Plant Physiol. (1983) 73, 877–880
0032-0889/83/73/0877/04/$00.50/0

Translocation of Carbohydrates and Proline in Young Grapefruit Trees at Low Temperatures[1]

Received for publication May 3, 1983 and in revised form July 27, 1983

ALBERT C. PURVIS AND GEORGE YELENOSKY
University of Florida, IFAS, Agricultural Research and Education Center, Lake Alfred, Florida 33850 (A. C. P.); and United States Department of Agriculture, Agricultural Research Service, 2120 Camden Road, Orlando, Florida 32803 (G. Y.)

ABSTRACT

Girdling and defoliation of fruit-bearing grapefruit (*Citrus paradisi* Macf.) branches inhibited the accumulation of soluble carbohydrates and proline in fruit tissues during low temperature treatment of trees. These treatments did not inhibit hydrolysis of sucrose to reducing sugars. Flavedo and albedo tissues responded similarly to low temperatures but little or no change occurred in the juice. Therefore, soluble carbohydrates and proline do not appear to interchange between different tissues of the fruit at low temperatures but instead are translocated into the fruit from other parts of the plant. Girdling fruit-bearing branches immediately after low temperature treatments inhibited the accumulation of sucrose in fruit tissues at dehardening temperatures. Also, proline levels decreased rapidly in fruit on girdled branches at dehardening temperatures. This rapid decrease suggests proline may serve as a source for respiratory energy in grapefruit during rapidly changing temperatures that favor active growth and during recovery of citrus from environmental stress.

Grapefruit which have accumulated relatively high concentrations of carbohydrates and proline are less likely to be injured by low, nonfreezing temperatures (9–11). A similar avoidance of low temperature injury occurs in citrus leaves where the accumulation of carbohydrates has been attributed to lower rates of translocation and metabolism of photosynthate at low temperature (5). Free proline also accumulates in citrus leaves during cold hardening (12, 20, 21), but its specific role in cold tolerance is unknown.

Leaves appear to be the source of soluble carbohydrates which accumulate in grapefruit flavedo (colored portion of the peel) tissue during low temperature hardening of the trees (12). Leaves may also be the site of synthesis of proline which accumulates in the flavedo tissue at low temperatures. Proline accumulates in leaves prior to accumulating in flavedo tissue, and levels which accumulate in leaves exceed by 5-fold those levels which accumulate in the flavedo (12). Leaves have been implicated as the source of proline which accumulates in roots of other plant species during water and low temperature stress (14, 16, 24).

Increases in soluble carbohydrates and proline may be more a consequence of low temperature stress conditions rather than a direct factor in tissue hardening; but, sugars and proline levels, nevertheless, do correlate well with the chilling resistance of grapefruit (9–11) as well as with cold hardiness of other citrus tissues (12, 20–23). The objective of this study was to determine whether leaves contribute to the accumulation of soluble carbohydrates and proline in fruit tissues when young trees were exposed to a low temperature hardening regime. Our approach was to design experiments to elucidate the source, translocation, partitioning and utilization of carbohydrates and proline in citrus tissues during exposure to low temperatures and during the rewarming period.

MATERIALS AND METHODS

Low Temperature Regimes. Potted 'Marsh' grapefruit trees (*Citrus paradisi* Macf.) that averaged seven fruit, 7 to 9 cm in diameter, at the start of the experiment were used for all low temperature experiments. Three tree replicates were used in each experiment. Trees were exposed to the low temperature hardening regime in controlled environment walk-in chambers previously described (12). The chambers were equipped with fluorescent and incandescent lights which provided photosynthetic photon flux density of 400 μmol m^{-2} s^{-1} during 12-h photoperiods. Trees were acclimated in the chambers at 25°C for 1 week prior to the low temperature treatments. Temperature was decreased 5°C each week until a final temperature of 5°C was reached (4 weeks from 25°C). After 1 week at 5°C, the temperature was increased to 25°C and maintained at that temperature for 3 weeks. Temperatures were constant throughout the light-dark periods. These temperature regimes have been previously shown to harden and deharden the young grapefruit trees (12).

