

Water Relations and Osmotic Adjustment via Sucrose -Hexose Conversions in Citrus Fruit during Cyclic and Continuous Water Stress¹

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Summary

Calamondin trees (*Citrus madurensis* Lour.) were used in this research to clarify the relationship between altered water availability and fruit sugar, and to determine the extent to which changes in the latter are mediated by reduced sugar import, osmotic adjustment, or sugar exit via xylem back-flow. Responses to a soil moisture cycle of "stress:regular irrigation:stress" was compared to "continuous stress" and "continuous irrigation" to contrast changes in carbohydrate translocation and/or metabolism resulting from alterations in soil water availability.

The results showed that water and sugar parameters appeared to stabilize in fruit after approximately 9 days of water stress. Pre-stress osmotic status was regained 5 days after reirrigation, but lost after only 3 days of additional stress. "Sweeter" fruits produced under mild stress appeared to result from a decrease in fruit size, but sugar conversion occurred.

The most striking observations in alternating stress experiments were dramatic sucrose-hexose conversions in fruit apparently associated with osmotic adjustment. Under drought stress, sucrose content decreased 0.52g and reducing sugar content increased 0.57g per fruit. Calculated sucrose concentration in fruit decreased by 40%, reducing sugar concentration increased by 75% whereas total reducing sugar content per fruit increased 41%. This sugar conversion increased osmolality of the juice by 15%. The process was partially but rapidly reversed by reirrigation. Fruit volume change was the major factor contributing to alteration of osmolarity under cyclic water stress.

(Key words: Citrus, Water relations, Osmotic adjustment, Drought stress, Sugar conversion)

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Introduction

Moderate drought stress has been reported to improve "sweetness" of many fruit, such as citrus (Bielorai, 1982; Morshet et al., 1983), grape (Ruhl and Alleweldt, 1985), apple (Landsberg and Jones, 1981), strawberry (Gehrmann, 1985). However, mild drought stress does not necessarily promote increases in sugar concentration (McCarthy and Coombe, 1985). In addition, such stress may accelerate fruit maturation (Gehrmann, 1985), reduce color (Goode et al., 1978), and decrease postharvest storage life of the fruit (Landsberg and Jones, 1981). In grapefruit, summer water stress can also result in high acid levels, which may persist for up to 6 months (Levy et al., 1978).

Total sugar content in stressed fruit theoretically should be less than in fruit from wellwatered trees because of overall reductions in photosynthesis (Lakso, 1985), and increases in photoassimilate

partitioning to roots (Hall et al., 1988). Production of "sweeter" fruit under moderate stress probably results to a large extent from smaller fruit size, however this may not be the only contributing factor. Solute accumulation in response to water stress, or "osmotic adjustment", is another possibility. This process has been studied under drought stress in roots (Sharp and Davies, 1979), leaves (Jones and Turner, 1980; Michelena and Boyer, 1982), hypocotyls (Meyer and Boyer, 1981), and inflorescences (Morgan, 1980). Research on osmotic adjustment in fruit species has focused primarily on leaves, including those of apple (Lakso et al., 1984), citrus (Feres et al., 1979), peach (Young et al., 1982), and grape (During, 1985). Osmotic adjustment and potential sucrose-hexose conversion in fruit tissue appear not to have been explored, however.

An additional factor which could affect fruit sugar levels is water loss via xylem-back flow to transpiring leaves. Occurrence of xylem back-flow has been indicated in a variety of instances, and clearly demonstrated (Huang, 1994). When such a reverse flow occurs, it is possible that apoplastic sugars may be vulnerable. In soybean, Bennett et al., (1984) demonstrated that exogenously applied ^{14}C sucrose could exit from developing seed coats and be drawn to vegetative parts of the plant in the xylem. However, direct evidence is lacking as to whether or not sugar can leave fruit via this route in vivo.

The objectives of this research were to clarify the relationship between altered water availability and fruit sugar, and to determine the extent to which changes in the latter are mediated by reduced sugar import, osmotic adjustment, or sugar exit via xylem back-flow. Calamondin trees (*Citrus madurensis* Lour.) was used in this research due to its miniature size and the profuse fruit available at different development stages throughout the year. Responses to a soil moisture cycle of "stress: regular irrigation: stress" was compared to "continuous stress" and "continuous irrigation" to contrast changes in carbohydrate translocation and/or metabolism resulting from alterations in soil water availability.

Materials and Methods

Plant Material

Six-year-old calamondin trees (*Citrus madurensis* Lour.) were grown in a greenhouse using 10-liter containers with a "Metro Mix 500" medium (W. R. Grace Co., Cambridge, MA). Each bore 25 to 30 fruit, 10 to 15 of which were undergoing the third stage of fruit development (final maturation).

