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Freeze Resistance of Pacific Northwest Strawberry Flowers

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Abstract. **The roles of freeze avoidance and freeze tolerance in determining strawberry (*Fragaria ×ananassa*) flower freeze resistance were compared in laboratory freeze tests. Genotype, freezing point depression of expressed cell sap, and flower size were examined as potential sources of variation in freeze resistance. When ice was added as a nucleator to excised flowers, mean freeze damage was 97% at –3.0 °C, but in the absence of ice, flowers appeared to supercool and had only 15% damage at –4.0 °C. Without nucleation, cultivar differences in freeze damage were significant in three of four freezing temperatures, but the relative ranking of cultivar freeze damage was not consistent across temperatures. Cultivars that sustained the least amount of injury at –4 °C, were not necessarily the least injured at –7 °C. With an ice nucleator, damage occurred at warmer temperatures (–1.5 °C), but there was no relationship between percentage damage at –1.5 °C with nucleation and –4 °C without nucleation across cultivars. Freezing-point depression of expressed cell sap did not account for the variation in freeze resistance. In nucleated and nonnucleated treatments, larger flowers were more likely to be freeze damaged. Results of this research suggest that flowers of all cultivars are susceptible to freeze damage and survive spring frosts by freeze avoidance.**

Crop loss caused by spring freeze damage to strawberry flowers is a recurring problem for strawberry growers. The critical temperatures that have been reported to produce flower freeze damage are quite variable. In controlled freeze tests, flowers from 21 strawberry cultivars grown in the eastern United States were shown to vary 3 °C in temperatures producing 10% flower injury from –2.5 °C for 'Gala' and 'Jerseybelle' to –5.5 °C for 'Earlidawn' (Ourecky and Reich, 1976). Receptacles of 'Honeoye' and 'Earliglow' primary flowers at full petal first showed symptoms of freeze injury at –4.0 and –4.7 °C, respectively (Ki and Warmund, 1992). Boyce and Marini (1978) inoculated detached strawberry flowers to prevent supercooling and found the temperature at which 50% of the flowers were injured (T_{50}) ranged from –1.7 to –2.3 °C in five cultivars tested in June. They tested two everbearing cultivars in the fall and found flower T_{50}s ranged from –1.6 °C in early September to –3.4 °C in late October. Field observations by Havis (1938) and Darrow and Scott (1947) indicated there were cultivar differences in strawberry flower freeze damage following severe spring frosts. In North Carolina, Perry and Poling (1986) measured the flower temperature of four strawberry cultivars during freezing events in the field and determined that –3.1 °C was the critical temperature of the open blossoms of all cultivars.

Freezing resistance of the flowers has not been determined for many of the strawberry cultivars grown in the Pacific Northwest. Field observations of the Pacific Northwest cultivars 'Totem' and 'Hood' indicate that 'Totem' flowers are more susceptible to spring frosts. Spring phenology of the two cultivars is similar with 'Hood', an earlier ripening variety, flowering slightly earlier than 'Totem'. While there may be an inherent difference in flower freeze resistance, other factors such as canopy architecture may play a role in spring freeze survival of these two cultivars. 'Totem' flowers are held above the leaves and thus are more exposed to freeze damage than 'Hood' flowers, which are borne below and perhaps protected by leaves. Determining the method of strawberry flower spring frost survival is necessary if this trait is to be incorporated into a breeding program.

Because of the variation in reported critical temperatures producing strawberry flower freeze injury, a preliminary experiment was conducted to determine the optimal freeze test procedure for determining the critical temperatures of 'Hood' and 'Totem' flowers. In this experiment, when 15 replications of 'Hood' and 'Totem' flowers were nucleated with ice crystals and held at –1.5 °C for 1.5 h followed by 4 h at –3 °C, all flowers were killed. However, of the 15 replications where ice crystals were not added, all flowers were supercooled and survived. Based on this experiment, it was decided to test flower freeze tolerance, using ice crystals to nucleate tissue water, and flower freeze avoidance or supercooling, using nonnucleated flowers. The goals of this research were to 1) determine the relative importance of freeze avoidance and freeze tolerance mechanisms in strawberry flower freeze resistance (Levitt, 1980), 2) measure the limits of freeze avoidance and freeze tolerance in the flowers of Pacific Northwest strawberry cultivars, and 3) investigate potential causes of observed variation in strawberry flower freeze avoidance.

