Influence of Ice Nucleation Temperature on the Freezing of Peach Flower Buds

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Abstract. The freezing behavior of peach [Prunus persica (L.) Batsch] flower buds was influenced by the temperature at which ice formation was initiated. Buds seeded with ice just below 0°C were more likely to exhibit deep supercooling, and water in the primordia would supercool to lower temperatures than in unseeded excised flower buds. This effect was not always expressed and varied with the stage of acclimation. Researchers using differential thermal analysis to estimate bud hardiness will need to evaluate this effect. Seeding specimens with ice may be warranted to obtain results comparable with field conditions.

Dormant flower bud primordia of several species avoid freezing injury by deep supercooling (5, 9, 10, 12, 15, 18, 19, 23, 28). Water in these tissues freezes as two distinct components. Freezing begins in the bud axis and scales between −3° and −10°C, whereas water within the bud primordium supercools to much lower temperatures before freezing (4, 9, 10, 12, 13, 19, 23). Several studies have demonstrated a strong correlation between the temperature of the low temperature exotherm and the temperature at which bud primordia are killed (12, 20, 23). Techniques that use detection of a low temperature exotherm to evaluate bud hardiness have been reported (1, 3, 13, 18, 20, 24).

Researchers in plant cold hardiness routinely have seeded plant tissues with ice just below 0°C when conducting controlled freezing tests. The rationale for this practice has been that excessive supercooling would result in rapid rates of ice crystal growth and excessive tissue damage when ice formation eventually was initiated (16). Several experiments demonstrating this effect have been reported (2, 14, 17, 27, 29). Whether the initial nucleation temperature influences the extent of deep supercooling by dormant flower buds has not been evaluated. We recently reported that ice formation was initiated near −2°C in the woody tissues of peach (6, 7) and other woody species (8), and then spread throughout the plant. In contrast, ice formation in a typical differential thermal analysis (DTA) experiment with excised flower buds was more likely to be observed at lower temperatures (−3° to 10°C) (4, 9, 10, 12, 13, 19, 23). The purpose of this study was to determine whether the temperature of the initial nucleation event would influence the extent of deep supercooling in peach flower buds.

Peach flower buds were obtained from mature trees in the orchards of the Appalachian Fruit Research Station between Nov. 1985 and Mar. 1986. One-year-old twigs were harvested on the morning of each experiment, put into plastic bags, and kept on ice until processed. Buds were prepared in three different ways for thermal analysis. The first involved excising buds from twigs so that a small portion of adjacent bark tissue was left attached. These buds were not seeded with ice during the experiments, and this protocol was typical of many DTA studies (1, 3, 4, 9, 12, 18, 28). The second involved leaving a single bud attached to a 4-cm twig piece and removing all other buds. The base of the twig piece was placed in 75 μL of distilled water containing a small chip of ice. The third method involved excising buds and passing a cotton thread through the subtending bark tissue and into 75 μL of distilled water containing a chip of ice. The cotton thread served as a wick and allowed ice to spread from the ice-water slurry into the bud.

Tissues in individual glass test tubes were placed in a circulating bath (Neslab Instruments, Portsmouth, N.H.). Bath temperature was lowered at 5°F/hr. The junction of a 36-gauge copper–constantan thermocouple was attached to the surface of each bud with a small piece of masking tape. Temperatures were monitored at 1-min intervals with a datalogger (Fluke 2200B, John Fluke Manufacturing, Everett, Wash.) interfaced to a computer and graphics terminal. The freezing of water was detected as an abrupt increase in sample temperature, and exotherm temperature was noted as the sample temperature immediately prior to that increase. When trees are dormant, living and dead buds cannot be distinguished based on external appearance. Therefore, immediately after the thermal analysis experiments were completed, tissues were thawed, buds bisected, and examined. Buds already containing brown primordia had been killed in the field prior to the start of the experiment, and data from these buds were not included in further analyses.

The freezing behavior of excised and unseeded 'Loring' flower buds and buds attached to stem sections and seeded with ice were compared on several dates. Ice formation was initiated within the bud axis and scales of excised buds between −4° and −7°C. Water within the primordia of excised buds was less likely to deep supercool than water in the primordia of buds attached to a stem section and seeded with ice just below 0°C. Significant effects were noted on 12 Nov., 6 and 7 Mar., and 12 and 13 Mar. (Table 1). In addition, on the 3 Mar. and 6 and 7 Mar. sampling dates, the primordia of excised buds did not supercool to as low a temperature as the primordia of attached and seeded buds. No differences in freezing behavior were noted on 26 Feb. and 20 Mar. On 26 Feb., buds were quite hardy, and both treatments exhibited deep supercooling. In contrast, on 20 Mar. buds were swollen, and neither treatment exhibited supercooling (Table 1).

