Project Title: Increased Sampling for the PNW Sweet Cherry Bud Phenology and Cold Hardiness Model

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Report Type: Final Project Report

Project Duration: 1-Year

Total Project Request for Year 1 Funding: $33,398

Budget 1
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Item	Type year of project start date here
Salaries[1]	$13,408
Benefits	$8,424
Wages	$0
Benefits	$0
Equipment	$0

Supplies	$680
Travel[2]	$504
Miscellaneous	$0
Plot Fees	$0
Total	$23,016

Footnotes:
[1] Salary and Benefits include 0.20 FTE Bio Sci Research Tech.
[2] Travel budgeted for travel to field sites for sampling and phenology assessment.

Budget 2
Co PI 2: Clark Kogan
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Contract Administrator: Katy Roberts
Telephone: 509-335-2885
Contract administrator email address: ARCGrants@wsu.edu

Item	Type year of project start date here
Salaries	$7,663
Benefits	$2,319
Wages	$0
Benefits	$0
Equipment	$0
Supplies	$0
Travel	$0
Miscellaneous	$400
Plot Fees	$0
Total	$10,382

Footnotes:
[1] Salary and Benefits include statistician time provided by the WSU Center for Interdisciplinary Statistical Education and Research.
[2] Miscellaneous includes facilitation of weather data and processing by AWN.

Recap of Original Objectives

The main purpose of this project was to increase the amount of cold hardiness data collected in the 2020-2021 season. Additional data, particularly from OR, was deemed critically important once the analysis of all previously collected data began to indicate that it was unreliable, and it became apparent that the model would be constructed using the 2020-2021 data, only being collected in WA. A second goal of this project was to directly compare the two methods that had been used for determining lethal temperature (LT); a differential thermal analysis (DTA) method that had been used for previous data, and a “freeze and dissect method” (F&D), that was being used for 2020-2021. The goal of this comparison was to 1 – potentially understand discrepancies in the previous data, and 2 – determine which method would be the best to use in any future data collections. Specific areas of interest included how well predicted LTs from both methods matched, whether one method had greater error in its prediction, and whether high temperature exotherms (HTEs) could be used to estimate LT, as has been indicated in recent publications.

Objective 1. Increase the amount of lethal temperature and relative water content data collected in spring 2021, in order to support the completion of an accurate model this year.

Objective 2. Compare the lethal temperature results obtained from the Differential Thermal Analysis (DTA) method and the traditional freezing/cutting method.

Significant Findings

Objective 1
Completion = 100%

- Data collected from Sweetheart, Chelan, Bing (The Dalles, OR) and Regina (Hood River, OR and The Dalles, OR) was combined with WA data to construct cold hardiness models.

- When plotted against growing degree days (GDD), OR and WA data was tightly correlated, supporting the legitimacy of the methods used.

Objective 2
Completion = 100%

- DTA and F&D methods showed strong correlation (.97 for LT25, 0.96 for LT50, 0.97 for LT25) overall.

- Mean absolute difference between DTA and F&D methods is 1.08°C (1.94°F)

- DTA data reliability degrades (error increases) as the season progresses, to a greater degree than F&D data.

- Buds appear to begin to lose the ability to super cool as early as stage 1.

- High temperature exotherms (HTEs), do not appear to be useful for calculating LT.

Methods

Objective 1: Increase the amount of lethal temperature and relative water content data collected in spring 2021, in order to support the completion of an accurate model this year.

The four cultivars currently being collected (Sweetheart, Regina, Chelan and Bing) in Washington or Hood River will also be sampled in The Dalles, OR, starting at the beginning of February and extending through the end of April. Collections will be made once a week to thoroughly cover the transition from dormancy to full bloom.

