Expansion and Osmotic Adjustment of Strawberry Fruit during Water Stress

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ADDITIONAL INDEX WORDS. water potential, solute potential, *Fragaria ×ananassa*

ABSTRACT. Expansion of green-white and red fruit in control (watered) and water-stressed greenhouse-grown strawberry (*Fragaria ×ananassa* Duch. 'Brighton') plants was monitored with pressure transducers. Expansion of green-white fruit in control plants was rapid, showing little diurnal variation; whereas in water-stressed plants, fruit expansion occurred only during dark periods and shrinkage during the day. Red fruit were mature and failed to show net expansion. The apoplastic water potential (ψ_w), measured with in situ psychrometers in control plants was always higher in leaves than in green-white fruit. In stressed plants, ψ_w of leaves was higher than that of green-white fruit only in the dark, corresponding to the period when these fruit expanded. To determine the ability of fruit to osmotically adjust, fruit were removed from control and water-stressed plants, and hydrated for 12 hours; then, solute potential at full turgor (ψ_s^{100}) was measured. Water-stressed green-white fruit showed osmotic adjustment with a ψ_s^{100} that was 0.28 MPa lower than that of control fruit. Mature leaves of water-stressed plants showed a similar level of osmotic adjustment, whereas water stress did not have a significant effect on the ψ_s^{100} of red fruit. Fruit also were severed to permit rapid dehydration, and fruit solute potential (ψ_s) was plotted against relative water content [RWC = (fresh mass - dry mass ÷ fully turgid mass - dry mass) × 100]. Water-stressed, green-white fruit had a lower ψ_s for a given RWC than control fruit, further confirming the occurrence of osmotic adjustment in the stressed fruit tissue. The lack of a linear relationship between turgor pressure and RWC prevented the calculation of cell elasticity or volumetric elastic modulus. Osmotic adjustment resulted in about a 2.5-fold increase in glucose and sucrose levels in water-stressed green-white fruit. Although green-white fruit on water-stressed plants showed osmotic adjustment, it was not sufficient to maintain fruit expansion during the day.

Expansion of fruit requires, among other factors, an adequate flow of water to the organ and sufficient turgor to drive cell enlargement. The water status of fruit or other plant organs may be characterized by the water potential (ψ_w), which is frequently considered the sum of turgor potential (ψ_p) and solute potential (ψ_s), ignoring the gravitational component (i.e., $\psi_w = \psi_p + \psi_s$) (Kramer and Boyer, 1995; Nobel, 1983). Assuming an unrestricted path, water moves along gradients toward lower ψ_w . The ψ_w gradients between organs and tissues are created by differences in evaporational losses, solute accumulation, and water demand caused by cell expansion (Nonami and Boyer, 1993). Diurnal fluctuations in fruit size have been reported in many species, including apple (*Malus domestica* Borkh.) (Trump, 1984) and peach [*Prunus persica* (L.) Batsch.] (Berger and Selles, 1993) and are related to the difference in ψ_w between the stem and fruit. Johnson et al. (1992) showed that a ψ_w gradient favoring solution flow into tomato fruit coincides with a rapid increase in fruit diameter, while inverting the gradient causes the fruit to shrink.

Positive cell turgor is required for plant growth (Kramer, 1988; Meyer and Boyer, 1981). Since ψ_p is equal to ψ_w minus ψ_s , the accumulation of solutes in cells and lowering of ψ_s maintains ψ_p , allowing turgor-dependent processes to continue at progressively lower ψ_w . Increased elasticity of cell walls is a passive mechanism that allows decreases in cell volume with dehydration, slowing the

rate of turgor loss with decreasing ψ_w . Osmotic adjustment, which is the maintenance of positive cell turgor through the active accumulation of solutes within cells, is often a strategy by which plants tolerate water or salt stress (Morgan, 1984). Hexoses, amino acids, and inorganic salts usually accumulate in plant tissues that osmotically adjust (Kramer and Boyer, 1995; Meyer and Boyer, 1981; Wang et al., 1995; Zhang and Archbold, 1993b). Osmotic and cell wall elastic adjustment together can maintain symplastic volume and positive cell turgor in plants diurnally and during soil drying (Saliendra and Meinzer, 1991). Jones et al. (1985) suggested osmotic adjustment likely occurs in fruit tissues'; although to our knowledge, it has not been reported in the literature. Yakushiji et al. (1996) reported that ripening fruit of water-stressed Satsuma mandarin (*Citrus unshiu* Marc.) trees accumulated higher levels of glucose and fructose, coinciding with a decrease in ψ_s than comparable fruit of well-watered trees. They did not determine whether there were differences in cell volume and wall elasticity in water-stressed and watered fruit.