This response was not unique to 'Loring'. In mid-March, the responses of two other cultivars were examined. Only one of nine excised and unseeded 'Reliance' buds exhibited deep supercooling, compared to nine of 10 attached and seeded buds. Likewise, three of 10 excised and unseeded 'Boone County'...
buds deep supercooled, compared to all 10 attached and excised buds.

It was important to determine whether the difference between the excised and attached treatments was due to an ~5°C difference in the initial ice nucleation temperature or the presence of the woody tissue. To distinguish these possibilities, comparisons were made between attached buds seeded via the woody tissue, excised buds seeded at the same temperature via a cotton wick, and excised buds that were not seeded. The experiment was conducted during the early bud swell period. A much lower proportion of excised and unseeded buds exhibited deep supercooking than was observed with the other treatments (Table 2). In addition, the low temperature exotherms of those buds that did supercool occurred at warmer temperatures than those observed in the two seeded treatments. The similar responses of the attached and seeded buds compared to excised and deep supercooled buds (Table 2) suggested that the seeding of tissues, rather than the presence of the twig, accounted for differences observed in Table 1.

When dormant peach flower buds freeze, ice forms in the bud axis and scales and there is a redistribution of moisture within the bud (4, 11, 25, 26). The importance of this initial freezing event in the bud axis and scales for the subsequent supercooling of water in the primordium has been reported in peach (4, 25, 26) and other species (15). Several factors that influence the extent of supercooling in peach, such as bud moisture content (26), cooling rate (4, 26), and previous temperature history (26), appear to exert their effect by influencing the redistribution of moisture during freezing. The influence of initial nucleation temperature as reported here probably had a similar effect. The excessive amount of supercooling noted in excised buds would lead to rapid freezing rates when ice formation finally was initiated. Pruppacher (21, 22) demonstrated that the rate of ice crystal growth increased exponentially as the extent of supercooling increased. Therefore, if ice formation was initiated at warmer temperatures, as it would be under field conditions (6, 7), the rate of crystal growth would be slower. Presumably, a slower freezing rate would facilitate the redistribution of water to ice in the bud axis and scales and promote supercooling of water within the primordium.

Although this is the first report, to the best of our knowledge, of the initial nucleation temperature affecting the extent of deep supercooling, it is well-known that the initial nucleation temperature can affect plant cold hardiness (2, 14, 16, 17, 27, 29). For this reason, plant tissues routinely are seeded with ice just below 0°C during hardiness determinations.

It is not clear why excised buds did not exhibit less deep supercooling than attached and seeded buds at all sampling dates. No differences were observed when buds were hardy in February, or in late March, when buds had acclimated. However, between these dates, the effect of excision was significant and may be related to changes in bud moisture content. If bud excision promoted rapid ice crystal growth upon freezing and thereby prevented the redistribution of water to ice in then scales, then one could postulate that, in hardy buds, when bud moisture content was low, the freezing rate in both excised and seeded buds was not in excess of that required to allow for the redistribution of water to ice in the scales. As the buds begin to swell, additional time would be required to complete freezing, and the accelerated freezing rate exhibited in the excised buds then might be too fast to allow for the redistribution of water. Likewise, with further bud swelling, neither rate of freezing is accommodating, or it may be that buds have developed so that regardless of freezing rate, no deep supercooling would be exhibited (4).

Regardless of the reason for the effect, the observation that initial nucleation temperature affected whether buds would deep supercool and the extent of the supercooling was of practical significance. Many researchers have used differential thermal analysis of excised buds to evaluate cold hardiness: a discussion of the molecular causes of injury with particular reference to deep supercooling of water, p. 199–226. In: H. Mussell and R.C. Staples (eds.). Stress physiology in crop plants. Wiley, New York.


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Inhibition of growth or removal of reproductive organs or other sinks for photosynthates decreased net CO₂ assimilation (A) for many plants (1, 3, 4, 6, 7, 13, 14). Fruit removal results in an accumulation of starch grains in the chloroplast (4, 5, 12). One possible explanation for the effect of fruit removal on reduced A rates is the interference of chloroplast absorption of light or disruption of thylakoid membranes due to starch accumulation in leaves (12).

