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COLD HARDINESS COMPARISON OF YOUNG TREES OF 'AMBERSWEET' WITH 'VALENCIA' ORANGE DURING CONTROLLED FREEZES

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Abstract. **Two-year-old 'Ambersweet' (*Citrus reticulata* Blanco × [*C. paradisi* Macf. × C. reticulata]) × midseason orange, *C. sinensis* (L.) Osb. trees on three rootstocks, sour orange (*C. aurantium* L.), Cleopatra mandarin (*C. reticulata*), and Carrizo citrange (*C. sinensis* × *Poncirus trifoliata* (L.) Raf.) growing in 15-liter pots were exposed to cold-hardening regimes in controlled-temperature rooms and freeze-tested at –6.7°C and colder for three hours. The ability of 'Ambersweet' to cold harden did not exceed that of control variety trees, 'Valencia' orange (*C. sinensis* (L.) Osb.) based on degree of freeze injury, freezing behavior, and biochemical changes in the tissues. Differences in the above factors were significant only between hardened and unhardened trees of both varieties. Results suggest 'Ambersweet' is a moderately cold-hardy type similar to 'Valencia' orange.**

The 1989 citrus release, 'Ambersweet' orange hybrid, developed in the ARS/USDA breeding program at the A. H. Whitmore Foundation Farm (2), is expected to be planted extensively throughout the Florida citrus industry in the next few years. The performance of 'Ambersweet' trees to date has been encouraging in both productivity and freeze survival. However, years of observations will be needed to make final evaluations on the role and performance of 'Ambersweet' under different field conditions and stresses in the industry. Until such time, initial observations on performance of young trees, as new plantings are established, are extremely important to help make decisions in managing 'Ambersweet' in the Industry. This report describes the freeze tolerance of young 'Ambersweet' trees during a series of freeze tests for the purpose of expanding freeze survival information critical to site selection for new plantings.

Materials and Methods

Trees. Test trees were developed on 3 different rootstocks, Carrizo citrange, Cleopatra mandarin, and sour orange, which were used in the original development of 'Ambersweet' trees (2). Single trees propagated from A. H. Whitmore Foundation Farm budwood on rootstock seedlings from open-pollinated seed were grown in 15-liter plastic pots containing Astatula fine sand Florida field soil. Single-stem trees were maintained in a 50% shaded greenhouse under natural-day conditions. Air temperatures in the greenhouse ranged from 32°C during the day to a 20°C minimum at night. Maximum photosynthetic photon flux density (PPFD) was approximately 1000 $\mu mol \cdot s^{-1} m^{-2}$ at the top of the trees, and relative humidity (RH) ranged from a low of 35% during the day to 98% at night. Trees were watered every 2 days and fertilized monthly with a solution of 12N-2.6P-5K liquid that contained micronutrients. Test trees ranged in height from 95 to 112 cm, and 0.9 to 1.1 cm in stem diameter, half the length of the scion above the budunion.

Cold hardening. Equal number and uniform appearing trees of each scion variety on common rootstocks were arbitrarily assigned simultaneously to 4 weeks of cold hardening and 4 weeks of nonhardening in separate programmed temperature rooms. Cold hardening consisted of 20°C 12-hr days with approximately 450 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PPFD at the top of the trees and 10°C nights for 2 consecutive weeks, followed by 2 weeks of 15°C days and 4°C nights. Nonhardening was at similar light conditions with 30°C days and 20°C nights for 4 weeks. Relative humidity fluctuated from 40% to 60% in both rooms during hardening treatments, and trees were watered daily.

Immediately following the 4-week cold hardening, a similar group of trees was used in a 1-week cold hardening scheme where temperature was abruptly lowered 5°C every 24 hr, going from 30°C to 2°C in 6 days. All other conditions remained the same except a greenhouse was the nonhardening treatment in this instance. This treatment was imposed to estimate the responsiveness of the two varieties to a sharply contrasting temperature regime than the standard 4 weeks known to induce significant cold hardiness in container-grown citrus trees (7). Respective greenhouse trees, in addition to those used above, were used as controls throughout the study.

Freeze tests. Trees of each variety on common rootstocks from different cold-hardening treatments were tested at –6.7°C and –8°C for 3 hr, and –10°C for 1 hr. Tests were done in a separate controlled-temperature room with 40% to 60% RH and no light. Trees were temperature equilibrated at 2°C for 3 hr immediately before temperature was lowered at a rate of 5°C per hr to minimum lows and durations, and thawed at 5°C per hr to original starting temperature of 2°C. Freeze-tested trees remained at room temperature (approximately 25°C) for 3 or more hours before being returned to the greenhouse for 5 weeks of observations on freeze injury. Tests were done on successive days, at temperatures of –6.7°C, –8°C, and –10°C, respectively. Injury was based on the number of leaves that were killed or abscised from the original number and the length of wood dieback relative to total length of scion. The approximate temperature of the scion-stem and apparent moment that ice started to form in the wood (nucleation temperature) were based on the release of latent heat of freezing (exotherm). This was indicated by copper-constantan thermocouples (36 gauge, 0.12 mm diameter) inserted 2 to 3 mm into the central vascular system of a leaf trace at mid-stem level. Thermocouples were connected to an automated data collection system accurate to ± 0.1°C at 22°C ambient and a resolution of 0.015° (8).