Experimental Treatments and Sample Timing

Trees were divided into 3 groups. The first was subjected to a cycle of "stress: regular irrigation: stress". The second was exposed to "continuous stress", and the third received "regular irrigation" throughout. Group I plants were exposed to mild drought stress for 10 days by supplying

each with 100 ml of water at 3-day intervals. Solute concentration peaked 8 to 10 days after drought stress. Water supply was then increased for 5 days, during which containers were saturated daily. Mild stress was resulted thereafter. Plants under "continuous stress" were maintained throughout with 100 ml of water at 3 day intervals, and those receiving "regular irrigation" were watered to saturation daily. Fruits from continuous watering regimes were sampled in concert with those receiving intermittent drought stress; the day prior to onset of the first stress, again immediately before rewatering, at subsequent intervals 3, 4, and 5 days after renewal of regular irrigation, and finally, 3 days after the onset of a second stress. All fruits were sampled between 1:00 and 1:30 pm.

Water Relations

Fruits with pedicels (about 7 to 8 cm long) were harvested and sealed in plastic bags to prevent desiccation during transport from the greenhouse to the laboratory. Whole fruit water potentials were measured by pressure bomb within 10 mins after excision. Fruit diameter and fresh weight were measured thereafter.

Water potential and osmotic potential of juice tissues were determined using a thermocouple psychrometer (Model HR-33T, wescor Inc. Logan, Utah). Individual, intact juice sacs were carefully dissected and two were placed in each chamber. A 4-hour period was found to provide sufficient equilibration prior to water potential measurements. Three such samples from each fruit were measured in separate psychrometers, and 5 fruits were harvested from each treatment at each sampling time.

For determination of osmotic potentials, small pieces of juice tissue with about 10 juice sacs each, were expressed inside a 1 ml syringe. Filter paper discs(0.8 cm diameter)were soaked with the resulting fluid and sealed in sample chambers. Equilibration and replications were as above. Pressure potential was calculated as the difference between water potential and osmotic potential of immediately adjacent juice sacs.

Sugar Analysis

Juice samples were boiled for 45 to 50 seconds to deactivate endogenous invertase and other enzymes with the potential to alter sugar compositions. Little or no non-enzymatic breakdown of sucrose occurs if these extracts are boiled for periods of less than 1 min(Koch and Avigne, 1984). Reducing sugars were quantified using the Park-Johnson method (Chaplin and Kennedy, 1986). Total sugars were measured using the same procedure following a 4-hour incubation with invertase (40 units invertase: 1 μ mol sucrose) Sucrose levels were determined by subtraction. The Park-Johnson method is sensitive to low levels of reducing sugars(1 to 5 μ g) and sucrose does not interfere(preliminary experiment, data not shown). Sugar content per fruit was computed by multiplying the concentration of total sugars by the fresh weight of each fruit(peel of calamondin accounts for ca 10 to 12% of fruit fresh weight).

Results

Solute concentration of juice increased slowly during the first 8 days of drought stress, but remained relatively stable for the duration of the 12-day experiment. Eventual decreases in osmotic potential were indicated by a rise in osmotic potential of juice after 4 weeks of stress (Preliminary experiment, data not shown).

Whole fruit water potential decreased from -0.9 Mpa to -2.1 Mpa during the first 10 days of stress, and again appeared to show minimal change during the remainder of the 18-day period examined (Fig. 1). Variation in mean values were non-significant and paralleled those of fruit from regularly irrigated trees. Rewatering stressed trees at day 10 increased fruit water potential, however, peak values remained significantly less than those of fruit from regularly irrigated trees. Fruit water potential decreased rapidly when water supply was reduced the second time. No significant difference was observed between final water potentials of fruit from trees exposed to a cycle of "stress:regular irrigation:stress" and those receiving "continuous stress" for 18 days (Fig.1).