Materials and Methods

Cultivar experiments. Freezing resistance of flowers from 11 strawberry cultivars ('Benton', 'Bountiful', 'Honeoye', 'Hood', 'Puget Beauty', 'Puget Reliance', 'Rainier', 'Redcrest', 'Shuksan', 'Sumas', and 'Totem') was tested in a series of experiments conducted in Spring 1993 and 1994. Flowers used in this research were collected from three sources: 1) strawberry plants in containers in a heated greenhouse, 2) strawberry plants in containers in an unheated screenhouse, and 3) strawberry plants in the field at Washington State Univ. Puyallup Research and Extension Center. Results of preliminary freeze tests indicated there was no difference in the freeze resistance of flowers collected from these sources (data not shown).

Primary, secondary, and tertiary flowers were cut from plants at the full-petal stage of development (Ki and Warmund, 1992) with about 3 mm of pedicel attached. Flowers were immediately

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Table 1. Freeze damage to strawberry flowers nucleated with ice at –1.0 °C.

Cultivar	Percent of flowers damaged			
	–1.5 °C[z]	–2.0 °C[z]	–2.5 °C[y]	–3.0 °C[x]
Benton	65	100	95	100
Honeoye	75	95	90	100
Hood	44	55	74	93
Puget Beauty	55	85	100	100
Puget Reliance	70	85	90	87
Rainier	80	90	93	100
Redcrest	95	95	100	100
Shuksan	70	95	100	93
Sumas	95	95	95	100
Totem	85	80	100	100
χ^{2w}	26.4**	27.4**	16.7^{NS}	7.6^{NS}

[z]n = at least 20 flowers per cultivar.
[y]n = 10 to 20 flowers per cultivar.
[x]n = 5 to 15 flowers per cultivar.
[w]Chi-square value and level of significance for each temperature.
^{NS,**}Nonsignificant and significant at $P \leq 0.01$, respectively.

placed in covered containers at 100% relative humidity (RH) and transported to the laboratory where individual flowers were placed in 20 × 200-mm Kimax screw-top test tubes. In experiments designed to test flower freeze tolerance, 2 mL deionized (DI) water was added to the test tubes before insertion of the flower. When experiments were designed to test flower freeze avoidance, no water was added to the tubes. The tubes were then placed randomly in an ethylene glycol freezing bath (model 2425 CH/P; Forma Scientific, Marietta, Ohio) set at 0 °C. Temperatures were monitored by inserting copper–constantan thermocouples (30-gauge, 0.2546 mm in diameter) in and near the receptacles of flowers in additional tubes placed at random in the bath. The thermocouples were connected to a programmable datalogger (CR7X; Campbell-Scientific, Inc., Logan, Utah) and the bath temperature was lowered to –1 °C. During each freeze test, tubes containing unfrozen control samples were held at 3 °C.

Flowers tested for freeze tolerance were placed in the tubes containing 2 mL DI water, immersed in the bath, and nucleated with finely crushed ice at –1.0 °C. The bath temperature was lowered to –1.5 °C, held overnight, then lowered 0.5 °C every 2 h. Tubes were removed at 0.5 °C intervals between –1.5 and –3.0 °C.

Table 2. Freeze damage to strawberry flowers that received no artificial ice nucleation.

Cultivar	Percent of flowers damaged			
	–4 °C[z]	–5 °C[y]	–6 °C[x]	–7 °C[x]
Benton	7	18	20	72
Bountiful	7	14	44	92
Hood	14	11	28	68
Puget Reliance	7	14	28	96
Rainier	39	46	44	64
Redcrest	7	7	20	76
Shuksan	21	25	52	64
Sumas	25	15	28	64
Totem	11	11	16	60
χ^{2w}	21.3**	21.3**	14.8^{NS}	16.9*

[z]n = 28 flowers per cultivar.
[y]n = 27 or 28 flowers per cultivar.
[x]n = 25 flowers per cultivar.
[w]Chi-square value and level of significance for each temperature.
^{NS,*,**}Nonsignificant and significant at $P \leq 0.05$ and 0.01, respectively.

