Differential Thermal Analysis and Freezing Injury in Cold Hardy Blueberry Flower Buds

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Abstract. Freezing injury of highbush blueberry flower buds (Vaccinium corymbosum L. cv. Rancocas and an experimental hybrid) were investigated after natural freezing and by differential thermal analysis (DTA). Terminal buds were less hardy than median and basal buds on the same twig. Apical, more mature florets were also less hardy than median or basal, more mature buds within each bud. DTA analysis of intact flower buds showed that a rate independent free water exotherm was followed by numerous rate dependent floret exotherms. Floret lethality was associated only with the rate dependent low temperature exotherms. Buds were held over water-glycerol solutions in desiccators so that they came into equilibrium with a relative humidity of 100%, 98% or 96% at 4°C. Artificially hydrated buds were hardy to $-10^\circ$C, and artificially dehydrated buds were $15^\circ$C harder. A mean survival temperature of the florets (MSTF) from DTA analysis, derived at slow cooling rates related closely to lowest survival temperature ($LST_{66}$) from artificial freezing hardiness tests. In mid-winter, under environmental conditions of low temperature and low relative humidity, flower buds of a hardy hybrid lost all DTA exotherms and were hardy to at least $-40^\circ$C. Tissue dehydration and accompanying loss of floret low-temperature exotherms was associated with extreme cold tolerance of blueberry flower buds with the hardy experimental hybrid.

Supercooling occurs in cells of many plant tissues (3, 4, 5, 6, 7, 8, 9, 15, 16, 17, 18, 19) and has been shown to be a freezing avoidance mechanism in apple stem, ray parenchyma tissues (17). Differential thermal analysis (DTA) has been successfully used to observe and study freezing in many plant tissues. Low-temperature exotherms have been observed in Cornus stolonifera Michx. stem tissue (2, 3), and flower buds of Rhododendron (6, 7), Prunus (16), Vaccinium (1), and Vitis (15). Occurrence of low-temperature exotherms has been correlated with absolute hardiness in apple stem tissue (17) and also appears to be associated with woody plant distribution in the northern hemisphere (5).

Plant tissue dehydration has been noted as a secondary freezing stress in several conifers and sufficient dehydration may cause cell death (9). However, certain cold hardiness research has shown that a degree of dehydration may be of benefit in aiding plant tissue to survive low temperatures. McKenzie (10) has noted that acclimated plants of C. stolonifera are less hydrated than non-acclimated plants. Bittenbender and Howell (2) showed that hydration influenced blueberry bud hardiness and that hydration and temperature may act independently.

Sakai (20) has observed the effects of tissue dehydration on various plant material. Survival at low temperatures was increased by slowly pre-freezing plant cells to $-30^\circ$C before immersion in liquid nitrogen. Apparently, the removal of intracellular water to extracellular ice nuclei, thus avoiding development of large intracellular ice crystals, may be responsible for the ability of live tissue to survive extremely low temperatures.

Flower buds of 'Rancocas' highbush blueberry and an unnamed Vaccinium seedling were used to test the following hypotheses: 1) bud dehydration results in an increase in cold hardiness, 2) bud age (position on stem) is related to hardiness potential, and 3) increased age of florets within a bud is associated with an increase in floret hardiness. Differential thermal analysis was employed as the principal research tool to test these hypotheses.

Materials and Methods

Flower buds of V. corymbosum 'Rancocas', grown at Excelsior, were collected at biweekly intervals during the winter of 1974, the fall and winter of 1975 and the winter of 1976. A hardy unnamed Vaccinium seedling grown in St. Paul was used for the dehydration tests because plants of 'Rancocas' were severely damaged by low temperatures in early January 1976. No injury was observed in the seedling exposed to the same temperatures. Plant materials were stored outdoors in snow-filled plastic bags for less than 1 week between collection and artificial freezing for determination of absolute hardiness and for exotherm studies by DTA. Buds which were used for dehydration tests were collected from the plants and prepared for desiccation on the same day. They were excised from the stem to aid dehydration. Buds from each stem were deposited sequentially on tape strips in their original order and the strips placed in open tubes and stored in sealed desiccators.