Water accounts for ≈90% of the total mass of a ripe strawberry fruit (Darrow, 1966), and therefore, even mild drought stress can greatly reduce fruit yield (Dwyer et al., 1987; Renquist et al., 1982a). Osmotic adjustment has been reported in leaves of the cultivated strawberry (*Fragaria ×ananassa* Duch.) (Renquist et al., 1982b; Save et al., 1993) as well as in its progenitor species, *F. chiloensis* Duch. (Archbold and Zhang, 1991; Zhang and Archbold, 1993a) and *F. virginiana* Duch. (O'Neill, 1983). Archbold and Zhang (1991) suggested that the greater degree of osmotic adjustment shown in *F. chiloensis* than *F. virginiana* contributes to continued growth and survival of *F. chiloensis* during drought. To our knowledge, no information is available on whether strawberry fruit undergo osmotic adjustment as a means to maintain cell expansion under water stress.

To distinguish between osmotic adjustment and passive changes in cell solute contents, such as increased concentration of solutes

Received for publication July 1, 1996. Accepted for publication 26 Aug. 1996. Oregon Agricultural Expt. Station Technical Paper no. 10,979. We thank the anonymous reviewers of this journal for their comments on an earlier draft of this manuscript. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

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through dehydration, ψ_s should be measured at a particular ψ_w or water content. For convenience and purposes of comparison, the level of osmotic adjustment is usually determined by contrasting the ψ_s of excised tissues from water-stressed and watered plants after a hydration step to bring tissue to full turgor (i.e., $\psi_w = 0$) (Jones and Turner, 1978; Turner, 1988). The degree of osmotic adjustment that occurs in plants in response to a water deficit also can be assessed by rehydration of control and water-stressed plants, followed by rapid dehydration and measurement of tissue ψ_s and relative water content (RWC) (Jones and Turner, 1978; Morgan, 1984). A change in RWC of plant tissue represents the change in cellular volume (Morgan, 1984). The ψ_s of tissues from water-stressed and unstressed plants is frequently plotted against RWC, and comparisons are made at a given RWC or cellular volume. If in such a comparison, ψ_s from stressed plants is more negative than that from an unstressed treatment, osmotic adjustment is considered to have occurred in that tissue (Jones and Turner, 1978). Plotting the change in calculated turgor pressure (i.e., $\psi_p = \psi_w - \psi_s$) vs. the change in RWC allows an estimate of cell elasticity or volumetric elastic modulus [$\epsilon = (\Delta\psi_p / \Delta\text{RWC}) \times 100$].

The measurement of osmotic adjustment in growing plant tissues, however, adds an additional complication. In expanding organs, such as growing fruit, cell walls yield under relatively low turgor pressure so that cell volume may increase during the rise to full turgor in the hydration step. If growing cells of water-stressed fruit tissue at harvest have a higher concentration of solutes than comparable nonstressed cells, they may take up more water osmotically during hydration and expand to a larger volume. This would dilute the accumulated solutes, increasing ψ_s and masking evidence of osmotic adjustment. Milad and Shackel (1992) found that expanding fruit from prune trees before irrigation showed a greater increase in volume and ψ_s on hydration compared to fruit hydrated 24 h after irrigation. Hydration of growing tissues may not always prevent the detection of osmotic adjustment. Jones and Turner (1980) reported that, after a hydration period that would permit dilution of cell solutes, the ψ_s in expanding leaves of water-

stressed sunflower plants was still lower at the same RWC than that of watered control plants. If cell expansion is metabolically dependent, lowering the temperature of tissue during the hydration step might reduce dilution of cell solutes and the concomitant rise in ψ_s . Lower temperatures during fruit hydration also would reduce respiration rates and use of solutes that contribute to ψ_s of the tissue.

Understanding the relationship between ψ_w and fruit expansion and the role of osmotic adjustment in growth maintenance could contribute to genetic or cultural manipulations to optimize fruit growth and yield. Our objectives were to determine whether fruit expansion in strawberry was related to the difference between leaf and fruit ψ_w and if fruit undergo osmotic adjustment during water-stress.

