Supercooling and Cold Hardiness in Sour Cherry Germplasm: Flower Buds

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ABSTRACT. Flower buds of two sweet cherry (*Prunus avium* L.), 12 sour cherry (*Prunus cerasus* L.) and one ground cherry (*P. fruticosa* Pall.) were collected monthly from Aug. 1990 to Mar. 1991, and subjected to freeze tests to determine the level of cold hardiness. LT₅₀ values (temperatures at which 50% of the flower buds were killed) summed over all months were significantly correlated ($r = 0.6844$, $P \leq 0.01$) to the flower bud low temperature exotherms (LTEs). Correlation of LTEs to LT₅₀ values was highest, $r = 0.85$, $P \leq 0.01$ for the acclimation and midwinter period, November to February collections. During this period the average LT₅₀ occurred before and within 2.5 °C of the LTE, indicating tissue injury before the LTE occurrence. During deacclimation, represented by the March collection, the LT₅₀ began within 2.0 °C, on average, of the LTE, and in 11 of 12 cultivars and seedlings preceded the LTE. In March, the correlation of LTEs to LT₅₀ values was less, $r = 0.69$, $P \leq 0.05$, indicating possible changes flower bud deep supercooling. LTE values were selected as a measure of flower bud hardiness in sour cherry. Exotherms were not detected in the flower buds of all germplasm tested on all evaluation dates, but were the best means of separating selections. While LTE analyses expressed significant differences in November, December, and March at $P \leq 0.01$, the LT₅₀ analyses expressed differences only in December and February at $P \leq 0.05$. The relationship between ambient temperatures and floral tissue hardiness indicated that November and March are two critical times for flower bud injury. November injury would occur in years when sudden low temperatures occur without sufficient pre-exposure to freezing temperatures. March injury would occur in years when sudden freezing temperatures follow warm days. This type of injury would be most pronounced in southern genotypes. Spring freeze injury could be significantly reduced by the selection of cultivars and seedlings that have delayed deacclimation. Exotherm occurrence and bud volume were correlated ($r = 0.95$, $P \leq 0.05$). In January, when exotherms were least prevalent, they were generally present only in the five cultivars and seedlings with large bud volumes. The LTEs in midwinter, occurred within 3 °C of the reported average annual minimum temperature for the northern range of *Prunus* commercial production (Zone 6). The results of the principal component analysis of flower bud LTEs indicated that other selection criteria as flowering time might have played a more significant role in the hardiness range of sour cherry than simply geographic origin. The first principal component (PC1), which accounted for 77% of the total variance was used to separate among cultivars and seedlings. Selections at the positive end of PC1 had flower buds that were more cold susceptible than selections at the negative end of PC. This concurs with other research showing that flower bud hardiness is related more to commercial range (i.e., the range of commercial production) than to geographic distribution.

Sour cherry yields in Michigan are reduced significantly one out of three years by spring freeze injury to floral tissues. The lack of consistent crop availability, from year to year, is a major factor limiting market development (Flore and Howell, 1987). The temperature at which flower buds are injured depends on the stage of development (Iezzoni and Hamilton, 1985). One analytical procedure frequently used in cold hardiness and freezing injury studies is exotherm or differential thermal analysis (DTA). In DTA, heat of fusion is detected by recording the temperature difference between a dry reference sample and a wet sample during freezing. The type of freezing observed by DTAs occurs in plants that resist freezing by supercooling. The DTA profile in species which deep supercool indicates two exotherms. The first is associated with extracellular freezing that results from heterogeneous nucleation and is called the high temperature exotherm (HTE). The second exotherm is associated with the

tissues that supercool to the homogeneous nucleation temperature and is termed the low temperature exotherm (LTE) (Ketchie and Kammereck, 1987; Quamme, 1985). In many *Prunus* flower buds, the location of ice formation is dispersed between different tissue types and is referred to as “extraorgan” freezing (Quamme et al., 1982). DTA evaluations cannot stand-alone. The DTA data are correlated with qualitative viability tests. Visual browning was the viability test chosen for this study. Unlike the relationship between geographic distribution and twig supercooling, there has been no obvious relationship found between origin and flower bud hardiness (Burke and Stushnoff, 1979).

The finding that deep supercooling occurs in *Prunus* flower buds has implications to breeding programs in which the objective is to improve cold hardiness. Exotherm analysis can be used to measure varietal differences in flower bud hardiness (Quamme, 1985). Knowledge of the expression of deep supercooling mechanisms in different species and cultivars should allow better identification of the germplasm required for making the improvements.

