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FREEZE TOLERANCE VERSUS FREEZE AVOIDANCE IN CITRUS LEAVES

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Abstract. **The leaf-freezing-point (LFP) technique was evaluated as an estimate of leaf cold hardiness in citrus. Freezing points and exotherm maxima, the temperature increases resulting from heat of fusion (values reported by LFP technique), were related to leaf size. Exotherm maxima were too low to correspond to melting point depression of the tissue solution. The LFP technique estimated freeze avoidance (supercooling) rather than freeze tolerance or actual winter hardiness (tolerance of extracellular ice formation). Application of the electrolyte leakage viability test to citrus demonstrated that 'Hamlin' orange leaves [**Citrus sinensis **(L.) Osb.] cold acclimated to tolerate ice formation.**

The leaf-freezing-point (LFP) technique has been used to estimate the cold hardiness of citrus leaves (4, 6, 7). When detached leaves are cooled, a sudden rise in temperature (exotherm) is detected when freezing occurs. The exotherm is a result of the heat of fusion released when supercooled solutions in the leaf turn to ice. The warmest temperature reached during this heat release (exotherm maximum) has been reported as the 'leaf freezing point' and was considered indicative of the killing temperature (4, 6).

Citrus trees exposed to cool temperatures cold acclimate and can survive freezing (2, 10). For example, 'Satsuma' mandarin (*C. reticulata*) leaves cold acclimate to tolerate temperatures 7°C colder than the freezing temperature, i.e., they tolerate being frozen and then being cooled to –8°C (2). Therefore, frost resistance is a complicated property involving the initiation of freezing, the ice growth in the tissue, the minimum temperature to which the plant is exposed, and the state of the plant cold acclimation.

The objective of this study was to demonstrate the validity of using the LFP technique as a means of assessing the cold hardiness of citrus.

Materials and Methods

Freeze avoidance. 'Orlando tangelo' (*Citrus reticulata* Blanco x *C. paradisi* Macf.) leaves were randomly selected from a grove in Gainesville, Florida for leaf freezing point determinations. Leaves were placed in a domestic freezer at –15°C and exotherms (heat of fusion from ice formation) were sensed by a thermister circuit as previously described (4). The leaf temperature was recorded at 15 sec intervals.

Freeze tolerance. Mature 'Hamlin' orange leaves (on sour orange (*C. aurantium* L.) rootstock) were randomly selected from a grove in Gainesville, Florida and rinsed with deionized water. Leaves were placed in large test tubes and immersed in a 190-liter refrigerated glycol bath set at –3°C. Samples were frozen by dropping chips of ice into the tubes. Leaves were held overnight at –3°C to ensure equilibrium freezing. The bath temperature was lowered at a rate of 1°C/hr. Replicates taken out at each test temperature were allowed to thaw slowly, in an ice bath. Leaves were sectioned into 1 cm strips, placed in 15 ml deionized water and incubated at 24°C for 24 hr. The conductivity of the decanted leachate was determined using a standard conductivity meter (Model CDM3, Radiometer Copenhagen). The leaf strips were heat killed before the leachate was returned to the original tubes. The samples were incubated for 24 additional hr. after which the conductivity was once again measured. The initial readings were divided by final readings and multiplied by 100 so that the damage was expressed as percentage electrolyte leakage vs. test temperatures. This yielded sigmoidal curves with the inflection point being the killing temperature.

Results and Discussion

Freeze avoidance. LFP analyses were carried out with tangelo leaves of various size (Fig. 1). There was an apparent relationship between leaf size and supercooling. The smaller leaves generally reached lower temperatures before freezing when compared with the medium size leaves which, in turn, froze at lower temperatures than large leaves. The