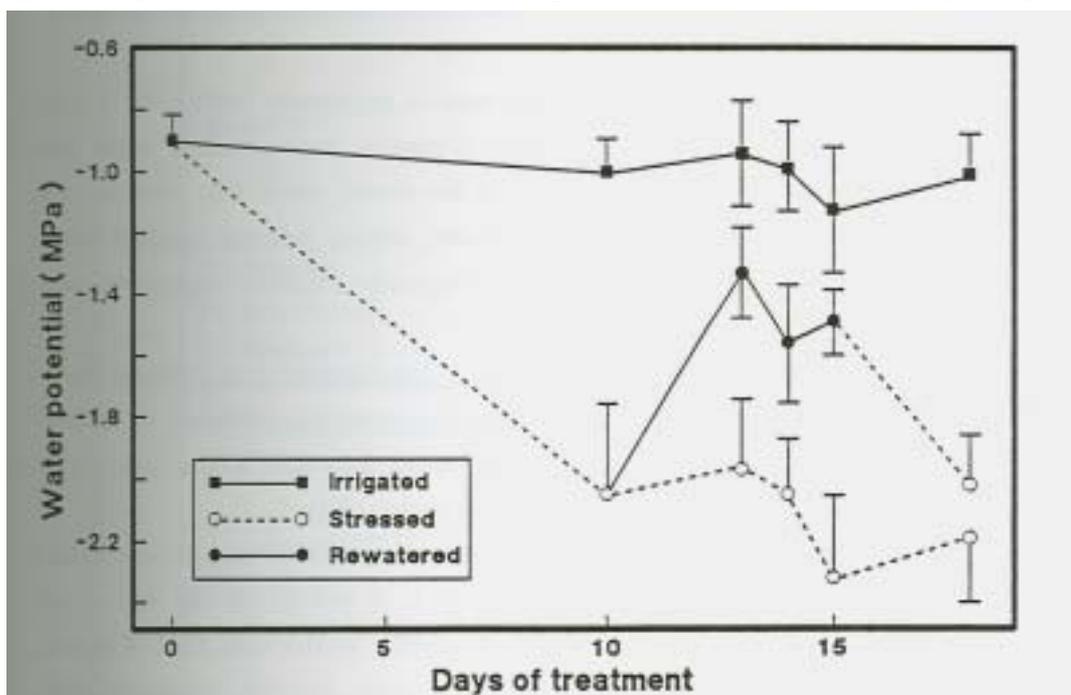


Fig. 1. Water potential of calamondin fruit under alteration of soil water availability. Experimental trees were subjected to either a cycle of "mild stress, reirrigation, mild stress", "continuous mild stress", or "regular irrigation". Solid and dashed lines represent regular irrigation and drought stress, respectively. Fruit were nearing final maturation (stage III of development). Water potential was measured by pressure chamber. Vertical bars denote the SE of 5 samples.

Water potential of juice sacs rose and fell significantly in response to stress, however, these changes did not exactly parallel those of whole fruit (Fig.2, A vs Fig.1). After 10 days of stress, whole fruit water potential decreased to a greater extent than did that of juice sacs, indicating that

the bulk of the fruit interior may not readily equilibrate with the rest of the fruit. In addition, whole fruit water potentials peaked within 3 days after rewatering, whereas 5 days were required before juice sacs water potential reached pre-stress levels. Water potentials of whole fruit and juice sacs both dropped rapidly after the onset of the second stress, and within 3 days reached levels equivalent to those observed at the end of the first 10-day stress. Water potentials of juice sacs from continuously stressed trees had dropped to their minimum within the first 10 days of stress and remained at this level for the 18-day duration. These values were consistently greater than those of whole fruit. In fruit of regularly irrigated trees, water potential of whole fruit were essentially equal to those of juice sacs and remained relatively constant throughout the experiment (Fig. 1 vs Fig. 2, A).

Osmotic potential decreased significantly in juice sacs of calamondin fruit after 10 days of drought stress. Five subsequent days of regular irrigation were necessary before prestress osmotic status was regained. Only 3 days of the second stress fully reversed this progression. In juice sacs of continuously stressed trees, osmotic potential remained essentially constant after 10 days of stress. Values for regularly irrigated counterparts were similar throughout (Fig. 2, B).

Pressure potentials were approximately 0.1 MPa in juice tissues of all irrigated trees, and slightly above 0 MPa under stress. Mean pressure potentials continued to increase during rehydration of juice sacs from stressed trees, rising from 0.03 MPa at day 10 to 0.08, 0.09, and 0.11 MPa on days 13, 14, and 15 respectively (Fig. 2, C).

The concentration of reducing sugars in juice tissues increased significantly under drought stress whereas that of sucrose decreased (Fig. 3, A and B). At the start of the experiment, the level of reducing sugars in fruit was slightly greater than that of sucrose, 49 mg ml⁻¹ vs 36 mg ml⁻¹. Within 10 days of drought stress, however, the concentration of reducing sugar rose to a mean of 86.3 mg ml⁻¹ and sucrose levels dropped to 22.3 mg ml⁻¹. The ratio of reducing sugars to sucrose had thus changed from 3/1 to about 4/1 during this period. The apparent sucrose to hexose conversion could have accounted for approximately 28% of the total increase in osmolarity of juice sacs, with the remaining 72% resulting from volume reduction during dehydration (calculated from data in Table 1).

