Table 4. Zinc concentration in various trunk tissues of blight-affected 'Valencia' orange trees on rough lemon rootstock.

	Mean Zn concn	
Sample tissue	dry wt (ppm)[z]	µg/patch area
Outer bark patch	26a	37
Inner bark patch	330b	340
Cambial zone	367b	42
Outer wood	24a	25

[z]Mean separation by Duncan's multiple range test, 5% level.

source for the high Zn levels of the phloem.

Trunk phloem Zn levels were low in all healthy seedling trees of the various citrus species commonly used as rootstocks and scions. In budded combinations, the trunk phloem Zn levels were usually higher, especially above rough lemon rootstock. The levels of phloem Zn were always elevated in diseased trees, but, again, the rootstock influenced the increase. The greater Zn accumulation in the trunk phloem of budded trees than of the seedling rootstock, the different Zn level depending on rootstock, and the extent of Zn increases in diseased trees depending on rootstock may indicate degrees of compatibility to phloem flow across the bud unions of various scion-rootstock combination. Results indicated a considerable decrease in Zn across the bud unions in trees on rough lemon rootstock, but none for healthy trees on trifoliate rootstock.

Zn and other mineral elements in the phleom may exist primarily as organic complexes in *Citrus* as in *Ricinus* (7, 8). Knowledge of the Zn status in the phloem would be very useful in studying the upset in blight-affected trees and some aspects of graft compatibility to phloem translocation to roots.

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HortScience 16(2):160–161. 1981.

Free Proline in Peel of Grapefruit and Resistance to Chilling Injury during Cold Storage[1]

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Additional index words. Citrus paradisi, postharvest disorders

Abstract. **Peel samples of 'Marsh' grapefruit (*Citrus paradisi* Macf.) from 2 separate chilling injury (CI) experiments conducted during the 1979-80 season were analyzed for proline. Proline levels were highest in the peel of grapefruit after the seasonal night temperatures reached their minimum and levels declined after night temperatures increased in the spring. The greatest resistance of grapefruit peel to CI during postharvest cold storage coincided with high proline concentrations. Peels of unexposed interior canopy fruit had higher proline contents and were also more resistant to CI than peels of exposed exterior canopy fruit. Proline accumulation may be a consequence of an environmental stress rather than a cause of hardening to the stress or a mechanism of resistance.**

Grapefruit harvested during midseason (February to March) are generally more resistant to CI than fruit harvested either earlier or later in the season (5, 8). Unexposed interior canopy fruit are also more resistant to CI than exposed exterior canopy fruit (7). The cause of variation in susceptibility of grapefruit to CI during cold storage is not known. Midseason resistance has been related to a high level of reducing sugars which accumulate in the peel at low grove temperatures (5, 8), but no differences were observed between sugar levels in the peels of unexposed interior canopy and exposed exterior canopy fruit (7).

Proline accumulation has been associated with increased freezing and drought resistance of several species including citrus (1, 2, 3, 6, 14, 15). However, a relationship between preharvest proline accumulation in grapefruit peel and resistance of grapefruit to CI during cold storage has not been reported. Therefore, peel samples from 2 separate CI experiments conducted during the 1979-80 season were analyzed for proline content and the levels were related to peel resistance to CI.

The samples analyzed for proline consisted of the flavedo (colored) portion of the peel of randomly selected fruit from each lot of fruit placed in cold storage. One set of samples came from a seasonal experiment in which grapefruit were randomly harvested from throughout the canopies of 5 trees at about 2-week intervals beginning October 14, 1979 and ending May 12, 1980. The other set of samples came from unexposed fruit harvested from inside the leafy canopy and exposed exterior canopy fruit maintained as separate lots. The samples were stored in polyethylene bags at –22.2°C until the end of the season when they were all analyzed simultaneously for proline content.

The peel samples were cut into small pieces (ca. 0.5 mm^2) and subsamples of 5 g each were extracted with boiling 80% ethanol as previously described except that the resin step was omitted (8). Proline content in the ethanol extracts was

[1]Received for publication September 15, 1980. Florida Agricultural Experiment Stations Journal Series No. 2603.

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ence and Technology.

[2]The author gratefully acknowledges Dr. R. C. J. Koo for supplying the temperature data and Mr. J. D. Rice for his technical assistance during the study.

determined by the procedure of Ting and Rouseff (13).

The seasonal trend in proline content of the peel of grapefruit is shown in Fig. 1. Proline increased in the peel when the mean minimum daily air temperature for the 30 days prior to picking approached the season minimum and declined after the temperature increased in the spring.