Deep supercooling of the flower bud appears to depend on water content (Kadir and Proebsting, 1994a; Kovacs et al., 2002). In many *Prunus* flower buds, the location of ice formation is dispersed among different tissue types and is referred to as “extraorgan” freezing (Sakai, 1979). At least one barrier to external nucleation of the supercooled flower primordium is known to occur in many *Prunus* species; a “dry region” at the primordia

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bases originates and prevents the ice boundary from spreading into the scales during the initial stages of freezing (Quamme, 1995). The formation of the "dry region" at the primordium base results when water is withdrawn from the bud axis to freeze in the bud scales; therefore, water is not withdrawn from the flower primordium (Quamme, 1978). Quamme et al. (1995) have shown that at least the bottom two bud scales are essential for supercooling to occur. Water freezes at higher temperatures in the bud scales and lower bud axis because these external tissues of the flower bud are lower in solute concentrations.

Determinations of cold hardiness must also involve an assessment of the seasonal changes in the hardiness values exhibited by different germplasm selections. Quamme (1983) conducted hardiness determinations on peach (*Prunus persica*) flowers from mid-October through early March. Much of the fluctuation in flower bud hardiness during midwinter appears to result from changes in air temperatures that cause redistribution of water during freezing and thawing and affect deep supercooling (Quamme, 1983). Andrews and Proebsting (1987) also examined the relationship between ambient temperatures and hardiness of dormant and deacclimating sweet cherry flower buds. They concluded that ambient temperatures influenced water content regulation in the flower primordia and in supercooling. Kadir and Proebsting (1993) found that water compartmentalization continued even after death of the floral tissue.

At the whole plant level, resistance to cold is a complex quantitatively inherited character (Cummins and Aldwinkle, 1983). Principal Component (PC) analysis is a multivariate statistical technique which generates linear combinations of variables (Llorens et al., 2002; Rao, 1964) that has been used successfully to understand the response of complex traits to imposed treatments or evolutionary pressures (Iezzoni and Pritts, 1991). When a large number of measurements are available in the data set, PC analysis provides a means of reducing the data to the variables of most interest without loss of significant information (Llorens et al., 2002; Rao, 1964; Tausz et al., 1998). When variables are highly intercorrelated, PC analysis can be used to separate plant material based on performance or loading on the PCs (Lo and Abrahamson, 1996).

Understanding cold resistance heritability and environmental influence in sour cherry is critical to cultivar development and future germplasm collection. A diverse sour cherry germplasm collection was established at Michigan State Univ. (MSU), Clarksville Horticultural Experiment Station, Clarksville, Mich. (Hillig and Iezzoni, 1988). The scope and diversity of this germplasm collection provided an excellent opportunity to investigate supercooling and cold hardiness in sour cherry. The four objectives of this study were: 1) to compare differential thermal analysis (DTA) and visual browning for measuring minimum hardiness levels of sour cherry floral buds; 2) to define the critical injury times within germplasm from November to March; 3) to investigate possible

relationships of bud volume to exotherm occurrence, and 4) to use PC analysis to investigate possible relationships of flower bud survival temperature to geographic origin.

Material and Methods

PLANT MATERIAL. The plant material included two *Prunus avium* selections, one *P. fruticosa* selection, and 12 *P. cerasus* selections chosen to represent different geographical origins (Table 1). All studies were conducted on flower buds from detached shoots of the previous season's growth collected at the MSU, Clarksville Horticultural Experiment Station. A three-way treatment structure of taxa, freeze temperature and month of evaluation was used in a completely randomized design with three subsamples and four replications per treatment, i.e., 12 twigs per treatment were evaluated. Two studies were conducted, one in Jan. 1990 and an 8-month study from Aug. 1990 to Mar. 1991. All twigs were cut in the field and placed in polyethylene bags for transit and storage. When collections were conducted at 0 °C or below, the polyethylene-bagged samples were placed in ice-packed coolers to prevent tissue thawing.