Several studies have found a decrease in leaf conductance (gᵢ) concomitant with decreased A rates after sink removal (3, 7). Therefore, another proposed explanation for the influence of fruit on A is via a feedback response from the fruit to the leaves controlling the stomatal mechanism (7, 8). The effect of fruit removal on net gas exchange characteristics of avocado has not been reported to the best of our knowledge. The purpose of this study was to test the effect of fruit removal on leaf dry weight per area (Wₛ), A, gᵢ, and internal CO₂ concentration (Cᵢ) of avocado leaves.

Eight-year-old 'Booth 7' avocado trees growing at the Tropical Research and Education Center, Univ. of Florida, in southern Florida were used for this experiment. During June 1986, at the time of early fruit development, fruit-bearing branches were girdled at the base and all but 10 current-year's leaves per branch were removed. For this cultivar, anthesis occurred from April to May, and fruit ripened in late summer. Girdled branches were divided into two treatments: a) branches with all fruit removed and b) branches with one fruit allowed to remain. At the time of initial fruit removal, the average fresh weight of the fruit was 30.3 g, which was ~7% of the fresh weight of a mature fruit of this cultivar. Each treatment consisted of two branches on five separate trees, totaling 10 replications.

Two current-year's leaves of similar ages between treatments were tagged on each branch at the time of initial fruit removal (day 0). Prior to initial fruit removal (day 0) and at 14-day intervals after fruit removal, A, gᵢ, and Cᵢ were determined between 9:00 and 11:30 AM from random branches of each treatment. Wₛ was determined from two leaves each of 10 additional branches for each treatment. Twenty-eight days after initial fruit removal, all fruit were removed from the branches that had one fruit remaining. Average fresh fruit weight at that time was 90.7 g. Net CO₂ assimilation, gᵢ, Cᵢ, and Wₛ then were determined for leaves of branches of each treatment 14 days later.

Net CO₂ assimilation, gᵢ, and Cᵢ were determined from CO₂ and H₂O vapor fluxes in an enclosed leaf chamber connected to a portable gas analyzer (Analytical Development Co., Haddesdon, Herts. U.K.) as previously described (15). The chamber contained a fan (to minimize boundary layer resistance), a humidity sensor, and thermocouples for sensing leaf and air temperatures. Outside air was supplied to the chamber at a flow rate of 6.25 ml·s⁻¹ by a mass flow controller. The air stream was first passed through silica gel to absorb some of the moisture and maintain relative humidity in the chamber at 30±1%. Although air temperature in the chamber increased over the measurement period, temperature remained between 31°C and 33°C during determinations. All A, gᵢ, and Cᵢ determinations were made well above saturating light levels (P₄₀₀ > 1500 µmol·m⁻²·s⁻¹) determined by a quantum sensor connected to a LI-1000 data logger (LI-COR). Leaf dry weight per area was determined from the dry weight of five 0.32-cm² leaf disks from each of two leaves per branch. Fourteen days after initial fruit removal (day 14), leaf sections were taken from mid-lamina of leaves in each treatment, preserved in formalin–acetic acid–alcohol (FAA), dehydrated in an ethanol–xylene series, and embedded in paraffin. Cross-sections (4 µm) were stained with I₂KI and the number and size of starch grains was determined. Starch grains were counted from five random 767.3-µm² microscope fields per section at x 1000.

Prior to initial fruit removal (day 0), there were no differences in A, gᵢ, Cᵢ, and Wₛ between treatments (Fig. 1). Fourteen and 28 days after initial fruit removal, Wₛ was 25% greater for branches with all fruit removed than branches with one fruit remaining (Fig. 1a). Cᵢ was slightly higher for branches with all fruit removed than those with one fruit remaining 14 and 28 days after

**Effect of Fruit Removal on Net Gas Exchange of Avocado Leaves**

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**Abstract.** All but 10 current-year leaves were removed from girdled branches of avocado (Persea americana Mill.) trees having one remaining fruit or all fruit removed. Removing all fruit resulted in increased leaf dry weight per area (Wₛ), a 250% increase in the number of starch grains in leaves, and a reduction in leaf conductance (gᵢ) and net CO₂ assimilation (A). Internal CO₂ concentration (Cᵢ) was lower for leaves of branches with fruit than for leaves of branches without fruit. The results suggest that the accumulation of starch in defruited, girdled branches results in an inhibition of A. The data suggest that the increased gᵢ associated with the presence of avocado fruit is possibly a result of increased A and reduced Cᵢ levels.