AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2021– 2023

TITLE: Developing molecular markers to determine dormancy status in Pacific Northwest cherry cultivars.

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COOPERATORS: N/A

SUMMARY/ABSTRACT:

Bud dormancy is an adaptation in perennial trees that allows floral buds initiated in summer to survive cold winter conditions, then successfully bloom the following spring. Dormancy is separated into three major stages (Lang et al., 1987). Paradormancy occurs in the late summer and fall, and is characterized by dormant buds that are kept inactive by apical dominance - signals sent by the growing tips of the branches. The next stage, endodormancy, is controlled by endogenous signals within the buds that are present until the tree meets its chill requirement, or the number of cold-but-not-freezing hours that is species and cultivar-specific. Endodormant buds are characterized by low water content, and by the fact that they will not "break" (swell and then bloom), even if the environment becomes favorable for flowering. When chill requirements are met, buds go through "dormancy release" and enter a phase of ecodormancy. In this phase, buds are no longer being internally repressed, and instead development is halted by environmental cues. As winter advances into spring, buds leave ecodormancy, reinitiate development and begin to swell, eventually leading to bloom.

Sweet cherry (*Prunus avium* L.) is a perennial tree fruit species of great economic importance in the state of OR as well as throughout the Pacific Northwest region, with 90% of the >$90 million dollar US sweet cherry production centered in WA, OR and CA (*Cherries | Agricultural Marketing Resource Center*). Understanding the current dormancy stage of a tree is an important element in many types of cherry research, including determining chill requirements for specific cultivars, understanding the physiological effects of cold damage, and analyzing the effects of climate change on cherry production. These types of studies are almost always geared directly towards helping cherry growers make informed decisions regarding the planning and care of their orchards.

Determining the dormancy status of cherry buds is usually accomplished with a combination of methods, including dissecting and making developmental observations, measuring moisture content, and forcing branches collected and maintained in spring-like temperature, light and humidity conditions until bud break can be assessed. All of these methods, however, are very time, labor and space intensive and often need to be done in combination to ensure accuracy. A promising possible improvement on these methods is the use of molecular markers to determine dormancy stage. Such molecular markers are often genes that have a very specific expression patterns, usually correlating with a developmental

stage or trait. By analyzing gene expression the stage or trait can be known. There are a number of recent publications which identify genes with expression patterns that vary based on bud dormancy status. Yet, little research has been done to analyze these genes as molecular markers for cherry dormancy, and thus far has almost exclusively been done with cultivars that are not grown in the Pacific Northwest (Vimont et al., 2019; Wang et al., 2020). I propose to identify promising molecular markers from gene expression data reported in recent publications and characterize the ability of each to determine bud dormancy in two Pacific Northwest cultivars, 'Bing' and 'Lapins.'

OBJECTIVES:

Objective One: Identify and test the accuracy and efficiency of potential molecular markers for use in 'Bing' and 'Lapins.'

Objective Two: Use molecular markers to predict dormancy stage in cherry buds collected from initiation in summer through bud-break the following spring.

Objective Three: Test actual dormancy by quantifying the number of buds that will bloom on a parallel collections of branches.

PROCEDURES:

Objective One:

Potential molecular marker genes will be chosen from published literature and will be selected based on expression patterns that show distinct changes in response to transition from one dormancy state to the next, i.e., para- to endodormancy, endo- to ecodormancy, and ecodormancy to the beginning of bud swell. Preliminary candidate genes include (but are not limited to): *PavSRP* and *PavTCX2*, which decrease as flower buds transition from para- to endodormancy, *PavDAM5*, which decreases when buds transition from endo- to ecodormancy, or *PavAP3*, which increases as the bud leaves ecodormancy (Vimont et al., 2019; Wang et al., 2020). Additional genes involved in hormone metabolism, dormancy control, or with unknown function may be selected if they exhibit correlative expression patterns.

Primers to amplify candidate genes will be either taken from the literature, or designed using published cherry genomes or transcriptomes. Primers will be tested for accuracy by amplifying from cDNA (coding DNA) synthesized from the RNA of pooled buds from each cultivar to verify that the correct gene is being amplified. Primers will be tested for efficiency by performing a quantitative PCR (qPCR) standard curve analysis on serial dilutions of the same cDNA. Ideally, at least two sets of primers that correspond to genes with expression shifts spanning each of the three major transitions will pass accuracy and efficiency tests, resulting in six molecular markers to advance to Objective Two.

Objective Two:

Primer sets that pass accuracy and efficiency tests will be used to predict dormancy status of buds collected once a month starting in August (when new floral buds have set and are

paradormant) until February (when buds will likely be close to "dormancy release" the transition from endo- to ecodormancy). Sampling will increase to every two weeks from February through bud break, in order to more finely map dormancy release as well as the initial re-activation of flower development that occurs prior to bud swelling – the first visible indication that bloom is beginning.

