Plant Physiol. (1975) 56, 540–543

Cold Hardening in Citrus Stems

Received for publication May 19, 1975 and in revised form June 23, 1975

GEORGE YELENOSKY
Agricultural Research Service, United States Department of Agriculture, Orlando, Florida 32803

ABSTRACT

Stem cold hardening developed to different levels in citrus types tested in controlled environments. Exotherms indicated ice spread was more uniform and rapid in unhardened than in cold-hardened stems. All attempts to inhibit the functioning of citrus leaves resulted in less cold hardening in the stems. Citrus leaves contribute a major portion of cold hardening in the wood.

Citrus trees are relatively cold-tender, woody plants. As with cold hardy plants, different tree parts differ in tolerance of freeze stresses. Cold resistance and acclimation develop less in citrus fruit than in the leaves, and leaves tend to be less cold hardy than the wood (5). Citrus fruit do not cold-harden to any appreciable degree below −3 C, but citrus leaves do (4, 18, 19). Less is known about citrus stems.

The extent that regenerative tissue of citrus stems can tolerate freezes largely determines hardiness of the whole tree. The ability of stems to cold-harden largely influences tree survival during severe freezes. This report includes relative cold hardiness of stems of different citrus selections exposed to controlled cold hardening and freezes, freeze profiles of the stem surfaces, and the effects of different foliar treatments on cold hardening of citrus stems.

MATERIALS AND METHODS

Plants. Citrus selections were 12- to 14-month-old seedlings from open-pollinated seed of sweet orange, *Citrus sinensis* (L.) Osbeck cv. Valencia; grapefruit, *C. paradisi* Macf. cv. Duncan and Marsh; sour orange, *C. aurantium* L.; and rough lemon, *C. limon* (L.) Burm. f.

Plants were grown in a standard soil mix in 3.8-liter containers and under natural daylight in a greenhouse. Temperatures averaged 30 C days and 23 C nights. Single stem plants were maintained. Profiles of freezing in the stems and rates of longitudinal ice spread were determined on 2-year-old Marsh grapefruit trees on Carrizo citrange rootstock.

Cold Hardening and Freeze Tests. Tests included selected uniform groups of 10 plants of each selection. Plants were cold-hardened in walk-in, controlled-environment chambers, 4 × 3 × 1.8 m. The chambers had a mylar barrier with 86% input wattage of cool white fluorescent lighting and 14% incandescent. Air temperatures were controlled at ±0.3 C and relative humidity at ±5%. Cold-hardening regimes included (*a*) unhardened plants directly from the greenhouse, and (*b*) moderate cold hardening at 21.1 C days and 10 C nights for 2 weeks, and an additional 2 weeks of 15.6 C days, and 4.4 C nights. Light intensity at the top of the plants was maintained at 200 μeinsteins/m²·sec measured with a Lambda L1-185 meter[1]. Air was circulated at 60 m/min. Automatic steam injection maintained relative humidity at 60 ± 5%.

Freezing was tested in a 3 × 3 × 1.8 m chamber in the dark at 50 ± 5% relative humidity. The standard freeze began at 4.4 C for 2 hr, followed by a 1.1 C/hr decrease to −6.7 C, which was maintained for 4 hr; temperature was then returned to 4.4 C at 1.1 C/hr. After freeze tests, plants were kept at 23 C for 3 hr and then were returned to the greenhouse for five weeks of injury observations. Plants were rated for percentage of dieback of the main stem.

Freeze Profiles of the Wood. The onset of ice formation and the rate of vertical ice spread in the wood of the main stem of intact trees was determined by continuous monitoring of exotherms as they developed along the stem height. Freezing was indicated by 36-gauge copper-constantan thermocouples attached firmly against the stem surface with plastic clips. Thermocouples were spaced 5 to 20 cm apart along the entire stem. Thermocouple leads were connected to potentiometer-type recorders. Multipoint connecting switches enabled any thermocouple to register within 5 sec of the other. To obtain continuous freeze curves of the stem, we connected thermocouple leads to digital multimeters with a resolution of 1 μv/digit. The rated accuracy was ±1 digit and less than 2 sec settling time. The reference junction was an insulated ice bath stable at 0 ± 0.2 C. Digital multimeters were connected to 0- to 100-mv, variable-speed, strip-chart recorders.