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Chlorophyll, PEP carboxylase, Rubisco, Photosynthesis, and Carbohydrates. Determinations were made at the end of the 4-week cold-hardening treatment immediately before freeze tests. Fully matured leaves from 3 trees of each of the 2 varieties on common rootstocks were sampled and analyzed according to procedures in previously published reports (4, 5). Chlorophyll concentrations were determined colorimetrically, PEP carboxylase and Rubisco enzyme activities were assayed with ^{14}C incorporation and liquid scintillation spectrometry, photosynthesis was determined on single-attached leaves using the LI-COR 6200 Portable Photosynthesis System, and carbohydrates analyzed included soluble sugars and starch assays using enzyme degradation of di- and polysaccharides and final colorimetric determinations of monosaccarides (5).

Results and Discussion

There were no indications that young trees of 'Ambersweet' are any more cold hardy than young trees of 'Valencia' orange. Freeze injury was similar for both varieties regardless of cold hardening, rootstocks, and freeze intensity (Table 1). Cold hardening for 4 weeks did not save the leaves but stems survived with little to no injury at –6.7°C and –8°C for 3 hr. This stem tolerance was largely lost at –10°C for 1 hr and dieback was more than 80% of scion height. The influence of rootstocks on freeze injury was not discernible in statistical analyses (Duncan's multiple range test and comparison of means, *t* test) and data were combined. The influence of rootstock apparently increases with tree age (7), and rootstock separation, though possible (6), is difficult without sufficient number of single tree replicates. In this study, lack of rootstock separation probably reflects too few trees tested at –6.7°C and even fewer trees at –8°C and –10°C. During the 1983 freeze at the Foundation Farm, 9-year-old 'Ambersweet' trees on Carrizo citrange rootstock had twigs killed at the top of the trees while those trees on Cleopatra mandarin and sour orange rootstocks were not injured (personal observation of C. J. Hearn). The influence of heaters that were used in the immediate area is not known. Similar rootstock associations were noted by C. J. Hearn on 16-year-old 'Ambersweet' trees in unheated areas at Lake Wales during the 1989 freeze.

The ability of the trees to cool 4°C to 7°C below the freezing point of water (0°C) before ice apparently started to form in the wood (N_t in Table 1) supports previous reports on supercooling in citrus (7). The general trend of lower nucleation temperatures in cold hardened than in nonhardened trees associates longer freeze avoidance with cold hardening, which remains unclear in field situations. In this study, freeze injury was noticeably greater when ice was in the wood for 2 or more hours. Benefits of longer freeze avoidance in less freeze injury seemingly are more likely to occur in freezes where temperatures do not go below –6°C (21°F) and minimums persist for 1 hr or less. Supercooling limits in this study do not identify any unusual ability of 'Ambersweet' to avoid ice in the tissues. It is not known whether similar behavior occurs in the field.

The 1-week cold-hardening scheme, where starting temperature of 30°C was abruptly lowered 5°C every 24 hr to a minimum of 2°C over 6 days, did not appreciably cold harden 12 trees (6 each of 'Ambersweet' and 'Valencia', 2 trees on each of 3 rootstocks) tested at –6.7°C for 3 hr. For all practical purposes, the 1-week cold hardening was significantly inferior to the 4-week program in that no leaves survived and stem kill averaged 75 ± 25%. Damage to varieties regardless of rootstocks was not significantly different, although 2 'Valencia' trees on sour orange rootstock survived with no stem injury. This may or may not have been a probable chance event of random supercooling associated with young citrus (7). In this instance, the observation was not considered sufficient to discriminate rootstocks. Regardless, there were no apparent signs that suggested young trees of 'Ambersweet' were better than 'Valencia' in responding to a relatively quick temper-

Table 1. Freeze tolerance of 2-yr-old 'Ambersweet' and 'Valencia' orange trees during controlled freeze tests.