ACCLIMATION AND FREEZING TREATMENTS. All twigs with flower buds were subjected to one of two artificial hardening procedures, depending on the hardiness of the material at the time of collection (Sakai, 1982). For December through March collections, twigs were hardened at -3 °C for 3 weeks followed by -5 °C for 1 week and -10 °C for 5 d and for August through November, 0 to -1 °C for a maximum of 20 d (Sakai, 1982). These artificial hardening treatments were used to induce uniform hardiness in order to minimize environmental variation (Gusta et al., 1997; Sakai and Weiser, 1973) and are used in woody plant cold hardiness evaluations to replace replications over years (Quamme, 1976; Sakai and Weiser, 1973). After artificial hardening samples were prepared according to McKenzie and Weiser (1975) and frozen in a converted ultralow Revco freezer (Mathers et al., 1991). The freezing treatment involved two stages: a holding period at -3 °C for 10 h followed by a temperature drop of 5 °C/h from -5 to -50 °C with the aid of a programmable temperature

Table 1. Names, abbreviations, presumed origins, and type designations of the cultivars and seedlings evaluated.

Name	Abbr.	Presumed origin	Type
<i>Prunus avium</i>			
Emperor Francis	F	Unknown	Southern
Schmidt	S	Unknown	Southern
<i>Prunus cerasus</i>			
Cigany Meggy o.p.	G	Hungary	Southern
Csengodi Csokras o.p.	K	Hungary	Southern
English Morello	E	Germany	Northern
English Morello X Sumadinka	U	Germany X Yugoslavia	Northern
Fructbare von Michurin	V	Moldavia	Northern
Meteor	R	Montmorency X Russian Seedling	Northern
Montmorency	M	France	Northern
Oblacinska o.p.	O	Yugoslavia	Southern
Pandy 114 o.p.	A	Hungary	Southern
Pitic de Iasi o.p.	I	Moldavia	Northern
Spaniole X Crisana o.p.	C	Spain X Romania	Southern
Wolynska X Sumadinka	W	Poland X Yugoslavia	Northern
<i>Prunus fruticosa</i> 586-1	P	Unknown	Northern

controller (Micron 82300; Research Inc., Minneapolis). At each 5 °C interval test temperature, four replicates were removed and thawed at 0 °C for 16 h.

FLOWER BUD HARDINESS DETERMINATIONS AND VOLUME DETERMINATIONS. Flower bud hardness was determined by examining cross sections of three freeze-thawed buds per twig under a dissecting 40× microscope and expressing survival as the temperature at which 50% of the flower buds were killed (LT₅₀). Means of the three flowers buds per twig were used for the statistical analyses. The LT₅₀ values were calculated according to the method of Bittenbender and Howell (1974). Flower bud volumes were determined by water displacement, measured in mL and converted to mm³. Analyses of variance were conducted using SAS procedure General Linear Model (GLM) (SAS Institute, Cary, N.C.). Fisher's least significance difference test was used to compare means (SAS Institute, 1989).

LTE DETERMINATIONS FOR FLOWER BUDS. For LTE determinations in dormant flower buds, a small shield of vegetative tissue bearing the flower bud and subtending leaf scar was excised from the shoot and attached to thermoelectric modules (4.2 × 4.2 mm) (FC 0.6-8-05L; Melcor Materials Electronic Products Corp., Trenton, N.J.). The samples were affixed to each module with the aid of a thermally conductive paste (Omegatherm 201; Omega Engineering, Stamford, Conn.) (Andrews et al., 1983). Four samples per cultivar and seedling were evaluated at each of five collection dates. LTE values presented represent the means of three florets per bud and four samples per collection date. Analyses of variance were conducted using SAS procedure GLM. Fisher's least significant difference test was used to compare means (SAS Institute, 1989).

PC ANALYSIS. Principal component (PC) analysis was performed using the flower bud LTEs from each of 5 months and the PRINCOMP procedure in SAS (SAS Institute, 1989). Since PC analysis is intended to reveal common principles in the data sets (Tausz et al., 1998), data were pooled over evaluation temperatures and replications. Factor loading can be interpreted as correlations between the respective variables and components (Llorens et al., 2002). To determine which of the first two PCs in each analysis accounted for the greatest portion of the variance for each month, the eigenvectors of the two PCs were compared (Currie et al., 2000; Llorens et al., 2002). The most variation attributed by each month was determined by identifying the eigenvectors with the highest absolute value for that month (Llorens et al., 2002).

ORCHARD AIR TEMPERATURES. Air temperatures were measured in an instrument shelter 1.5 m above the ground and at maximum, 350 m from the orchard at Clarksville, Mich. The equipment used was a Campbell Scientific HM-P 35C Temperature and Relative Humidity Probe (Campbell Scientific, Logan, Utah) calibrated annually against readings from a National Weather Service thermometer.