Only one tree could be accommodated at one time for stem-freeze profiles. Thus plants had to be spaced at least 1 day apart in cold hardening to ensure uniform cold-hardening time. Stem-freeze profile tests began at 1.7 C for 1 hr, followed by a 5.5 C/hr decrease until freezing was identified by heat release. Air temperature at that moment was maintained for the remainder of the test. Stem temperature lagged about 1 C behind air temperature. Thawing rate was 10 C/hr to 4.4 C. This thawing rate did not affect the percentage of freezing injury in preliminary trials associating different rates of thawing with resulting freeze injury. These trials also indicated that stem freezing usually begins at a single point near the top or bottom of the stem. Multipoint freezing was not found in 20 single trials, and only one midstem froze first. Midstem freezing can be demonstrated readily by properly spaced thermocouples. These freezing characteristics were evident also in the freezing of solutions in small bore-glass tubes exposed to identical conditions. Thermocouple spacing in this study was determined from preliminary trials and considered adequate to determine the rate of ice spread from elapsed time between onset of freezing at different points along the stem length.

Foliar Treatments. Different foliar treatments were applied to

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

determine the contribution of foliage to stem cold hardening. Treatments included defoliation, dehydration, and blocked stomata. Leaves were removed manually, water stress was imposed by reduced watering, and stomata were blocked with easily peelable, nonphytotoxic white latex paint and soaking sprays of antitranspirants Foliguard, Wiltpruf, All Safe, and Mobil Leaf. Relative water stresses in the leaves were determined by various stages of wilt and by the Schardakov dye method (9). In the stems, water stress was determined by electrical resistance adapted from freeze injury studies (8, 15). Porometer readings confirmed blocked stomata. Stomata blocked with paint were unblocked for freeze tests when we peeled off the paint. We saw no paint residue or physical damage under the microscope. Antitranspirant films were not removed.

RESULTS AND DISCUSSION

In this study as in previous studies (17), citrus stems tolerated freezing temperatures without noticeable injury if ice did not form in the tissues. Ice, and not low temperatures, kills plant cells when plant tissues are frozen under the microscope (12). Ice formation appears readily as heat release in freeze studies. Enough heat was released to show an average temperature increase of 1.2 C for cold-hardened and 1.6 C for unhardened grapefruit trees (Table I). Ice spread considerably slower in cold-hardened trees, even when supercooling was more than 0.5 C greater and air temperatures about 0.6 C lower than for unhardened trees. Increased supercooling and lower air temperatures did not result in more rapid ice spread. Ice spread could be slowed in citrus by some of the changes reported to occur in plants during cold hardening. These changes include increases in sugars, sap concentration, bound water, colloid stability, and a decrease in total water (21). In Table I, slower ice spread apparently would have contributed to less freeze injury in cold-hardened trees. In a model cell system, when ice crystals grew slower, ice crystals penetrated less through pores of membranes (11). Such penetration is a mechanism for ice spread from cell to cell, and many observations have substantiated increased leakage from freeze-damaged citrus tissues. This leakage indirectly indicates considerable damage to citrus cell membranes as a result of ice crystals. Such damage has been determined with electron microscopy (23).

Table I. *Average Freeze Profiles of Stems of 2-year-old Marsh Grapefruit Trees on Carrizo Citrange Rootstock*

Ice duration was 1 hr for all plants. Air temperature when ice started to form was −7.6 C for cold-hardened stems and −7 C for unhardened stems. Data are the average of 10 samples.

	Cold-hardened[1]		Unhardened	
	Scion	Root-stock	Scion	Root-stock
Start of ice formation (C)[2]	−7.1	−6.6	−6.4	−6
Rate of ice spread (mm/sec)	4.1		12.4	
Peak temperature (C)[3]	−5.9	−5.9	−4.8	−5.2
End temperature (C)[4]	−6.7	−7.3	−6.3	−5.9
Leaf kill (%)	98		100	
Scion dieback (%)	21		99	
Rootstock dieback (%)	0		14	

[1] Two weeks each successively of 21.1 C days and 10 C nights and 15.6 C days and 4.4 C nights.

[2] Stem surface temperature.

[3] Maximum stem surface temperature reached as a result of heat release.

[4] Stem surface temperature 1 hr after ice started to form.