Defoliation and Girdling. The source of metabolites was eliminated by removing the leaves from trees prior to initiating the low temperature treatments. After the low temperature treatment, some branches bearing fruit were girdled before the rewarming phase began in an attempt to change the partitioning and utilization of internal metabolites. To study translocation during low temperature treatment, branches bearing fruit were girdled before initiation of low temperature treatments. Defoliation consisted of manually removing all leaves including the petioles. Branches were girdled by removing a 1-cm-wide strip of bark, including phloem tissue, 6 to 8 cm from the fruit using a sharp scalpel. All leaves between the girdle and the fruit were removed.

Tissue Analyses. For carbohydrate and proline analyses, four leaves were harvested from each tree, cut into 1-cm sections and a 3-g subsample was extracted in boiling 80% ethyl alcohol as previously described (10, 11). Analyses were made on tissues from individual fruits. The flavedo was removed in 5-mm strips. The strips were cut into 5-mm lengths and 3-g subsamples were extracted in boiling 80% ethyl alcohol. Juice was extracted with a Sunkist hand reamer and immediately killed in boiling 80% ethyl alcohol. The albedo (white portion of the peel) tissue was cut into small pieces and extracted in boiling 80% ethyl alcohol. Sugars were analyzed as previously described (11, 12). Proline was analyzed by the methods of Ting and Rouseff (19).

[1] Florida Agricultural Experiment Stations Journal Series No. 4705.

RESULTS

Effect of Defoliation on Flavedo Metabolites. Removing the leaves from grapefruit trees prior to imposing a low temperature hardening regime altered the accumulation of carbohydrates and proline in flavedo tissue of fruit (Table I). Although total soluble carbohydrates, reducing sugars, and proline all increased in flavedo tissue of fruit on foliated trees, only proline increased in flavedo tissue of fruit on defoliated trees. Soluble carbohydrate levels remained unchanged in flavedo tissue of fruit on defoliated trees exposed to low temperatures.

When the temperature was increased to 25°C, total soluble carbohydrates declined and sucrose increased in flavedo tissue of fruit on foliated trees. Little change occurred in proline level. Girdling the fruit-bearing branch after imposing a low temperature hardening regime altered the pattern of accumulation of constituents during the dehardening regime. Sucrose did not increase in flavedo tissue of fruit on girdled branches during the dehardening process. In contrast to fruit on ungirdled branches, proline decreased in fruit on girdled branches. Girdling defoliated trees prior to the dehardening process resulted in further decreases in total soluble carbohydrate, sucrose, and proline concentrations.

Effect of Defoliation on Albedo Metabolites. Albedo tissue responded similarly to flavedo tissue with respect to defoliation and low temperature hardening treatments (Table II). However, soluble carbohydrate levels were considerably higher and proline levels were lower in albedo tissue than in flavedo tissue of the same fruit. Sucrose accounted for a larger proportion of carbohydrates in albedo tissue and defoliation resulted in a large decrease in sucrose content during the low temperature treatments.

Total soluble carbohydrate and reducing sugar levels declined in albedo tissue during the dehardening process of foliated trees. Girdling the fruit bearing branch prior to the dehardening process also produced results in albedo tissue similar to those for flavedo tissue. Total soluble carbohydrate, sucrose, and proline levels all declined sharply in girdled branches. Even greater declines in both sucrose and proline levels in albedo tissue were observed for fruit on girdled branches of defoliated trees.

Effect of Defoliation on Juice Metabolites. Defoliation and low temperature hardening treatments had no effect on soluble carbohydrate levels in the juice of grapefruit (Table III). Carbohydrate levels were similar to those found in albedo tissue and higher than those found in flavedo tissue. Low temperature hardening treatments did cause an increase in proline concentration of the juice, but defoliation was without effect. Proline levels were lower, however, than those found in flavedo or albedo tissues. Flavedo tissue had the highest proline content of all the fruit tissues analyzed. Little or no change occurred in the juice constituents during the dehardening process, and girdling prior to the dehardening process had no effect.