Materials and Methods

PLANT MATERIAL AND STRESS IMPOSITION. Plants of the day-neutral strawberry (*Fragaria × ananassa* Duch.) 'Brighton', were grown during winter and early spring months in 3.8-L plastic pots in 1 soil : 1 coarse sand : 1 peat (by vol.) in a shaded greenhouse maintained at 30 °C day/18 °C night in which high-pressure sodium lamps (HPS) provided supplemental irradiance ($175 \pm 25 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at the top of the canopy) and a 14-h photoperiod, from 0730 to 2130 HR. The single primary flower on each inflorescence was self-pollinated with a small paint brush and was tagged, and all competing flowers or buds at lower orders were removed. Plants were watered daily and fertilized with 20N-8.6P-16.6K, with N at 400 mg·L⁻¹, twice weekly. At the start of each experiment, water was withheld from plants designated as water stressed, whereas control plants were watered daily.

FRUIT EXPANSION. Fruit expansion at green-white [15 to 20 d after anthesis (DAA)] and red (25 to 35 DAA) stages was monitored using linear variable differential transformers (LVDT) (model LD400-2.5; Omega Engineering, Stamford, Conn.) in conjunction with a micrologger (model 21X; Campbell Scientific, Logan, Utah). Each LVDT was secured in a custom-built, U-shaped plexiglas platform that was placed over a single attached fruit positioned horizontally on a small stand. The plunger of the LVDT rested on the fruit from above at about fruit midlength, and its mass was sufficient to maintain contact without damaging the fruit surface. An excitation voltage of 10 vdc was maintained using an in-line voltage regulator. Expansion measurements were recorded diurnally every 10 min. Day measurements were corrected for a slight, but consistent, sensitivity of LVDTs to the HPS lamps. Green-white and red fruit expansion was examined over 3 d and was replicated on three separate occasions. In a another experiment, the expansion of green-white fruit on control and water-stressed plants was monitored over 24 h on four separate days, beginning 4 d after water was withheld from stressed plants. Apoplastic water potential (ψ_{aw}) measurements on leaves and green-white fruit were conducted on similar plants during the same 24-h time periods.

WATER RELATION COMPONENTS. ψ_{aw} , which is considered to represent ψ_w of a tissue (Nobel, 1983), was measured in fruit as described by Pomper and Breen (1995) using temperature-corrected in situ psychrometers (Dixon and Tyree, 1984) that were calibrated according to the manufacture's specifications (Plant Water Status Instruments, Guelph, Ont., Canada) and were used in conjunction with a microvoltmeter (model HR-33T; Wescor, Logan, Utah). Psychrometers were attached with silicone grease to a site on a fruit where achenes had been removed with a forceps or to leaves where the cuticle was removed with fine sandpaper (600

Fig. 1. Expansion of green-white and red 'Brighton' fruit on control (---) and water-stressed (—) plants recorded over 3 d with pressure transducers. Water was withheld for 4 d before the start of monitoring expansion. Data are from individual fruit from one representative experiment. Bars on x-axis indicate dark periods. Arrow indicates the beginning of wilting of leaves on stressed plant.

grit), and the surface was rinsed with deionized water and wiped dry. Plants had 10 to 15 leaves, of which one fully expanded, mature leaf was randomly chosen for ψ_{aw} measurement. Three hours was adequate for fruit tissue to reach equilibrium with the air in the psychrometer chamber (data not shown).

To monitor the diurnal fluctuation in ψ_{aw} , psychrometers were attached to a leaf and a green-white fruit on the same plant under water-stressed or control conditions at least 5 h before the first measurement was taken in the evening (2100 HR); subsequent measurements were at predawn (0600 HR) and in mid-afternoon (1500 HR). Water was withheld from stressed plants for 5 to 7 d before attaching psychrometers. HPS lamps did not create an electrical interference with psychrometer measurements. Diurnal leaf and fruit ψ_{aw} measurements were replicated on four separate cloudy days over several weeks. The same leaf or fruit was subjected to multiple ψ_{aw} measurements during a given day. Leaf and fruit ψ_{aw} data were subjected to analysis of variance using a repeated measures design blocked by day. Leaf stomatal conductance and quantum flux were measured with a steady state porometer (model LI-1600; LI-COR, Lincoln, Neb.) using a broadleaf chamber (aperture set at 2 cm²) as specified by the manufacturer.