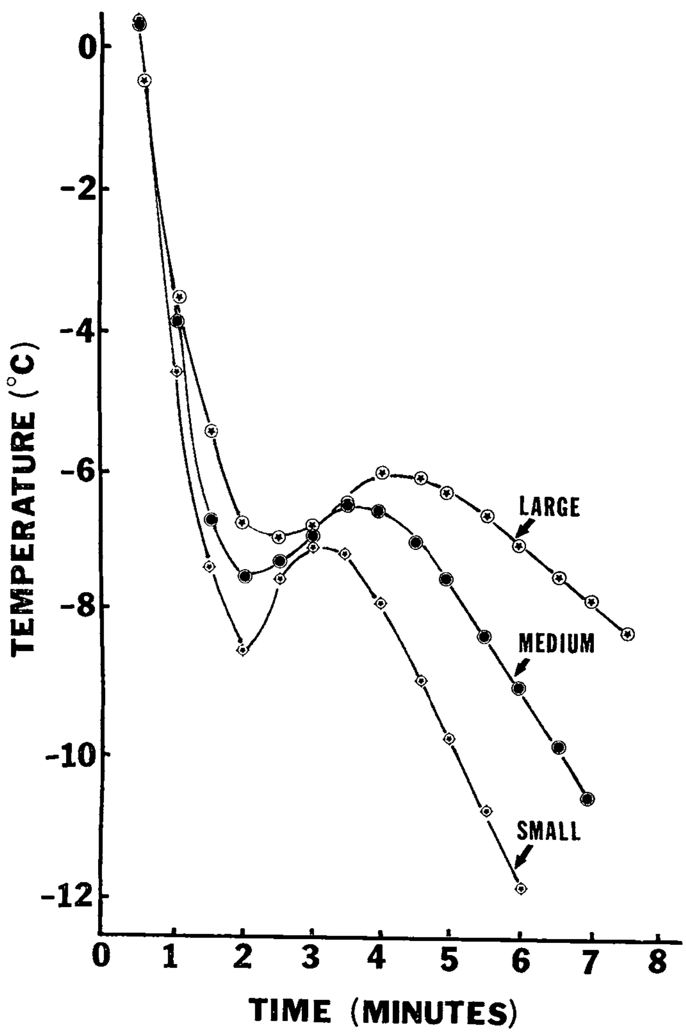

Fig. 1. Cooling curves with exotherms for small, medium, and large 'Orlando' tangelo leaves.

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leaf sizes in the figure are relative. Such data demonstrates that small sample size favors supercooling.

The same relationship held for the maximum (temperature reached during the exotherms (Fig. 1). The values for a small, medium and large leaf were –7.1°C, –6.5°C, and –6.0°C, respectively.

The maximum temperature reached during the exotherm is a function of how much heat is removed by the freezing chamber in relation to the amount of heat liberated by the freezing process. Ideally, the temperature should rise to a point determined by the concentration of solutes in the cell solution, about –2 to –1°C in the case of citrus leaves (2, 5). The chamber temperatures used for the LFP technique are too cold to allow the leaf reaching the potential maximum of the exotherm. In addition to this, different chamber temperatures result in different values of the exotherm maximum (9).

Freeze tolerance. Evidence that differences in freezing behavior do not account for differences in hardiness between *Citrus* species came from equilibrium freezing curves (2). The amount of tissue freezing at progressively lower temperatures was determined for citrus leaves varying in hardiness. All species (lemon, grapefruit, orange, and mandarin) were found to exhibit similar freezing behavior although they differed by as much as 7°C in hardiness. Mandarin leaves were found to be hardier than lemon leaves because they tolerate freezing of a greater fraction of tissue water, not as a result of differences in freezing behavior.

Further evidence that leaf freezing points do not necessarily correspond to killing points arises from the observation that acclimated leaves can become water soaked (an indication of freezing) without being killed (2, 10). This is a result of tolerance of ice formation in acclimated tissue. The LFP technique provides no information on freeze tolerance. For this reason, LFP's showed a poor correlation with whole tree responses to cold (3).

To determine the degree of freeze tolerance of acclimated leaves, the electrolyte leakage viability test was adapted for use with citrus. Methods used were the same as the standard test (8) but the leaves were sectioned into 1 cm strips because the waxy cuticle proved to be a major barrier to diffusion of electrolytes from frost injured tissue. This treatment does not introduce significant error (1). This procedure (electrolyte leakage test following controlled freezing) was used to monitor the cold hardiness of 'Hamlin' orange leaves on sour orange rootstock. A representative hardiness evaluation is shown in Fig. 2. The killing temperature on this date (January 5, 1981) was –7°C the inflection point of the plot of percent conductivity vs. temperature.