Effect of Girdling on Flavedo Constituents during Exposure to a Low Temperature Hardening Regime. Girdling fruit-bearing branches prior to imposing the low temperature hardening regime completely negated the effect of low temperature on the accumulation of total soluble carbohydrates, reducing sugars, and proline in grapefruit flavedo tissue (Fig. 1). Sucrose levels declined in flavedo tissue of fruit on girdled branches of trees

Table I. *Carbohydrate and Proline Levels in Flavedo Tissue of Grapefruit from Foliated and Defoliated Trees Which Were Exposed to a Low Temperature Hardening Regime*

Initial temperature was 25°C. Temperature was decreased 5°C at weekly intervals. Final temperature was 5°C. After the low temperature treatment, some fruit-bearing branches were girdled; others were left ungirdled and the temperature was increased to 25°C for 3 additional weeks. Means of individual fruit from three separate trees ± SD.

Treatment	Total Soluble Carbohydrates	Reducing Sugars	Sucrose	Proline
	mg/g fresh wt			*μg/g fresh wt*
Initial (25°C)	41.6 ± 3.1	20.5 ± 3.0	14.0 ± 2.2	1094 ± 325
After low temperature regime				
Foliated	68.4 ± 3.2	30.1 ± 5.0	19.6 ± 4.5	2739 ± 441
Defoliated	44.1 ± 3.7	21.4 ± 1.9	11.7 ± 3.6	2002 ± 202
After 3 weeks at 25°C				
Foliated				
Ungirdled	45.5 ± 6.3	17.5 ± 1.0	28.1 ± 6.7	2419 ± 164
Girdled	43.6 ± 6.5	29.7 ± 5.2	13.9 ± 1.4	1378 ± 138
Defoliated				
Ungirdled	40.1 ± 5.2	25.3 ± 2.4	14.9 ± 6.5	1533 ± 268
Girdled	34.4 ± 1.3	27.1 ± 1.9	7.2 ± 0.8	1066 ± 253

Table II. *Carbohydrate and Proline Levels in Albedo Tissue of Grapefruit from Foliated and Defoliated Trees Which Were Exposed to a Low Temperature Hardening Regime*

Initial temperature was 25°C. Temperature was decreased 5°C at weekly intervals. Final temperature was 5°C. After the low temperature treatment, some fruit-bearing branches were girdled; others were left ungirdled and the temperature was increased to 25°C for 3 additional weeks. Means of individual fruit from three separate trees ± SD.

Treatment	Total Soluble Carbohydrates	Reducing Sugars	Sucrose	Proline
	mg/g fresh wt			*μg/g fresh wt*
Initial (25°C)	65.7 ± 8.1	30.8 ± 4.4	33.4 ± 8.3	811 ± 248
After low temperature regime				
Foliated	86.0 ± 9.0	40.8 ± 6.8	34.0 ± 5.8	1174 ± 433
Defoliated	66.1 ± 1.4	31.5 ± 1.9	18.2 ± 5.0	1219 ± 114
After 3 weeks at 25°C				
Foliated				
Ungirdled	68.8 ± 4.1	31.3 ± 5.0	37.4 ± 2.1	1436 ± 44
Girdled	49.3 ± 2.1	35.8 ± 2.3	13.5 ± 0.4	958 ± 82
Defoliated				
Ungirdled	49.8 ± 5.3	34.0 ± 5.0	15.8 ± 2.9	1181 ± 318
Girdled	45.4 ± 1.8	34.4 ± 1.0	9.8 ± 0.6	785 ± 225

Table III. *Carbohydrate and Proline Levels in the Juice of Grapefruit from Foliated and Defoliated Trees Which Were Exposed to a Low Temperature Hardening Regime*

Initial temperature was 25°C. Temperature was decreased 5°C at weekly intervals. Final temperature was 5°C. After the low temperature treatment, some fruit-bearing branches were girdled; others were left ungirdled and the temperature was increased to 25°C for 3 additional weeks. Means of individual fruit from three separate trees ± SD.