The traditional freezing and dissecting method will be used to determine lethal temperatures, in an identical manner as the data currently being collected in Prosser, WA and Hood River, OR. Spurs from randomly selected shoots on trees of similar age and rootstock will be collected from cooperating growers in The Dalles, OR, in locations with proximity to AgWeatherNet (AWN) stations. Forty spurs will be frozen at decreasing 1^{o} C increments using the Tenney Temperature Cycling Test Chamber at MCAREC, to a temperature low enough to guarantee 100% flower death. Four spurs will be removed in each of the last ten increments in the freeze series, and allowed to thaw slowly in the refrigerator (4^{o} C) for a minimum of one hour. They will then be incubated at 21^{o} C for 24 hours to allow enzymatic activity to result in oxidative browning. Two to three buds from each cluster, for a total of ten buds from each temperature, will be dissected to determine individual flower mortality within buds. An additional 25 unfrozen buds will be dissected to assess the level of field mortality present before our experiments and will be used to adjust mortality rates. Every bud will have date, site, phenology, and flower mortality documented.

In addition to phenology assessments made by visual inspection, relative water content will be measured for all collections. This will help identify when the buds leave dormancy and begin to develop, even if visual clues (e.g. bud swelling) are not apparent. Fifty buds will be weighed fresh, dried for four days at 130° C and then weighed again to calculate water content.

This data will be combined with weather data from the closest AWN stations and used to construct statistical models to estimate sweet cherry bud phenology and predict related lethal temperatures

Objective 2: Compare the lethal temperature results obtained from the Differential Thermal Analysis (DTA) method and the traditional freezing/cutting method.

The sweet cherry bud phenology and cold hardiness model will utilize two types of lethal temperature data: current measurements collected by the traditional method of freezing and dissecting buds, as well as past measurements made by differential thermal analysis (DTA). Both of these methods are used widely to determine lethal temperature of floral buds in many species, and each has its own set of disadvantages. The traditional dissecting method is time consuming and labor-intensive, requiring at least three consecutive days of set-up, sample removal, and dissection/data collection. The DTA method in comparison is much easier, requires only one day of set up, and can use a much larger sample size. However, the DTA method has long been considered inappropriate in certain species or bud stages that do not supercool. In sweet cherries, the ability to supercool is thought to be lost by first swell, and so DTA data collected in previous years for the Cherry Cold Hardiness Model has not been used past this stage.

In recent years, some research on fruit tree cold hardiness has indicated that the DTA method is actually a viable method in older, more developed buds and even open flowers, if the single exothermic peak produced upon freezing is used to determine the floral death point. If this could be shown to be true in sweet cherry, it would allow a larger amount of previously-collected data to be used in the model. Perhaps more importantly, it would justify the use of this more efficient method in any future data collection as the model is validated and expanded for other cultivars and locations.

In order to compare these two methods, DTA will be performed simultaneously alongside the freeze/dissection run. For each cultivar, 60 buds will be removed from 20 spurs collected from the same trees and placed in four modules on two DTA plates. These will be run at the same time, in an identical Temperature Cycling Test Chamber, using the same freeze protocol as in Objective 1. At the end of the run, DTA data will be gathered and statistically analyzed to determine whether it correlates to the lethal temperature data gathered by the freeze/dissection method throughout development.

Results and Discussion

Both objectives of this project were accomplished in 2021. We collected F&D data from three cultivars (Sweetheart, Chelan, Bing) from The Dalles, OR, and one cultivar (Regina) from Hood River and The Dalles, OR, starting at the beginning of February and extending throughout full bloom in April. These collections were combined with the data from the three cultivars (Sweetheart, Chelan, Bing) gathered in Prosser, WA, giving us two datasets for each. This data was used for model construction (see final report entitled *Modeling PNW sweet cherry bud phenology and cold hardiness* for more details regarding the models).

