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Capacity of Citrus Flowers to Supercool

G. Yelenosky
Agricultural Research Service, U.S. Department of Agriculture, 2120 Camden Road, Orlando, FL 32803

Additional index words. freeze avoidance, ice nucleation, exotherms, pistil, style, ovary, *Citrus sinensis*

Abstract. **'Hamlin' orange [*Citrus sinensis* (L.) Osbeck] flowers readily supercooled on young trees tested in a controlled-temperature room. Differential thermal analysis (DTA) determinations with thermocouples inserted into different flower parts indicated ice nucleation occurred from −3.8° to −6.1°C when flowers were attached to trees and from −8.1° to −11.9°C when flowers were detached. Similar supercooling levels also were noted in ovaries and young leaves. Ice-nucleation-active (INA) bacteria apparently were not involved based on total bacteria counts and flower wash extracts sprayed on flowers. Supercooling of citrus flowers was comparable to flowers of deciduous fruit trees in temperate climate zones. Data indicate a degree of freeze avoidance not previously recognized in citrus reproduction organs.**

Supercooling in citrus by some freeze avoidance standards does not appear to be of practical consequence because of limitations in relative degree of supercooling. For example, the apparent role of deep supercooling in woody plants (8) and horticultural crops other than citrus (4) is not apparent in −7°C lethal temperatures for citrus trees (16). However, the potential that does exist in citrus (17) is adequate to account for occasional uninjured trees (escapes) that are seen after injurious freezes in Florida. As yet, supercooling potential cannot be used for practical advantage during natural freezes in citriculture, largely because the event is uncontrollable, with no assurance that it will occur in trees at different freeze temperatures or persist for ≥ 1 hr. The importance of ice-nucleating agents, such as bacteria (15) or intrinsic internal factors (3), have not been resolved. It apparently is possible to modify partially the role of nucleation sites, since both cold-hardening temperatures (13) and water stress (14) increase supercooling in citrus.

The detachment of plant parts or a break in vascular continuity also suggests that nucleating sites can be modified successfully for practical advantage (2, 5). The objective of this reported work was to determine the supercooling potential in citrus flowers. Flowers are especially vulnerable to injury during natural freezes, suggesting a very low level of supercooling, probably not much below −2°C, a level of efficient ice nucleation based on activity of various INA agents (15). Such information would help to identify supercooling limits in different citrus tissues and provide useful data for modeling whole-tree survival without injury during freezes at different stages of growth and development.

Citrus trees. Thirty uniform and healthy 2.5-year-old 'Hamilin' orange trees on *C. macrophylla* rootstock from a registered Florida citrus nursery were the source trees of all flower tests. Trees in a high organic soil mix in 20-liter pots kept outdoors were watered daily with monthly applications of 3 liters of a 60:1 (v/v) dilution of 15N–3P–6K complete fertilizer (Sunniland Corp. Sanford, Fla.). Tests were done during flowering months of Feb. through Apr. 1985. Trees were 1 to 1.5 m tall above soil level, trunk diameters ranged from 2.5 to 3.5 cm 10 cm above the bud union, and 5- to 10-cm long flower-bearing stems were <2 mm at mid diameter.

INA agents. Of primary concern were INA bacteria implicated in freezing of citrus (12). A random sample of 100 flowers, open for at least 1 day, were excised from the 30 trees immediately before the first and last tests. Subsamples of 20 flowers were immersed in 20 ml of sterile glass-distilled H_2O and shaken for 30 sec on a vortex shaker, and 10 ml was serial-diluted on King's B agar containing 40 $\mu g \cdot ml^{-1}$ cycloheximide (11). Plates were incubated for 3 days at 20° ± 1°C and total bacteria counts expressed as colony-forming units (cfu) per flower. The remaining 10 ml were misted with a hand sprayer on isolated flower clusters and checked for early freez-

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Table 1. Capacity of 'Hamlin' orange flowers, isolated ovaries, and single leaves to supercool.

