FINAL PROJECT REPORT

Project Title: Managing acclimation, hardiness and bacterial canker of sweet cherry

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Total Project Request: **Year 1:** $43,657 **Year 2:** $43,820 **Year 3:** $44,503

Other funding sources
None

Budget 1: Hubbard
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Item	2015	2016	2017	2018
Salaries[1]	19,750	20,343	20,953	
Benefits[2]	10,107	10,177	10,250	
Supplies[3]	8,500	8,000	8,000	
Travel[4]	1,800	1,800	1,800	
Miscellaneous [5]	3,300	3,300	3,300	
Plot Fees[6]	200	200	200	
Total	43,657	43,820	44,503	No-cost extension

Footnotes: [1]Salary is for graduate student (D. Hubbard) at 0.25FTE and postdoc at 0.2FTE. A 3% increase is factored into years 2 and 3; [2] Benefits are based on a graduate student static cost and the actuals of a postdoc rate; [3]supplies include lab consumables, nursery stock & supplies and several chest freezers and rates for microscopy lab use at OSU-Corvallis; [4]travel is for # trips to Corvallis at 0.565 cents per mile and travel to research plots in The Dalles; [5]shipping and nutrient analysis (factor $25/ship date for shipping fees and $12/sample x # of samples per date); [6]greenhouse space at 0.21 cents/sqft/mo and cold room space at 0.94 cents/sqft/mo

Objectives:

1. Examine the role of acclimation and induced early winter damage on infection by *Pseudomonas syringe* pv *syringe* (*Pss*) and subsequent bacterial canker formation.

2. Determine the location of epiphytic populations & infection points of *Pss* on sweet cherry tissues using microscopy techniques.

3. Evaluate commercial & experimental plant growth regulators for their ability to induce defoliation and increased cold hardiness.

4. Evaluate the effects of defoliating compounds on nutrient remobilization and tissue content during dormancy and early spring development.

Significant Findings:

Objective 1

- Regrowth of plant tissue subjected to varying freezing temperatures showed only a slight reduction in overall growth in inoculated treatments for both the natural and artificially acclimated plant tissue.
- Inoculation after the freeze event and having free water does appear to increase disease development as opposed to prior to.

Objective 3

- All defoliation treatments were efficient at abscising leaves, though only on a single sampling date was an increase in acclimation observed.
- A reduced rate of ABA applied multiple times gave compelling evidence of both enhancing remobilization of nutrients and defoliating trees several weeks ahead of the control.
- Inoculated can yard experiment yielded stunning results of defoliant treatments with presence of the pathogen. Most treatments did not yield the expected outcome, but the Lime Sulfur treatment appeared to be a complete success.

Objective 4

- Leaves showed significant Nitrogen remobilization from one of the treatments, while the rest had little to no time for sufficient reuptake
- Zinc and Boron showed rapid remobilization in all treatments, with the exception of lime sulfur which burned the leaves far too quickly in 2016, but ACC had similar effects in 2017.

Results & Discussion

Objective 1

2015: Gisela 6 rootstocks were received from North American Plants (NAP) in McMinnville, OR in late August. Plants were roughly 9 inches tall. These plants were segregated by the following acclimation treatments: 1) Naturally acclimated under ambient, outdoor conditions 2) Non-acclimated in a greenhouse (75^{o}F daytime and 60^{o}F nighttime) and 3) artificially induced to acclimate by exposing plants to low night time temperatures within a cold storage unit and moved outdoors during the day. After sufficient cooling was achieved, plants were again divided equally into inoculated & non-inoculated treatments. Inoculations were carried out prior to exposure to freezing. A suspension (3.1×10^{8}) of a local *Pss* isolate was applied to run-off and bagged immediately to maintain high humidity and held at ambient temperature (68^{o}F) for 24 hrs. Tissue washes conducted on plants after incubation showed an average recoverable *Pss* population of 1.4×10^{7}. Based on direct measurements, we identified sub-freezing temperatures that generated an increasing level of tissue injury until the kill points were reached for each of the three acclimation treatments (Table 1).