In addition to gathering additional datasets using the F&D method, we also simultaneously ran DTA analyses on samples collected at the same time, from the same trees. This allowed us to directly compare the outputs of both methods. In previous years, raw DTA data gathered at MCAREC was processed in excel in order to determine low temperature exotherms (LTEs) and high temperature exotherms (HTEs). LTEs are designated as voltage peaks that occur when water inside a floral initial that is capable of super-cooling freezes. HTE voltage peaks occur when water outside of the floral initial

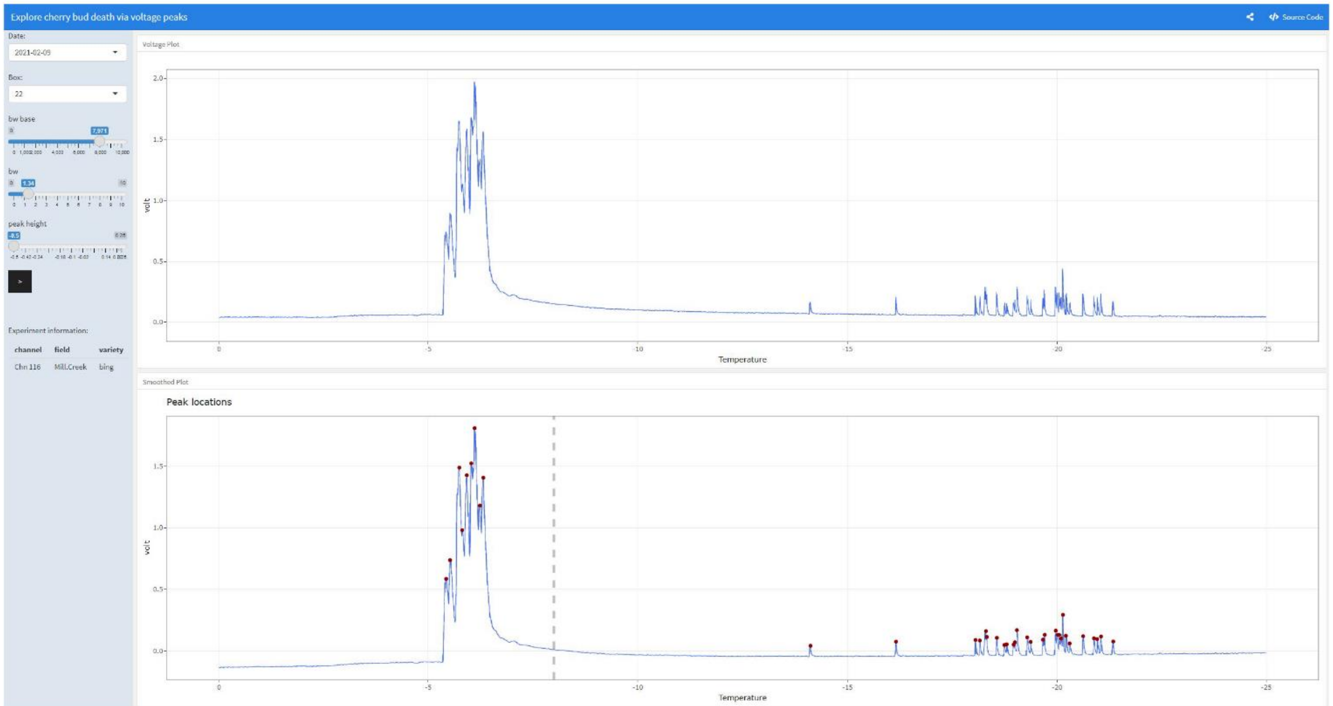

Figure 1. Example of the R program designed to call LTE and HTE peaks, for one sample of Bing collected on February 9th, 2021. Top panel shows the smoothed voltage curve. Bottom panel shows the peaks that are designated either LTEs (lower than -8°C (17.6°F)) or HTEs (higher than -8°C). User inputs include sliders to adjust the degree to which the curve is smoothed and the threshold for voltage height that is considered a peak.

freezes. The previous excel program performed a number of functions, including LTE and HTE peak calling, but it had a complicated user interface and the calculations it used were complex and in some cases, too cryptic for us to be confident in the results. For this reason, we developed a new, R-based GUI with adjustable inputs to take raw voltage data, apply baseline subtraction, smoothing and peak detection, and extract values for LTEs and HTEs (Fig. 1).