Desiccating solutions consisted of water and glycerol at ratios of 100:0 (100% relative humidity, RH), 95:5 (98% RH), and 90:10 (96% RH) prepared according to directions by Osborne and Bacon (14). The relative humidity of each solution was tested with an Abeon hygrometer placed in each desiccator for 24 hr at 4.4°C before readings were taken. These relative humidity readings are equivalent to vapor pressure gradients of 0.0125 and 0.251 mm Hg and water potentials of 0.255 and -51.60 bars, respectively.

Freezing patterns of buds and florets. The material to determine freezing pattern was collected January 1974, following a low temperature of $-35^\circ$C. One hundred branches with stems bearing intact flower buds were selected at random from above and below the snowline.

Flower buds from stems with 2, 3, 4 and 5 buds were scored dead or alive by excising with a razor blade and observing discoloration through a dissecting microscope. The terminal bud on each stem was numbered B1.

Florets within each bud were observed and rated in arbitrary groups as basal (B), median (M) and terminal (T).
Differential thermal analysis. The differential thermal analysis techniques used in these tests were very similar to the methods described by Quamme et al. (17) and by George et al. (6). Flower buds were removed from storage and all stem tissue was excised from the base of each bud. (The time interval between removal from storage and the beginning of the DTA test was always less than 30 min). Each bud was placed in a small aluminum foil cup. The end of a 0.08 mm, chromel-constantan thermocouple was placed within the cup. The foil was crimped to prevent slipping of the thermocouple and to insure thermocouple-bud contact.

Samples were then inserted into individual glass lined wells in an aluminum block. The block acted as a heat sink and temperature stabilizer. Each well opening was sealed tightly with a styrofoam plug and reference thermocouples were inserted into a 4th well, which contained only air. A heavily insulated styrofoam box surrounded the aluminum block and an inlet tube fed a constant supply of cold nitrogen into the box.

Samples were cooled at a constant rate of 8°C per hr in all tests, except in tests for rate dependency where rates of 65°, 33°, 8° and 6.5°C per hr were used.

An average of the floret low temperature DTA exotherms was calculated to score blueberry buds for hardness. This temperature will be referred to as mean survival temperature of the florets (MSTF). This method was used in lieu of an LT50 because blueberry buds are racemose, each containing from 5 to 20 potential florets which may vary in developmental age. A bud may have 50% of its florets injured by cold and still put forth 50% of its original floral complement in the spring. The first large, free water exotherm was not used in the MSTF because at slow cooling rates and at this stage of maximum cold tolerance, it does not depict floret death.

Differential scanning calorimetry. Buds were prepared for differential scanning calorimetry in the same way as for DTA tests. A Perkins-Elmer DSC-2 was used to verify the DTA exotherm data. The cooling rate was 0.31°K per min (0.31°C per min) or 18.6°K per hr (28.6°C per hr) and samples were cooled at a lower limit of 223°K (— 50°C).

Lowest survival temp (LST66). Test material included 3 stems for each temperature and 3 buds per stem (9 buds per test temperature). Preparation for this test began as soon as buds were brought indoors. Foil cylinders containing stems with buds were placed in a thermos bottle and sealed with wet cheesecloth extending from the closed mouth of the vacuum bottle to insure the presence of external ice nucleation. The insulated thermos bottles regulated the temperature drop at 6-8°C per hr. A bottle was removed at each of the following temperatures: 0°, —5°, —10°, —15°, —20°, —25° and —30°C.

Blueberry flower buds were tested every 2 weeks throughout the fall and winter, within one day of collection from the field, to determine the LST. LST tests were also run in conjunction with dehydration tests before dehydration and after one week of dehydration.

Buds were sectioned after freezing and visually rated for browning of florets. LST was determined as the lowest temperature at which at least 2 out of 3 buds survived for each stem position (LST66). Buds used in dehydration tests were prepared and tested in the same way, except the buds were excised from stems and placed on tape strips.