Table 1. Test temperatures to achieve similar freeze damage for each of three different acclimation levels (ºC)

	Acclimation level		
	Non-Acclimated	Artificially Acclimated	Naturally Acclimated
UTC	---	---	---
Temp 1	-2º	-4º	-4º
Temp 2	-6º	-8º	-8º
Temp 3	-8º	-15º	-13º
Temp 4	-12º	-17º	-15º

Freeze runs with rootstocks began daily on 1 November with 2 reps of each of the 30 treatments per day. To accommodate all treatment x replicate combinations required 4 days of freezing. We segregated the inoculated and non-inoculated populations between two identical programmable freeze chambers in order to minimize transfer of bacteria between treatments. The temperature was reduced at a rate of 1ºC per hour to better simulate natural freeze events. These plants were removed from the chambers after a minimum of 1 hr exposure to the designated temperatures. Once these plants were removed, they were held in isolated growth chambers at 60ºF until the 4 days of freezer runs were complete. These plants were then held in a walk-in cooler at 34ºF for one week before temperatures were reduced to 32ºF for the remainder of the winter. Plants were removed from the walk-in on 15-April and allowed to break bud and grow in a controlled climate greenhouse for the 2016 season. Plants were measured upon removal and then again when growth had terminated in September 2016.

Growth data showed a slight reduction in growth of inoculated compared to non-inoculated in both the natural & artificially acclimated tissues, but puzzlingly, not in the non-acclimated treatment (Figure 1). However, these data do not account for buds that did not break and grow shoots in the spring due to tissue death (which was markedly more pronounced as temperatures decreased). These data, therefore, will be re-analyzed to capture this effect. Additionally, disease symptoms did not appear to develop over the 2016 growing season, which requires modification of our inoculation protocol for fall 2016/winter 2017. We intended on conducing additional freeze runs during the month of November, but due to equipment malfunction, heat was lost in the greenhouse and all non-acclimated tissue was lost due to exposure to multiple days of low temperatures (i.e., acclimation).

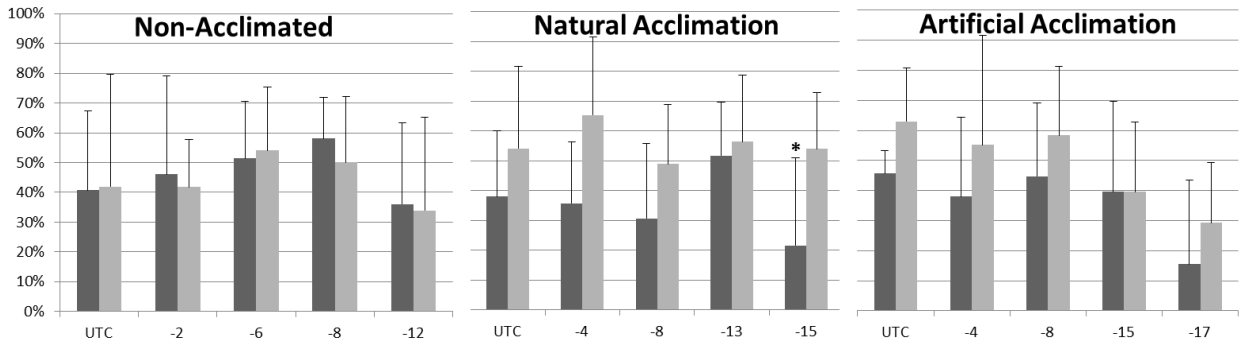

Figure 1. Percentage of relative regrowth of Gisela 6 rootstock of 3 levels of acclimation after being subjected to differing injurious temperatures. Dark bars represent inoculated treatments, while light bars are non-inoculated. Lines are top of bars are ± one standard deviation. * represents significance (P = 0.05)

2016: Mazzard rootstocks, rather than Gisela, from NAP in early September. These plants have not completed their growth for the season and are presently being hardened off. Several artificial freeze tests are planned for this season to evaluate the role of acclimation and non-acclimation on freeze injury with and without inoculation. Modifications to the inoculation procedure will also be tested this year. The freeze procedure will remain in place with additional temperature mapping of the freeze chamber to account of variability within the unit. Heating equipment in the Greenhouse has been restored to working

order and will be monitored to ensure that the environment remains controlled. This experiment was repeated 4 times over the course of 3 weeks.