Results

CORRELATION OF SUPERCOOLING TO TISSUE INJURY. Only five selections (Csengodi Csokas o.p., Pandi 114 o.p., 'English Morello' × Sumadinka, Cigany Meggy o.p., and 'Montmorency') consistently exhibited LTEs during November through March (Tables 2–5). The fewest number of selections exhibiting LTEs occurred in January (data not shown). Despite the reduced number of selections exhibiting exotherms, flower bud LTEs were significantly different among selections when sampled in November (Table 2), December (Table 3), and March (Table 5).

Table 2. Comparison of a November collection of 15 cherry cultivar/seedlings evaluated by low temperature exotherm (LTE) analysis conducted at –5 °C/h and by visual injury of floral bud cross sections frozen to –35 °C at –5 °C/h and presented as survival temperatures at which 50% of the flower buds were killed (LT₅₀).

Cultivar/seedling	Flower bud LTE	Flower bud LT ₅₀
<i>Prunus avium</i>		
Emperor Francis	---	–11.0 a
Schmidt	---	–12.0 a
<i>Prunus cerasus</i>		
Csengodi Csokas	–12.3 b ^z	–16.0 a
Spaniole X Crisana	---	–17.0 a
Pandi 114	–10.7 b	–13.0 a
Pitic de Iasi	–16.5 c	–18.3 a
Wolynska X Sumadinka	–12.3 b	–16.0 a
English Morello X Sumadinka	–8.0 a	–17.5 a
Oblacinska	–17.5 c	–20.0 a
Cigany Meggy	–11.3 b	–13.3 a
Montmorency	–16.0 c	–18.0 a
Fructbare von Michurin	–17.5 c	–18.0 a
English Morello	---	–17.0 a
Meteor	---	–23.0 a
<i>P. fruticosa</i> 586–1	–18.5 c	–15.0 a

^zMeans in columns, based on three subsamples, per four replicates, followed by different letters are significantly different ($P=0.05$) according to Fisher's least significant difference test.

Table 3. Comparison of December collection of 15 cherry cultivar/seedlings evaluated by low temperature exotherm (LTE) analysis conducted at –5 °C/h and by visual injury of floral bud cross sections frozen to –50 °C at –5 °C/h and presented as survival temperatures at which 50% of the flower buds were killed (LT₅₀).

Cultivar/seedling	Flower bud LTE	Flower bud LT ₅₀
<i>Prunus avium</i>		
Emperor Francis	---	–14.0 a
Schmidt	---	–15.0 a
<i>Prunus cerasus</i>		
Csengodi Csokas	–12.5 b ^z	–15.5 b
Spaniole X Crisana	---	–18.3 b
Pandi 114	–12.3 b	–15.0 a
Pitic de Iasi	---	–18.3 b
Wolynska X Sumadinka	–17.5 c	–19.3 b
English Morello X Sumadinka	–14.0 b	–16.5 a
Oblacinska	–17.5 c	–20.5 b
Cigany Meggy	–10.7 a	–13.7 a
Montmorency	–17.0 c	–20.0 b
Fructbare von Michurin	–15.0 c	–18.0 b
English Morello	---	–18.0 b
Meteor	---	–23.0 b
<i>P. fruticosa</i> 586–1	–14.0 b	–17.0 b

^zMeans in columns, based on three subsamples, per four replicates, followed by different letters are significantly different ($P=0.05$) according to Fisher's least significant difference test.

In November, there were significant differences in the flower bud LTEs but not in the LT₅₀ values (Table 2). In December, there were significant differences in the flower bud LTEs and the LT₅₀ values but the LTEs and LT₅₀ values gave different results (Table 3). With the LT₅₀ values, the majority of northern genotypes were significantly different from southern genotypes except for 'English Morello' × 'Sumadinka.' In contrast, LTE values in December separated northern genotypes into two significantly different

Table 4. Comparison of a February collection of 15 cherry cultivar/seedlings evaluated by low temperature exotherm (LTE) analysis conducted at -5°C/h and by visual injury of floral bud cross sections frozen to -45°C at -5°C/h and presented as survival temperatures at which 50% of the flower buds were killed (LT_{50}).