Ten buds from each of three randomly selected trees per cultivar will be collected, bud scales will be removed and they will be pooled and homogenized. RNA will then be extracted. Pooling buds will give a more average indication of bud status, as individual buds can differ slightly in degree of development. cDNA synthesis and qPCR will be performed in a one-step reaction and the resulting expression levels of each gene will be analyzed and graphed throughout development. This data will be compared to published values, in order to determine the transitions in dormancy.

Objective Three:

In order to test whether the markers are performing as expected, actual dormancy stages will be tested in branches collected at the same time and from the same trees as the buds for qPCR. Three branches with floral buds will be collected randomly from each replicate, placed in water, tipped in order to relieve the apical dominance that controls paradormancy, then incubated in forcing conditions (25°C, 16hr light, 8 hr dark, 70% humidity) for ten days. After ten days, the number of breaking buds will be counted; greater than 50% bud break will indicate that either buds are paradormant (early collections) or that dormancy has been released (later collections). Bud break less than 50% will indicate that the branches are endodormant. Data from Objectives Two and Three will be compared to determine the feasibility of using the selected molecular markers to determine dormancy status in 'Bing' and 'Lapins' cherry trees.

SIGNIFICANT ACCOMPLISHMENTS TO DATE:

Primers have been identified, by analyzing published gene expression patterns. Two genes, *PavSRP* and *PavTCX2*, that have been shown to decrease as cherry buds transition from paradormancy to endodormancy will be used. Two genes, *PavDAM4* and *PavDAM5*, that decrease as buds transition from endodormancy to ecodormancy, and one gene, *PavMEE9*, that increases during this transition will be used. One gene, *PavAP3*, that increases as buds transition out of endodormancy will also be used. In order to perform qPCR, reference genes also needed to be identified – we will use *PavACTIN*, *PavRPII*, and *PavEF1*. All primers have been obtained.

Tissue collection for gene expression analysis began in October of 2021. No qPCR has been performed yet. A lyophilizer has just recently been obtained, and will be used to lyophilize tissue prior to RNA extraction, pending lyophilization optimization.

Branches have been collected and forced once a month, starting in October 2021. We changed the protocol to include five branches, and extend to 21 days (previously 10), based on published studies indicating that 10 days were frequently insufficient to observe budbreak. We are measuring bud length and width at collection, and again at the 21 day mark, in order to quantify bud swell. So far, we have been surprised to see that buds have not entered endodormancy on either cultivar – the most recent collection from the beginning of January has flowered and produced leaves 21 days later (Figure 1). Hood River experienced and

abnormally warm early-winter period, and the lack of cold may have postponed the natural transition of cherries in this region into endodormancy.

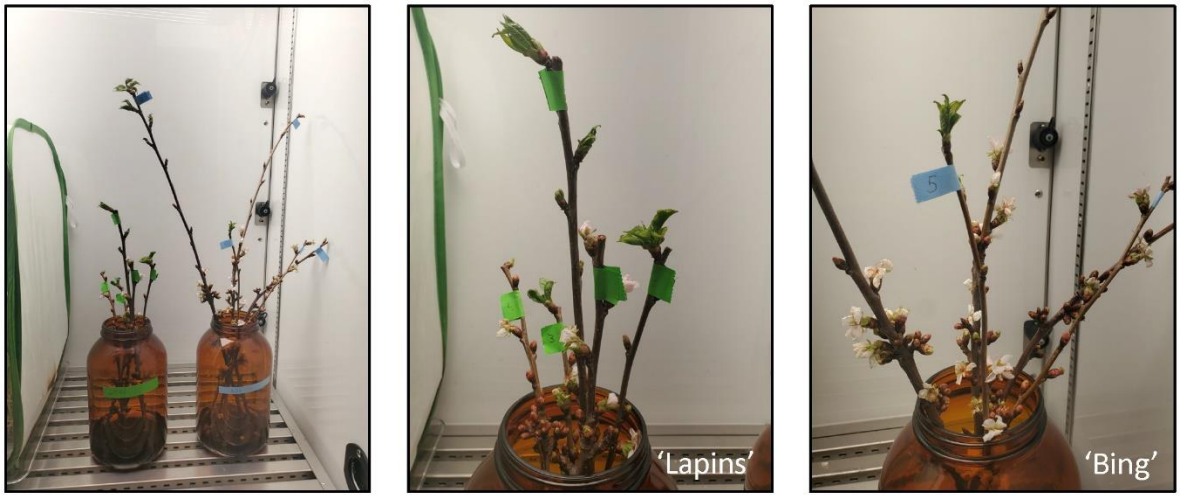

Figure 1. Five branches of first- and second-year wood from 'Lapins' and 'Bing' sweet cherry trees, after a 21-day incubation period in forcing conditions. Reproductive and vegetative buds from both varieties have "broken", indicating that branches have not entered endo-dormancy.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

A closely related project aimed at modeling the cold hardiness of sweet cherry buds was funded by the Oregon Sweet Cherry Commission and the Washington Tree Fruit Research Commission in 2022, for a total of $87,341.

FUTURE FUNDING POSSIBILITIES:

Future funding sources could potentially include the Oregon Sweet Cherry Commission, the Washington Tree Fruit Research Commission or the Columbia Gorge Fruit Growers Association.