Results

Examination of injured buds and florets in 'Rancocas' following the —35°C outdoor freeze revealed a general pattern wherein terminal buds were injured most frequently and a trend toward less injury occurred with basipetal position along stems (Fig. 1), similar to results reported by Bittenbender and Howell (1).

Similarly, within a bud, % kill increased slightly from the

![Fig. 1. The relationship between bud position on the stem and % bud kill (mean ± SE).](image_url)

![Fig. 2. The relationship between floret position within a bud and floret kill (mean ± SE).](image_url)
basal to terminal group of florets (Fig. 2), corresponding to their reported acropetal pattern of developmental maturity (23).

**DTA.** Floret exotherms from terminal buds collected from one 'Rancocas' plant exhibited rate dependence when buds were frozen at rates of 65°C/hr, 33°C/hr, 9°C/hr and 6.5°C/hr. At least one exotherm appeared at each rate as shown in Fig. 3, but discrete floret exotherms were not detectable at the fastest cooling rates. At fast rates (33°C and 65°C/hr) one large exotherm with smaller peaks superimposed appeared beginning at about −10°C, which probably represents freezing of extracellular, free water as reported by Graham (7). Small peaks often occurred on the large exotherm which probably indicates that the florets froze along with the bulk water at rapid freezing rates. At the slow rates (6.5°C, 8°C and 9°C/hr), one free water exotherm was followed by numerous smaller peaks. The small peaks represent the sequential freezing of individual florets, similar to reports by Graham (7) and by George et al. (6) with azalea. DTA tests at slow rates were used to simulate, as closely as possible, rates occurring in nature. The most workable freezing rate for testing purposes in our studies was 8°C per hr, which was thus used in subsequent trials (Fig. 4).

During the winter of 1976, only the free water exotherm was observed at slow freezing rates in 10 out of 11 'Rancocas' buds tested January 9 and 13, following outdoor exposure for several days to −30°C min temperatures. Numerous buds were sectioned and examined before and after the DTA tests were run indicating that injury had occurred several days before the DTA tests. Therefore, at slow freezing rates, the presence of a free water exotherm and the absence of small low temperature floret exotherm peaks was an indication of dead florets.

**Bud dehydration.** The hypothesis that bud hydration affects hardiness was tested in 2 stages with the cold hardy hybrid: January (stage 1) and February (stage 2), 1976.

Buds tested immediately after collection from the field exhibited DTA profiles which were similar to the profile shown in Fig. 5 for field conditions. Each had a large free water exotherm followed by numerous floret exotherms which had MSTF's distributed from −24.2°C to −24.7°C.

Buds tested after 1 week of storage at specified relative humidity conditions exhibited DTA profiles as shown in Fig. 5. The DTA, MSTF’s were different for each relative humidity regime (Table 1). The DTA profile for 100% RH buds had a large free water exotherm followed very closely by large floret peaks. The LST66 calculated from artificial freezing and visual ratings of dead florets and the MSTF calculated from DTA analysis of a separate set of buds from 100% RH were closely related and were hardy to −10°C (Table 1, Fig. 5). The average moisture content for buds from this regime was 106.5% (per unit dry weight).

In contrast, buds from the 98% RH regime were about 10° hardier by both determinations. Their DTA profiles (Fig. 5) were spread out and the free water exotherm was reduced in size. These buds were hardy to −21.9°C by MSTF rating and this hardness rating agreed closely with the LST66 of −20°C. Buds from the 98% RH regime had an average moisture content of only 52%.

Dehydration under 96% relative humidity resulted in no appreciable change in MSTF (−23.9°C) and a slight increase to −25°C by LST66. DTA profiles from this humidity regime were also spread out with a flattened free water exotherm. The average moisture content of buds from this treatment was 44.8%.

Buds of the hardy hybrid were also tested for DTA exotherms in February following severe outdoor cold conditions when the min temperature was −29°C and the average RH was 64%. Differential thermal analysis at 8°C/hr was carried to −43°C. Of 6 buds taken from these conditions, none showed exotherms. Both free water and floret exotherms were absent.