Plants were frozen at temperatures noted prior, then inoculated following a 24hr incubation period, bagged for humidity in a growth chamber at 50ºF. These were held on a day/cycle for 96hrs, then placed into a cold storage room at 32ºF. During late winter, the defroster unit malfunctioned for some period of time (sending the cold room between 32ºF and 65ºF several times a day), desiccating the tissue to a point of mortality. Once this was discovered, plants were promptly removed and placed into the greenhouse to regrow, but the drought injury was severe enough, no meaningful data could be recovered.

2017: For fall 2017 experiments, Gisela rootstocks were acquired from NAP in August due to availability. These plants needed to be regrown after a severe powdery mildew outbreak immediately following arrival at MCAREC defoliated many of them. Once growth had terminated, they were graded and placed into either a natural acclimation state (a covered, unheated structure), a growth chamber to be acclimated systematically or left in the greenhouse at an ambient temperature of ~65ºF. This experiment was conducted twice on Gisela 6 and once Mazzard. Though we did not experience significant mortality at lower temperatures as we had hoped, we did begin to have effects at lower temperatures with significance being found at the lowest temperatures between inoculated and non-inoculated (Figure 2).

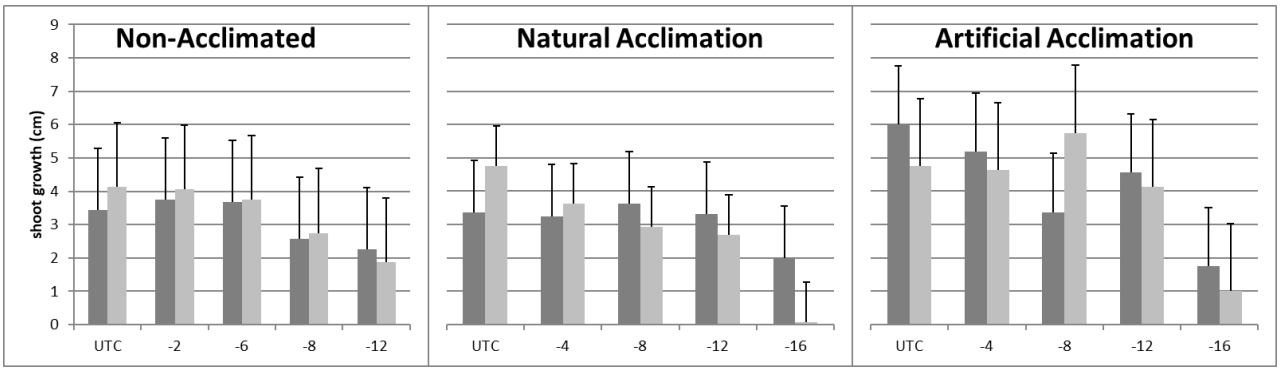

Figure 2. Growth of Mazzard rootstock of 3 levels of acclimation after being subjected to differing injurious temperatures. Dark bars represent non-inoculated treatments, while light bars are inoculated. Lines are top of bars are ± one standard deviation.

Objective 2

Due to the lack of disease development in plants from objective 1, tissue immersed in fixative was not assessed via SEM as locations of damage were unknown. This year, tissue, once hardened off, will be subjected to artificial freeze assays without inoculum and inspected with a light microscope to better understand locations & signs of damage. Once these locales are identified, tissue from these areas will be fixed and saved for SEM inspection over the winter of 2017. From what little disease symptoms did develop on 2015 tissue, it was far too general to isolate specific areas for microscopy. Similar results were seen in 2016, leading us to abandon this objective. Leftover funding will be returned to OSCC following the completion of existing objectives.

Objective 3

2015: In a grower collaborator orchard, treatments of elemental (lime sulfur) or commercial & experimental plant growth regulators (ABA and ACC) were evaluated for their ability to induce early defoliation and cold hardiness. Defoliation efficiency was examined objectively as the percentage of leaves to senesce and abscise (4 shoots per rep). ABA and lime sulfur applications were made on 7 October followed by ACC applications on 21 October. All PGR treatments included 0.1% Simulaid. Overall, all treatments significantly sped up the process of defoliation, whether it be by chemically burning leaves (Lime Sulfur) or seemingly, by increasing the rate of natural abscission (Table 3).