One interesting pattern we noticed when collecting LTE peak data in this way, was the surprisingly early shift out of super-cooling (Fig. 2., Fig 3.), indicating that the buds were leaving dormancy and initiating development earlier than we had anticipated. This was evident in both a decrease in the number of LTEs we obtained (Fig. 2) and in shifts in the distribution of the LTEs (Fig. 3). While this phenomenon has been reported to occur in early March[1,2], it occurred in our collections prior to any obvious changes in outward bud appearance. For example, on February 23rd, Sweethearts were at stage 1=20% and 2=80%, while all other cultivars were at 100% stage 1, but all cultivars show some evidence of leaving dormancy.

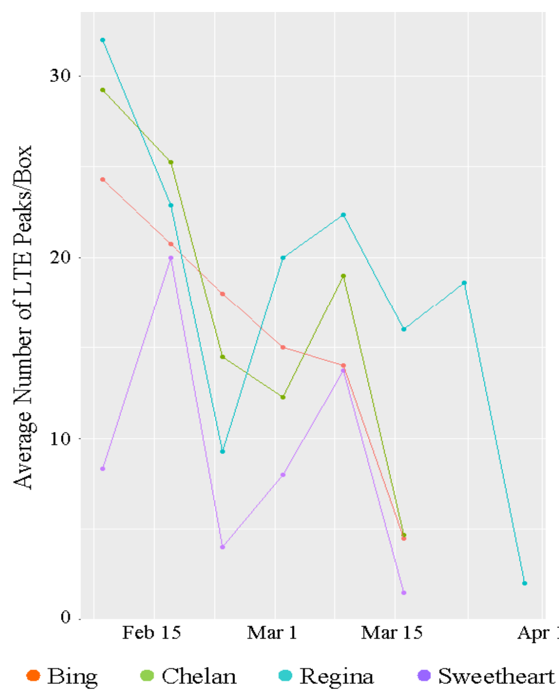

Figure 2. Average number of LTEs obtained from each sample for each cultivar throughout the season. Averages decrease as floral initials leave dormancy because they do not produce an LTE upon freezing. Apparent increase in mid-March are possibly caused by re-acclimation following cold temperatures at that time.

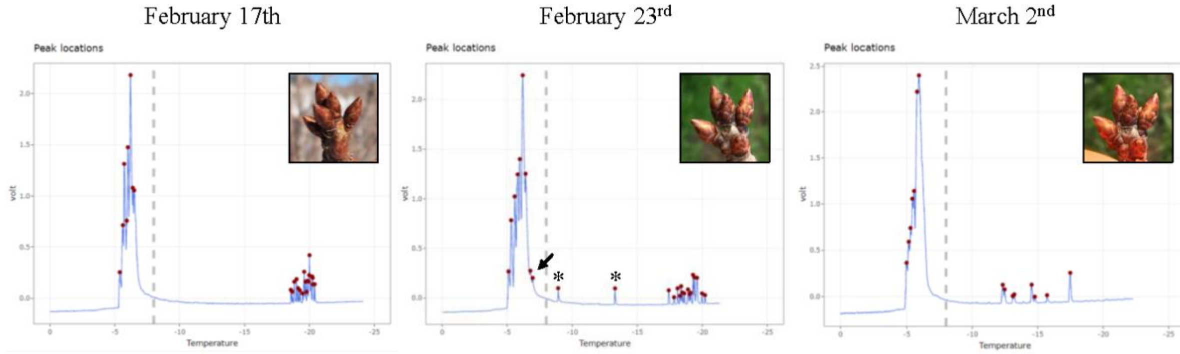

Figure 3. Comparison of LTE peak distribution on three dates, for Bing. On February 17th, LTEs are tightly clustered at a low temperature (~ -20°C (-4°F)), by February 23rd, some LTEs are shifted to higher temperature (asterisks), and are beginning to combine with HTE peaks (arrow). This indicates a general progression out of dormancy. By March 2nd, there are far fewer LTEs, indicating that many buds have already lost the ability to super-cool, even though bud phenology stage appears to be in between 0-1.