Cultivar/seedling	Flower bud LTE	Flower bud LT_{50}
<i>Prunus avium</i>		
Emperor Francis	-17.0 a^z	-20.0 a
Schmidt	---	-20.0 a
<i>Prunus cerasus</i>		
Csengodi Csokas	-22.0 a	-23.5 b
Spaniole X Crisana	-16.7 a	-19.0 a
Pandy 114	-20.0 a	-23.0 b
Pitic de Iasi	-19.0 a	-21.7 b
Wolynska X Sumadinka	-18.0 a	-20.3 a
English Morello X Sumadinka	-17.5 a	-20.5 a
Oblacinska	-20.0 a	-22.0 b
Cigany Meggy	-18.3 a	-20.3 a
Montmorency	-22.0 a	-25.0 c
Fructbare von Michurin	-20.0 a	-24.0 b
English Morello	-22.0 a	-25.0 c
Meteor	---	-26.0 c
<i>P. fruticosa</i> 586-1	---	-27.0 c

^zMeans in columns, based on three subsamples, per four replicates, followed by different letters are significantly different ($P=0.05$) according to Fisher's least significant difference test.

Table 5. Comparison of a March collection of 15 cherry cultivar/seedlings evaluated by low temperature exotherm (LTE) analysis conducted at -5°C/h and by visual injury of floral bud cross sections frozen to -40°C at -5°C/h and presented as survival temperatures at which 50% of the flower buds were killed (LT_{50}).

Cultivar/seedling	Flower bud LTE	Flower bud LT_{50}
<i>Prunus avium</i>		
Emperor Francis	-13.0 a^z	-10.0 a
Schmidt	---	-18.0 a
<i>Prunus cerasus</i>		
Csengodi Csokas	-18.0 b	-18.0 a
Spaniole X Crisana	-20.0 c	-14.7 a
Pandy 114	-15.3 b	-16.3 a
Pitic de Iasi	-22.3 c	-20.3 a
Wolynska X Sumadinka	-21.0 c	-20.3 a
English Morello X Sumadinka	-22.0 c	-19.5 a
Oblacinska	-20.0 c	-20.0 a
Cigany Meggy	-21.0 c	-15.3 a
Montmorency	-19.0 c	-20.0 a
Fructbare von Michurin	-18.0 b	-18.0 a
English Morello	-22.0 c	-18.0 a
Meteor	---	-27.0 a
<i>P. fruticosa</i> 586-1	---	-19.0 a

^zMeans in columns, based on three subsamples, per four replicates, followed by different letters are significantly different ($P=0.05$) according to Fisher's least significant difference test.

hardiness classes (Table 3). Again, flower bud LTEs expressed more differences among cultivars and seedlings than LT_{50} values. The differences in LTEs were significant at $P \leq 0.01$ in December, whereas, differences in LT_{50} values were significant at $P \leq 0.05$. In January, significant differences were not found in flower bud LTEs or LT_{50} values (data not shown). In February, only LT_{50} values indicated differences among cultivars and seedlings (Table 4). In March, however, only LTEs showed cultivar and seedling differences (Table 5).

Most northern type cultivars and seedlings had significantly smaller buds, except for 'English Morello' x 'Sumadinka', 'Pitic de Iasi' and 'Spaniole' x 'Crisana' (Table 6). Csengodi Csokras o.p. had significantly larger buds than any other cultivar or seedling evaluated (Table 6). Southern genotypes ranged in size from 0.76 to 0.32 mm^3 with an average size of 0.43 mm^3 . Northern genotypes ranged from 0.53 to 0.19 mm^3 with an average size of 0.34 mm^3 . No relationship appeared to exist between geographic origin and number of bud scales or number of florets per bud (Table 6). Exotherm occurrence and bud volume were correlated ($r = 0.950$, $P \leq 0.05$).

For the acclimation and midwinter period, November to February collections, correlation of LTEs to LT_{50} values was strongest, $r = 0.85$, $P \leq 0.01$. During this period the average LT_{50} occurred before and within 2.5°C of the LTE, indicating tissue injury before the LTE occurrence (Tables 2–4), January (data not shown). In deacclimation, represented by the March collection, the LT_{50} began within 2.0°C , on average, of the LTE, and in 11 of 12 cultivars and seedlings preceding the LTE (Table 5), as in November to February evaluations. In March, the correlation of LTEs to LT_{50} values was less, $r = 0.69$, $P \leq 0.05$, indicating possible changes in the flower bud reducing deep supercooling occurrence.