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Fig. 3. Typical DTA profiles of 4 'Rancocas' flower buds taken from field grown plants and cooled at 4 rates: 65°C/hr, 33°C/hr, 9°C/hr and 6.5°C/hr.

Fig. 4. Typical DTA profile of a 'Rancocas' flower bud cooled at 8°C per hr.
FIELD CONDITIONS

Table 1. The relationship between flower bud survival determined by mean survival temperature of the florets (MSTF) calculated from differential thermal analysis (DTA); lowest survival temperature (LST\text{66}) calculated from visual ratings under artificial freeze regimes and moisture content.

<table>
<thead>
<tr>
<th>Relative humidity</th>
<th>DTA (MSTF/bud)</th>
<th>Average moisture content of 4 buds/unit dry weight</th>
<th>LST\text{66}/9 buds</th>
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<tbody>
<tr>
<td>100%</td>
<td>-10.5°</td>
<td>106.5%</td>
<td>-10°</td>
</tr>
<tr>
<td>98%</td>
<td>-11.1°</td>
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<td>96%</td>
<td>-12.4°</td>
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<td>98%</td>
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<tr>
<td>Overall (\bar{x}) = &amp; -11.2° ± 0.75</td>
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<td>98%</td>
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<td>Overall (\bar{x}) = &amp; -11.2° ± 0.75</td>
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<td>96%</td>
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<td>Overall (\bar{x}) = &amp; -11.2° ± 0.75</td>
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Fig. 5. Typical DTA profiles of 'Rancocas' flower buds from 4 relative humidity regimes: field conditions (January, 1976) and after 1 week at 100%, 98% and 96% RH.

and the DTA profile remained totally flat. Exotherms would not be likely to appear below this temperature because homogenous nucleation takes place at about \(-40^\circ\) and is the last exotherm to be seen in most plant tissues.

Buds taken from this freezing test were then held at 100% RH for 6 days and sectioned. Five out of the 6 buds were still alive even after cooling to this low temperature. A corresponding determination of floret hardiness by artificial freezing was carried to only \(-25^\circ\)C and all buds were viable.

Buds taken from the same field conditions which were held for 1 week at RH of 100%, 98% and 96% also did not show DTA exotherms. Free water exotherms and floret exotherms were absent in all cases. Buds from each RH regime were sectioned 6 days after the DTA test. All buds from the 100% RH regime were killed by DTA freezing to \(-40^\circ\)C. The corresponding LST hardiness test was run only to \(-20^\circ\) at which point buds were still alive. Therefore, buds from the 100% RH regime were killed somewhere between \(-20^\circ\) and \(-40^\circ\). Two buds from the 100% RH regime were also tested in the differential scanning calorimeter (DSC). No exotherms appeared, which indicated an absence of freezable water. These 2 buds had moisture contents of 33% and 37% respectively.

Buds which had been held at 98% and 96% RH also did not show exotherms. However, almost all buds remained viable even after freezing to \(-40^\circ\)C, which contrasted with the death of buds from the 100% RH regime. The LST hardiness test showed that buds from 98% and 96% RH were hardy to at least \(-30^\circ\), the lower limit for this test.

**Discussion**

Examination of buds by sectioning and observing visual browning revealed that terminal buds are least hardy. Terminal florets were also slightly less hardy when compared to medially and basally located florets. The basal florets were hardest. Routine hardiness tests of blueberry should take this difference into account to avoid undue variation of results.

DTA of blueberry flower buds revealed profiles very similar to those reported for azalea (3, 6, 7) and several Prunus species (16). The configuration of the DTA profile is rate dependent. This is also a characteristic of azalea buds (6). Fast freezing rates showed profiles with one major free water exotherm, often with smaller peaks initiated on top of the large peak. Apparently, ice growth was very fast and the free, extracellular ice "seeded" the floret cells, resulting in their death. A slower freezing rate produced "spread out" profiles with one large free water exotherm followed by smaller floret exotherms. Fig. 3 shows that the profile for 9°/hr is more spread out than the profile for 6.5°/hr. This may be explained by variability in hardiness of terminal buds sampled and by the small rate difference between 9°/hr and 6.5°/hr compared to the larger difference between 33°/hr and 9°/hr. Therefore, results for the 2 slowest rates and 8°/hr (Fig. 4) may be expected to overlap.
An average of the floret exotherms or MSTF provided an assessment of cold resistance which was similar to an assessment from scoring artificially frozen buds by floret browning, LST66.