Figure 4. Graphs showing estimated LT25, LT50 and LT75 values for each method, for each cultivar collected in OR. DTA = orange, F&D = blue. Error bars = 95% confidence intervals. The absence of confidence intervals for DTA indicate that only a single box had usable data. DTA and F&D collections occurred on the same day, data points are slightly offset for better visibility. p-values associated with the comparison of mean LT between methods are listed above each date. Given the assumptions for both methods hold, small p-values can suggest a lack of correspondence between DTA and F&D. However, small p-values do not necessarily indicate that the discrepancy between methods is large, only that it is less plausibly zero. LT values for the last three dates (four for Chelan) are absent for DTA, as LTEs were no longer present in any of the voltage graphs as all buds had lost the ability to super-cool.

In order to compare the performance of the DTA and F&D methods, average lethal temperature values and their respective errors were calculated for each method. For the F&D method, average LTs were estimated by logistic regression and confidence intervals were obtained with cluster bootstrapping on the spur level using the quantile method. For the DTA method, average LTs were estimated by extracting 0.25, 0.5 and 0.75 quantiles for each box and then taking the mean across boxes. Confidence intervals were obtained assuming normality of the quantiles across boxes. Average LT25, LT50 and LT75 for each method were then plotted by date (Fig. 4), and correlation between the LT values was calculated across dates and cultivars. Overall, we observed a strong correlation between the average LTs estimated by each method (0.97 for LT25, 0.96 for LT50, 0.97 for LT25) (Fig. 4). However, it was impossible to obtain LTs for the DTA method past the end of March, because there were no longer any LTEs being produced. We also noticed an apparent increase in the error surrounding the DTA-predicted average LTs as the season progressed, which was in large, a result of a reduction in the number of boxes with any LTEs near the beginning of April. When error is averaged for each method and compared, this general pattern holds true (Fig. 5).

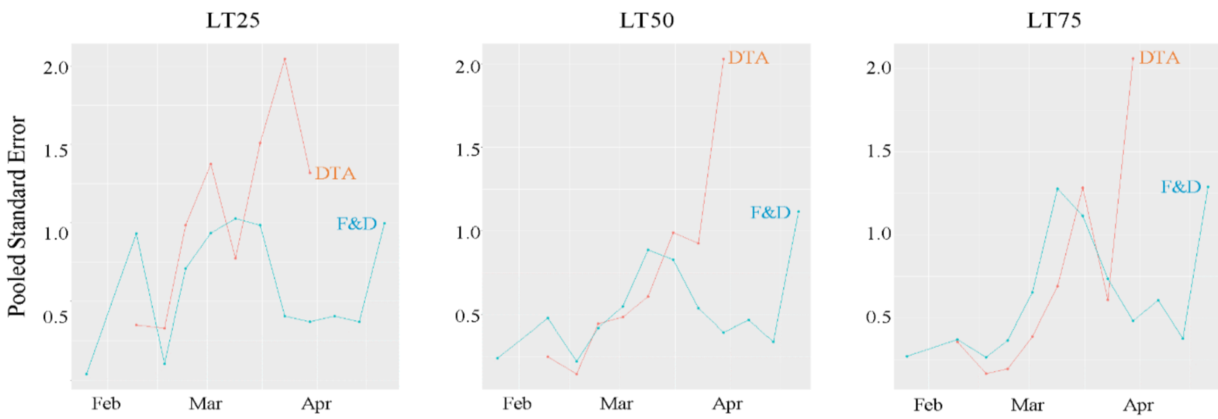

Figure 5. Average standard error values for all cultivars, for each method, compared throughout the season. DTA = orange, F&D = blue.