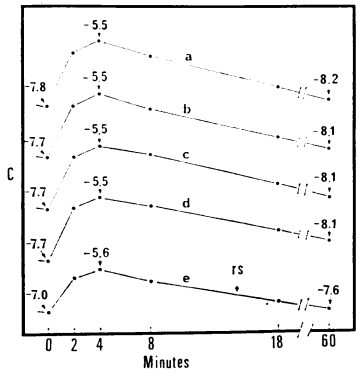

FIG. 1. Surface temperature (C) of the stem of a 2-year-old unhardened Marsh grapefruit tree on Carrizo citrange rootstock (rs) exposed to a controlled freeze. Air temperature decreased 5.5 C/hr and was −8.2 C when freezing was first indicated in the stem (0 min). This −8.2 C was maintained for 1 hr. Temperatures were simultaneously measured at different stem heights (cm above soil level) of (a) 85, (b) 65, (c) 40, (d) 20, and (e) 5.

The peak temperatures in Table I are maximum tissue temperatures resulting from heat release when air temperatures stayed at supercooling levels. These levels are indicated as the start of ice formation. Peak temperatures indicate a higher freezing level for unhardened tree stems, regardless of differences in supercooling. These peak temperatures are not regarded as true freezing points, because supercooling and tissue mass differ. The liquid phase in the stem was not completely frozen 1 hr after ice started to form. This prolonged freezing is suggested by the higher stem than air temperatures at the end of the 1-hr freeze. Slow rates of heat loss after freezing starts would help to lessen freeze injury during short, damaging freezes.

Exotherms, simultaneously measured at different stem heights above soil surface, showed a relatively uniform and rapid type of freezing in unhardened stems. In one example (Fig. 1), the thermocouples were at various stem heights, and 0 min was the moment of the first sign of heat release. Although not apparent in Figure 1, freezing was first detected at the 85-cm stem height, and within 45 sec had progressed to the 5 cm height. This rate of ice spread averaged more than 1.7 cm/sec from the top to the bottom of the stem. Ice propagation rates exceed 15 cm/min at −5.2 C in lemon (10). Exotherms along the stem were so similar that a composite could represent the entire unhardened stem. Exotherms were different for cold-hardened stems (Fig. 2). In Figure 2, freezing was first detected at the top of the stem, progressed rapidly for about 60 cm down the stem, and then slowed considerably. Ice was not indicated in the rootstock until about 5 min after the start of freezing at the top of the stem. Ice spread averaged slightly more than 0.2 cm/sec or about 1/8 of the rate for the unhardened stem in Figure 1. In contrast to the uniform type of freezing for unhardened stems, Figure 2 shows a very irregular type of freezing for hardened stems. This irregularity suggests some inhibition of ice spread not observed in unhardened stems. Figures 1 and 2 also illustrate that sometimes supercooling is less in hardened than unhardened stems. Exotherms substantiate that freezing points were surpassed in all instances. In Figure 1, the rootstock at −7 C was warmer than the scion at −7.7 C largely because of being closer to the soil and having wood somewhat larger in diameter.

The cold-hardening process in citrus stems probably fits into the general schemes proposed by others for cold acclimation of nondeciduous plants, for leaves are apparently essential (13). This essentiality has been indirectly shown in studies on citrus

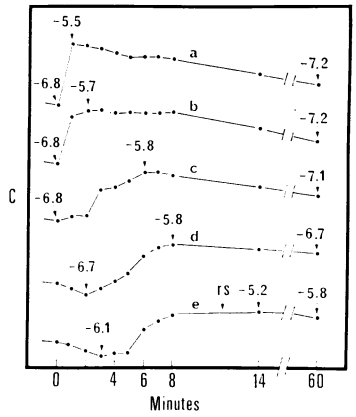

FIG. 2. Surface temperature (C) of the stem of a 2-year-old cold-hardened Marsh grapefruit tree on Carrizo citrange rootstock (rs) exposed to a controlled freeze. Air temperature decreased at 5.5 C/hr and was −7.6 C when freezing was first indicated in the stem (0 min). This −7.6 C was maintained for 1 hr. Temperatures were simultaneously measured at different stem heights (cm above soil level) of (a) 100, (b) 80, (c) 40, (d) 15, and (e) 5.

