

Water Potential Components in Growing Citrus Fruits¹

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ABSTRACT

Growing navel orange fruits (*Citrus sinensis*) 5.4 to 5.7 centimeters in diameter were used as a model system to determine the effects of transpiration and carbohydrate translocation on water and osmotic potentials in fruit tissues. Evidence supported the hypothesis that osmotic potential in the vesicles would be affected little by changes in transpiration or carbohydrate translocation because the vesicles are anatomically isolated from the transpiration stream and are at the end of the carbohydrate translocation pathway. In the mesocarp tissue, which contains a vascular network, osmotic potential decreased during the daytime when environmental conditions favored transpiration and increased at night. Exocarp water potential followed a similar pattern. Girdling of the stem above the fruits 5 days before sampling caused an increase of osmotic potential in the mesocarp but had no effect on exocarp water potential. Neither diurnal changes in transpiration nor girdling of the stem affected the osmotic potential of the vesicles.

Osmotic potentials in all tissues of the fruit were in the range of -10 to -15 bars. Measurements of osmotic potential at 16 locations along a longitudinal plant through the fruit axis showed that osmotic potential increased from the stem to the stylar end, but it decreased from the pericarp tissues to the vesicles. As exocarp water potential decreased during a 20-day period after watering, osmotic potential decreased in the vesicles and exocarp. Turgor pressure, calculated as the difference between water and osmotic potentials, decreased with water potential in the vesicles but not in the exocarp. The lack of decrease of turgor pressure in the exocarp may result from a measurement error caused by pectins or from osmotic adjustment related to carbohydrate accumulation at low water potentials.

Many experiments on growth and physiology of tissue have emphasized the study of water relations or carbohydrate translocation. Only a few reports, however, provide information about the effects on growth of inter-relationships among water potential and its components, carbohydrate translocation, and carbohydrate interconversion. Even less is known about the interaction between these physiological aspects and physical characteristics of tissue such as strength and extensibility.

Because it is an isolated organ and has a number of separated types of tissue, a growing citrus fruit is useful as a model system for studying the combined effects of water relations, carbohydrate translocation and interconversion, and tissue strength on growth.

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Anatomical evidence suggests that water moving through a citrus fruit follows the vascular bundles in the mesocarp, diffuses through the exocarp, and evaporates on the surface. Carbohydrates also move through the vascular network in the mesocarp. Those carbohydrates ultimately reaching the vesicles first cross the endocarp underlying the mesocarp and then pass through the vesicle stalk into the vesicle. The vesicles, located within the fruit, are not involved in the water transport pathway in fruit transpiration and are located at the end of the carbohydrate translocation pathway. In addition, no vascular connections exist between the vesicles and the phloem or xylem in the pericarp. Based upon these anatomical considerations, it can be hypothesized that factors affecting transpiration or translocation will have little effect on the water relations of the vesicles. This hypothesis is supported by the work of Rokach (11), which suggests that diurnal changes in water content of the fruit result from changes of water content of the pericarp rather than of the pulp.

This paper examines the effects of changes in transpiration and disruption of translocation in an attempt to learn more about water and carbohydrate flux in specific parts of a growing citrus fruit. Osmotic potential was measured at many locations to determine local differences among tissues, and several experiments were designed to learn if osmotic potential changes equally in all tissues under certain experimental conditions. These experiments provide a basis for subsequent studies on carbohydrate translocation and conversion in similar fruits. Studies on physical strength of citrus fruit tissue are the subject of another paper in preparation.

METHODS AND MATERIALS

All experiments were performed on growing navel orange fruits (*Citrus sinensis* var. Washington navel) from mature orchard trees in Riverside, California. Fruits were 17 to 18 cm in circumference (5.4-5.7 cm diameter), corresponding to stage II in development (1), and were collected between September 24 and October 29, 1969. Fruits selected for measurement were brought into the laboratory in plastic bags. All subsequent handling and sampling were carried out in a humid chamber within 1.5 hr of collection unless otherwise noted.

Measurement of Water Potential. Water potential was measured with a Richards and Ogata thermocouple psychrometer, with a correction for heat of respiration (2). Measurements were made on convex discs of exocarp about 2 mm thick at the center and 2.5 cm in diameter. The discs were taken from the fruit near its equator. No correction was made for tissue resistance to water vapor transfer (5). Preliminary measurements of water loss from fruits during transpiration indicated that tissue resistance was less than 1.5 or 2.0 sec·cm⁻¹ even in the dark. Therefore, the resistance error in psychrometer measurements was probably low. In contrast, errors caused by leaf resistance for citrus ranged from 11.3% of the measured water potential for young leaves to 18.0% for old leaves (6).

Measurement of Osmotic Potential. Osmotic potential meas-

urements were made on tissue collected from various portions of the fruit depending on the particular experiment (see below). Samples were sealed in small vials, frozen at -16 to -20 C, and then thawed for extraction of sap. Cell sap was expressed with a small press fitted with a cloth filter which was replaced after each sample. Extraction pressures were normally less than 4 bars. Samples of solution ranged from 25 to 150 μ l in volume.

Osmotic potentials were measured at 37 C with a vapor pressure osmometer (Hewlett Packard, model 302B). The osmometer was calibrated by using NaCl solutions of known potential (9) on the thermistor. A steady temperature differential normally was achieved within several minutes. Reproducibility on any one sample was generally within 0.2 bar, although for some of the more viscous samples (from endocarp, mesocarp, or central axis tissue) the error may have been greater. The greater viscosity of these samples probably is caused by a higher proportion of pectin (12) and more cell fragments from lobed parenchymal cells in the expressed sap.