Table 3. Defoliation efficiency of chemical compounds over 6 weeks beginning 14 days post application in 2015

		Evaluation Date						
Treatments	Rate	10/20	10/27	11/3	11/12	11/15	11/23	12/1
UTC	---	4% a[#]	7% b	8% c	10% d	37% c	56% c	100% a
ABA	500ppm	22% a	41% a	52% b	59% c	74% b	85% b	100% a
ABA	1000ppm	23% a	64% a	71% ab	75% b	84% ab	96% ab	100% a
ABA then ACC	500ppm 500ppm	19% a	48% a	77% ab	87% ab	97% a	98% a	100% a
ABA then ACC	1000ppm 1000ppm	20% a	53% a	99% a	100% a	100% a	100% a	100% a
ACC	500ppm	8% a	10% a	78% ab	93% ab	100% a	100% a	100% a
Lime Sulfur	10% (v/v)	21% a	79% a	85% a	88% ab	92% a	92% a	100% a

Means within a column followed by the same letter do not differ significantly (P=0.05) based on significant difference

Flower buds of the aforementioned treatments were evaluated for their hardiness by differential thermal analysis (DTA). DTA detects freeze events (i.e., exotherms) that signify flower death. Buds were evaluated biweekly beginning prior to applications, at which time no exotherms were detectable (implying that flowers were not acclimated). Exotherms were observed 3 weeks after the initial applications. An increase in the number of exotherms was seen for all treatments with subsequent sampling dates (Table 4). Despite numerical differences in the percentage of kill points observed among treatments, high variation led to insignificant differences among treatments. The first frost event of the fall occurred 4 November.

Table 4. Percentage of recoverable flower exotherm peaks on a series of evaluation dates

		Evaluation Date			
Treatments	Rate	10/28/15	11/11/15	11/25/15	1/8/16
UTC	---	10.86%	11.51%	86.18%	100.00%
ABA	500ppm	18.21%	20.92%	89.67%	100.00%
ABA	1000ppm	37.50%	17.43%	88.16%	100.00%
ABA then ACC	500ppm 500ppm	29.89%	36.68%	94.02%	100.00%
ABA then ACC	1000ppm 1000ppm	39.02%	39.63%	100.00%	100.00%
ACC	500ppm	26.69%	55.83%	92.64%	100.00%
Lime Sulfur	10% (v/v)	57.41%	42.90%	84.57%	100.00%

2016: Beginning on 6 October, initial samples were taken with applications of ABA & Lime Sulfur subsequently following. Treatments were altered for 2016, including multiple applications of lowered rates of ABA to address the short-lived nature of the molecule *in vivo*. Shoots were marked and counted similar to 2015 (Table 5). Contrary to 2015's trial, floral peaks could be found on the initial DTA assays. This occurrence of peaks so much earlier than 2015 did not allow the testing of floral buds to confirm they are expiring in the mass ice nucleation mentioned prior. Following similar protocols as mentioned in 2015, spur samples were taken weekly and analyzed via DTA. Detection of peaks is represented similar to last year with some modifications of treatments (Table 6). Similar results to prior years regarding the high variation of detectable peaks and a relatively stable LT50 of buds across sampling dates, insignificant differences were found across treatments. Possible explanations for the stable LT50's could be due to a constant temperature between transportation and subsequent processing of buds prior to being placed in the freezer units.

Table 5. Defoliation efficiency of chemical compounds over 4 weeks beginning 12 days post application in 2016

		Evaluation Date								
Treatments	Rate	10/18	10/21	10/25	10/28	11/4	11/11	11/15	11/18	11/21
UTC	---	2% a[#]	3% a	3% a	4% a	6% a	18% a	47% a	71% a	96% a
ABA (x3)	500ppm	9% a	21% ab	28% ab	44% bc	55% b	87% b	97% b	100% b	100% a
ABA (x3)	250ppm	10% a	32% ab	45% b	63% c	73% bc	94% b	97% b	100% b	100% a
ABA then ACC	500ppm 1000ppm	5% a	51% a	75% c	85% d	86% b	91% b	98% b	98% b	96% a
ABA then ACC	1000ppm 1000ppm	20% a	67% b	73% c	89% d	92% b	98% b	100% b	100% b	100% a
ACC	1000ppm	5% a	8% a	23% a	57% b	68% bc	90% b	99% b	100% b	98% a
Lime Sulfur	10% (v/v)	50% b	58% c	71% c	81% d	82% b	88% b	93% b	94% b	97% a

Means within a column followed by the same letter do not differ significantly (*P*=0.05) based on significant difference