Fruit solute potential (ψ_s) was determined after ψ_{aw} of fruit was measured at 1500 HR. Fruit were cut longitudinally with a razor blade, and a portion was placed in 1.5-mL microfuge tubes (Fisher Scientific, Pittsburgh). The capped tubes containing the tissue were submerged in liquid N for 15 min, then left to thaw for 20 min at room temperature. Cell sap was obtained by centrifugation (3000× *g* for 5 min) at 4 °C. After 3 min at room temperature, an aliquot of cell sap was put on a 0.32-cm² filter paper disk (Wescor) that was inserted into a sample holder provided by the manufacturer, and ψ_s was determined psychrometrically. The average cell ψ_p of a fruit was calculated (i.e., $\psi_p = \psi_{aw} - \psi_s$).

OSMOTIC ADJUSTMENT. Two different methods were used to determine whether fruit underwent osmotic adjustment in plants subjected to water stress. In the first method, to determine whether osmotic adjustment occurred in water-stressed leaves and red fruit, control and slightly wilted water-stressed plants used in ψ_{aw} measurements were watered at 1500 HR and were allowed to recover for 3 h in black plastic bags away from light (at which point leaves of stressed plants appeared fully turgid). Red and green-white fruit and fully expanded mature leaves (usually two per plant) were severed while still in plastic bags and taken to the laboratory. Fruit pedicels and leaf petioles were recut under water and placed in beakers with deionized water. Water-saturated paper towels were placed around the beakers, which then were covered with a black plastic bag to maintain a water saturated atmosphere. Fruit were weighed at 0, 1, 3, 8, 10, and 12 h after the start of hydration. Based on fruit mass and water displacement data, fruit volume (in milliliter per milliliter) in 'Brighton' increases linearly with increasing fresh mass ($y = -0.264 + 1.225x$, $r = 0.99$) from small green to red stages. This regression equation was used to determine increases in fruit volume after hydration. After 12 h, a leaflet or portion of a fruit was put in a capped microfuge tube (1.5 mL), placed in liquid N for 30 min, and thawed for 20 min at room temperature; cell sap was obtained by centrifugation, and solute potential at full turgor (ψ_s^{100}) was determined psychrometrically (Jones and Turner, 1978). The ψ_s^{100} of mature leaves (15 leaves per treatment) and red fruit (four fruit per treatment) was determined

Table 1 Leaf temperature, photosynthetic photon flux (PPF), and stomatal conductance measured during leaf water potential and fruit expansion experiments.

Time (HR)	Leaf temp (°C)	PPF ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Stomatal conductance ($\text{cm}\cdot\text{s}^{-1}$)	
			Control	Water-stress
2100	21.1 ± 0.3 ^a	137.0 ± 10.5	0.16 ± 0.02	0.02 ± 0.01
0600	16.2 ± 0.3	0	0	0
1500	24.1 ± 0.8	228.0 ± 58.3	0.53 ± 0.05	0.13 ± 0.02

^aMeans derived from four replicate experiments; ±SE.

on three separate days and subjected to analysis of variance in a randomized block design, blocked by day. During hydration, green-white fruit accumulated appreciable amounts of water (i.e., increased in fresh mass) raising concerns about a possible differential increase in cell volume between water-stressed and control fruit (data not shown).

In another experiment, to minimize green-white fruit expansion during hydration, low temperature was used to reduce fruit expansion during the hydration step. Green-white fruit were cut in half lengthwise with a razor blade, and half of each pair was placed in deionized water in a petri dish at 4 or 20 °C. Fruit halves were weighed before hydration and after 1, 3, 8, 10, and 12 h. Using fruit mass and water displacement regression information, increases in fruit volume (milliliter per milliliter) after hydration were calculated. At the end of 12 h of hydration, a portion of the fruit was frozen in liquid N, and ψ_s^{100} was determined psychrometrically as previously described. This experiment was replicated on 3 d, with four half-fruit used for water-stressed and hydration treatment combinations in each replicate. Data for volume change and ψ_s^{100} were subjected to analysis of variance using a split-plot design blocked by day.