Item	Temperature (°C)			
	Attached to trees		Detached from trees	
	Nucleation	Range	Nucleation	Range
Open flowers	−4.5 ± 0.3[z] a[y]	−3.6 to −6.1	9.9 ± 0.9 b	−8.1 to −11.9
Isolated ovaries	−5.2 ± 0.2 a	−4.3 to −5.4	8.9 ± 0.7 b	−6.7 to −11.1
Leaves	−4.2 ± 0.5 a	−3.6 to −5	−10 ± 0.5 b	−9.5 to 10.6

[z]Mean ± SD, n = 19–21.
[y]Significant at 1% level, comparison of means between attached and detached tissues.

Table 2. Capacity of 'Hamlin' orange flowers and isolated ovaries to supercool when bearing stem is detached from tree.

Item	Temperature (°C)			
	Attached to tree		Detached with bearing stem[z]	
	Nucleation	Range	Nucleation	Range
Open flowers	−5.0 ± 0.2[y] a[x]	−4.6 to −5.5	−6.2 ± 0.2 b	−6.1 to −6.9
Isolated ovaries	−5.2 ± 0.5 a	−4.8 to −5.8	−7.2 ± 1.1 b	−6.7 to −8.3

[z]Five to 10 cm long and 2 mm at middiameter.
[y]Mean ± SD, n = 17.
[x]Significant at 1% level, comparison of means between attached and detached tissues.

ing in contrast to nonsprayed flowers. Bacteria were not tested for ice nucleation activity in separate freeze trials such as test tube freezing (3).

Freeze tests. Supercooling limits were defined daily throughout the flowering cycle period in single tree tests in a controlled-temperature room (17) and at 50% + 5% RH without light. Moment of freezing was determined by differential thermal analysis (DTA). Equilibration was at 2°C for 1 hr, followed by a steady 5°/hr decrease to the end of the test. Supercooling levels were identified by abrupt increases in tissue temperature. The flowers tested were scattered widely throughout the canopy of the tree to minimize induced freezing from neighboring tissue. Flowers and flower-bearing stems were razor-detached after temperature equilibration to minimize possible dehydration of excised parts. Dehydration was neither visible nor found in moisture content determinations during preliminary trials using oven-dry weights. Copper–constantan thermocouples (36 gauge) were inserted into open flowers through the top of the stigma and into the style (Fig. 1), into closed flowers through the petals, into the side of ovaries, and taped to abaxial side of leaves. Thermocouple leads were connected to 24-gauge wire extensions attached to a 15-channel multipoint recorder accurate to ±0.1°. Two additional thermocouples were connected to digital multimeters (1 μV/digit resolution), which were connected to variable strip-chart recorders, 0 to 100 mV, for graphical visualization using an insulated ice bath stable at ±0.1° for the reference junction. The reference thermocouple was enclosed firmly in an oven-dried 'Hamlin' orange pistil.

Viability determinations. Flowers that apparently escaped freeze injury through supercooling based on DTA were tested further in a differential respirometer at 30°C for O_2 uptake compared to frozen flowers. Single flowers were placed in flasks containing 0.2 ml 10% KOH in the center well with a filter paper wick. Flasks were oscillated at 100 strokes/min.

The results of DTA determinations indicated freezing from −3.6° to −6.1°C, with an average of −4.5° ± 0.3°, in 'Hamlin' orange flowers (Table 1). Critical concentrations of INA bacteria (9) apparently were not a determining factor, since total bacteria counts in this study were less than 1×10^2 cfu/flower, and flower washed extracts did not promote early freezing in sprayed flowers in contrast to unsprayed flowers. The apparent noninvolvement of highly active ice-nucleating agents also is supported in this study by significant increases in supercooling achieved through detachment of citrus tissues (Table 1). The effect of detachment on supercooling of citrus flowers was less than when flowers were attached to detached stems (Table 2). Greater supercooling in detached than attacked tissues also has been observed for fruit trees other than citrus (10, 11). However, the reason(s) for greater supercooling is unclear. In this study, greater supercooling of detached citrus flowers only compared to flowers on detached stems supports the inverse relationship with sample mass (3) and the assumption that flowers were nucleated as a result of xylem freezing in attached stems (10).