Grapefruit were moderately susceptible to CI during the entire 1979-80 season except for a brief period of resistance in early March 1980 (Fig. 1). Resistance to CI coincided with a high proline content in the peel.

Resistance to CI was correlated with peel proline content over the season ($r = 0.647$, $P < 1\%$) and for unexposed interior and exposed exterior canopy fruit ($r = 0.996$, $P < 1\%$) (Fig. 2). Interior canopy fruit were more resistant to CI and had higher proline contents than exterior canopy fruit from the same trees.

Proline has been implicated in freezing and drought resistance of several species, including citrus (1, 2, 3, 6, 14, 15). The data presented here indicates that a relationship also exists between proline and resistance to CI.

Proline accumulates in plant tissues during low temperature stress (2, 14) and water stress (1, 3, 6, 15). The increased free proline content is due to both a stimulation of proline synthesis and an inhibition of its utilization, rather than due to concentration during dehydration of the tissue (4).

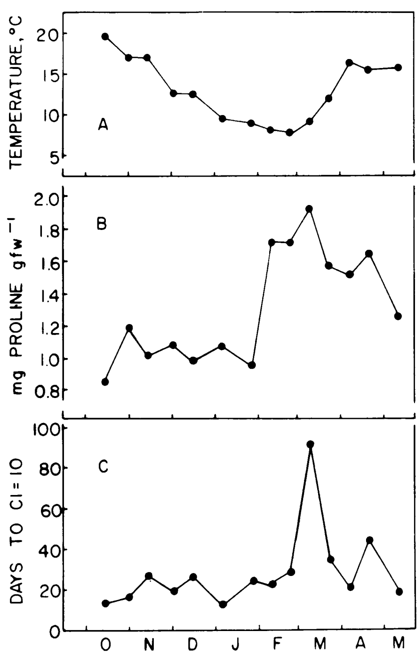

Fig. 1. Seasonal changes in proline, resistance to CI determined by holding fruit at 4.4°C, and mean minimum field temperature (°C) for the 30 days preceding harvests. CI = 10 is the days in storage before the onset of CI is apparent to a trained observer.

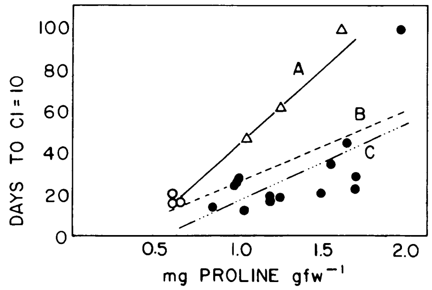

Fig. 2. Relationship between resistance of 'Marsh' grapefruit to CI at 4.4°C and free proline content in the peel. Open circles (o) and open triangles (Δ) represent fruit from outside and inside tree canopy, respectively and maintained as separate samples. Closed circles (●) are fruit harvested from outside and inside canopies collectively at approx 2-week intervals throughout the season. Regression line A (----) is for outside and inside fruit maintained as separate samples ($Y = -28.74 + 0.072X$; $r = 0.996$). Regression line B (——) is for all samples ($Y = -8.65 + 0.034X$; $r = 0.582$.). Regression line C (____. . . .) is for fruit harvested at 2-week intervals with no separation of outside and inside canopy fruit ($Y = -20.64 + 0.037X$; $r = 0.647$). All correlations are significant at the 1% level.

Low grove temperatures are probably responsible for the seasonal accumulation of proline in grapefruit peel and perhaps also the increased proline levels in the peels of interior canopy fruit. Unexposed interior canopy fruit have lower temperatures throughout much of the day than the exterior fruit which are exposed to direct solar radiation (12). On the other hand, the exposed exterior fruit are more subject to water stress than the unexposed interior fruit (12). However, any water stress experienced by the exterior fruit is more apt to occur during the time when the daily temperatures reach their maximum. Hence, the effect of water stress may be opposed by temperature.

Whether proline accumulation is of adaptive value to grapefruit is not known. Several beneficial roles for proline as an osmoticum (11), a desiccation protectant (9, 10), a nitrogen and reducing power sink during stress (1), and a source of energy during recovery from stress (3) have all been suggested for freezing and drought resistance. However, it is unlikely that the level of proline alone is responsible for resistance of grapefruit to CI since the seasonal accumulation of proline occurred well in advance of any increase in resistance to CI. Nevertheless, the correlation between proline content and resistance of grapefruit to CI during the season and with canopy position suggest that proline plays a role, either directly or indirectly in resistance to CI. Until the mechanism of CI is elucidated, the role of proline in resistance to CI is still speculative.

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