A second method, basically that described by Jones and Turner (1978), except that fruit ψ_{aw} was determined psychrometrically, was used to determine whether green-white fruit underwent osmotic adjustment. Briefly, water-stressed and control plants with green-white fruit were watered in early evening, taken to the laboratory, placed in plastic bags, and allowed to recover for 6 h, by which time wilted leaves of stressed plants were turgid. Psychrometers were attached to fruit as previously described, and ψ_{aw} measurements started after a 3-h recovery period. After an additional 3 h, fruit that were clamped securely to psychrometers were severed from plants. The ψ_{aw} of detached fruit was measured every 6 h, after which one previously water-stressed and control fruit was sacrificed for ψ_s and RWC measurements. ψ_s was determined psychrometrically with one section of a fruit, a second section (II) was weighed and then dried, and a third section (III) was placed in deionized water for 3 h at 4 °C and then weighed and oven-dried. RWC was calculated as $[(FW_{II} - DW_{II}) \div (TW_{III} - DW_{III})] \times 100$, where FW_{II} was initial fresh mass of the second portion, TW_{III} turgid mass of portion III after 3 h hydration, and DW the dry mass of oven-dried portions of the fruit. ϵ was determined by plotting the calculated turgor pressure for each green-white fruit from water-stressed and control plants vs. its RWC.

SUGAR ANALYSIS. Glucose and sucrose contents were measured in control and water-stressed green-white strawberry fruit. In an effort to avoid cell damage that could induce hydrolysis of sucrose, fruit were not sectioned and were rehydrated in deionized water as previously described. Instead, water-stressed and control plants were rewatered and placed in black plastic bags for a 6-h recovery period, after which green-white fruit were harvested. One section of fruit then was used to determine ψ_s as previously described, and another section was placed in boiling 70% ethanol for 5 min. The ethanol extract was taken to dryness, reconstituted in deionized

H₂O, and glucose and sucrose levels were determined as described by Pomper and Breen (1995).

Results

FRUIT EXPANSION. In control plants, green-white fruit continually expanded, increasing at $\approx 2.4 \text{ mm} \cdot \text{d}^{-1}$ over the 3 d of the experiment, whereas control red fruit expanded slightly at first but showed shrinkage by the last day (Fig. 1). As water-stress progressed, it greatly diminished the growth of green-white fruit, and they showed increasingly conspicuous diurnal fluctuations in expansion. Although the nighttime expansion rate of green-white fruit was similar for water-stressed and control fruit, growth of stressed fruit ceased during daylight hours, and shrinkage often was recorded. Shrinkage in green-white fruit was especially marked on the third day of measurement when water-stressed plants first wilted. Red fruit on water-stressed plants failed to show net expansion but did exhibit a slight diurnal pattern of expansion and contraction.

WATER RELATIONS. Fruit expansion and fruit and leaf ψ_{aw} of control and water-stressed plants were determined over the same 24-h period. Leaf temperatures and light levels (photosynthetic flux) were fairly consistent day to day during measurement periods in this study (Table 1). Their values, as well as those of stomatal conductance, were highest at the mid-afternoon (1500 HR) sam-

pling time. As expected, stomatal conductance during daylight was much lower in stressed than control plants. Fruit on control plants showed rapid growth during day and night, whereas expansion of water-stressed fruit occurred only at night and shrinkage was generally observed during daylight hours (Fig. 2A). Diurnal fluctuation in ψ_{aw} was greater in leaves than fruit (Fig. 2B). ψ_{aw} of water-stressed leaves was significantly more negative than those of control plants. Leaf ψ_{aw} in both treatments significantly increased during the night and declined by 1500 HR. Water stress also lowered fruit ψ_{aw} , but the effect was not significant. Fruit ψ_{aw} did decline significantly from 0600 to 1500 HR in control and water-stressed plants. Water-stressed plants showed wilting by 1500 HR, and their green-white fruit expanded little during light periods; indeed, there was shrinkage.

Water flow to and expansion of strawberry fruit may be associated with the differences in ψ_{aw} between leaves and fruit. The difference in ψ_{aw} between leaves and green-white fruit was influenced significantly by water-stress and the time of day (Fig. 3). In control plants, the differences were always positive (i.e., ψ_{aw} of the leaf was higher, or less negative, than that of fruit), with the smallest difference occurring in mid-afternoon. This positive difference corresponded with the relatively rapid expansion of green-white fruit on control plants (Fig. 2A).

In stressed plants, differences in ψ_{aw} between leaf and fruit were negative (i.e., ψ_{aw} of the leaf was less than that of fruit) at the two daytime measurements (2100 and 1500 HR) when fruit shrinkage occurred (Fig. 3). However, the difference was positive at predawn (0600 HR), when expansion of fruit on stressed plants was rapid. Green-white fruit from control plants in mid-afternoon had a positive ψ_p of 0.33 MPa, which was significantly higher than that of fruit from water-stressed plants in which the calculated ψ_p was negative, -0.88 MPa (independent *t* test; $P < 0.05$).