In a preliminary experiment, we determined that placing the flower pedicel in 2 mL of DI water and placing finely crushed ice in direct contact with the top portion of the receptacle was a more efficient method of nucleation than wrapping flowers in moist Kimwipes, which were nucleated with ice at –1.0 °C.

Flowers tested for freeze avoidance were placed in tubes without water and immersed at random in the bath. No ice was added. The bath temperature was lowered to –4.0 °C, held overnight, then lowered 1.0 °C every 4 h. Tubes were removed at 1.0 °C intervals between –4.0 and –7.0 °C.

All samples were thawed overnight at about 3 °C then incubated for 48 h at 100% RH and room temperature. Freeze damage was determined at 24 and again at 48 h by visual evaluation of tissue browning of the styles and receptacle. Flowers were rated as damaged if oxidative browning injury was observed. Replication numbers of each cultivar at each freeze test temperature are indicated in Tables 1 and 2. A chi-square test for independence in a contingency table was calculated using counts of damaged vs. undamaged flowers (Gomez and Gomez, 1984).

Freezing-point depression experiment. Receptacles of 'Hood' and 'Totem' flowers were excised just above the anthers, blotted to ensure that there was no free water present, and inserted in 1.5-mL screw-top polypropylene microfuge tubes. The microfuge tubes were sealed in a glass jar and frozen at –60 °C. There were three replications consisting of five receptacles for each cultivar. Immediately after thawing, receptacles in each test tube were macerated with a glass rod, and the osmolality of 10-µL samples of expressed liquid was measured with a vapor-pressure osmometer (model 5100C; Wescor, Inc., Logan, Utah). The freezing point depression was calculated and cultivars were compared with a Student's *t* test.

Flower position experiments. Freezing tolerance and freezing avoidance of primary, secondary, and tertiary flowers were compared for 'Hood', 'Sumas', and 'Totem.' Flowers from field plots were collected as described above. When testing for freeze tolerance, flowers were placed in tubes containing 2 mL DI water, immersed in the bath at 0 °C, and nucleated with finely crushed ice when the bath reached –1.0 °C. Then the bath was lowered to –1.5 °C and held constant overnight, and the following morning all tubes were removed. There were 13 replicate flowers from each position in the inflorescence for 'Hood' and 'Sumas' and 18 replications for 'Totem'. In the freeze avoidance tests, flowers were placed in dry test tubes and immersed in the bath at 0 °C, and the temperature was lowered at 1 °C·h^{-1} to –6 °C. After remaining at –6 °C overnight, samples were removed from the bath. Each inflorescence position was represented by 11 'Hood', 18 'Sumas', and 20 'Totem' flower replications.

All flowers were thawed and evaluated for damage as in the cultivar experiment. After the visual browning evaluation, each flower was carefully blotted to remove visible water and fresh mass was immediately determined. The relationship between mean freeze damage and mean flower mass was determined by simple linear regression and correlation analysis (Gomez and Gomez, 1984).

Results and Discussion

Cultivar experiments. Chi-square analysis indicated that freeze damage of nucleated flowers was influenced by cultivar at –1.5 and –2.0 °C but not at –2.5 and –3.0 °C (Table 1). At –1.5 °C, 'Hood' with 11 of 25 flowers damaged appeared to be the most freeze-tolerant cultivar, while 'Redcrest' and 'Sumas' with 19 of 20 flowers injured were the least freeze-tolerant cultivars. 'Hood'