RESPONSE DIFFERENCES IN TISSUE. The analysis of variance (ANOVA) of flower bud LTEs showed significant differences due to the main effects of taxa and month and the interaction of taxa x month (data not shown). The ANOVA of the LT_{50} data showed significant differences due to the main effects of taxa and month and the interaction of taxa x month (data not shown). The significant interaction in the LT_{50} analyses reflects differential hardiness changes among cultivars and seedlings over time (Fig. 1). In December (Table 3) and February (Table 4) LT_{50} means indicated significant differences among cultivars and seedlings; however, there were no significant differences in LT_{50} means in November (Table 2) and March (Table 5). The industry standard *P. cerasus* 'Montmorency' was harder than all the *P. cerasus* seedlings evaluated, summed over all evaluation dates (Fig. 1).

RELATIONSHIP OF AMBIENT TEMPERATURES. High temperatures in the deacclimation period reduced the capacity of flower buds to avoid freezing. This was particularly evident in the southern genotypes (data not shown). *Prunus cerasus* 'Meteor', 'Pitic de Iasi', 'Wolynska' x 'Sumadinka', 'Oblacinska' and 'Montmorency' deacclimated significantly later in March (Table 5) than *P. avium* 'Emperor Francis'.

FLOWER BUD PC ANALYSIS. Given the complexity of the data collected over 5 months of evaluation, data were subjected to PC analysis, which generates linear combinations of variables that contain most of the variability (Llorens et al., 2002; Rao, 1964). Linear combination, named "principal components" (PCs), allow for calculating the scores of each component for every individual measurement (Llorens et al., 2002). In this study, in the analysis of the flower bud LTEs, the first two PCs account for 89% of the total variance among cultivar and seedling means, i.e., for 77% (PC1) and 12% of the variance (PC2), respectively. The original variables (month) can then be expressed as linear combinations of these components (Llorens et al., 2002). Factor loadings can be interpreted as correlations between the variables (months) and components (Tausz et al., 1998). Data from all 5 months, November 1990 to March 1991, contributed to the variation along PC1 (data not shown). Selections at the positive end of PC1 had flower buds that were more cold susceptible than selections at the negative end of PC1 (Fig. 2). 'Meteor' (R) and 'Emperor Francis' (F) are situated at the extremes of PC1, representing

Table 6. Names, type designations, bud volumes, number of scales per bud, and number of flowers per bud for the cultivars and seedlings evaluated.

Name	Type designation	Vol. of bud (mm ³)	No. of bud scales	No. of flowers/bud
<i>Prunus avium</i>				
Emperor Francis	Southern	---	---	---
Schmidt	Southern	0.36 c ^z	6	3
<i>Prunus cerasus</i>				
Cigany Meggy	Southern	0.37 c	6	3
Csengodi Csokras	Southern	0.76 a	7	3
English Morello	Northern	---	---	---
EM ^y X Sumadinka	Northern	0.53 b	6	4
Fructbare von Mic. ^y	Northern	0.27 d	7	3
Meteor	Northern	0.28 d	6	3
Montmorency	Northern	0.23 d	6	3
Oblacinska	Southern	0.37 c	6	3
Pandy 114	Southern	0.43 b	6	3
Pitic de Iasi	Northern	0.46 b	6	3
Spaniole X Crisana	Southern	0.32 c	6	3
Wolynska X Sum. ^y	Northern	0.40 c	7	3
<i>Prunus fruticosa</i> 586-1	Northern	0.19 d	6	3

^zMeans in columns, based on five replicates, followed by different letters are significantly different ($P = 0.05$) according to Fisher's least significant difference test.

^yEM, Mic. and Sum. represent English Morello, Michurin, and Sumadinka, respectively.

their divergence in floral hardiness. 'Meteor' is a hardy outlier, with the closest cultivar with similar hardiness being its maternal parent 'Montmorency'. Proceeding from negative to positive values of PC2, the cultivars and seedlings decrease in hardiness in February. February represents the time of maximum floral cold resistance for all selections evaluated (Table 4). Ground cherry (P) and 'Spaniole' x 'Crisana' (C) are situated at the extremes of PC2, representing their differences in magnitude of floral midwinter hardiness. Seven of the 12 *P. cerasus* cultivars and seedlings evaluated, and all of the *P. avium*, are situated between *P. fruticosa* and Spaniole x Crisana; i.e., between 0 and -1.5 on the PC2 axis (Fig. 2).

