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Survival of Young Cold-hardened 'Hamlin' Orange Trees at – 6.7C

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Abstract. Potted greenhouse-grown, 1-year-old 'Hamlin' orange [*Citrus sinensis* (L.) Osbeck] trees on 1.5-year-old rough lemon (*C. jambhiri* Lush.) rootstock were temperature-conditioned for 6 consecutive weeks in a controlled-environment room to test cold-hardening ability. Holding at $15.6 \pm 0.6C$ during 12-hr days [$425 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ photosynthetic photon flux (PPF) at top of trees] and $4.4C$ during nights resulted in 100% tree survival and no leaf loss "after 4 hr of – 6.7C in a dark freeze test room. Unhardened greenhouse trees were killed to rootstock. Solute efflux ($\text{dS}\cdot\text{m}^{-1}$) from unhardened frozen leaves was > 20-fold that from frozen leaves on hardened trees and nonfrozen leaves on unhardened trees. Oxygen uptake was not significantly impaired in frozen hardened leaves. No O_2 uptake was evident for frozen unhardened leaves.

'Hamlin' orange is one of the world's major early maturing (October-January) sweet orange cultivars and is second to the late-maturing (March-June) major juice orange, 'Valencia,' in hectareage and number of trees in Florida. The 1988, commercial citrus tree inventory (Fla. Agr. Stat. Ser., 1988) showed about a 31% increase in 'Hamlin' plantings to $\approx 61,400$ ha from a low of 46,900 in 1986 and to 16 million from 12 million trees. Much of this increase is in replanting of areas devastated after freezes in the 1980s. The choice of 'Hamlin' for replanting frozen-out areas apparently was partly based on its high yield potential (Fla. Agr. Stat. Ser., 1989; C.J. Hearn, personal communication); early maturing of fruit, which decreases the risk of total loss to freezes; and wide acceptability in fresh fruit and processed juice where blending with 'Valencia' orange increases color acceptability (Wutscher and Bistline, 1988). However, there are few published reports on cold hardiness of 'Hamlin' (Hearn et al., 1963) because it is a relatively new cultivar in extensive plantings of freeze-risk areas.

The objective of this study was to test the cold-hardening ability of 'Hamlin' on a cold-sensitive rootstock in a severe freeze situation to obtain basic information on the freeze survival of this cultivar and its potential in low-temperature stress tolerance (Ashworth, 1986; Barrett, 1981).

Forty-five trees, matched for uniform growth and appearance, were selected from a greenhouse population of 100 one-year-old

'Hamlin' orange trees on 1.5-year-old rough lemon rootstock. Single trees were grown in 2.5-liter plastic pots containing Pro-Mix (shredded sphagnum peat with equal parts of vermiculite and perlite and major and minor elements added; Premier Brands, Inc., New Rochelle, N.Y.). Rootstock were from open-pollinated seed from a single source tree near Leesburg, Fla. 'Hamlin' scions were from state-registered budwood from a single source tree near Dundee, Fla. Buds were grafted, 10 cm above soil level, into 5-month-old rootstock. Single-stem trees were maintained in a 50% shaded greenhouse under natural day conditions. Air temperatures in the greenhouse ranged from $32C$ during the day to $15C$ at night. Relative humidity ranged from 35% during the day to 98% at night. Trees were watered every 2 days and each tree was fertilized monthly with 12N-5P-4.3K liquid fertilizer containing microelements at 100 ml per pot. Trees averaged 88 ± 4 cm in total height, 0.7 ± 0.2 cm stem diameter 10 cm above budunion, 1.4 ± 0.1 cm mid-diameter of rootstock, and 29 ± 2 leaves.

Fifteen trees were exposed to a cold-hardening regime of 6 consecutive weeks of 12-hr days ($425 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ PPF at the top of the trees) with $15.6C$ days and $4.4C$ nights, and 30 trees were left in the greenhouse. Hardened trees were frozen along with 15 unhardened trees in a controlled-freeze chamber (Yelenosky, 1975). In controlled freezes, temperatures were lowered $1.1C/hr$ from $4.4C$ to $-6.7C$ for 4 hr, and then returned to $4.4C$ at $1.1C/hr$. Temperatures were measured to $\pm 0.6C$; relative humidity was $60\% \pm 5\%$. Stem temperatures were measured with 24-gauge T-type thermocouples taped to the main stem of five trees and connected to a 15-channel digital recorder, and were within $1C$ of ambient air before trees froze. Leaf freezing was noted every hour by observing water soaking in the leaves. Soil temperatures did not drop below $1C$. Trees were kept at $\approx 25C$ for 3 hr after re-

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Table 1. Freeze injury 5 weeks after freezing of 'Hamlin' orange trees on rough lemon rootstocks after 4 hr at $-6.7 \pm 0.6^\circ\text{C}$ in a controlled-temperature chamber.

Treatment	Leaves ^z					Dieback of main stem (%)
	Trees (No.)	Killed (%)	Injured ^y (%)	Leakage (%)	O ₂ uptake ^x ($\mu\text{l}\cdot\text{hr}^{-1}\cdot\text{g}^{-1}$ dry wt)	
Cold-hardened, frozen	15	0	3 ^y	0.9 a	163 $\pm$ 3 b	0
Non-hardened, frozen	15	100	---	20 b	0 a	100
Not frozen	15	0	0	0.9 a	190 $\pm$ 9 c	0

^xMean separation in columns by LSD, 5% level.