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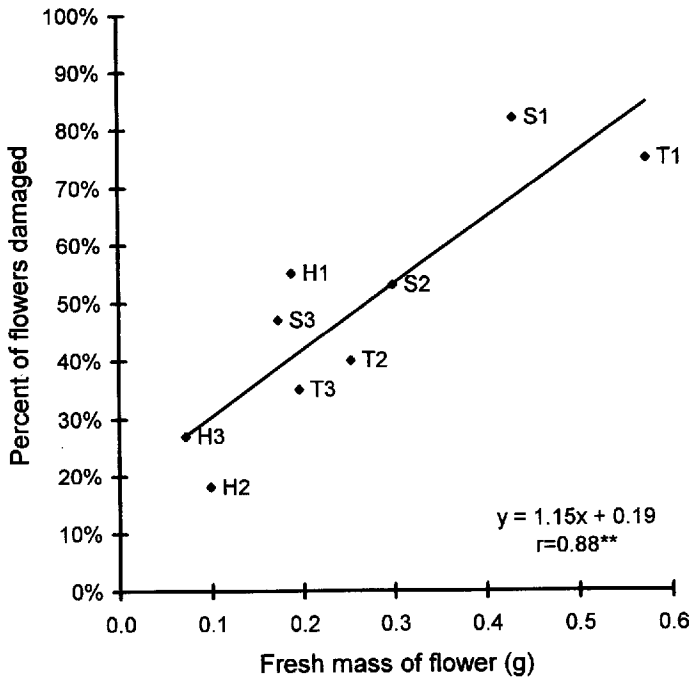

Fig. 1. Relationship between percent damage and fresh mass of primary (1), secondary (2) and tertiary (3) flowers from 'Hood' (H), 'Sumas' (S), and 'Totem' (T) strawberry cultivars nucleated with ice at –1.0 °C and held overnight at –1.5 °C. **Significant at $P \leq 0.01$.

was also the least damaged cultivar at –2.0 °C. Of 'Totem' flowers, 17 and 16 were damaged at –1.5 and –2.0 °C, respectively. As the temperature decreased, flower damage increased until, at –3.0 °C, only 1 of 15 'Hood' and 'Shuksan' and 2 of 15 'Puget Reliance' flowers were undamaged. At temperatures below –2.0 °C cultivar differences were not significant.

Cary and Mayland (1970) studied supercooling in tender plants and found 68% of corn plants (*Zea mays* L.) survived for 2 h at –4.5 °C with snow crystals on the leaves, while 7% of tomato (*Lycopersicon esculentum* Mill.) and 28% of corn plants survived for 1 h at –3.0 with a light covering of snow on the leaves. Their results indicated placement of ice crystals in contact with the tissue did not ensure nucleation and freezing of tissue water in every plant. Although ice crystals were placed on the receptacles in an effort to nucleate tissue water and test the susceptibility of strawberry flowers to freeze injury, it seems likely that the observed cultivar differences (Table 1) were due to nucleation failure and continued supercooling of the surviving flowers.

When strawberry flowers were allowed to supercool in the absence of ice crystals, cultivar differences were significant at –4, –5, and –7 °C but not at –6 °C (Table 2). At –4.0 °C 'Benton', 'Bountiful', 'Puget Reliance', and 'Redcrest' flowers were least damaged and 'Rainier' flowers were most damaged. This ranking of the cultivars for flower supercooling was not consistent at all test temperatures. 'Rainier', which had the most freeze damage at –4 °C, was among the least damaged cultivars at –7.0 °C, while 'Bountiful' and 'Puget Reliance', two of the least damaged cultivars at –4.0 °C, were the two most damaged cultivars at –7.0 °C. Supercooling of 'Hood' and 'Totem' was similar and generally intermediate to the other cultivars at all temperatures.

The lack of consistent cultivar performance in supercooling across temperatures seems to indicate that no individual strawberry cultivar had flowers with superior supercooling capability. The observed variation may be due to the random nature of the supercooling point, which is not a constant value but varies even in repeated tests on the same solution (Levitt, 1980). Other factors such as flower size, barriers to ice propagation, and external and/or intrinsic ice nucleators may be involved. In their study of 'Honeoye' strawberry flowers, Warmund and English (1994) found no association between freezing injury and colonization by ice-nucleation-active bacteria and concluded that other, nonbacterial nucleators may be involved in ice nucleation of strawberry floral tissue. Anderson and Smith (1989) found a significant cultivar difference in the freezing temperature of flowers and shoots from three peach [*Prunus persica* (L.) Batsch] cultivars and indicated that differences in either quantity or quality of intrinsic ice-nucleating agents could be the cause. Additional studies are required to determine the biological significance of the observed cultivar differences in strawberry flower supercooling.