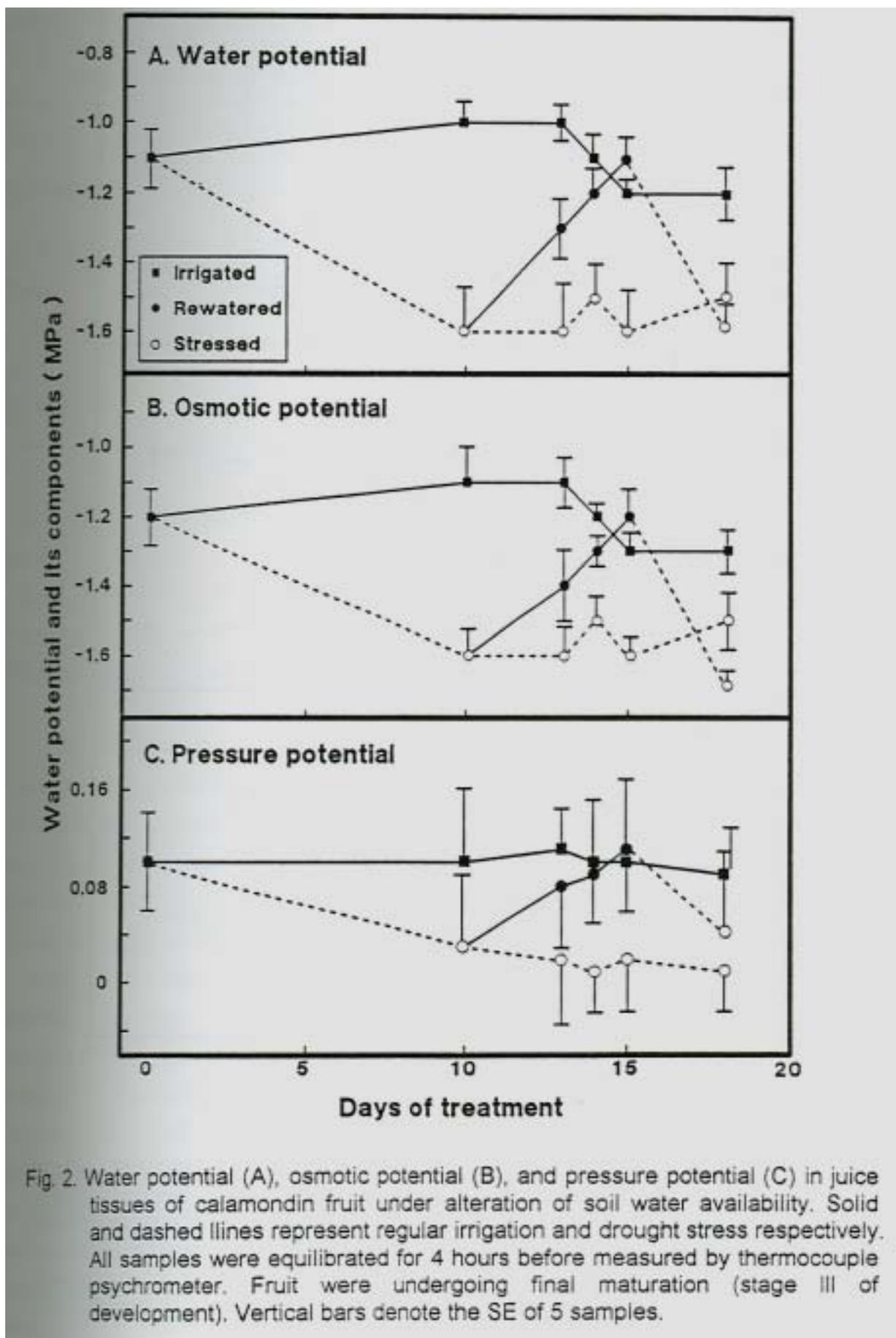


Fig. 2. Water potential (A), osmotic potential (B), and pressure potential (C) in juice tissues of calamondin fruit under alteration of soil water availability. Solid and dashed lines represent regular irrigation and drought stress respectively. All samples were equilibrated for 4 hours before measured by thermocouple psychrometer. Fruit were undergoing final maturation (stage III of development). Vertical bars denote the SE of 5 samples.