The free water exotherm was observed at about —10°C, regardless of freezing rate. Thus, extracellular ice formation probably behaved similarly to that in lettuce seeds (8) where it neither caused injury nor entered into hardness determinations except with florets which were seeded at very fast freezing rates. In contrast, floret exotherms were rate dependent and were the only peaks used to determine hardness by MSTF. Graham (7) has noted that the exotherm initiation point of excised Rhododendron florets is neither rate dependent nor correlated to its natural freezing point. Bittenbender and Howell (1) have tested excised florets for freezing point determinations. Their results show that the exotherm initiation point is not rate dependent and they have concluded that the Average Exotherm Temperature (AET) is unsuitable for hardness tests. Since they used excised florets for hardness determinations by AET, the following should be considered. An excised floret of azalea exhibits one exotherm which is not rate dependent nor correlated to lethality (7). Our research shows that each intact floret within an intact blueberry flower bud exhibits one low temperature exotherm which is rate dependent and is correlated to death at slow freezing rates. The exotherm of an excised floret, described by Bittenbender and Howell (1), may be analogous to the free water exotherm which is seen while freezing an intact bud and which is not necessarily analogous to a low-temperature exotherm. Neither is rate dependent and neither determines the absolute hardness of the florets. DTA of intact buds at “slow freezing rates” and calculation of MSTF provides an accurate indication of hardness and does correspond to hardness determination by floret browning, LST66.

Previously killed buds exhibited DTA profiles with one large exotherm and no floret exotherms. Quamme (16) speculated that exotherms are related to a structural feature of the cells. Levitt (9) has stated that supercooling is partly a function of cell membrane continuity and compartmentalization. Dead floret cells have probably lost membrane structure; therefore, supercooling does not take place and floret exotherms do not appear. Buds which had been dead for a few days always exhibited the free water exotherm.

Bud hydration appears to be an important factor in flower bud hardiness. Blueberry buds may enter the winter in a dehydrated state. In the fall, dehydration may be initiated by short days as McKenzie et al. (11) have shown with Cornus stolonifera, root resistance increased and stomatal resistance decreased coincidentally with the first stage of acclimation. Dehydration of tissues also probably takes place by extracellular ice formation during freezing temperatures. Artificial tissue hydration in midwinter had a dehardening effect on blueberry buds as can be seen in Fig. 5 for the 100% RH regime. Artificial dehydration in an environment of 98% RH resulted in a 10°C hardiness increase over fully hydrated buds. Dehydration by a relative humidity of 96% increased hardiness even further. Hardiness increased linearly as bud hydration decreased.

Under field conditions, blueberry flower buds dehydrated naturally and at the same time showed an increase in hardiness to at least —40°C. Apparently, low temperatures and low atmospheric relative humidity cause buds to lose all freezeable water, as evidenced by loss of DTA and DSC exotherms. It is interesting to note that an increase in hardness by dehydration may be accomplished by either extracellular freezing or low relative humidity.

Buds which were excised from their supporting twigs did not rehydrate, as evidenced by failure of exotherms to reappear, even when stored under 100% RH for 1 week. Rehydration probably occurs from the stem to the bud via vascular tissues in an undisturbed situation.

It may also be noted that although buds which were held at 100% RH for 6-7 days did not redevelop floret exotherms, they also did not remain viable to —40°C as they were prior to the rehydration treatment. Buds which were exposed to 98% and 96% RH also did not redevelop exotherms and were hardy to —30°C as measured by LST tests. This is probably related to the inability to rehydrate following excision from the stem even under 100% RH. The loss of 10°C hardness without the reappearance of exotherms during the attempted rehydration treatment, is apparently related to some process other than hydration of cellular components which can be detected by DTA.