Treatment	Total Soluble Carbohydrates	Reducing Sugars	Sucrose	Proline
	mg/g fresh wt			*μg/g fresh wt*
Initial (25°C)	71.1 ± 8.5	35.7 ± 2.9	23.1 ± 2.8	238 ± 65
After low temperature regime				
Foliated	73.7 ± 7.5	32.5 ± 3.5	28.6 ± 5.4	482 ± 21
Defoliated	73.2 ± 1.9	33.5 ± 2.3	20.4 ± 1.3	402 ± 21
After 3 weeks at 25°C				
Foliated				
Ungirdled	78.0 ± 8.4	50.7 ± 3.4	27.3 ± 5.7	457 ± 46
Girdled	78.8 ± 4.3	50.6 ± 0.7	28.2 ± 4.3	403 ± 26
Defoliated				
Ungirdled	73.7 ± 0.9	40.3 ± 3.0	29.0 ± 3.1	453 ± 60
Girdled	74.2 ± 6.6	39.7 ± 1.7	28.0 ± 5.6	336 ± 46

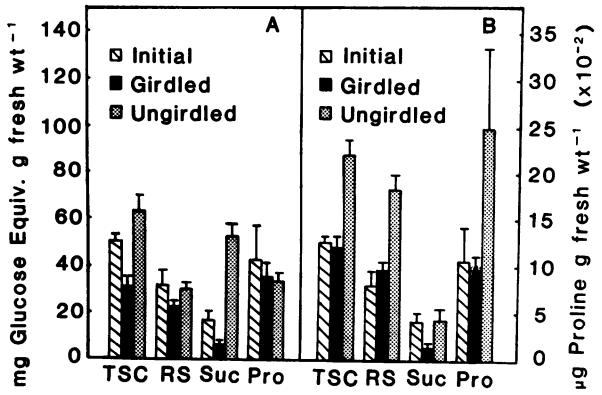

FIG. 1. Carbohydrate and proline levels in flavedo tissue of grapefruit from girdled and ungirdled branches of trees. Initial samples were taken prior to temperature treatments. A, Trees were maintained at 25°C for the entire experimental period (4 weeks). B, Trees were subjected to a low temperature regime with the temperature being decreased 5°C each week. Final temperature was 5°C. Branches were girdled after initial samples were taken. Means of individual fruit from three separate trees ± SD. Total soluble carbohydrates (TSC), reducing sugars (RS), sucrose (Suc), proline (Pro).

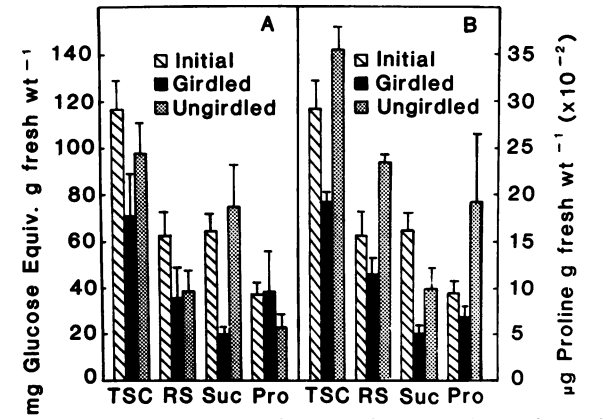

FIG. 2. Carbohydrate and proline levels in albedo tissue of grapefruit from girdled and ungirdled branches of trees. Initial samples were taken prior to temperature treatments. A, Trees were maintained at 25°C for the entire experimental period (4 weeks). B, Trees were subjected to a low temperature regime with the temperature being decreased 5°C each week. Final temperature was 5°C. Branches were girdled after initial samples were taken. Means of individual fruit from three separate trees ± SD. Total soluble carbohydrates (TSC), reducing sugars (RS), sucrose (Suc), proline (Pro).

which were exposed to low temperatures as well as those which were maintained at 25°C. Girdling the fruit-bearing branches of trees maintained at 25°C also resulted in decreases in total soluble carbohydrates and reducing sugars but had no effect on the proline level.