OSMOTIC ADJUSTMENT. Osmotic adjustment occurred in mature leaves of water-stressed plants, their ψ_s^{100} being 0.27 MPa lower than control leaves (Table 2). Water-stress also caused significant osmotic adjustment in green-white but not red fruit (Table 2; independent *t* test; $P < 0.05$). The ψ_s^{100} of green-white fruit from water-stressed plants was 0.28 MPa lower than that of fruit from control plants (Table 2). Hydration did not significantly affect the final ψ_s^{100} of green-white fruit, so data were pooled for statistical analysis.

The volume increased in red fruit during hydration was not significant ($0.001 \pm 0.002 \text{ mL} \cdot \text{mL}^{-1}$) in either control or water-stressed treatments. However, fruit halves of control and water-stressed green-white fruit significantly increased in volume during hydration at 20 and 4 °C, reaching maximum volume by 10 h (data not shown). Although both treatment (control or water stress) and hydration temperature had significant effects on volume change of green-white fruit on hydration, they did not show a significant interaction. The volume increase in control green-white fruit, based on mass gain after hydration was significantly less at the lower hydration temperature, with mean values of 0.14 and 0.09 $\text{mL} \cdot \text{mL}^{-1}$ for 20 and 4 °C, respectively (means separated by Student-Neuman-Keuls, $P < 0.05$). Similarly, the volume of water-stressed green-white fruit on hydration increased to 0.26 $\text{mL} \cdot \text{mL}^{-1}$ at 20 °C but only 0.18 $\text{mL} \cdot \text{mL}^{-1}$ at 4 °C.

The ψ_s did not decrease with a reduction in RWC with green-white fruit of control or water-stressed plants using the method of Jones and Turner (1978) (Fig. 4A). However, ψ_s of water-stressed green-white fruit was usually less than -1.4 MPa and more negative than that of control fruit at a given RWC, indicating that osmotic adjustment had occurred in water-stressed fruit. The lack of a linear relationship between turgor pressure and RWC pre-

Fig. 2. (A) Expansion of green-white 'Brighton' fruit in control (---) and water-stressed (—) plants recorded over 24 h with pressure transducers. Data are from individual fruit from one representative experiment. (B) The apoplastic water potential (ψ_{aw}) of leaves and green-white fruit in control and water-stressed plants. Values are means $\pm \text{SE}$ of four daily replicates.

vented the calculation of cell elasticity or ϵ (Fig. 4B). Calculated ψ_p was negative for most control and water-stressed fruit after dehydration.

SUGAR ANALYSIS. The ψ_s of green-white fruit from water-stressed plants was -1.41 MPa, which was significantly more negative than that of control plants at -0.82 MPa ($P < 0.05$ mean separation using independent t test; $n = 5$). Sugar concentrations in green-white fruit were calculated on a water content of 90% at full turgor (based on fruit fresh and dry mass data not shown). The concentration of glucose was 3.0 mM and that of sucrose at 4.6 mM in fruit of control plants, whereas values were significantly higher in fruit from water-stressed plants at 13.9 and 13.4 mM for glucose and sucrose respectively (independent t test; $P < 0.05$, $n = 5$).

Discussion

Strawberry fruit expansion was sensitive to changes in plant water status, even 3 d before leaf wilting (Fig. 1). The decreased expansion of green-white fruit on water-stressed plants likely reflected increased water demand by leaves (even with reduced opening of stomata) and limited soil moisture. In a laboratory study, Antoszewski (1974) reported that illuminating strawberry leaves caused contraction in green-white and red fruit, coinciding with opening of leaf stomata. However, in that study, the water status of plants used was not defined. In our study, red fruit had reached final size and showed little diurnal expansion or contraction.

The shrinkage of green-white fruit on water-stressed plants may reflect movement of water back into the plant crown or that the rate of water transport to the fruit was less than the rate of water lost from its surface. Fruit shrinkage under water stress has been reported in other species and has been taken to represent back flow of water into the stem (Berger and Selles, 1993; Johnson et al., 1992; Trump, 1984). Water flux from apple fruit toward the trunk during plant water stress was demonstrated by Yamamoto (1983) using a heat-pulse method. Even in well-watered plants, tomato fruit enlarge during the night and shrink during the day (Grange and Andrews, 1995).