Table II. *Average Percentage of Stem Dieback of Sour Orange Seedlings Differing in Relative Water Stress and Exposed to Cold Hardening and Freezing*

Foliar Wilt	Water Potential in Leaves	Electrical Resistance in Stems	Stem Dieback[1]	
			Cold-hardened[2]	Unhardened[3]
	bars	*ohms × 10⁵*	%	
None	−3 to −6	3.3 ± 0.1	3 b[4]	99 a
Moderate	−20 to −26	5.7 ± 0.1	6 b	100 a
Severe	−28 to −34	7.0 ± 0.1	42 a	96 a

[1] Freeze damage after −6.7 C for 4 hr.
[2] Two weeks each successively of 21.1 C days and 10 C nights and 15.6 C days and 4.4 C nights.
[3] Greenhouse controls.
[4] Mean separation by Duncan's multiple range test at the 5% level (n = 10).

Table III. *Average Percentage of Stem Dieback for Rough Lemon Seedlings Soaked with Antitranspirants and Exposed to Cold Hardening and Freezing*

Antitranspirant	Stem Dieback[1]	
	Cold-hardened[2]	Unhardened[3]
1:4 dilution	%	
None	66 c[4]	100 a
Foliguard	87 b	100 a
Wiltpruf	91 b	100 a
All Safe	92 b	100 a
Mobil Leaf	100 a	100 a

[1] Freeze damage after −6.7 C for 4 hr.
[2] Two weeks each successively of 21.1 C days and 10 C nights and 15.6 C days and 4.4 C nights.
[3] Greenhouse controls.
[4] Mean separation by Duncan's multiple range test at the 5% level (n = 10).

cold hardening in which some citrus plants do not cold harden in total darkness (20), whereas others respond to a temperature-light interaction (16). For deciduous plants, some data indicate synthesis of a cold-hardiness promoter via the phytochrome system in the leaves (2, 3).

All my attempts to inhibit the functioning of citrus leaves resulted in less cold hardening in the stems. Leaf dehydration imposed by reduced watering especially harmed stem cold hardening when leaves wilted severely and water potential measured −28 to −34 bars in the leaves (Table II). In addition to curtailing severely the function of the leaves, imposed dehydration seemed to increase dehydration stresses as a result of ice in the stems. On the other hand, antitranspirants also reduced stem cold hardening (Table III). Antitranspirants form impermeable plugs that abruptly and severely inhibit gas exchange and water loss through stomata (1). The apparent effect is to maintain the plant status at the time of spray. This inhibition is not the same as the reduced photosynthesis and increased leaf-diffusion resistance measured by others during cold hardening of citrus (22). Such effects are a gradual slowing response to lower temperatures and reflect an adjustment stage. Antitranspirants cause an abrupt cessation of activity, as does latex paint.

Table IV. *Average Percentage of Stem Dieback of Citrus Seedlings with Leaf Surfaces Covered with Latex Paint and Exposed to Cold Hardening and Freezing*

Covered Leaf Surfaces	Stem Dieback[1]			
	Valencia orange		Duncan grapefruit	
	Cold-hardened[2]	Unhardened[3]	Cold-hardened	Unhardened
None	13 c[4]	100 a	26 c	100 a
Top	49 b	98 a	36 b	100 a
Bottom	97 a	99 a	93 a	100 a
Bottom and top	100 a	100 a		

[1] Freeze damage after −6.7 C for 4 hr.
[2] Two weeks each successively of 21.1 C days and 10 C nights and 15.6 C days and 4.4 C nights.
[3] Greenhouse controls.
[4] Mean separation by Duncan's multiple range test at the 5% level (n = 10).

Table V. *Average Percentage of Stem Dieback of Valencia Orange Seedlings Manually Defoliated to Different Degrees and Exposed to Cold Hardening and Freezing*

Defoliation	Stem Dieback[1]	
	Cold-hardened[2]	Unhardened[3]
%		
0	7 d[4]	100 a
25	15 cd	100 a
50	33 c	100 a
75	50 b	100 a
100	80 a	100 a

[1] Freeze damage after −6.7 C for 4 hr.
[2] Two weeks each successively of 21.1 C days and 10 C nights and 15.6 C days and 4.4 C nights.
[3] Greenhouse controls.
[4] Mean separation by Duncan's multiple range test at the 5% level (n = 10).

Total leaf coverage with latex paint in this study resulted in reduced cold hardening of the stem, and cold hardening was reduced more when the underside of the leaves were covered than when the top sides were (Table IV). When leaves were manually removed, and defoliation was increased, stem injury increased (Table V). All of these results indicate that citrus leaves contribute to cold hardening of the stems. Similar results have been reported by others for plants more cold-hardy than citrus (2, 6, 7).