One additional important question that we wished to answer with this data analysis was whether HTEs could be used, later in the season, to estimate LT. This has been proposed recently for a number of stone fruits, including sweet cherry[3–5], with the premise that once floral initials lose the ability to supercool, all water within a bud sample freezes at the same time, which simultaneously kills the bud. We hypothesized that if we could show that LTs predicted from HTEs correlate well with the F&D method, that it would be possible to use DTA throughout the entire season. However, we did not find a strong correlation ($\rho_{25} = 0.13$, $\rho_{50} = 0.19$, $\rho_{75} = 0.28$) between LTs generated from the HTEs and LTs generated from the F&D method on the last three dates of sample collection (Fig. 6). Furthermore, we noted large mean absolute errors between the methods ($MAE_{25} = 2.22$°C, $MAE_{50} = 3.27$°C, $MAE_{75} = 4.29$°C). This indicates that this will not be a useful method for later collections in the future.

Conclusions

Overall, this project supported the ultimate goal of the development of a sweet cherry cold hardiness model in a number of ways. The additional five datasets we collected in Oregon made it possible to construct models for Sweetheart, Regina, Chelan and Bing. In comparing the performance of the two methods, we found a large amount of congruity in the predicted LTs overall, which was reassuring that both methods are legitimate and capable of providing quality data. We were surprised to see evidence of buds leaving dormancy so early – within bud stage 1 and 2. This observation confirms the idea that a large amount of development is occurring inside the bud, without visible changes to the outside, supporting the value of a model based on GDD and not strictly on bud phenology. We did not observe a strong correlation between HTE-derived LTs and F&D-derived LTs, which discourages us from using this method later in the season. All-together, the early loss of LTEs in the DTA method, the increasing error throughout the season for DTA, and the fact that it remains unusable in later stages, confirms our plans to use the F&D method for any and all future data collection.