The levels of supercooling that were found in 'Hamlin' flowers significantly exceeded the expected −2°C based on the presumption of low supercooling in citrus flowers. The insertion of thermocouples into the tissues was of no apparent consequence, although insertion of thermocouples has appeared on occasion to be associated with early freezing in citrus wood and leaves (personal observation). The preferred method of attachment, rather than insertion of thermocouples, to lessen the risk of promoting early freezing was not applicable to citrus flowers in this study. Although the levels of supercooling found in attached citrus flowers are less than those noted in stems of young citrus plants (17), they are equal to and even exceed levels associated with flowers of deciduous fruit trees (5, 11). Supercooling of citrus ovaries in this study compares favorably with supercooling of young, developing fruit in deciduous fruit tree orchards (10). In a study on freeze survival in peach and prune flowers, it was found that ovaries may supercool even if ice is in the stem of the flower (7).

In instances where apparent citrus flower escapes were identified for exceptional supercooling at −6°C or greater, based on no exotherm and no visible watersoaking, additional tests on O_2 uptake helped to confirm that there was little to no injury in supercooled flowers in contrast to frozen flowers (Table 3). Trees with apparently uninjured flowers during freeze tests as low as −8° set and developed young fruit equal to that of control trees not exposed to freezing temperatures. Apparently, supercooling had no deleterious effects on postfreeze fruit set and development.

Factors contributing to supercooling of

Table 3. Dark respiration at 30°C for frozen and nonfrozen flowers on a Hamlin orange tree after a −8° freeze.

Time (min)	Respiration (μl O_2/flower)	
	Frozen	Nonfrozen (supercooled)
0	0	0
15	1 ± 0.6[z]	43 ± 10
30	6 ± 2	96 ± 24
45	15 ± 2	151 ± 30
60	24 ± 2	190 ± 29

[z]Mean ± SD, n = 3.

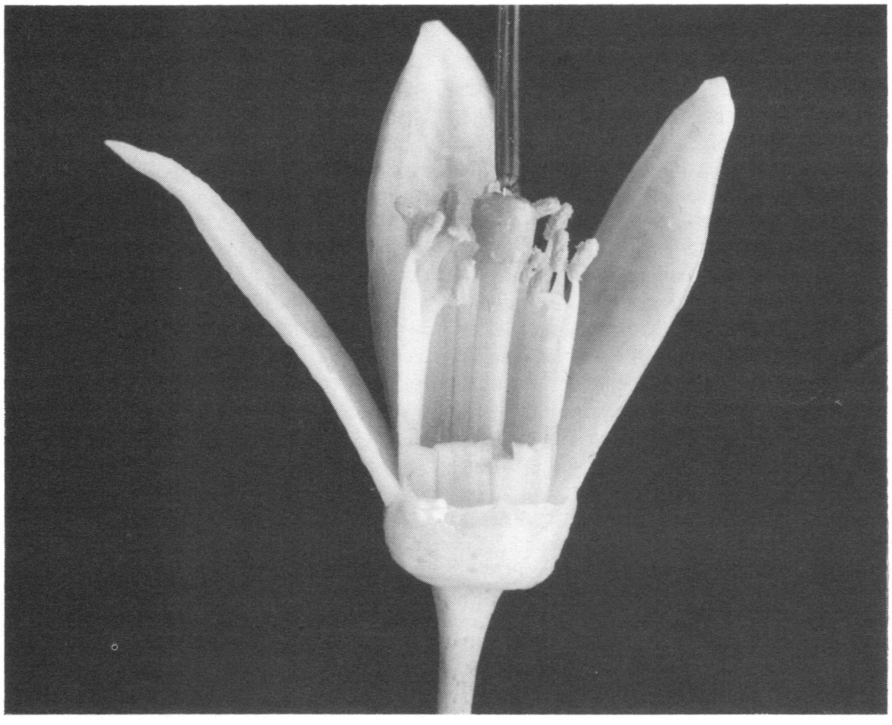

Fig. 1. Thermocouple placement in open flowers on 'Hamlin' orange trees during tests on supercooling.