The mean flower damage for all strawberry cultivars was 73%, 87%, 94%, and 97% at –1.5, –2.0, –2.5, and –3.0 °C, respectively, in the nucleated treatment (Table 1), while nonnucleated flowers were 15%, 18%, 31%, and 73% damaged at –4.0, –5.0, –6.0, and –7.0 °C, respectively (Table 2). This demonstration of strawberry flower injury at higher temperatures when supercooling is limited by nucleation corroborates the results of Boyce and Strater (1984) and may explain the difference between 'Honeoye' freeze resistance in the present study and that reported by Ki and Warmund (1992). When 'Honeoye' flowers at full petal stage were nucleated, 75% of the flowers were damaged at –1.5 °C (Table 1). When flowers at full-petal stage were not nucleated, the first freeze damage to receptacles occurred at –4.0, –4.2, and –5.8 °C for primary, secondary, and tertiary flowers, respectively (Ki and Warmund, 1992). Due to lack of flowers, 'Honeoye' was not included in our freeze avoidance experiments. Using whole plants without nucleation, Ourecky and Reich (1976) observed <50%

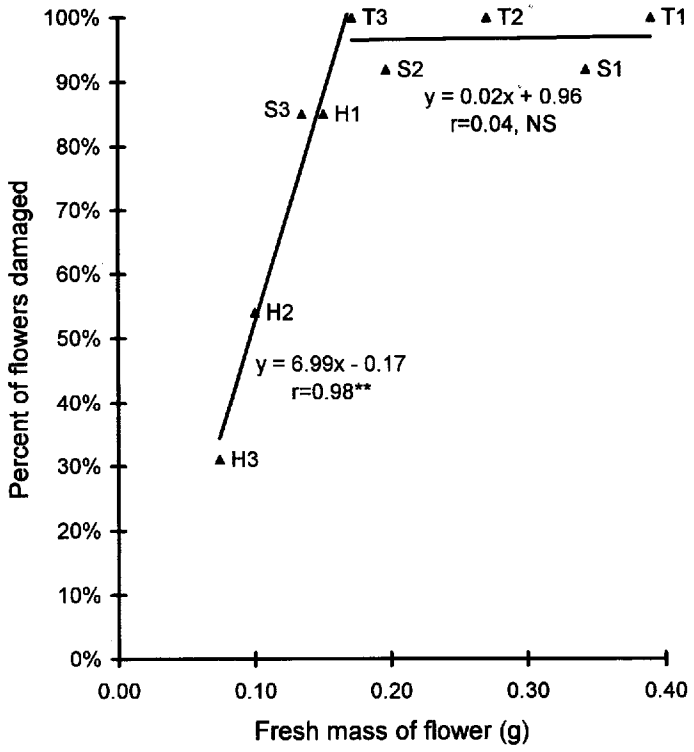

Fig. 2. Relationship between percent damage and fresh mass of primary (1), secondary (2), and tertiary (3) flowers from 'Hood' (H), 'Sumas' (S), and 'Totem' (T) strawberry cultivars held overnight at –6.0 °C without artificial nucleation. NS,**Nonsignificant and significant at $P \leq 0.01$, respectively.

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injury at –5.5 °C to flowers of 9 of 21 cultivars tested. Anderson and Whitworth (1993) determined average freezing temperatures of –5.1 °C for leaf petioles and –6.1 °C for flower pedicels in nonnucleated, flowering 'Arking' strawberry plants. They also reported that individual leaves froze independently and observed barriers to ice propagation in the strawberry plants.