^yLeaves developed bleached areas between major veins.

moval from the freeze chamber and returned to greenhouse for 5 weeks of observation for injury. Trees were rated for freezing injury using percentage of leaf kill and stem dieback.

Tolerance to internal ice was determined in leaves using electrolyte leakage, O₂ uptake, and permanent visual damage. Two hours after the freeze test, one leaf, selected randomly from each of five trees per treatment, was detached with a razor cut at the end of the petiole. Whole leaves were immersed to the depth of the winged petiole in 25 ml 2 $\times$ distilled, deionized water and placed at 4.4°C in the dark for 24 hr. Electrolyte leakage, before and after 5 min of boiling, was determined at 24°C (Markson Conductivity Meter, Del Mar, Calif.). Specific ion leakage, using the same methods and atomic absorption analysis, was done 72 hr postfreeze on three leaves per treatment to estimate membrane damage in leaves that developed surface bleaching but no necrosis.

Oxygen uptake was measured on leaf disks (6 mm in diameter) immediately after the freeze test as another index of membrane integrity and cell viability. Composite disk samples consisted of two disks per leaf from each of four leaves from the upper one-third of each of five trees per treatment. Samples were replicated three times with one 40-disk sample in each of three flasks per treatment. Oxygen consumption was measured at $30 \pm 0.2^\circ\text{C}$ with a differential respirometer (Gibson Medical Electronics, Middleton, Wis.) and 0.2 ml of 10% KOH with paper wick in the center well. Readings were taken at 10-min intervals (flasks closed) for 1 hr after 30 min of equilibration. Oxygen uptake was calculated per gram of oven-dry weight of leaf tissue. Data were analyzed by linear regression and correlation.

These young 'Hamlin' orange trees survived -6.7°C for 4 hr with little or no injury (Table 1), as did 'Valencia' orange on Rusk citrange rootstock and a different hardening regime (Yelenosky, 1978). Thus, both 'Hamlin' and 'Valencia' express an adequate degree of potential to survive severe freeze conditions in Florida, although different cold-hardening regimes and rootstock were used (Whitney, 1985). Optimum cold-hardening conditions are not established, but -6.7°C for 4 hr with little or no injury is considered

close to maximum cold-hardening potential for *C. sinensis* (Yelenosky, 1985). However, this level of cold hardening to survive -6.7°C is not well-established under natural conditions.

In this study, temperature exotherms indicated freezing in the stems between -4.9 and -6.2°C , and all hardened and unhardened trees were observed to have extensive water-soaking (Young and Peynado, 1967) in the leaves when temperature reached -6.7°C during the freeze. Internalize (presumably intercellular, especially for cold-hardened trees) apparently was extensive in leaves and bark for at least 4 hr at -6.7°C ambient air and for another 3 to 4 hr as temperatures returned to 4.4°C. The presence of ice for 7 to 8 hr apparently was not injurious to membrane integrity of hardened trees, as determined by leakage and O₂ uptake (Table 1).

Leakage, as percentage of conductivity of postfreeze electrolyte efflux to total efflux after boiling leaves, was > 20 times that for hardened, frozen leaves, for either unhardened unfrozen controls, or cold-hardened, frozen leaves. Three percent of total leaves observed were bleached 1 day after the freeze test. Atomic absorption analysis of efflux identified a slight increase in leakage of K⁺ and Zn²⁺ in bleached vs. nonbleached leaves. Bleaching did not result in leaf kill or abscission and was not reversed during 5 weeks of observation. Chlorophyll destruction in citrus leaves apparently can be caused by chilling injury (Yelenosky, 1982), internal ice, and surface frost during natural freezes with high dewpoints (personal observation).

The 14% reduction in O₂ uptake in cold-hardened, frozen leaves in contrast to unhardened, unfrozen controls (Table 1) and lack of significant electrolyte leakage suggest some alteration in the respiratory process/enzyme system without major injury to membrane integrity. The measured respiratory activity was linear with time ($r = 0.99$) during the 1-hr determination with a slope of 2.8 for cold-hardened, frozen leaves and 3.2 for unhardened nonfrozen leaves, and a y-intercept of zero. The rate of O₂ uptake, membrane leakage and visual observations coincide to illustrate considerable ice tolerance in leaves of cold-hardened 'Hamlin' orange, similar to that in 'Valencia' orange.

One of the prevailing views in plant cold hardiness is that internal ice is intercellular and a major obstacle for freeze survival is lack of tolerance to ice-induced cell plasmolysis (Anderson et al., 1983).

The results of this and a related study (Yelenosky, 1988) suggest that 'Hamlin' is similar to 'Valencia' in its potential to cold harden. The freeze tolerance of 'Hamlin' during the severe 1962 freeze in Florida, however, was rated slightly better than 'Valencia' (Hearn et al., 1963) and 'Hamlin' trees also had better recovery following the freeze (Cooper et al., 1969).

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