It appears that the loss of floret low-temperature exotherms under extreme cold and low RH and the associated increase in hardness represent a survival mechanism, unique to the cold tolerant hybrid, which is not present in ‘Rancocas’. Therefore, fruit breeders who are working with cold tolerance in blueberries may wish to consider DTA as a rapid means of identifying this mechanism.

Snow cover plays an important role in insulating prostrate plants, such as strawberries, from winter cold and it has also been observed that blueberry flower buds will survive very cold winters if the flower buds have been covered by snow, even if they are up to 1 m above the soil surface. It is difficult to rationalize that the uppermost buds are being protected by insulation of soil heat as is the case with low growing plants. The observations regarding dehydration and bud hardiness, however, lead to the speculation that snow-ice crystals may protect by providing a stable vapor pressure gradient in the immediate vicinity of the buds. Thus, water may move out of the bud to external ice nuclei in the snow at a gradual rate or come to equilibrium as opposed to being exposed to wide, diurnal fluctuations in temperature and RH of the air above the snow line.

Literature Cited


Materials and Methods

Rootstocks were Swingle citrumelo (C.P.B. 4475) (Citrus paradisi × Poncirus trifoliata (L.) Raf.); Morton and Troyer citrange (Citrus sinensis (L.) Osbeck × P. trifoliata; rough lemon (C. jambhiri Lush.); Colombian sweet lime (Citrus limettoides Tan.); sour orange (C. aurantium L.); Smooth Seville or Australian sour orange (C. aurantium ?); Nansho daidai (Citrus taiwanica Tan. & Shim.); Cleopatra mandarin (Citrus reshni Hort. ex Tan.); Tachibana orange (Citrus tachibana (Mak.) Tan.); Precoce de Valence and Saccari sweet orange (Citrus sinensis (L.) Osbeck); C58-229 (Rangpur lime × Troyer citrange); Yuzu (Citrus junos Sieb. ex Tan.); Karna Khatta (Citrus karna Raf.); Ortanique (probable hybrid Citrus sinensis × C. reticulata Blanco); Scarlet Emperor, Soh Siem, and Sanguinea mandarins (C. reticulata); Orlando tangelo (Citrus paradisi × C. reticulata); Assam lemon (Citrus assamensis Dutta & Bhatt.); and cuttings of California nucellar ‘Redblush’ (CES #3) grapefruit.

The fruit was harvested in mid-December each year. All fruit was removed from the trees at harvest and size 96 or larger was designated as commercially acceptable fruit. The size 96 is the minimum commercially acceptable fruit in South Texas. The yield of trees on nine rootstocks may have been affected by phytophthora foot rot.

Results and Discussion

Trees on Swingle had the highest yields, and the 5-year mean yield was significantly (5% level) greater than from other rootstocks. Earlier reports were on the first production year when tree size was still increasing. Texas is experiencing the longest period without a major freeze since the rootstock research program began. Catastrophic losses occurred as a result of freezes in 1951 and 1962. In 1963, trees on rootstocks showing potential for high yield and tolerance to tristeza virus were included in a test planting at the Texas A&M University Research and Extension Center, Weslaco. The first 6-years production (1967-1972) from this planting have been reported (3, 7). Yield and tree performance of nucellar ‘Redblush’  (CES #3) grapefruit on these rootstocks after 14-years of evaluation and their adaptability for use in South Texas are presented here.

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2 Assistant Professor and Associate Professor, respectively.


Performance of Mature Nucellar ‘Redblush’ Grapefruit on 22 Rootstocks in Texas.1

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Abstract. Five-year mean yield from 14-year-old nucellar ‘Redblush’ (CES #3) grapefruit (Citrus paradisi Macf.) on 22 rootstocks was significantly greater on Swingle citrumelo 4475, C58-229 (Rangpur lime × Troyer citrange) and Troyer citrange than on the standard sour orange used in Texas. Percentage of commercially desirable fruit (size 96 and larger) was greatest on rootstocks having the highest yield. The yield of trees on nine rootstocks may have been affected by phytophthora foot rot.
