



## Variation in cold hardiness of sweet cherry flower buds through different phenological stages



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### ABSTRACT

Freezes during early spring can damage flower buds of sweet cherry (*Prunus avium* L.). The reduction in sweet cherry production due to cold injury to floral tissue can be significant and is a common and serious problem for growers and commercial producers in the Pacific Northwest and other regions of the US. The critical temperatures affecting the bud and the associated damage are variable and unknown for most of the new cherry cultivars. The goal of this study was to identify the critical temperatures of cherries buds at different development stages, using differential thermal analysis (DTA) and a traditional cold exposure method for assessing cold damage. Three sweet cherry cultivars including Bing, Chelan and Sweetheart were tested during the late winter to early spring seasons of 2012 and late fall to early spring season of 2013. The DTA showed that the temperature causing freezing injury was related to the initiation of the low temperature exotherms (LTE's), the value of the lethal temperature changes depending on the cultivar and bud development stage. High (HTE) and low temperature (LTE) exotherms were clearly identified for the different cherry cultivars that were evaluated during the early stages of bud development. Cold hardiness increased from late fall through mid-January, followed by deacclimation for the remainder of the winter months. During deacclimation, DTA was not an effective method for detecting LTE in cherry buds. Therefore, a traditional approach that exposes the tissue to different cold regimens was used for the remaining period of bud and flower development. To determine the total number of buds and flowers that were killed due to cold exposure, a simple evaluation using dissections under a stereoscope was conducted. Logistic models were developed for the freeze-survival data for both methods. Differences in hardiness were found for all the cherry cultivars that were evaluated as well as between different sampling dates. The information that was generated in this study will provide a better understanding of cherry cold hardiness and will support growers in decision-making of frost control practices.

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### 1. Introduction

Sweet cherry buds and blooms are often damaged due to frost events that occur during early spring (Dexter, 1933; Smeeton, 1964; Rodrigo, 2000; Cittadini et al., 2006). The frequency of frosts in early spring and temperatures below freezing during and after budbreak has the potential to not only injure buds but also flowers, developing fruits and shoots. From dormancy to fruit set the reproductive bud transforms through a number of developmental stages that are associated with a progressive increasing vulnerability to low

temperatures (Rodrigo, 2000; Longstroth, 2013). Most of the orchard management decisions depend upon the potential for low temperature injuries or are in response to previous injury from low temperatures (Proebsting and Mills, 1971; Longstroth and Perry, 1996). Spring growth begins with the onset of warm temperatures resulting in the swelling of both floral and vegetative buds. At the onset of this swelling the bud starts to lose their ability to tolerate cold (Longstroth and Perry, 1996; Rodrigo, 2000).

During the spring, cold-hardiness is a function of both environment and bud stage (Mexal et al., 1991; Longstroth, 2013) and the resistance of fruit crops to freezing temperatures is variable (Rodrigo, 2000; Caprio and Quamme, 2005; Longstroth, 2013). The year-to-year variation and differences among cultivars seem to indicate that there is a strong impact of weather conditions and genotype in sweet cherry fruit set (Choi and Andersen, 2001; Garcia-Montiel et al., 2010). Environmental conditions prior to or

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immediately after a low temperature occurrence greatly influence the degree of freezing injury. A spring frost that coincides with the stage of bud break can cause severe injury, while at the more advanced stages of bud break the buds and small flowers become increasingly susceptible.

During spring frosts, the cellular damage is usually produced by extra-cellular freezing and results in a series of anatomical effects that can be reflected in diverse morphological changes (Rodrigo, 2000). In cherries, freeze damage may occur during late autumn and during the early winter months before buds are acclimatized and especially during the deacclimation as the buds swell and progress toward anthesis. During this period, buds vary considerably in their level of hardiness. Reproductive buds of cherries are more susceptible to freezing injury than vegetative buds, while the blossoms of the growing cherry trees are extremely susceptible to frost damage and frost resistance varies within the tree itself in the same extent varies within orchard cultivars, flowers of cultivars in the same phenological stage (Proebsting and Mills, 1978a). The resistance of the flower bud to low temperatures changes rapidly and predictably in response to both temperature and the stage of floral development (Proebsting and Mills, 1978b; Westwood, 1993; Iezzoni, 1985). In dormant flower buds, the effect of freezing temperatures is not uniform, because ice crystals formed in only some floral tissues. If the temperature decreases gradually, a tree is in a better condition to resist injury and can stand surprisingly low temperatures when compared to a steep drop in temperature from warm to cold, which causes most of the injuries. How different tree species react to freezing temperatures depends on where growth is taking place, where growing points are, and if the cells have been built in anti-freeze systems to prevent ice crystal formation. The tolerance can also be influenced by the hardening off process (Mazur, 1969; Ashworth and Wisniewski, 1991; Palonen and Buszard, 1997).