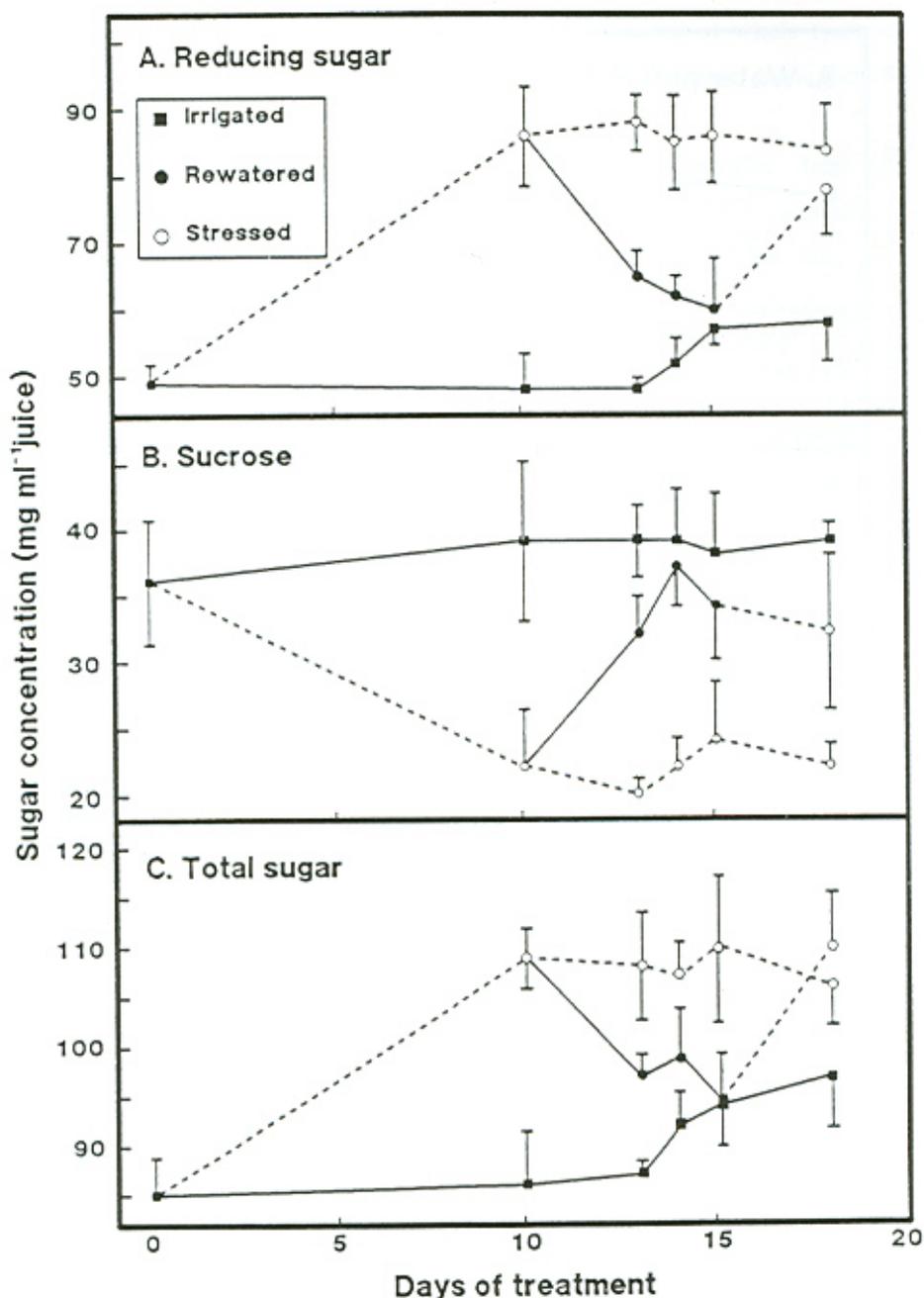


Fig. 3. Concentration of reducing sugar (A), sucrose (B), and total sugar (C) in juice tissues of calamondin fruit under alteration of soil water availability. Solid and dashed lines represent regular irrigation and drought stress respectively. Calculation of reducing sugars and sucrose were determined by subtraction using the Park-Johnson method before and after a 4-hour incubation with invertase. Fruit were undergoing final maturation (stage III of development). Vertical bars denote the SE of 5 samples.

Table 1. Change in sugar composition and osmolarity in juice sacs of calamondin under cyclic water stress. Sugars were quantified by Park-Johnson method. Fruit in development stage (maturation) were determined.

Days of	Change in sugar composition	Change in osmolarity
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treatment	Reducing sugar	sucrose	From sugar conversion	From volume change	Total
Stress	g fruit ⁻¹		mM		
0-10	+0.57	-0.52	+62	+160	+222
Reirrigation					
10-13	-0.26	+0.35	-25	-117	-142
10-14	-0.30	+0.47	-20	-123	-143
10-15	-0.30	+0.44	-20	-140	-163
Stress					
15-18	+0.17	-0.24	+11	+126	+137