Table 6. Percentage of recoverable flower exotherm peaks on a series of evaluation dates

		Evaluation Date					
Treatments	Rate	10/06/16	10/14/16	10/20/16	10/27/16	11/03/16	11/18/16
UTC	---	48.15%	22.78%	100.00%	100.00%	94.44%	100.00%
ABA (x3)	500ppm	29.44%	54.07%	98.89%	100.00%	100.00%	100.00%
ABA (x3)	250ppm	27.41%	42.78%	100.00%	100.00%	91.11%	100.00%
ABA then ACC	500ppm 1000ppm	12.78%	50.00%	88.33%	100.00%	100.00%	100.00%
ABA then ACC	1000ppm 1000ppm	43.33%	51.11%	100.00%	96.11%	100.00%	100.00%
ACC	1000ppm	37.22%	30.00%	94.44%	100.00%	85.00%	100.00%
Lime Sulfur	10% (v/v)	43.33%	52.22%	87.22%	89.44%	87.78%	100.00%

2017: Following promising results from the nutrient remobilization and defoliation data of 2016, treatments were altered for the current year. Treatments were replicated in 2 more cherry blocks and in a potted tree experiment, all 3 of which are in the Hood River Valley, in addition to the mature 'sweetheart' block used in The Dalles, OR. Results from this trial are in table 7.

Initial sampling made 28 September in the Dalles trial and no peaks were present. The following sampling date, 5 October, did have peaks present. The following day, buds from the same orchard were run again with the experiment ending shortly before peaks had been seen the day prior. These buds were dissected and visually inspected for ovary mortality and it was found ~95% of these were dead following the mass ice nucleation seen, answering the question of where the "invisible peaks" were in prior experiments.

For the defoliation trial, rates were similar to what had been seen before. Lime Sulfur began defoliation first but slowed once a period of time had been met. Treatments containing ACC were rapid once applications were made, although slightly faster if ABA had been applied prior to. ABA treatments did increase defoliation and were rate dependent. As seen in prior years, the number of recoverable exotherm peaks were insignificant across treatments with no differences in either the number or LT50 of the buds.

Table 7. Defoliation efficiency of chemical compounds over 4 weeks beginning 10 days post application in 2017

		Evaluation Date								
Treatments	Rate	10/10	10/17	10/24	10/31	11/3	11/7	11/14	11/21	11/28
UTC	---	3%	3% a[#]	4% a	5% a	30% a	39% a	48% a	82% a	100%
ABA (x3)	500ppm	3%	2% a	32% c	66% c	94% c	99% c	99% b	99% b	100%
ABA (x3)	250ppm	4%	2% a	16% b	39% b	68% b	71% b	79% b	93% b	100%
ABA then ACC	500ppm 1000ppm	3%	13% ab	93% e	96% d	100% c	100% c	100% b	100% b	100%
ACC	1000ppm	3%	3% a	4% a	95% d	100% c	100% c	100% b	100% b	100%
Lime Sulfur	10% (v/v)	5%	18% b	62% d	74% c	84% c	94% c	96% b	96% b	100%

Means within a column followed by the same letter do not differ significantly (*P*=0.05) based on significant difference

Two commercial 'sweetheart' cherry blocks roughly 4 miles SE of Odell, OR were chosen based on late harvest dates and age of blocks, one is young, but of bearing age and the other is still immature and vegetative. The 2 commercial blocks will be evaluated for potential winter damage from freeze events next spring and evaluation of *Pss* symptoms. Results from these experiments were inconclusive as little to no winter injury or Pss symptoms developed

The young potted 'Bing' trees are also being used. Treatments on the three new locations are Multiple applications of 250ppm ABA, 1000ppm ACC and a solution of 10% Lime Sulfur. The young potted trees also received these treatments in addition to 2 chemical treatments (LMA – 2% and Oxytetracycline – 200ppm + buffer). for *Pss* 2 days before and 2 days after inoculation occurred on November 7th, 2017. The potted tree experiment shows promise.