Discussion

The observation that floral tissue browning occurred after the LTE is in agreement with previous studies of *Prunus* flower bud supercooling (Andrews and Proebsting, 1987; Quamme et al., 1982). Andrews and Proebsting (1987) reported that LT₅₀ values occurred from 0.1 °C to 2.8 °C after the LTEs. Quamme et al. (1982) observed LT₅₀ occurrences 3 °C after the LTEs, averaged over seven species and cultivars. Exotherm occurrences in February were similar to that reported by Andrews and Proebsting (1987) for *P. avium*. Andrews and Proebsting (1987) reported *P. avium* LTEs at -17.3 °C in 1983 and -19.9 °C in 1984, compared to the observed LTE occurrence at -17 °C. In March, their *P. avium* LTEs were again similar occurring at -14.5 °C in 1983 compared to my -13 °C. The observed *P. cerasus* 'Montmorency' LTE occurrence (-19 °C) was 12 °C higher than the *P. cerasus* 'Montmorency' LTEs (-31 °C) reported by Quamme et al. (1982). However, in my study *P. cerasus* cultivar LTE occurrences in February, representing maximum acclimation, were within 3 °C of -25 °C. The normal extent of flower bud supercooling is -25 °C and approximately coincides with the average annual minimum temperature at the northern range of *Prunus* commercial production (Quamme, 1978). My *P. cerasus* LTEs were also within 2.7 °C, on average, of those reported by Rajashekar and Burke

(1978). Other researchers have discovered significant correlations between LT₅₀ values and LTEs (Andrews and Proebsting, 1987; Quamme, 1976) and this study concurs with these cited studies.

The relationship between ambient temperatures and floral tissue hardiness indicates two critical times for flower bud injury, November and March. November injuries would occur in years when sudden low temperatures occur without sufficient pre-exposure to freezing temperatures. March injuries would occur in years when sudden freezing temperatures followed warm days. This type of injury was most pronounced in southern genotypes. Rodrigo (2000) indicates low temperatures occurring after maximum rest has past or deacclimation is occurring are usually more important than low winter temperatures in limiting crop production and distribution of deciduous tree fruits.

It has been noted in various plant species, that small tissue sections tend to test harder than large sections (Ashworth, 1990; Scarasascia-Mugnozza and Valentini, 1989). Kadir and Proebsting (1994b) noted a relationship between small bud volumes and lack of LTE occurrences in January that concurs with the findings of this study. They found that *Prunus* species with small buds had low water contents and lacked exotherms in December and January after 2 d at -7 °C. Kovacs et al. (2002) speculated that the relationship between tissue size and cold hardiness might reflect the effect of water evaporation.

Spring freeze injury could be significantly reduced by the selection of cultivars and seedlings that have delayed deacclimation. *Prunus cerasus* 'Meteor', 'Pitic de Iasi', 'Wolynska' x 'Sumadinka', 'Oblacinska', and 'Montmorency' deacclimated later in March than southern genotypes and *P. avium* cultivars. Gelvonauskis et al. (2000) found that winter hardiness of *Malus* sp. (Mill.) was controlled predominantly by additive gene action. Therefore, selection of parents based on their phenotypic response to cold would be an effective method for development of winter hardy apple cultivars. The results of this study indicate selection of the five *P. cerasus* northern type cultivars listed above with delayed deacclimation appears to make them advantageous choices for a sour cherry cold resistance breeding program based on Gelvonauskis et al. (2000) findings.

The lack of significant differences found among selections with either LTE evaluations or LT₅₀ evaluations, in January, corresponds to findings presented by Quamme (1986) for grapes. Quamme (1986) indicated that grape cultivars responded differently to preconditioning temperatures and that preconditioning may be used to improve cultivar separation. Exposure of buds to low temperatures for 3 d improved the separation of three grape cultivars in autumn when they were similar in hardiness. In midwinter, however, after they had already been exposed to low environmental temperatures there was no separation improvement (Quamme, 1986). We found a similar effect of preconditioning in sour and sweet cherry.

Kadir and Proebsting (1994a) reported that DTA of *P. avium* floral buds separated selections that clearly differed in floral bud hardiness from December to March. My findings are similar for *P. cerasus* except that preconditioning minimized cultivar

Fig. 1. Comparison of survival temperatures at which 50% of the flower buds were killed (LT_{50}) values in *P. cerasus* 'Montmorency' and three representative *P. cerasus* seedlings over all evaluation dates, Nov. 1990 to Mar. 1991.

