GBG52-G3PDH F
GBG54-G3PDH F
GBG55-G3PDH F
HA05-M13F(-21)
HA11 G3PDH CONSENS
LP01-G3PDH F
LP03-G3PDH F
MS09-M13F(-21) copy
SP33 G3PDH CONSENS
SP34-G3PDH F
SP36-G3PDH F copy
SP37-G3PDH F
Botrytis aclada MUCL8415
SP30-G3PDH F
NP19 G3PDH CONSENS
MS04 G3PDH CONSENS
FH04 G3PDH CONSENS
NP28-G3PDH F copy
GBG57-G3PDH F
GBG03-G3PDH F
Botrytis prunorum KP339985
NP24-G3PDH F copy copy
NP18-G3PDH F copy
MS01-G3PDH F
MS01b-G3PDH F
GO03-G3PDH F
GBG19-G3PDH F
EL01-G3PDH F
GBG35 A-G3PDH F
PIO03 G3PDH
BP17b-G3PDH F
Botrytis convoluta MUCL11595
BP22-M13F(-21)
BP18-G3PDH F
SH01-G3PDH F
SH02 G3PDH CONSENS
SH06 G3PDH CONSENS
PIO02-G3PDH F
NP27-G3PDH F copy
NP16-G3PDH F
HA29b-G3PDH F copy
HA18-G3PDH F copy
HA06 G3PDH CONSENS
GO02-M13F(-21) copy
GBG06-G3PDH F
BP21 CONSENS
BP18b CONSENS
Botrytis hyacinthi MUCL442
Botrytis croci MUCL436
Botrytis ranunculi CBS178.63
Botrytis elliptica BE9714
Botrytis deweyae CBS134649
Botrytis ficariarum MUCL376
Botrytis squamosa MUCL1107
Botrytis sinoallii HMAS250008
Botrytis mali EF367129
Botrytis narcissicola MUCL2120
Botrytis gladiolorum MUCL3865
Botrytis byssoidea MUCL94
Botrytis tulipae BT9830
Botrytis polyblastis CBS287.38
Botrytis sphaerosperma MUCL21481
Botrytis globosa MUCL444
Botrytis galanthina MUCL435
Botrytis fabiopsis BC2
HA13 G3PDH CONSENS
Botrytis caroliniana CB15
Botrytis porri MUCL3234
COOL03-G3PDH F
COOL02-G3PDH F
COOL08-M13F(-21) copy
CR05-G3PDH F copy
HA24-G3PDH F
SP02-G3PDH F
Botrytis sinoviticola GBC-3-1c
NP21 G3PDH CONSENS
GBG49b-G3PDH F
Botrytis calthae MUCL2830
AP05-M13F(-21)
AP04-G3PDH F
Botrytis pseudocinerea 10091
AB03-M13F(-21)
GBG13-M13F(-21)
Botrytis fabae MUCL98
AP06-M13F(-21)
Botrytis pelargonii CBS497.50
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EL03 G3PDH CONSENS
HA08 G3PDH CONSENS
MS05-M13F(-21) copy
NP38-G3PDH F
SP05b-G3PDH F
GBG61-G3PDH F
GBG09-G3PDH F
NP29-G3PDH F
NP39-G3PDH F copy
PIO01-G3PDH F copy
PP11 G3PDH CONSENS
SP27 G3PDH
SP32-G3PDH F
Botrytis cinerea MUCL87
CC06-M13F(-21)
DL07-M13F(-21)
GO04-G3PDH F copy
HF07-G3PDH F copy
MS12-M13F(-21) copy
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SP28 G3PDH CONSENS
SP05-G3PDH F
PP08-G3PDH F
MS10-G3PDH F
HA14-G3PDH F copy
EL05-G3PDH F
CR06-G3PDH F copy
BP30-G3PDH F
BF08-G3PDH F
THF04-M13F(-21) copy
SP03-G3PDH F
NP22-G3PDH F
MS11-G3PDH F
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FH01-G3PDH F
SH03 G3PDH CONSENS
CC09-G3PDH F copy
Monilinia fructigena 9201
CC07-M13F(-21)
CC04-G3PDH F
Sclerotinia sclerotiorum 484
GBG20-G3PDH F
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