Effect of Girdling on Albedo Constituents during Exposure to a Low Temperature Hardening Regime. Girdling also prevented the accumulation of total soluble carbohydrates, reducing sugars, sucrose, and proline in albedo tissue as in flavedo tissue (Fig. 2). Initial carbohydrate levels were higher than those found in the flavedo tissue and also higher than those in the albedo tissue in the previous experiment (Table II). However, seasonal differences in the concentration of constituents in fruit tissues are expected (9–11). Girdling also resulted in lower carbohydrate levels in albedo tissue of fruit on trees maintained at 25°C.

Effect of Girdling on Juice Constituents during Exposure to a Low Temperature Hardening Regime. Low temperature treatments did not alter the accumulation of total soluble carbohydrates, reducing sugars, and sucrose in the juice constituents of grapefruit (Fig. 3). Girdling did, however, inhibit sucrose accumulation in juice of fruit on trees which were exposed to low temperatures as well as those which were maintained at 25°C. Proline tended to increase in the juice of fruit on ungirdled branches of trees exposed to low temperatures.

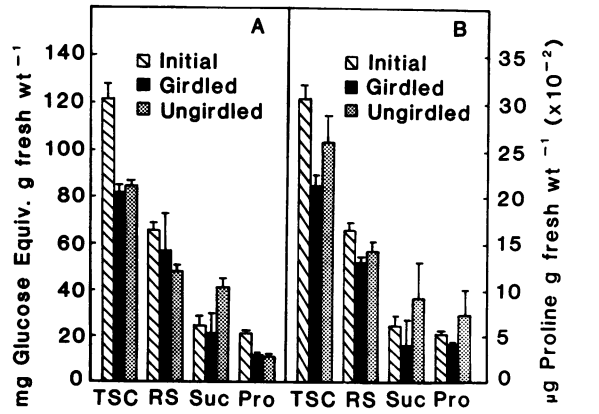

FIG. 3. Carbohydrate and proline levels in the juice of grapefruit from girdled and ungirdled branches of trees. Initial samples were taken prior to temperature treatments. A, Trees were maintained at 25°C for the entire experimental period (4 weeks). B, Trees were subjected to a low temperature regime with the temperature being decreased 5°C each week. Final temperature was 5°C. Branches were girdled after initial samples were taken. Means of individual fruit from three separate trees ± SD. Total soluble carbohydrates (TSC), reducing sugars (RS), sucrose (Suc), proline (Pro).

DISCUSSION

Data from this study support the concept that leaves are both the source of soluble carbohydrates as well as the primary site of synthesis of proline which accumulates in the tissues of grapefruit when young trees are exposed to a low temperature hardening regime. Defoliation prior to the low temperature treatments completely inhibited any increase in soluble carbohydrates and partially inhibited the accumulation of proline in fruit tissues during the low temperature treatments. However, girdling the fruit-bearing branches prior to the low temperature treatment completely prevented the accumulation of both soluble carbohydrates and proline in fruit tissues. Girdling the stems of citrus seedlings also prevented the accumulation of proline in root tissues (17). Furthermore, radioactive tracer studies indicated that a large proportion of the ^{14}C in the amino acid fraction was cycled into proline where it accumulated in large quantities in the leaves of girdled seedlings (17). Such observations do not necessarily mean that proline is entirely synthesized in citrus leaves since girdling also inhibits the translocation of sucrose and glutamate, the putative precursors of proline. However, proline does not accumulate in detached fruit containing a high level of sucrose and stored at low temperatures (12). Thus, citrus fruit tissues, like citrus root tissues (17) and root tissues of other species (1), may either lack the capacity or have a greatly reduced capacity to synthesize proline.

Therefore, the accumulation of proline in citrus fruit tissues (and root tissues [17]) during stress apparently results from a reduction in the utilization or oxidation of proline (15) which is translocated into them. Boggess *et al.* (3) and Sells and Koeppe (13) have shown that proline is oxidized in mitochondria. Proline oxidation rates by mitochondria isolated from water-stressed seedlings, decreased approximately 60% as seedling water potentials were decreased from −0.5 to −1.0 mPa while succinate,

exogenous NADH, and malate plus pyruvate oxidation rates decreased only 10% to 15% over the same stress range (13). Low temperatures may likewise result in decreased proline oxidase activity in mitochrondria of citrus fruit tissues.