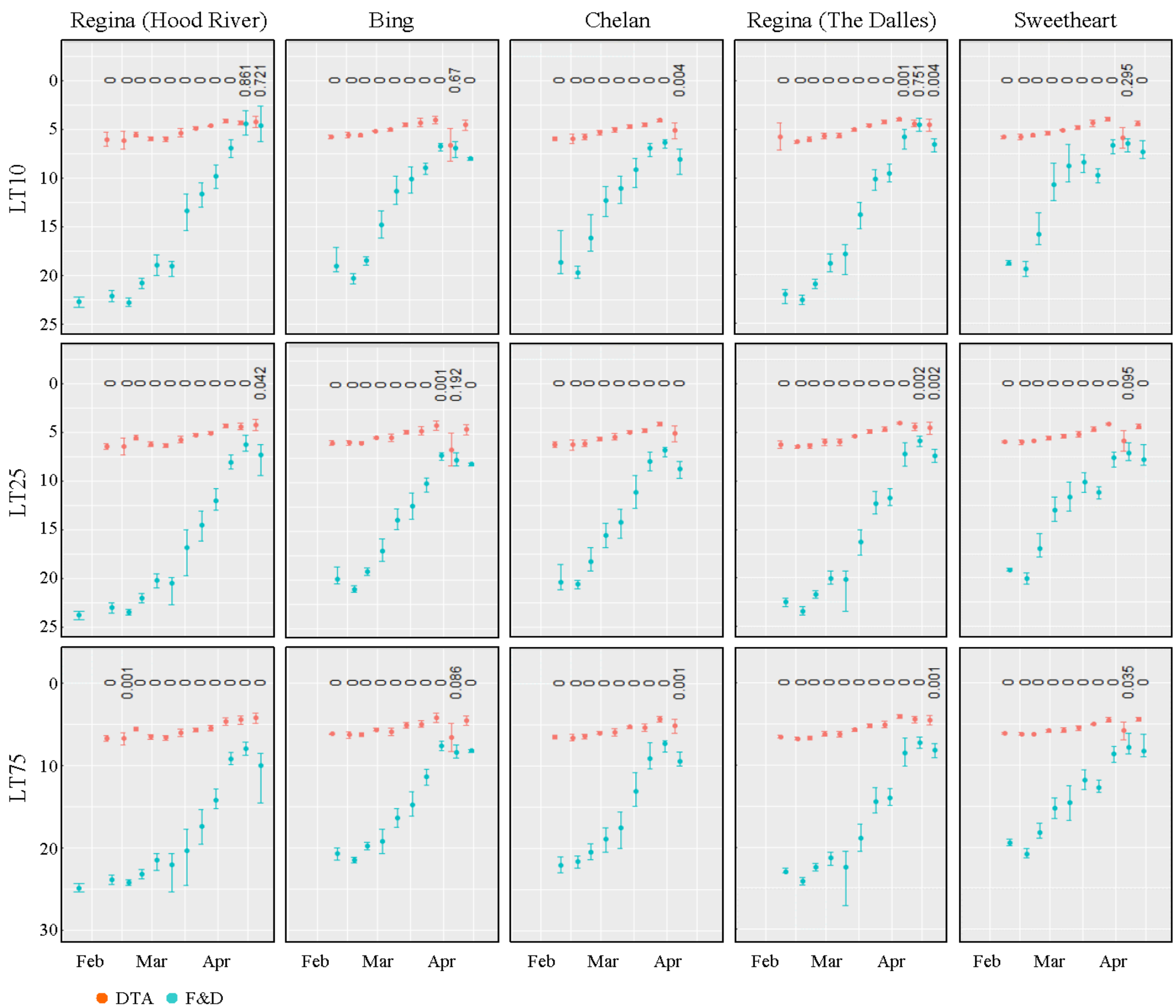

Figure 6. Graphs showing estimated LT25, LT50 and LT75 values for each method, for each cultivar collected in OR, except LTs for the DTA method are calculated from HTEs instead of LTEs. DTA = orange, F&D = blue. Error bars = 95% confidence intervals. The absence of confidence intervals for DTA indicate that only a single box had usable data. DTA and F&D collections occurred on the same day, data points are slightly offset for better visibility. p-values associated with the comparison of mean LT between methods are listed above each date. Given the assumptions for both methods hold, small p-values can suggest a lack of correspondence between DTA and F&D. However, small p-values do not necessarily indicate that the discrepancy between methods is large, only that it is less plausibly zero.

Executive Summary

Project Title: Increased Sampling for the PNW Sweet Cherry Bud Phenology and Cold Hardiness Model.

Key Words: *Prunus avium*, cold hardiness, model, dormancy, differential thermal analysis, DTA, frost, HTE, LTE.

Abstract:

In order to provide a weather-related decision-support tool to guide cherry growers in their response to cold weather events, we aim to develop a sweet cherry cold hardiness model capable of predicting lethal temperature (LT) based on growing degree days (GDD). This project was a sub-element of that larger goal, with two major objectives: increasing the amount of sampling done in the 2020-2021 season and directly comparing the two methods that had been used to determine LT. We successfully increased the sampling, from three datasets to eight, which were then used for model construction. These datasets showed high correlation across both states (OR and WA) when plotted against GDD. In comparing the two methods, we determined that differential thermal analysis (DTA) method and the freezing and dissecting (F&D) method had high correlation overall. However, we also determined that DTA error increases to a greater degree during the spring season, compared to F&D. This is likely related to the buds beginning to lose their ability to supercool in stages 1 and 2. When we tested the potential of using HTEs to estimate LTs late in the season, we did not see a strong enough correlation to the F&D-derived LTs to warrant using DTA in this way. Overall, we determined that for these reasons, F&D will be the best method moving forward as we gather additional cold hardiness data.