flowers in this study are not clearly identified. Tissue hydration may be significant, based on considerably lower-than-expected water content. The stigma–style component had a mean fresh weight of 6.8% or 13.9 mg H_2O per stigma–style. Ovaries averaged 13.8% H_2O or 31 mg H_2O per ovary. These levels of tissue hydration are considerably less than that assumed to exist in citrus flowers, often referred to as succulent tissues highly intolerant of freezes. However, citrus leaf callus supercools to −11°C, regardless of 94% to 97% H_2O content (unreported data).

The significant delay in freezing of detached citrus parts agrees with results of deciduous fruit trees (11) and is notable in at least two situations. One is the use of detached citrus leaves to determine lethal freezing points (1), and the second is in determining the role of xylem discontinuity in freeze survival (7). Apparently, correction factors are appropriate for determining critical freeze temperatures based on DTA of detached plant parts, and liquid phase discontinuity in the xylem may be as important in freeze tolerance of subtropical citrus trees as that inferred in temperature deciduous species (7). The factor of water column tension has not been addressed in citrus freeze trials, and the effect of detachment on tissue hydration is unknown. In peach trees, there appears to be a constitutive part of mature wood that limits supercooling to about −2°C (3).

The supercooling levels found in this study are adequate to accommodate the probability of freeze severity (6) that coincides with citrus bloom in central Florida, a major citrus area. However, the role of supercooling in citriculture is yet unclarified under natural freezes, and controlled supercooling is a formidable research challenge (4).

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Seedling Emergence Forces of Vegetable Crops

A.G. Taylor[1] and C.W. Ten Broeck[2]

Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456

Additional index words. soil crusting, soil compaction, seed vigor, force transducer, seed size

Abstract. **Individual seedling emergence forces were determined for nine vegetable crops with an universal testing instrument (Model TTCM Instron). The seed energy content also was calculated with an oxygen bomb calorimeter. Seed weights of the different crops were correlated with seedling forces and the seed energy content. The time required to achieve the maximum force varied among crops and the pressure exerted varied from 26 mN for table beet (*Beta vulgaris* L.) to 3400 mN for snap bean (*Phaseolus vulgaris* L.). Equipment was developed to measure the combined seedling emergence forces of 50 seedlings. Snap bean seeds were sized into three groups: 200, 275, and 350 mg per seed. Total force, percent seedling emergence, force per seedling, pressure exerted, and energy content increased in a linear trend as seed size increased. An inverse relation existed between the capacity of seeds to use reserve materials and seed size. Small-sized seeds were more efficient in using reserve materials than large ones.**

Sowing seeds (and their subsequent germination) and seedling establishment are annual events in vegetable crop production. A good plant stand is essential for maximum yield potential and harvest efficiency. To accomplish this goal, the seed must complete germination and then emerge through the soil surface. The soil can act as a physical barrier to seedling emergence and may decrease or even prevent seedling establishment, especially under conditions of soil crusting (7) or soil compaction (17).

The emergence ability (EA) of a seedling can be described by the following formula: EA = EF·Ch·Sp (8). In this equation, EF is the vertical elongation force or emergence force. Ch is the morphological characteristic of the seedling. Sp is the speed of elongation or the time to achieve maximum force. The morphological character is the shape, or, more specifically, the cross-sectional area of the emerging seedling. From this discussion, a monocot should have a greater emergence ability than a dicot, since the former has a smaller surface area to penetrate the soil surface than the latter.

Several methods have been described to quantify seedling emergence forces (15); however, the use of a force transducer interfaced with a chart recorder may be the best method (8). Goyal et al. (5) summarized seedling emergence forces recorded from 1950 to 1977. Recent work on emergence forces have been reported for leguminous crops (8), cotton (2), and soybean (13).

The purpose of this study was to quantify seedling emergence forces for several different vegetable crop seedlings. A method is

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[1]Associate Professor.

[2]Former graduate student.