Water is expected to flow to regions of lower ψ_{aw} in a plant, but this assumes an unrestricted path of water movement. There is evidence that this difference in ψ_{aw} may not be a good predictor of water transport in plants (Passioura, 1988). Clearly there are mechanisms that either isolate organs from the xylem stream or somehow provide preferential water transport to certain regions. Trolinder et al. (1993) reported that petals of cotton flowers continue to expand when leaf expansion is inhibited by water-stress, with ψ_w of petal tissue being consistently higher than that of subtending leaves and bracts. They suggested that petal expansion was obtained by an unknown active uptake mechanism that functions independent of the ψ_w gradient within the cotton plant. In wheat and barley, grain ψ_w remains ≈ 1 MPa higher than that of associated bracts during water-stress (Barlow et al., 1980; Brooks et al., 1982). Barlow et al. (1980) reported that the deposition of lipids and suberin in the chalazal end of the wheat kernel was enhanced by water-stress and may serve to isolate the grain hydraulically from the mother plant, preventing a fall in ψ_w in this organ under water-stress. They also suggested that the phloem may be the major path of delivery of water to the isolated grain.

Fruit expansion in strawberry occurred when leaf ψ_{aw} was higher than that of fruit, supporting the idea that water flows to regions of lower ψ_{aw} in the plant. The greater diurnal variation in ψ_{aw} in strawberry leaves than fruit (Fig. 2) is similar to that reported to by Johnson et al. (1992) in tomato, where fruit expansion and

Fig. 3. The difference between leaf and green-white fruit apoplastic water potential (ψ_{aw}) (e.g., $\Delta\psi_{aw} = \psi_{leaf} - \psi_{fruit}$) for control (□) and water-stressed (▨) 'Brighton' strawberry plants. Mean $\pm$ SE of four replicate experiments.

shrinkage were correlated, respectively, with the diurnal rise and fall in stem ψ_{aw} . Interestingly, strawberry fruit ψ_{aw} was significantly higher than that of leaves in stressed plants in the afternoon, suggesting that fruit were somehow buffered or isolated from extreme changes in ψ_{aw} of the xylem stream.

The ability of strawberry fruit to maintain a steady ψ_{aw} under plant water stress could be related to the development of a hydraulic barrier to the xylem stream. Pomper and Breen (1995), using a pressure chamber found evidence of an apoplastic barrier to solute movement from strawberry fruit when they were unable to retrieve a xylem mobile dye from the pedicel after it was injected into the body of the fruit. Antoszewski (1974) reported that cavitation greatly increases in the strawberry fruit pedicel on illumination of leaves, a process that could effectively isolate the fruit from the xylem stream of the rest of the plant. Possibly the phloem is an important vascular component for water delivery into strawberry as it is in other fleshy fruit (Findlay et al., 1987; Lee, 1989).

It was expected that turgor would be low in green-white fruit from water-stressed plants. However, the very negative turgor value (-0.88 MPa) calculated for such fruit in mid-afternoon, when turgor of control fruit was positive, probably resulted from an overestimation of fruit ψ_s and/or an underestimation of ψ_{aw} . The calculated turgor of severed fruit subjected to rapid drying also was negative (Fig. 4B). In tissues under water stress, negative ψ_p values can be calculated (Wenkert, 1980). The freeze-thaw step used to disrupt cells before measuring ψ_s permits dilution of cell solutes with apoplastic water, which may increase ψ_s and decrease calculated ψ_p values. Since the extent of dilution of cell solutes by solution in the apoplast in strawberry fruit was not estimated, ψ_s was not adjusted. Additionally, there may be ψ_w gradients within severely water-stressed strawberry fruit, namely the ψ_{aw} of the outer region of the fruit may be more negative than that of internal regions. In such a situation, the ψ_{aw} values obtained with in situ psychrometers attached to the surface of the fruit could be more negative than internal regions, thereby lowering calculated turgor values of stressed fruit (i.e., $\psi_p = \psi_{aw} - \psi_s$). Milad and Shackel (1992) found differences in ψ_s of cell sap measured between the stem and stylar ends of prune fruit. Differences in ψ_s of cells within the strawberry fruit also could result in negative calculated turgor

values for whole fruit. Regardless of the reason for the calculated negative turgor values, green-white fruit on water-stressed strawberry plants were likely at very low turgor.