Table 7. Efficacy of chemical compounds for control of Pss on sweet cherry

			Shoots	Length (cm)	
Treatment	Rate	% dead	Total #	Total	Individual
UTC	---	25% a	4.9 abc[#]	49.7 ab	7.9 b
ABA	250ppm	63% c	3.0 ab	31.7 ab	4.5 a
ACC	1000ppm	38% b	4.6 abc	36.1 ab	4.9 ab
Oxytetracyline	200ppm	69% c	2.4 a	20.7 a	2.6 a
LMA	2% (w/w)	19% a	5.7 b	99.2 b	13.5 b
Lime Sulfur	10% (v/v)	13% a	7.6 ac	257.6 c	32.0 c

Means within a column followed by the same letter do not differ significantly (*P*=0.05) based on significant difference

Objective 4

2015: Tissues were dissected into leaf, bud & spur as sampling occurred. These tissues have been sent to the lab for analysis following the decision to continue with the project, but due to a long queue, results were not completed over the course of a year. After results were returned, discrepancies in the data had us resubmit samples that had been withheld. Given the multiple years of this study, we do not expect for variation from the 2 subsequent years.

2016: As stated above, all tissue was dissected into separate parts and being dried in ovens to eliminate excess water. The tissue from 2016 were submitted and returned and subsequently analyzed. Remobilization charts were built to understand the rate of remobilization. Nitrogen appears to be the most limiting mineral for remobilization as it appears to be a slow process (Figures 1). Although the actual percentage removed from the leaves appears to be large, the actual nitrogen found in bud & spur tissue

appears to be similar, except for the lime sulfur treatment which, again, burned the foliage far too quickly to leave much viable tissue intact. The Zinc and Boron appeared to remobilize quickly once signaled from PGR treatments.

2017: Tissues were processed as in prior studies. As seen in 2016, Nitrogen remobilization appears to be the slowest (Figures 2). Upon analysis, we did not detect any significant differences for final concentrations of minerals in buds or spurs once dormancy was achieved.