The leaves cold acclimated –3°C in October to –7°C in January and deacclimated to –3°C by June (Fig. 3). Water soaking occurred at about –3°C throughout the experiment indicating an acquisition of freeze tolerance. This acclimation-deacclimation sequence was the result of prevailing temperatures in Gainesville. Under these conditions 'Hamlin' orange leaves acquired 4°C of hardiness in about 4 months. Similar evaluations might find use as a local predictive tool for the need of frost protection measures.

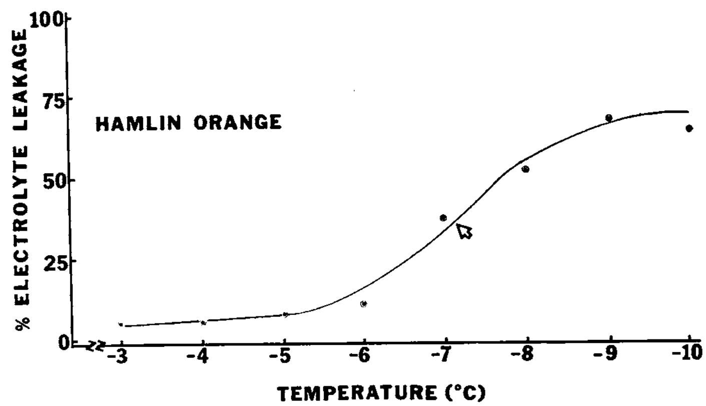

Fig. 2. Electrolyte leakage viability test for 'Hamlin' orange leaves. The killing temperature is indicated by the arrow.

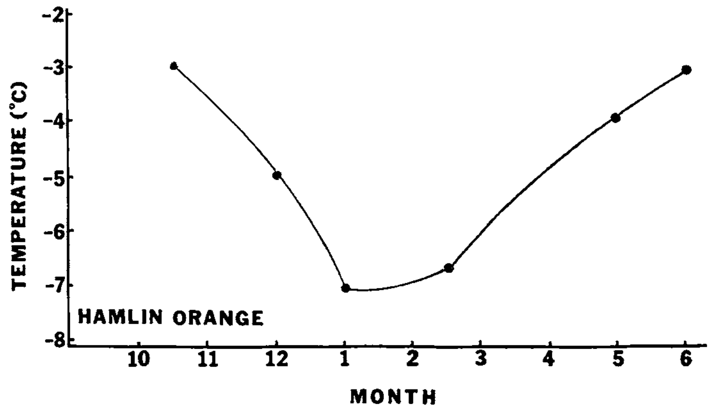

Fig. 3. Freeze killing temperature of 'Hamlin' orange leaves from October (10) to June (6) of 1981.

Literature Cited

1. Anderson, J. A. 1983. Ice nucleating agents involved in freezing of plant tissues. Ph.D. Dissertation, Univ. Florida.
2. Anderson, J. A., L. V. Gusta, D. W. Buchanan, and M. J. Burke. 1983. Freezing of water in citrus leaves. J. Amer. Soc. Hort. Sci. 108:397-400.
3. Davies, F. S., D. W. Buchanan, and J. A. Anderson. 1981. Water stress and cold hardiness in field-grown citrus. J. Amer. Soc. Hort. Sci. 106:197-200.
4. Gerber, J. F. and F. Hashemi. 1965. The freezing point of citrus leaves. Proc. Amer. Soc. Hort. Sci. 86:220-225.
5. Haas, A. R. C. and F. F. Hama. 1931. Sap concentration and inorganic constituents of mature citrus leaves. Hilgardia 5:407-424.
6. Hutcheson, D. E. and W. J. Wiltbank. 1972. The freezing point of detached leaves as a measure of cold-hardiness of young budded citrus plants. HortScience 7:27-28.
7. Rouse, R. E., W. J. Wiltbank, and W. S. Brey, Jr. 1982. Nuclear magnetic resonance of water in deuterated citrus leaf tissue. J. Amer. Soc. Hort. Sci. 107:1124-1128.
8. Sukumaran, N. P. and C. J. Weiser. 1972. An excised leaflet test for evaluating potato frost tolerance. Hort Science 7:467-468.
9. Young, R. H. 1966. Freezing points and lethal temperatures of citrus leaves. Proc. Amer. Soc. Hort. Sci. 88:272-279.
10. Young, R. and A. Peynado. 1967. Freezing and water-soaking in citrus leaves. Proc. Amer. Soc. Hort. Sci. 91:157-162.