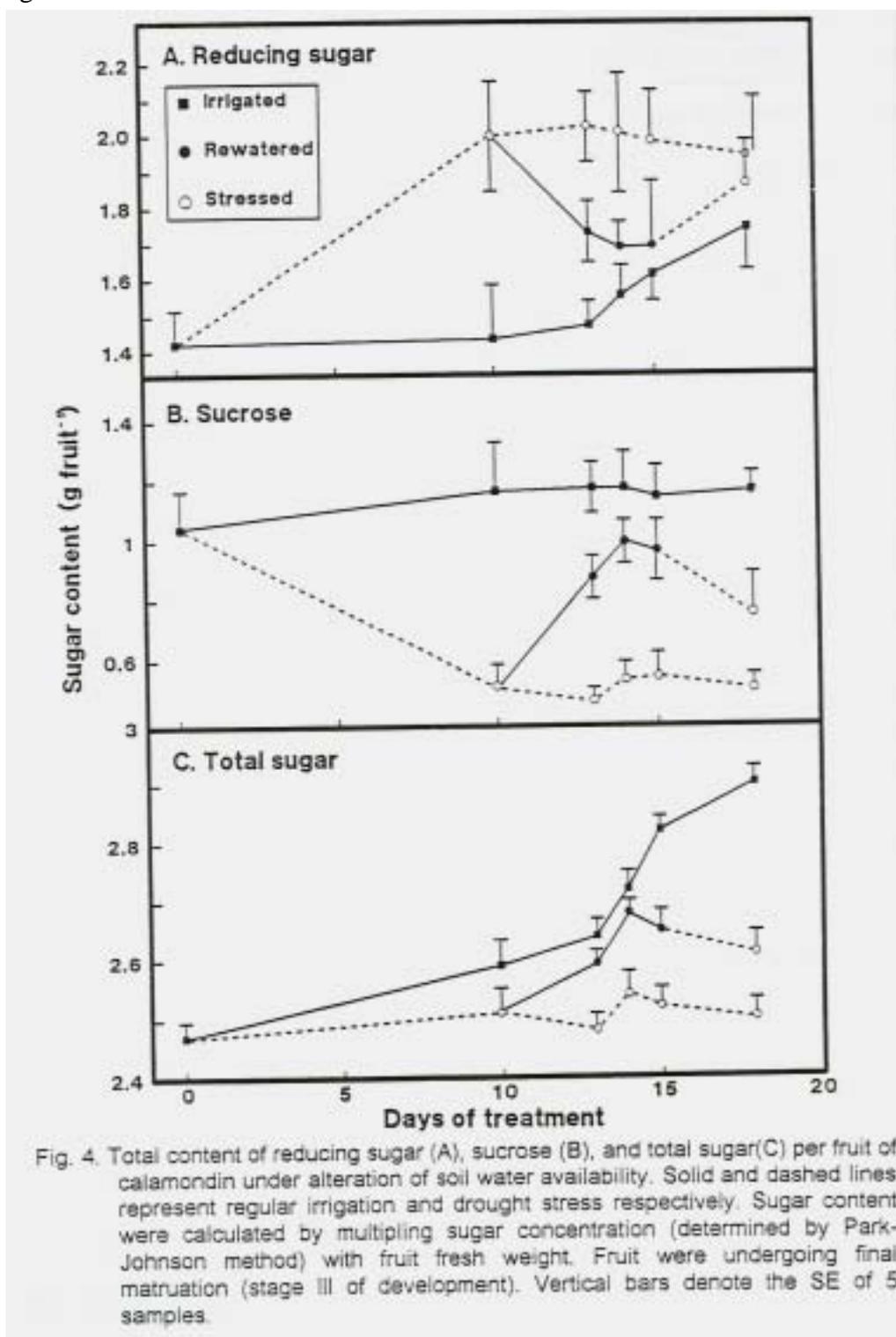
^Z Values are means of 5 samples±SE

Within 3 days after resumption of regular irrigation, the concentration of reducing sugars decreased significantly whereas that of sucrose increased. Sucrose reached its prestress level in 4 days after reirrigation(Fig. 3, B), but hexose concentration did not drop fully to original levels(Fig. 3, A). Concentration of reducing sugars again rose rapidly after the onset of the second drought stress. Fruit of continuously stressed trees maintained a reducing sugar concentration about 4-fold greater than that of sucrose (Fig. 3, A vs B).

Although total sugar concentration increased markedly after a 10-day stress period, calculated total sugar content per fruit did not change significantly (Fig. 4, C). The decreases of fruit fresh weight and volume (20% and 26% respectively after 10 days of stress) were the major factors resulting in this concentration increase. However, total sugar content in fruit of regularly-irrigated trees increased continuously and rose significantly above that of stressed trees if drought lasted 10 days or longer. Effects of stress on total fruit sugar content were not immediately evident if reirrigation began on day 10, however, a significant delay in sugar accumulation became apparent after only 3 days of a second drought stress. After a total of 18 days, sugar content was greatest in fruit from trees receiving "regular irrigation", and significantly less in fruit exposed to one cycle of "stress:regular irrigation:stress". Total sugar content was least in fruit receiving "continuous stress"(Fig. 4, C).

Similar to the change in reducing sugar and sucrose concentration, total reducing sugar and total sucrose content per fruit rose and fell significantly in response to water stress and reirrigation(Table 1). An increase of reducing sugar content by 0.57g per fruit was observed when trees were exposed to stress for 10 days, whereas sucrose content dropped 0.52g during the same period. When trees were reirrigated for 3 days, total reducing sugar content per fruit decreased

0.26g, but sucrose increased 0.35g. Again, the onset of a second stress resulted in an increase of reducing sugar content and decrease of sucrose content.



Osmolarity of the juice tissues increased a total of 222 mM after 10 days of stress. Sugar conversions contributed 62 mM of the total and the remainder resulted from changes in fruit volume. Osmolarity dropped in response to reirrigation, during which sugar conversions and volume increase contributed to ca 18% and 82% of the total reduction in osmolarity in a 3-day period.

The overall effects of sugar conversions and volume changes on alterations in fruit osmolarity were 16% and 84%, respectively, during the total 18-day period of this study.

Discussion

Together, data presented here indicate that changes in concentrations of fruit sugars under drought stress can be brought about not only by decreases in fruit volume, but also by sucrose-hexose conversion during osmotic adjustment. Cyclic alternation in water availability showed that the sucrose-hexose conversion was partially reversible, and that water relations of juice sacs changed fairly slowly during rehydration.

Analysis of water relations in whole fruit and individual sacs indicated progressive dehydration for the first 8 to 10 days of drought stress, followed by apparent stabilization for the remainder of the 12 to 18-day experimental periods (Fig. 1, and 2). Water-conserving attributes of calamondin orange fruit are minimal in comparison to other citrus fruit such as grapefruit. However, the possibility that juice sac water may be partially isolated from that in the fruit exterior and adjacent xylem is further indicated by the differences between water potentials of whole fruit from stressed trees (determined by pressure bomb shown in Fig. 1) and those of juice sacs (determined by psychrometer shown in Fig. 2). Such differences were not evident in fruit of regularly irrigated trees.

Water relations of fruit on trees undergoing a cycle of "stress:regular irrigation:stress" indicated that whole fruit (Fig. 1) and juice sacs (Fig. 2) were slow to regain prestressed water potentials and osmotic potentials. A total of 5 days was necessary for this process in juice sacs. However, whole fruit water potentials did not recover. The discrepancy may be due to the possible lack of readily available water from sacs during pressure bomb measurements of whole fruit under stress. Fresh weight increased rapidly during fruit rehydration yet remained significantly less than that expected for full recovery until 5 days after reirrigation. The slow reentry of water into juice sacs of calamondin fruits may be due in part to the structural isolation of juice sacs noted above.