Results in this study indicate that full and healthy tree canopies would help to develop maximum cold hardening in citrus stems during the winter. In cold-hardiness screening of citrus relatives, stem cold hardening is useful in separating selections that have a deciduous trait such as trifoliate orange and when all leaves are killed or when leaf injury is too overlapping to separate selections (14).

LITERATURE CITED

1. Davies, W. J. and T. T. Kozlowski. 1974. Short- and long-term effects of antitranspirants on water relations and photosynthesis of woody plants. J. Am. Soc. Hort. Sci. 99: 297-304.
2. Fuchigami, L. H., D. R. Evert, and C. J. Weiser. 1971. A translocatable cold hardiness promoter. Plant Physiol. 47: 164-167.
3. Fuchigami, L. H., C. J. Weiser, and D. R. Evert. 1971. Induction of cold acclimation in *Cornus stolonifera* Michx. Plant Physiol. 47: 98-103.
4. Gerber, J. F. and F. Hashemi. 1965. Freezing points of citrus leaves. Proc. Am. Soc. Hort. Sci. 86: 220-225.
5. Hendershott, C. H. 1962. The response of orange trees and fruits to freezing temperatures. Proc. Am. Soc. Hort. Sci. 80: 247-254.
6. Howell, G. S. and S. S. Stackhouse. 1973. The effect of defoliation time on acclimation and dehardening in tart cherry (*Prunus cerasus* L.). J. Am. Soc. Hort. Sci. 98: 132-136.
7. Hurst, C., T. C. Hall, and C. J. Weiser. 1967. Reception of the light stimulus for cold acclimation in *Cornus stolonifera* Michx. Hortscience 2: 164-166.
8. Ketchie, D. O. 1969. Methods of determining cold hardiness and cold injury in citrus. Proc. 1st Int. Citrus Symp. 2: 559-563.
9. Knipling, E. B. and P. J. Kramer. 1967. Comparison of the dye method with the thermocouple psychrometer for measuring leaf water potentials. Plant Physiol. 42: 1315-1320.
10. Lucas, J. W. 1954. Subcooling and ice nucleation in lemons. Plant Physiol. 29: 246-251.
11. Lusena, C. V. and W. H. Cook. 1953. Ice propagation in systems of biological interest. I. Effect of membranes and solutes in a model cell system. Arch. Biochem. Biophys. 46: 232-240.
12. Modlibowska, I. and W. S. Rogers. 1955. Freezing of plant tissues under the microscope. J. Exp. Bot. 6: 384-391.
13. Weiser, C. J. 1970. Cold resistance and acclimation in woody plants. Hortscience 5: 403-410.
14. Wilner, J. 1965. The influence of maternal parent on frost-hardiness of apple progenies. Can. J. Plant Sci. 45: 67-71.
15. Wilner, J., W. Kalbfleisch, and W. J. Mason. 1960. Note on two electrolytic methods for determining frost hardiness of fruit trees. Can. J. Plant Sci. 40: 563-565.
16. Yelenosky, G. 1971. Effect of light on cold-hardening of citrus seedlings. Hortscience 6: 234-235.
17. Yelenosky, G. and G. Horanic. 1969. Subcooling in wood of citrus seedlings. Cryobiology 5: 281-283.
18. Young, R. H. 1966. Freezing points and lethal temperatures of citrus leaves. Proc. Am. Soc. Hort. Sci. 88: 272-279.
19. Young, R. H. 1969. Cold hardening in citrus seedlings as related to artificial hardening conditions. J. Am. Soc. Hort. Sci. 94: 612-614.
20. Young, R. H. 1969. Cold hardening in 'Redblush' grapefruit as related to sugars and water soluble proteins. J. Am. Soc. Hort. Sci. 94: 252-254.
21. Young, R. H. 1970. Induction of dormancy and cold hardiness in citrus. Hortscience 5: 411-413.
22. Young, R. H. and W. D. Bell. 1974. Photosynthesis in detached leaves of cold-hardened citrus seedlings. J. Am. Soc. Hort. Sci. 99: 400-403.
23. Young, R. H. and M. Mann. 1974. Freeze disruption of sour orange leaf cells. J. Am. Soc. Hort. Sci. 99: 403-407.