Freeze	Variety	No. of Trees	Cold Hardened	Nucleation temp.[z] (°C)	Ice duration in scion wood (hr)	Leaves killed (%)	Wood Dieback Scion (%)	Wood Dieback Rootstock (%)
–6.7°C (20°F) for 3 hr	Ambersweet	9[y]	Yes	–6.0 ± 0.8[v]	2.8 ± 0.5	100	2 ± 2	0
		9	No	–5.4 ± 0.5	3.1 ± 0.1	100	100	10 ± 10
	Valencia	9	Yes	–6.2 ± 0.4	2.8 ± 0.2	89 ± 10	0	0
		9	No	–5.4 ± 0.6	3.0 ± 0.1	100	100	0
–8°C (17.6°F) for 3 hr	Ambersweet	3[x]	Yes	–5.2 ± 1.0	5.2 ± 0.3	100	5 ± 5	0
		3	No	–4.5 ± 0.7	5.4 ± 0.2	100	100	30 ± 30
	Valencia	3	Yes	–5.4 ± 0.4	5.2 ± 0.2	100	5 ± 3	0
		3	No	–5.3 ± 0.6	5.2 ± 0.1	100	100	33 ± 33
–10°C (14°F) for 1 hr	Ambersweet	1[w]	Yes	–7.3	2.0	100	94	0
		1	No	–4.7	2.8	100	100	70
	Valencia	1	Yes	–5.1	2.4	100	82	0
		1	No	–5.3	2.4	100	100	80

[z]Temperature when ice apparently started to form in the scion wood.
[y]Composite of 3 trees each on 3 rootstocks (Carrizo citrange, Cleopatra mandarin, Sour orange).
[x]Composite of 2 trees each on Carrizo citrange and 1 on Cleopatra mandarin.
[w]1 tree on Cleopatra mandarin.
[v]Mean ± standard deviation.

ature cold hardening which is a highly desirable trait under Florida's winter conditions.

We also did not find anything in analyses of leaf tissues that would target 'Ambersweet' as being more cold-hardy than 'Valencia'. Both varieties, on the 3 different rootstocks had similar rates of photosynthesis that approximated 4.5 ± 0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during low-temperature cold hardening and 10.2 ± 0.3 μmol during nonhardening temperatures. Lower rates of photosynthesis at lower temperatures are characteristic of citrus (4). In viewing other factors, only trees on sour orange rootstock were sampled. Chlorophyll (chl) concentrations in leaves were approximately 55% less in 'Ambersweet' than in 'Valencia' which averaged 3.1 ± 0.4 mg $chl \cdot g^{-1}$ fwt at nonhardening and cold-hardening temperatures. This might be expected because 'Ambersweet' trees have lighter green foliage than 'Valencia', regardless of conditions or time of the year (personal communication, C. J. Hearn). Activity of PEP carboxylase, an important enzyme in carbon metabolism, was also less in 'Ambersweet' and averaged about 62% of 53 ± 0.5 $\mu mol \cdot g^{-1}$ $fwt \cdot hr^{-1}$ found in 'Valencia' leaves during nonhardening and 154 ± 10 μmol during cold hardening. The approximately 3-fold increase in PEP carboxylase activity at the lower temperatures seems a common response in both varieties. Such a large increase with lower temperatures was not evident for the major carbon fixing protein, Rubisco. Rubisco activity in 'Ambersweet' leaves was about 84% of 319 ± 4 $\mu mol \cdot g^{-1}$ fwt hr^{-1} found in 'Valencia' at nonhardening conditions and 298 ± 6 μmol at cold-hardening conditions. Carbohydrate concentrations were also lower in 'Ambersweet' than in 'Valencia' leaves. Total sugars (reducing sugars plus sucrose) averaged 55 ± 3 $mg \cdot g^{-1}$ dry wt in nonhardened 'Ambersweet' leaves which was approximately 87% of that found in 'Valencia'. Starch concentrations of 41 ± 10 mg were 64% of that in 'Valencia'. In cold hardening, 'Ambersweet' leaves averaged 55 ± 3 $mg \cdot g^{-1}$ dry wt total sugars which was about 69% of that found in 'Valencia'. Starch concentrations of 24 ± 7 mg were about 34% of that in 'Valencia'. Data do not suggest anything unusual about enzyme activity or solute accumulation that would favor a high degree of cold hardiness potential in 'Ambersweet'. The seasonal fluctuations of physiological factors associated with cold hardiness in citrus are not known for 'Ambersweet' trees under natural conditions, and only limited work has been done for 'Valencia' (9).

In summary, the results of our limited study suggest that young trees of 'Ambersweet' do not have exceptional cold hardiness and probably should not be extensively planted in sites considered too cold for 'Valencia' or 'Hamlin' orange without some provisions for freeze protection. The developer, C. J. Hearn, rated 'Ambersweet' trees moderately cold-hardy based on field observations (2), and we found no basis to suggest a higher rating. There is some concern by owners of citrus nurseries that 'Ambersweet' has been "overhyped" in freeze tolerance (1, 3). In view of the developer's original rating and the results of our study, there appears to be some need for caution in developing 'Ambersweet' plantings in highly freeze-vulnerable areas and extending geographical limits too far north until more data are available on performance of 'Ambersweet' in commercial field plantings.

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