Fruit enlarged dramatically when irrigation was resumed. Fruit fresh weight increased 15.6% during the first 3 days after reirrigation whereas that of fruit on regularly irrigated trees remained essentially unchanged (Preliminary experiment, data not shown). Fruit from previously-stressed trees typically change volume faster than those from regularly irrigated trees upon reirrigation (Cohen and Goell, 1988; Mitchell et al., 1984). A relative increment of fruit volume change previously reported for grapefruit under cyclic water availability was as great as 124 to 165%. However, grapefruit are considerably larger and have a far thicker peel than do fruit of calamondin.

Changes in fruit sugar concentrations appear to be due primarily to the change in fruit water content (approximated by fruit volume). Reducing sugar and total sugar concentrations in fruit

increased markedly under drought stress and decreased significantly after reirrigation (Fig. 3). Total sugar content per fruit did not follow this trend, however, and did not increase in fruit of mildly stressed trees relative to regularly irrigate counterparts (Fig. 4). Previous studies have indicated that mild drought stress produces sweeter fruits in a wide range of species, including peach (Veihmeyer, 1972) apple (Jones et al., 1983; Lankes, 1985) , grape (McCarthy and Coombe, 1985), citrus (Koo and Smajstrla, 1984), tomato (Wight et al., 1962), muskmelon (Pew and Gardner, 1983; Wells and Nugent, 1980), plum (Nasharty and Ibrahim, 1961), watermelon (Doneen et al., 1939), pear (Raese et al., 1982), cantaloupe (MacGillivray, 1951), and strawberry (Kimbrough, 1930). Results of the present work are consistent with the hypothesis that the "sweeter" fruits produced under mild stress are likely to have resulted from high sugar concentration, due to low water content, and smaller fruit size, but not more sugar per fruit.

In addition, reirrigation of stressed trees reduced the total sugar concentration in fruit (Fig. 3, C), but increased the fresh weight relative to fruit of continuously stressed trees. Total fruit sugar thus increased significantly (Fig. 4, C). A dilution effect is evident in juice sacs during resumption of regular irrigation, and is consistent with reported changes in Brix after release of drought stress in citrus (Koo and Smajstrla, 1984).

Total sugar content per fruit showed no significant increase under continuous stress (Fig. 4, C), indicating that photosynthesis and/or translocation were inhibited or partitioning changed under these conditions. Photosynthesis typically declines when water is withheld (Ruan et al., 1988; Henson et al., 1989b), particularly as plant water potentials fall to between -1.0 and -1.5 MPa (Deng et al., 1990). Mild water deficits can decrease photosynthesis via stomatal closure alone (Flore et al., 1985; Bois et al., 1985), however, more severe stress can also decrease activation and concentration of RuBPCase (Vu and Yelenosky, 1988; Sharkey and Seemann, 1989). Large reductions in citrus leaf photosynthesis have previously been reported in stressed 'Valencia' orange trees by Vu and Yelenosky (1988).

Recovery of photosynthesis in stressed trees after reirrigation varies with species and the extent of stress. In orange, leaf water potential of mildly stressed trees recovers to the normal value in less than a week after reirrigation, whereas stomatal conductance does not fully recover in 2 months if trees are subjected to leaf water potentials below -5 MPa (Feres et al., 1979). In contrast, rewatering can result in a complete recovery of photosynthesis in grapevine after a 4-week drought, and subsequent rates may exceed those of well-watered controls by 10%. However, a permanent inhibition of photosynthesis reportedly occurs after an 8-week drought, resulting in a 22% reduction (Ruhl and Alleweldt, 1983).

Water stress is also known to reduce the export of photoassimilate from source leaves (Sheikholeslam and Currier, 1977), translocation rate (Lang and Thorpe, 1986), and alter the

partitioning of assimilates among different tissues (Hall et al., 1988; Beruter, 1989). The rate of fixed carbon export from leaves is sharply reduced in cacao, for example, as leaf water potential declines from -0.8 to -2.0 MPa (Deng et al., 1989). Water stress also commonly increases photoassimilate partitioning to the roots (Hall et al., 1988), leaving minimal sugars available for storage in fruit,

In the present study, effects of drought stress are not immediately evident in total sugars per fruit, however, a delayed response seems to occur even after rewatering (Fig. 4). Total sugar content per fruit did not differ significantly between fruit of regularly irrigated trees and those from stressed trees until after 13 days. When stressed trees were reirrigated at 10 day, fruit sugar accumulation continued without interruption at rates similar to those of regularly irrigated trees. It is possible that 10 days of mild drought stress do not critically reduce photosynthesis or translocation, however, a marked drop in rate of fruit sugar accumulation occurred later with only a short stress.

Previous research has indicated that carbohydrate export from source leaves to sink organs can continue at the expense of leaf carbon reserves even when photoassimilation falls to near 0 during periods of water stress (Deng et al., 1989). Cohen and Goell (1988) indicated that during prolonged periods of drought, dry matter accumulation in fruit of grapefruit still continued, but at very low rates. This was observed even when increases in fruit volume ceased or became negative. Translocation into pear fruits proceeded similarly (Mitchell et al., 1984). Water stress may also induce changes of leaf and/or fruit composition of lipids (Hubac et al., 1989), proteins (Valluri et al., 1988) and/or acids (Levy et al., 1988). Carbohydrate supply may be reduced to some degree, though probably slight, to provide raw material or respiratory substrate for these conversions.