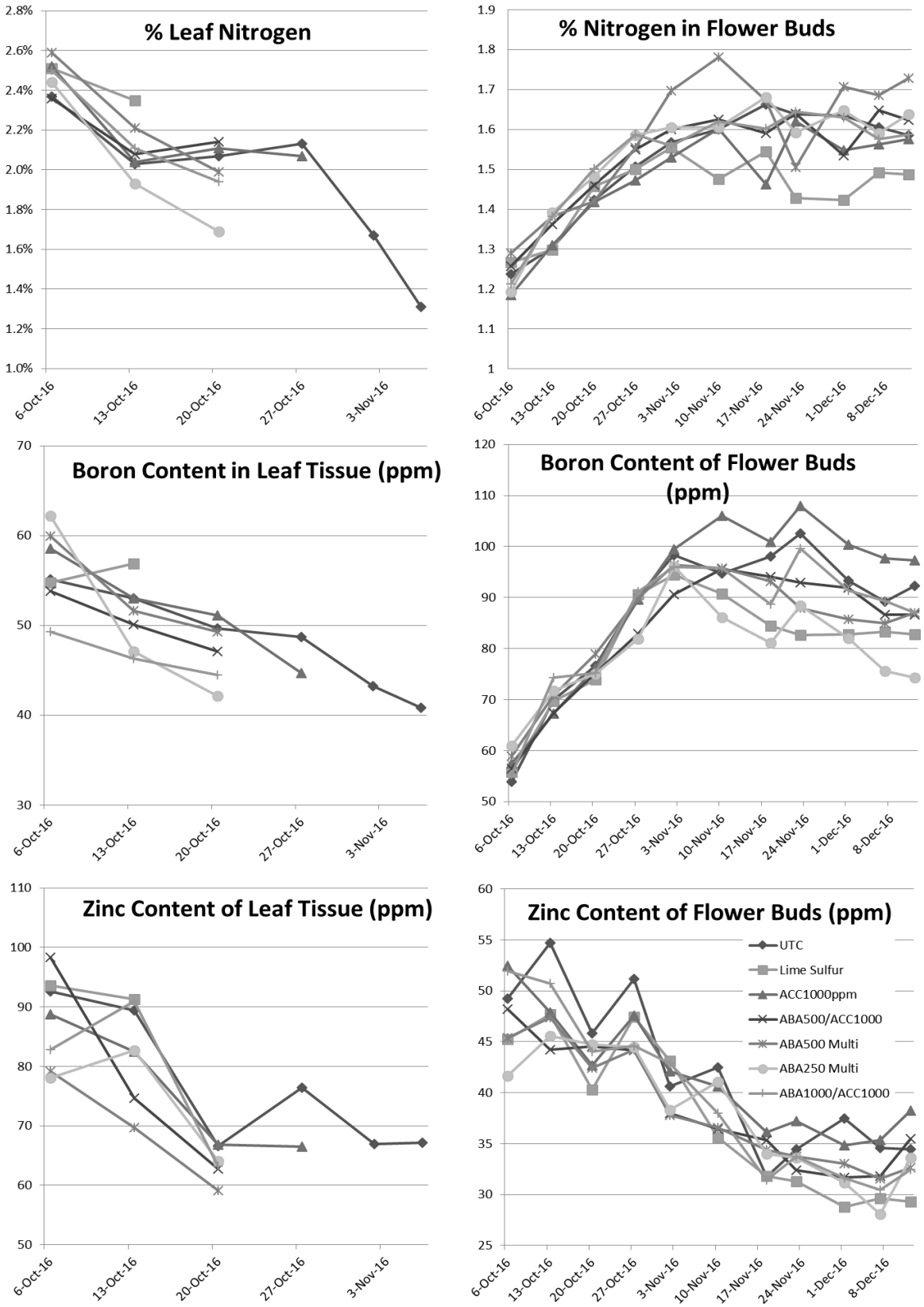

Figure 1. Nutrient levels in various 'Sweetheart' Sweet Cherry tissues from Fall 2016 (from left to right). Percent leaf Nitrogen. Percent Nitrogen content in flower buds. Leaf Boron content in leaf tissue in parts per million (ppm). Boron content (ppm) in flower buds. Leaf ZInc content (ppm) in leaf tissue. Zinc content (ppm) in flower buds. Treatment key in bottom left figure