Girdling fruit-bearing branches after the trees were exposed to low temperatures, but prior to exposure to dehardening temperatures, prevented the accumulation of sucrose in flavedo tissue during the subsequent dehardening process but had little effect on the level of reducing sugars. Thus, the sucrose accumulation in flavedo tissue after temperatures increase in the spring (10, 11) apparently results from increased translocation into the fruit rather than from the resynthesis of sucrose from reducing sugars already present in the tissue. Even greater reductions in sucrose accumulation were seen in albedo tissue of fruit on branches girdled prior to the dehardening treatment. Girdling the fruit-bearing branches after the low temperature treatments also resulted in large decreases in proline in flavedo tissue during the dehardening treatment. Thus, free proline accumulation during stress may reinforce the accumulation of reducing sugar to be used in meeting abrupt increases in energy demands during changing growing conditions and/or recovery from stresses. Blum and Ebercon (2) found that recovery of sorghum cultivars from water stress was positively correlated with proline accumulation during stress and with subsequent free ammonia concentration and dark respiration rates during recovery. They concluded that proline may serve as a source of respiratory energy to the recovering plant.

There is no indication that soluble carbohydrates and proline are transferred between the various fruit tissues (flavedo, albedo, and juice vesicles) at low temperatures. The patterns of soluble carbohydrate and proline accumulation in flavedo and albedo tissues during exposure of the trees to low temperatures were similar. Smaller increases in the concentration of both constituents were seen in albedo tissue than in flavedo tissue, and little or no change occurred in the juice components during the relatively short low temperature treatments imposed in this study. Compared to flavedo tissue, albedo tissue and juice vesicles have considerably lower respiratory rates (6) which indicates they also have lower metabolic activity. It is not surprising, therefore, that low temperature should have had less effect on the metabolism of juice vesicles than it had on the metabolism of the flavedo tissue.

The soluble carbohydrates in flavedo tissue are almost entirely accounted for by sucrose and the reducing sugars, glucose and fructose (10, 18). Sucrose and reducing sugars also account for most of the soluble carbohydrates in albedo tissue. Juice vesicles, however, appear to contain additional soluble carbohydrates since sucrose and reducing sugars accounted for only two-thirds to three-fourths of the total soluble carbohydrates in juice vesicles.

The decline in soluble carbohydrates in flavedo and albedo tissues of fruit on trees maintained in chambers at 25°C (12) indicates that photosynthesis was inadequate to support the tree with fruit. At lower temperatures, however, the maintenance respiration requirement of the leaves was likely decreased to the point that carbohydrates not only accumulated in leaves but were also translocated to fruit tissues. Decreasing the sink strength by girdling some of the fruit-bearing branches enhanced the accumulation of soluble carbohydrates in the other fruit on the ungirdled branches.

Whether soluble carbohydrate and proline accumulation is beneficial to citrus fruit at low temperatures still is not known. Proline accumulation in plants subjected to low temperatures (4, 20, 21) and water stress (14–16) appears to be a universal phenomenon. Although proline synthesis in leaves (14, 16, 24) and translocation to other plant tissues (24) enhance the involvement of proline in citrus fruit chillng tolerance, high levels of proline alone do not render plant tissue hardy to low temperatures or water stress (9, 15). Accumulation of soluble carbohydrates and proline are more likely prerequisites rather than causal factors of low temperature hardening and chilling resistance processes in plants (7, 8, 24).