Spring frosts can be a limiting factor for sweet cherry production. The association of spring frost with tree fruit damage was recognized by Proebsting (1963), who developed the concept of minimum hardiness level that provides guidance for bud hardiness modeling and forecasting of tree fruits, including some sweet cherries (Andrews et al., 1987; Andrews et al., 1984). There are historical cold hardiness data available on some species based on research conducted by Proebsting (1970), Proebsting et al. (1980) on flower buds of peach, apricot, cherry, grapes and plum trees and Ketchie and Beeman (1973) on apples, and Ketchie (1985) pears. Proebsting and Mills (1978a) established the critical temperatures for the various stages of flower development in the spring for Bing sweet cherry. However, this research was conducted long time ago. The current cherry bud charts are based on most of Proebsting research and are still being used today, even for new cultivars. Since plants adapt to the short term temperature environment and this influences the critical temperature at which damage can occur, extrapolation of these critical temperatures to a given crop and environment is questionable (Snyder et al., 2004). Knowledge of the critical temperatures and frost sensitivity of sweet cherry cultivars for each of the development stages that reflect the decrease in hardiness level during bud development in spring can aid in orchard design by placing most susceptible cultivars in the best sites, it is also very important as a guide for appropriate crop management, and especially for activating frost protection systems during the spring which should be started sooner on the more susceptible cultivars. Differential thermal analysis (DTA) is one of the common methodologies for determining cold hardiness in woody tissues of some species. In apple (Quamme et al., 1972a), (Quamme et al., 1972b), pear (Montano et al., 1987), and azalea (Graham and Mullin, 1976), a low temperature exotherm (LTE) of dormant woody stem sections detected by DTA, has been correlated with injury to both xylem and pith that occurred during cooling (Volk et al., 2009). The successful application of this approach to determine cold

hardiness for dormant cherry and apple buds has been demonstrated in previous studies that were conducted by Andrews et al. (1983) who determined the freezing injury of deacclimating peach and sweet cherry reproductive organs using DTA. The hardiness progression through winter dormancy and early spring for new cherry cultivars has not been well characterized and very little is known about the frost susceptibility of new sweet cherry cultivars. There is, therefore, a need to update this information using new commercial varieties. The overall goal of this study was to determine the variation in cold hardiness of cherry buds through different phenological stages for different cherry cultivars using DTA and a controlled freezing technique.

## 2. Materials and methods

### 2.1. Plant material

Flower buds were randomly sampled for two consecutive years. Data was collected for year one during the late winter (February) and early spring season (March) of 2012 and for year two from October 2012 throughout the complete spring season (April) of 2013, to determine the cold hardiness for different developmental stages of cherry buds. Bud stages were characterized following the charts developed by Ballard et al. (1982). Three sweet cherry cultivars including Bing, Chelan and Sweetheart were tested. The samples were taken from the experimental orchards of 6-year old trees located at the Washington State University Irrigated Agriculture Research and Extension Center in Prosser, WA. Complete spurs with dormant buds were collected weekly from three similar trees for each cultivar and the sample size ranged from 200 to 500 spurs. When samples were collected, they were placed in a container that had been previously cooled to the local air temperature, since it is very important that the samples remain as close as possible to the air temperature (Proebsting and Mills, 1971). The spurs were then separated and randomly assigned to five sets of 12 samples per cultivar. One complete set of each cultivar was kept as a control and was not frozen for visual evaluation of oxidative browning to check the variability and dead material that was present in the orchard. The remaining four sets were then used for the DTA analysis.

### 2.2. Differential thermal analysis

Differential thermal analysis is a technique used to quantify cold tolerance in plants, freezing episodes called exotherms can be identified as changepoints, local minima or selected inflection points of differential temperature (Gerard and Schucany, 1997). For DTA, each set of bud samples was comprised of 12 replicates per cultivar a total of seven to ten buds were used each time per replication. The samples were placed on four trays; each tray included nine thermoelectric modules (TEMs) that detect temperature gradients generated by the exotherms according with the methodology described by Mills et al. (2006). Five to ten buds (depending on the size of the bud) were covered in aluminum foil and placed directly on each TEM protected by foam insulation pads. A chamber lid was tightened to the tray and then loaded into a programmable freezer (Tenney Temperature Test Chamber, Model T2C-A-WF4 2.0 cu.ft. Watlow F4. Temperature range:  $-73^{\circ}\text{C}$  to  $200^{\circ}\text{C}$  with a resolution of  $0.3^{\circ}\text{C}$ , Thermal Product Solutions). The freezer was programmed for the standard cooling rate of  $4^{\circ}\text{C}/\text{h}$  decline which means that the freezer hold at  $4^{\circ}\text{C}$  for 1 h and then drop to  $-40^{\circ}\text{C}$  in 11 h, at that moment return to  $4^{\circ}\text{C}$  in 10 h, and then a DTA analysis was performed. The system that was used in our laboratory is the same as the one described by Mills et al. (2006), but with updated computer technology and brand-new trays. Thirty six TEMs were loaded per run (180–252 buds). The system recorded for each TEM

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