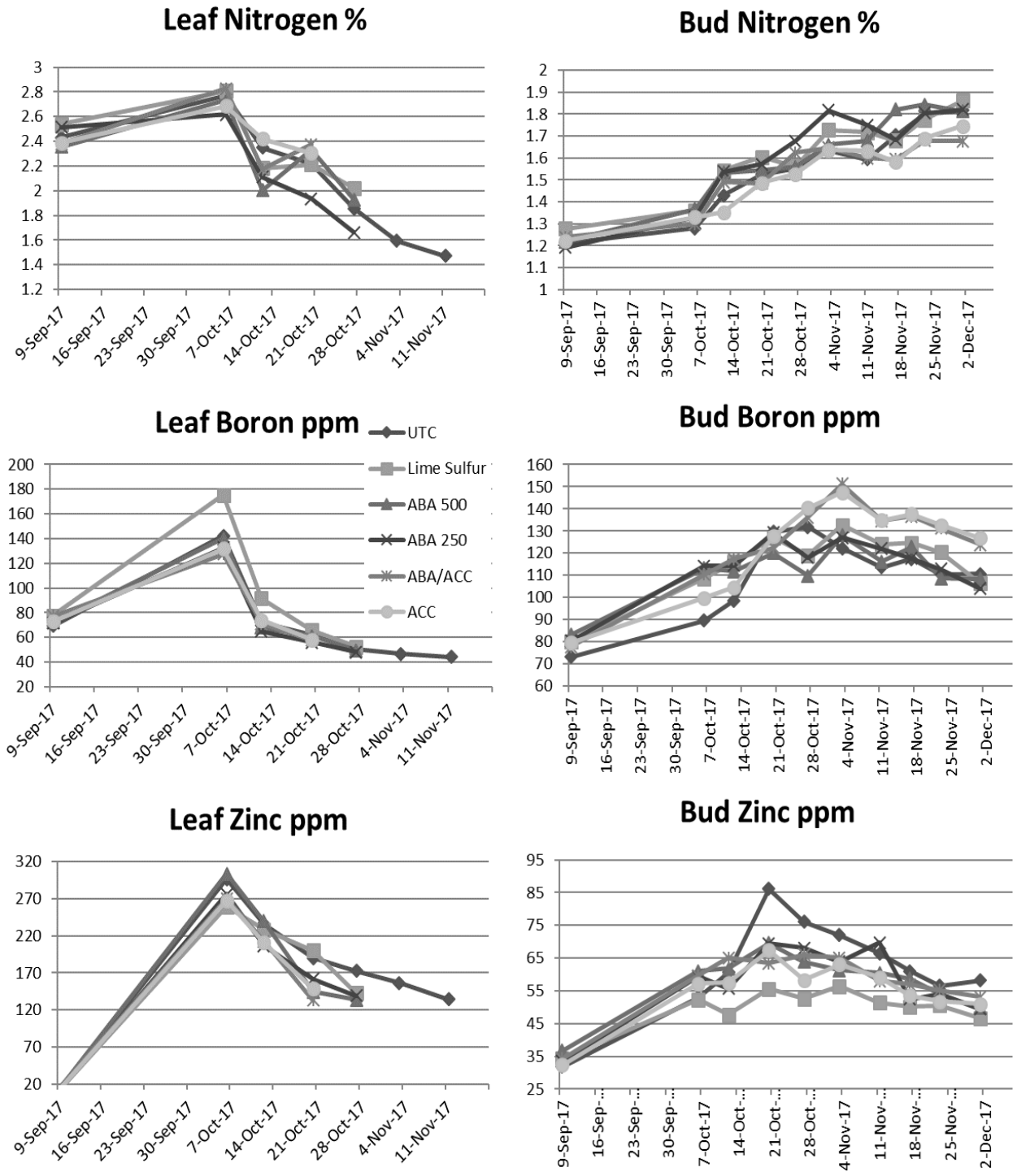

Figure 2. Nutrient levels in various 'Sweetheart' Sweet Cherry tissues from Fall 2017 (from left to right). Percent leaf Nitrogen. Percent Nitrogen content in flower buds. Leaf Boron content in leaf tissue in parts per million (ppm). Boron content (ppm) in flower buds. Leaf Zinc content (ppm) in leaf tissue. Zinc content (ppm) in flower buds. Treatment key in figure

Executive Summary

Objective 1) Development of our "model" system was wrought with problems, not all stemming from the experiment itself, but subsequent handling of plant material and inoculation protocols. These experiments are being conducted once more this year (no funding requested), so that we may better explain the variation seen across the experiments. Due to the challenge of working with *Pss*, these results were not surprising

Objective 3) These defoliation experiments should us a great deal in terms of effects from various compounds that had yet to be tested. We found ABA can induce something similar to natural defoliation and an slight increase in mineral remobilization. ACC shows great promise in future endeavors, though this compound has only recently begun to be studied in applied settings, future studies within plant science will develop it further. We did not find, however, any benefit in terms of gained cold hardiness from any one treatment. While this is discouraging, these data are only valid when using DTA to determine bud hardiness. Overall plant hardiness may still show a benefit.

The larger commercial plots, without bud sampling, showed there was no detriment to crop set the following year, but plots were harvested before samples could be taken for quality & mineral analysis. These experiments are being replicated this year, to answer that question (no funding requested). We also did not see any significant results from natural inoculation or winter damage in these plots (data not shown).

The small potted tree experiment did yield some startling results. Lime Sulfur appeared to have an, overall, positive effect on overall tree health & survival. Trees from all treatments had variation, but largely all grew very little the subsequent year and most had small, distorted foliage if they leafed out at all. LMA shows some promise, although the compound is neither available for commercial agriculture and it's residual for minimal applications will be another challenge going forward. The PGRs showed little in the way of a solution for bacterial canker, while ACC performed similar to the inoculated only treatment, the multiple applications of ABA showed an increased amount of infection and subsequent mortality. This, in combination with DTA data from the commercial experiments may show a brief lose in hardiness following an application, which disagrees with prior literature. This may be why mortality was so high. The treatment of Oxytetracycline is also troubling, as it had the highest mortality of the treatments. This could be for multiple reasons, but in prior experiments conducted in the Pacific Northwest it had performed well. We've recently discovered strains of the pathogen that are tolerant, if not resistant to the compound in the Mid-Columbia region, although not in the Hood River Valley. This may no longer be the case. Samples have been given to OSU in order to determine if this was the case. This experiment is also being replicated again, but in Corvallis, to corroborate the data seen from the prior year (no funding requested).

Objective 4) Nutrient remobilization has only been studied in sweet cherry, primarily for Nitrogen. These studies show us the movement of other minerals, such as Zinc & Boron moving from leaf into bud & spur tissue. We saw a saturation effected for these minerals in various tissues. This should aid in validating Fall applications of other minerals outside of Nitrogen.