Freezing-point depression experiment. Based on average osmolalities of 385 and 420 $mOs \cdot kg^{-1}$ ($mmol \cdot kg^{-1}$), the freezing point depression of the solutions collected from 'Hood' and 'Totem' receptacles averaged 0.72 and 0.78 °C, respectively. The difference between cultivars was not significant, indicating that differential solute concentration did not account for the observed difference in flower freeze damage (Table 1) to 'Hood' and 'Totem'.

Flower position experiments. The strawberry inflorescence is a compound dichasium that produces a hierarchy of flowers, typically with one primary, two secondary, four tertiary, and eight quaternary flowers (Darrow, 1966; Janick and Eggert, 1968). The number of pistils in the flower and fruit size is consistent with the flower position in the inflorescence: primaries are largest and secondaries and tertiaries are progressively smaller (Darrow, 1966; Janick and Eggert, 1968). Position in the inflorescence significantly influenced fresh mass of 'Hood', 'Sumas', and 'Totem' flowers, with tertiary flowers averaging 43%, 40%, and 38% of the primary flowers, respectively.

Following ice nucleation and exposure to –1.5 °C, the percent of damaged strawberry flowers increased with increasing flower size as measured by fresh mass (Fig. 1). The simple linear correlation coefficient, $r = 0.88$ ($P \leq 0.01$), indicated that, within the range of flower sizes tested, large flowers were more likely to be freeze damaged than small flowers. When flowers were held overnight at –6.0 °C without artificial nucleation, the relationship between percent damage and fresh mass, while nonlinear, was approximated with two separate linear regressions. The first linear regression indicated that there was a significant correlation between flower damage and flower fresh mass for flowers averaging 0.074 to 0.172 g (Fig. 2). In the second analysis, the r value for flowers of 0.172 g and larger was 0.04 and the regression line was nearly flat, indicating no linear relationship between flower size and percent freeze damage for the largest flowers tested. As demonstrated in other plants, citrus [*Citrus sinensis* (L.) Osbeck] flowers (Yelenosky, 1988), peach trees (Andrews et al., 1986 Ashworth and Davis, 1984; Ashworth et al., 1985), and tomatoes (Anderson and Ashworth, 1985), the ability of strawberry flowers to supercool depended on tissue mass, with larger flowers more likely to freeze. Ashworth and Davis (1984) and Andrews et al. (1986) reported a logarithmic relationship between sample fresh mass and nucleation temperature in peach stems, indicating that small samples supercooled to lower temperatures than large samples.

This relationship between sample size and supercooling may relate to Ki and Warmund's (1992) findings that primary 'Honeoye' and 'Earliglow' flowers were injured at higher temperatures than tertiary flowers and to the results showing greater kill of primaries when primary and secondary 'Honeoye' flowers were exposed to the same subfreezing temperatures (Warmund and English, 1994). Cultivar differences in flower size may also help explain why 'Hood' flowers survive field and laboratory (Table 1) freezes when 'Totem' flowers do not. The primary flower in the strawberry inflorescence is the largest (Darrow, 1966; Janick and Eggert, 1968), and 'Totem' flowers are larger than 'Hood' flowers. The average fresh mass for primary, secondary, and tertiary flowers was 0.481, 0.262, and 0.184 g in 'Totem' and 0.169, 0.099, and 0.073 g in 'Hood', respectively. Enhanced supercooling in the smaller 'Hood' and the smaller secondary and tertiary flowers may be sufficient to account for the observed differences in freeze survival.

This research indicates that flowers of the strawberry cultivars tested are susceptible to freeze injury and most likely rely on supercooling as a freeze avoidance mechanism for spring frost survival. The observed differences in freeze survival of strawberry flowers of different order in the inflorescence and of different cultivars may be determined by their size as it affects their supercooling capability. Thus, spring freeze tolerance is not a trait that can be directly incorporated into strawberry flowers by breeding and selecting for freeze-tolerant flowers. Rather, plant breeders must select for traits that would enhance flower spring frost avoidance like a protective canopy architecture and/or spring phenology. Selecting for small-flowered types, while potentially enhancing supercooling, could have a detrimental effect on fruit size.

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