Total sugar content per fruit did not increase in those from continuously stressed trees (Fig. 4, C), and within 13 days of stress, a significant difference was evident between sugar levels in these fruit and counterparts from irrigated trees. These data showed that carbon import into stressed fruit virtually ceased under experimental conditions.

Osmotic adjustment was indicated by sucrose-hexose conversion in fruit during onset and release of drought stress. Reducing sugars and sucrose ratios in mature fruit of grapefruit, orange, and lemon vary fairly widely, but are typically reported to be approximately 2/1, 1/1, and 1/1 respectively (Erickson, 1968). In the present experiments, the ratio of reducing sugars to sucrose in calamondin fruit changed from ca 1.3/1 in fruit of regularly irrigated trees to 4:1 in stressed counterparts (Fig. 3). This ratio returned approximately half-way to pre-stress levels when regular irrigation was resumed for 5 days (Fig. 3).

Particularly notable is that total hexoses per fruit and total sucrose per fruit shifted in a manner consistent with the occurrence of osmotic adjustment, and that these changes were greater than could be expected from altered influx of phloem sucrose alone. Some sucrose-to-hexose conversion is

standard during storage of sugars in juice sacs (Koch and Avigne, 1990), so that the balance would be expected to move in this direction if rate of sucrose entry decreased. It seems unlikely that a quarter of the hexoses in fruit of stressed trees could have arisen by this mechanism alone, however, in only 10 days out of 270 days needed for full maturation. Half of this quarter is again lost when stressed trees are reirrigated, indication that the associated drop in sucrose/hexose ratio is not simply the result of new sucrose entering as translocation is resumed.

In some tropical grasses, osmotic adjustment at full turgor can account for a 0.11 MPa per MPa decrease in total water potential (Wilson and Ludlow, 1983a). Maximum osmotic adjustment in these species is reportedly 0.66 MPa to 1.0 MPa (Wilson and Ludlow, 1983a, 1983b). This process leads to the maintenance of turgor potential and hence continuation of plant growth (Morgan, 1984). Fig. 2 shows an osmotic potential change of 0.47 MPa in fruits when calamondin trees are shifted from regular irrigation to drought stress. Presumably, this value does not represent the maximum osmotic adjustment possible in this tissues, because experimental trees receive only a mild stress, and are known to respond less markedly than field trees (During, 1985; Jones et al., 1985).

Little or no previous research has addressed the possibility that osmotic adjustment in growing fruit could substantially alter their sugar composition. The process itself has been investigated in a wide range of plants and leaves, from peach seedlings (Yung et al., 1982), and bean plants (Vassey and Sharkey, 1989), to peach leaves (Steinberg et al., 1989), cotton leaves (Ackerson, 1981), sorghum and sunflower leaves (Jones et al., 1980), wheat leaves (Munns and Weir, 1981), orange leaves (Vu and Yelenosky, 1989), grape leaves (During, 1985), and some tropical grasses (Wilson and Ludlow, 1983a, b). Sugars and amino acids are major constituents of osmotic adjustment in expanded organs of many species (Ackerson, 1981 Munns and Weir, 1981). In some reports, potassium, organic acids (malate, citrate), nitrate, sodium, magnesium and chloride ions have also been found to contribute (Morgan, 1984; Wilson and Ludlow, 1983b). Sugars were the focus of the present study, however, because storage cells in fleshy fruit have particularly large quantities of these solutes.

High apoplastic sugar has been found in some tissues and species such as: soybean seed coat (Hsu et al., 1984), pea, soybean and cucumber seedlings (Cosgrove and Cleland, 1983), and sugarcane stems (Welbaum and Meinzer. 1990). In sugarcane, sugar content in the apoplast of mature stalks was estimated to constitute as much as 21% of the total stored sucrose (Welbaum and Meinzer, 1990). In soybean, apoplastic sucrose at the interface between cotyledons and surrounding seed coat was greater than the overall sucrose concentration in the cotyledons and seed coat (Gifford and Thorne, 1985). Relatively little is known about apoplastic sugar in citrus fruit, and its significance is debated in many tissues. Further studies on apoplastic sugar will be helpful to the

understanding of carbohydrate translocation as well as sugar metabolism and osmoregulation in fruit.

Conclusions

The primary factor affecting elevated concentrations of fruit sugars under water stress was the change in fruit volume (water content). Data shown here were consistent with the hypothesis that "sweeter" fruits produced under stress result primarily from high sugar concentration, low water content, and small fruit size, but not more sugar per fruit.

Osmotic adjustment was indicated by sucrose-hexose conversions in fruit during onset and release of drought stress. Under drought stress, sucrose concentration in calamondin fruit decreased by 40 % whereas reducing sugar concentration increased by 75 %. This sugar conversion contributed to increase of osmolarity by 15 %. Fruit volume change was the major factor contributing to alteration of osmolarity under cyclic water stress.

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