Osmotic and elastic adjustment together may be important in maintaining symplastic volume and positive cell turgor in mature tissues of water-stressed plants (Bressan et al., 1990; Saliendra and Meinzer, 1991). However, we did not find a clear relationship between turgor pressure and RWC in green-white strawberry fruit (Fig. 4A) and, therefore, were unable to determine whether there are differences in ϵ of control and water-stressed fruit. Growth of green-white fruit during hydration, even at low temperature, likely caused unreliable measurements of RWC, and this may have been

Table 2. Solute potential at full turgor (ψ_s^{100})^z of leaves and red fruit hydrated at 20 °C and green-white fruit hydrated at 4 and 20 °C from 'Brighton' strawberry under water-stressed or control (watered) conditions.

Treatment	ψ_s^{100} (MPa)		
	Leaf	Red fruit	Green-white fruit
Control	-1.30 ^y	-0.94	-0.72
Water stressed	-1.57	-1.07	-1.00
Control—water stressed	0.27 ^a	0.13 ^{NS}	0.28 ^a

^zAfter hydration of tissue from control or water-stressed plants, sections of the material were placed in microfuge tubes, frozen in liquid N, and thawed; ψ_s^{100} was measured from the cell sap psychrometrically.

^ySeparation of means using an independent *t* test; $P < 0.05$, $n = 3$. ψ_s^{100} data of green-white fruit hydrated at 4 and 20 °C was pooled for statistical analysis.

^{NS}, ^aNonsignificant or significant at $P \leq 0.05$, respectively.

Fig. 4. (A) The relationship between solute potential (ψ_s) and relative water content (RWC) during rapid drying for green-white fruit from (○) control and (●) water-stressed 'Brighton' strawberry plants. The dashed line emphasizes that most fruit from water-stressed plants display ψ_s less than -1.4 MPa. (B) The relationship between turgor potential (ψ_p) and RWC during rapid drying for green-white fruit from (○) control and (●) water-stressed plants.

responsible for the lack of a linear relationship between ψ_p and RWC, as well as between ψ_s and RWC. The increase in volume in green-white fruit was $\approx 30\%$ less at a hydration temperature of 4 than 20 °C, suggesting that strawberry fruit expansion during hydration was in part metabolically dependent.

Osmotic adjustment resulted in about a 2.5-fold increase in the levels of glucose and sucrose in water-stressed green-white fruit. Since glucose and fructose are at equal levels in 'Brighton' fruit (Forney and Breen, 1986), the sum of the concentration of free sugars, essentially glucose, fructose, and sucrose, in green-white fruit can be estimated as 10.6 mM in control and 29.2 mM in water-stressed fruit. The sugar levels in control strawberry fruit were lower than that reported by Forney and Breen (1986) at the green-white stage, possibly because the green-white fruit used in our study were grown in midwinter in a greenhouse under low-light levels and also may have been less mature. ψ_s can be calculated using the vant' Hoff equation: $\psi_s = -CRT$, where C is the molality of the solution or osmolarity (moles of solute per kilogram of H_2O), R is the gas constant ($0.00831 \text{ kg}\cdot\text{MPa}\cdot\text{mol}^{-1}\cdot\text{T}^{-1}$), and T is the absolute temperature (K) (Salisbury and Ross, 1992). From this equation, the ψ_s of total sugars is calculated as -0.0262 and -0.072 MPa in control and water-stressed green-white fruit, respectively, which account for only $\approx 3\%$ of the total ψ_s of control fruit (-0.82 MPa) and $\approx 5\%$ of that of water-stressed fruit (-1.41 MPa). Because the hydration method used in this experiment (i.e., rewatering and covering plants for 6 h) may not have allowed complete rehydration of fruit, we report the solute contribution of sugars toward fruit ψ_s and not in terms of fruit ψ_s^{100} . Zhang and Archbold (1993b) found that the sum of glucose, fructose, sucrose, myo-inositol, and amino acids in leaves of *F. chiloensis* accounted for 24% and 39% of ψ_s^{100} in control and water-stress plants, respectively. They suggested that solutes they failed to measure accounted for the remainder of the ψ_s^{100} . Presumably solutes other than hexoses and sucrose are the major contributors to the ψ_s of strawberry fruit in our study.

In conclusion, strawberry fruit expansion was very sensitive to water-stress and only occurred when leaf ψ_{aw} was higher than that of fruit. Although, green-white fruit underwent osmotic adjustment when plants were subjected to water-stress, this was not sufficient to maintain fruit expansion during daylight hours when stress was greatest. Strategies that raise leaf ψ_{aw} above that of green-white fruit or stimulate additional osmotic adjustment by fruit tissues would be necessary to maintain fruit expansion during the daytime in water-stressed plants.

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