LITERATURE CITED

1. ASPINALL D, LG PALEG 1981 Proline accumulation: Physiological aspects. *In* LG Paleg, D Aspinall, eds, The Physiology and Biochemistry of Drought Resistance in Plants. Academic Press, Australia, pp 205–241
2. BLUM A, A EBERCON 1976 Genotypic responses in sorghum to drought stress. III. Free proline accumulation and drought resistance.Crop Sci 16: 428–431
3. BOGGESS SF, DE KOEPPE, CR STEWART 1978 Oxidation of proline by plant mitochrondria. Plant Physiol 62: 22–25
4. CHU TM, M JUSAITIS, D ASPINALL, LG PALEG 1978 Accumulation of free proline at low temperatures. Physiol Plant 43: 254–260
5. GUY CL, G YELENOSKY, HC SWEET 1981 Distribution of ^{14}C photosynthetic assimilates in 'Valencia' orange seedlings at 10° and 25°C. J Am Soc Hortic Sci 106: 433–437
6. HUSSEIN AA 1944 Respiration in the orange. A study of systems responsible for oxygen uptake. J Biol Chem 155: 201–211
7. PAQUIN R 1977 Effet des basses temperatures sur la resistance au gel de la Luzerne (*Medicago media* Pers.) et son contenu en proline libre. Physiol Veg 15: 657–666
8. PAQUIN R, P LECHASSEUR 1982 Acclimation naturelle de la Luzerne (*Medicago media* Pers.) au froid. II. Variations de la teneur en sucres totaux des feuilles et des collets. Acta Ecologia/Ecol Plant 3: 27–38
9. PURVIS AC 1981 Free proline in peel of grapefruit and resistance to chilling injury during cold storage. HortScience 16: 160–161
10. PURVIS AC, W GRIERSON 1982 Accumulation of reducing sugar and resistance of grapefruit peel to chilling injury as related to winter temperatures. J Am Soc Hortic Sci 107: 139–142
11. PURVIS AC, K KAWADA, W GRIERSON 1979 Relationship between mid-season resistance to chilling injury and reducing sugar level in grapefruit peel. HortScience 14: 227–229
12. PURVIS AC, G YELENOSKY 1982 Sugar and proline accumulation in grapefruit flavedo and leaves during cold hardening of young trees. J Am Soc Hortic Sci 107: 222–226
13. SELLS GD, DE KOEPPE 1981 Oxidation of proline by mitochondria isolated from water-stressed maize shoots. Plant Physiol 68: 1058–1063
14. SINGH TN, D ASPINALL, LG PALEG, SF BOGGESS 1973 Stress metabolism. II. Changes in proline concentration in excised plant tissues. Aust J Biol Sci 26: 57–63
15. STEWART CR, AD HANSON 1980 Proline accumulation as a metabolic response to water stress. *In* NC Turner, PJ Kramer, eds, Adaptation of Plants to Water and High Temperature Stress. John Wiley and Sons, New York, pp 173–189
16. STEWART CR, CJ MORRIS, JF THOMPSON 1966 Changes in amino acid content of excised leaves during incubation. II. Role of sugar in the accumulation of proline in wilted leaves. Plant Physiol 41: 1585–1590
17. STEWART I 1961 Nitrogen transformations in citrus trees. Soil Crop Sci Soc Fla Proc 21: 272–282
18. TING SV, EJ DESZYCK 1961 The carbohydrates in the peel of oranges and grapefruit. J Food Sci 26: 146–152
19. TING, SV, RL ROUSEFF 1979 Proline content in Florida frozen concentrated orange juice and canned grapefruit juice. Proc Fla State Hortic Soc 92: 143–145
20. YELENOSKY G 1978 Cold hardening 'Valencia' orange trees to tolerate −6.7°C without injury. J Am Soc Hortic Sci 103: 449–452
21. YELENOSKY G 1979 Accumulation of free proline in citrus leaves during cold hardening of young trees in controlled temperature regimes. Plant Physiol 64: 425–427
22. YELENOSKY G, CL GUY 1977 Carbohydrate accumulation in leaves and stems of 'Valencia' orange at progressively colder temperatures. Bot Gaz 138: 13–17
23. YOUNG R 1969 Cold hardening in 'Redblush' grapefruit as related to sugars and water soluble proteins. J Am Soc Hortic Sci 94: 252–254
24. VEZINA L, R PAQUIN 1982 Effet des basses temperatures sur la distribution de la proline libre dans les plantes de Luzerne (*Medicago media* Pers.). Physiol Veg 20: 101–109