Fig. 2. Position of Principal Component (PC) analysis scores for low temperature exotherms (LTEs) of flower buds of 15 cherry cultivar/seedlings evaluated over 5 months November to March on the first two PC axes. Abbreviations are as indicated in Table 1.

and seedling differences in midwinter (January to February) as previously discussed (Quamme, 1991). Because of correlation of flower bud LT_{50} values to their LTEs, and the LTE ability to separate selections in November, December, and March, LTE evaluations were the selected method for flower bud hardiness determinations in this sour cherry investigation.

PC analysis reveals common physiological (Tausz et al., 1998) or genetically inherited (Currie et al., 2000) principles in the data. This type of analysis assumes the presence of unobserved variables (PC factors) that underline the items or events originally measured (Tausz et al., 1998). PC analysis of flower bud LTE values evaluated for 5 months depict some gradations between

the northern and southern genotypes. However, human selective forces seem to have played a more significant role in the hardiness range of sour cherry flower buds than simply geographic origin or I would have seen a clearer separation between northern and southern genotypes along PC1. This is illustrated by the cultivar Meteor representing the extreme in midwinter cold resistance, a standard in the sour cherry industry. Also, this conclusion is further supported by my LTE determinations in midwinter, which occurred within 3 °C of the reported average annual minimum temperature for the northern range of *Prunus* commercial production (Zone 6). If midwinter hardiness were the major determinant in geographic origin, LTEs would have occurred close to known hardiness zonation values vs. commercial production values.

In February, the floral tissue reached its maximum cold resistance. Similar to reports by Kadir and Proebsting (1994a) for *P. avium*, floral hardiness decreased rapidly after early February. Kadir and Proebsting (1994a) showed that in early February, LTE occurrence in *P. avium* '7147-13' was at -19.8 °C. The LTE occurrence then decreased rapidly during late February through March to -13.9 °C. My February evaluations were conducted early, 9 Feb., and indicated *P. avium* 'Emperor Francis' LTE occurred at -17 °C. By the time I conducted the March collections, 8 Mar., the *P. avium* 'Emperor Francis' flower buds had deacclimated to -13 °C.

There was a large increase in hardiness from January to February. *Prunus fruticosa* increased in hardiness by 9 °C in this period and some of the *P. cerasus* selections by 5 and 6 °C. This increase in hardiness caused the high positive value on PC2 for February. If floral hardiness were related to geographic distribution, you would expect to see the clear separation along PC2 for southern and northern genotypes. This clear separation did not occur indicating, as Burke and Stushnoff (1979) reported, that flower bud hardiness is related to commercial range rather than geographic distribution. Sour cherry flower buds were hardy to Zone 6 in midwinter, but their critical injury times were during acclimation and deacclimation, limiting their commercial range. The cold susceptibility that I found in the flower buds, especially of southern genotypes in March, concurs with observations of Quamme et al. (1982) who considered that flower bud hardiness is probably more important to distribution of commercially grown fruits than those grown in home gardens or native fruit grown in the wild.

The first two PCs of the analysis of the flower bud LT_{50} data account for 77% and 12% of the variance, respectively (data not shown). 'Meteor' (R) and 'Emperor Francis' (F) are situated at the extremes of PC1, representing their divergence in floral hardiness. 'Meteor' is an outlier, with the closest cultivar being its maternal parent 'Montmorency'. Proceeding from negative to positive values of PC2, the cultivars and seedlings decrease in hardiness in February. February represents the time of maximum floral cold resistance for all selections evaluated. Stushnoff et al. (1985) discuss the presence of unilateral maternal inheritance in

Prunus. Nearly twice as many seedlings resistant to cold stress were obtained when the cold-hardy species, *P. americana*, was used as the female parent compared to the tender *P. salicina*. A maternal effect has been reported on the resistance of germinating apple seed to low temperature damage (Stushnoff et al., 1985). The effects along PC1 indicate some possible maternal inheritance with 'Meteor' an outlier, situated closest to 'Montmorency' its maternal parent.

One obvious requirement in any breeding program for hardiness is to be able to quickly develop hardy parental material with desirable commercial attributes. DTA evaluations can be used to accelerate breeding for cold tolerance in *P. cerasus*. The relationship between ambient temperatures and floral tissue hardiness indicates two critical times for flower bud injury, November and March. November injuries would occur in years when sudden low temperatures occur without sufficient pre-exposure to freezing temperatures. Spring freeze injury could be significantly reduced by the selection of cultivars and seedlings that have delayed deacclimation.

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