While the value measured with the vapor pressure osmometer is termed the osmotic potential, Wilson (16) pointed out that measurements on frozen tissue actually include a matric component along with the osmotic component and that these components may change upon freezing. Furthermore, he suggested that when sap is expressed from the frozen tissue the value obtained for the sum of the osmotic and matric components may differ somewhat from the sum for entire frozen tissue. Barrs (3) noted, however, that freezing avoids the possibility of membrane filtration when tissue is pressed. Since the size or even the existence of these changes caused by freezing or sap expression in the experiments reported here is not known, it is assumed that any such errors are negligible. The osmometer measurements on small amounts of expressed sap are likely to be considerably more accurate than those from other techniques with intact frozen and thawed tissue (*i.e.*, thermocouple psychrometer) because of better instrument resolution and the size of the tissue samples available. In addition, the osmometer is much more rapid and convenient. The possible existence of a matric component in the measured value of osmotic potential is recognized, particularly in tissue containing large amounts of pectin, and this is taken into consideration in the interpretation of observations. However, the matric effect is minimized when the matrix is filled with solution.

Tissue Samples. Citrus fruits are rather complex in structure—a variety of tissue types are found beginning early in the development of the fruit. A stage II fruit (1), *e.g.*, 5 to 6 cm in diameter, consists of three major parts: the pericarp, the pulp, and the central axis. The pericarp is divided into (a) the exocarp, or flavedo, containing an epidermis underlain by chlorophyllous parenchyma cells and oil glands; (b) the mesocarp, or albedo, containing white, lobed parenchyma cells with abundant intercellular spaces and a network of vascular bundles; and (c) the endocarp, an epidermis sheathing the outer, curved surface of the segments.

The pulp of the fruit consists of vesicles, or juice sacs, arranged on stalks attached to the endocarp, with septa radiating from the central axis and separating groups of vesicles into segments. The central axis contains a vascular bundle network to which ovules in the segments are connected. The vesicles are not connected to the central axis. The anatomy and morphology of citrus are described in more detail by Bain (1) and Schneider (13).

Samples were collected from the fruit at as many as 16 sites along a longitudinal plane through the central axis. These positions are shown in Figure 1. Sites A through H were in various parts of the pericarp: exocarp (A, D, and G), outer mesocarp (B and E), and inner mesocarp (C, F, and H). The pericarp was too thin near the stylar (navel) end to collect two mesocarp samples. Two positions were sampled in the endocarp, one

toward the stem end (I) and one toward the stylar end (J). Sites K through O were vesicles in various positions throughout the segment, and site P was in the central axis. Samples of exocarp and mesocarp tissue were generally 1.0 to 1.5 mm thick and 1.0 to 1.5 cm² in area. Endocarp samples were thinner and slightly larger in area. Samples of juice sacs and central axis averaged roughly 5 × 5 × 7 mm in size.

Experimental Treatments. In an initial analysis of osmotic potential, measurements were made at all positions shown in Figure 1. Based upon these observations, experiments were conducted to determine the effects of time of day, degree of water stress, and girdling on water and osmotic potentials in specific fruit tissues. Diurnal effects were determined by collecting nine fruits from nine different trees at 1:30 PM, 8:00 PM, and 7:30 AM. Fruits were collected from the south side of the trees where diurnal effects are likely to be the greatest, and tissue was sampled only from the exposed (south) side of each fruit. Tissue samples were taken from three sites within the orange, one in the mesocarp containing vascular tissue (site E, Fig. 1) and two in the pulp (sites K and O, Fig. 1). Exocarp water potential was measured on only five fruits (selected randomly) at each sampling time because of the limited number of sample chambers in the thermocouple psychrometer.

The effect of decreasing water potential on osmotic potential was determined by periodic sampling of 10 fruits from separate trees during a drying cycle between irrigations. Fruits were collected between 7:30 and 8:00 AM from the south side of trees. Tissue samples for osmotic potential measurements were taken from the exocarp (site D) and the vesicles (site M). Exocarp water potential was measured for each fruit using tissue collected near site D.

To determine the importance of a continued supply of carbohydrates to the fruit in maintaining an osmotic potential, six fruits on six trees were girdled. The phloem was removed from the stem between the 4th and 6th cm from the fruit and any leaves within 6 cm of the fruit were removed. The stripped xylem was coated with petroleum jelly and covered with aluminum foil. Fruits were girdled on the shaded north side of trees where fruit photosynthesis presumably is the lowest. Five days after girdling, the six girdled fruits and six adjacent control fruits on the same trees were collected at 8:00 AM. Osmotic potential was measured in the exocarp (site D), mesocarp (site F), and vesicles (site M). Exocarp water potential was measured on both the girdled and control fruits.

RESULTS

Thorough Analysis of Osmotic Potential. The results of a detailed analysis of osmotic potential from five fruits collected from separate trees are shown in Figure 1. Samples were collected at 8:00 AM, when the exocarp water potential was -5.5 ± 0.1 bars. Osmotic potential in all tissues of the growing orange fell in the general range of -10 to -15 bars. These values are typical for most mesophytic plant tissues. A trend of increasing osmotic potential (decreasing concentration of osmotically active solutes) was observed from the stem to the stylar end in each type of tissue examined. For instance, osmotic potential in the exocarp increased from -12.5 bars (site A near the stem) to -11.8 bars (site D at the fruit equator) and finally to -10.4 bars (site G near the stylar end), a gradient of about 0.4 bar/cm. A similar trend of increasing osmotic potential toward the stylar end was observed in the mesocarp, endocarp, and vesicles. There was no difference in osmotic potential among different layers of the pericarp at any position between the stem and stylar ends. However, osmotic potential in the vesicles was consistently lower than that of adjacent pericarp tissue in all parts of the fruit, with typical pericarp-to-vesicle gradients of 1 bar/cm.

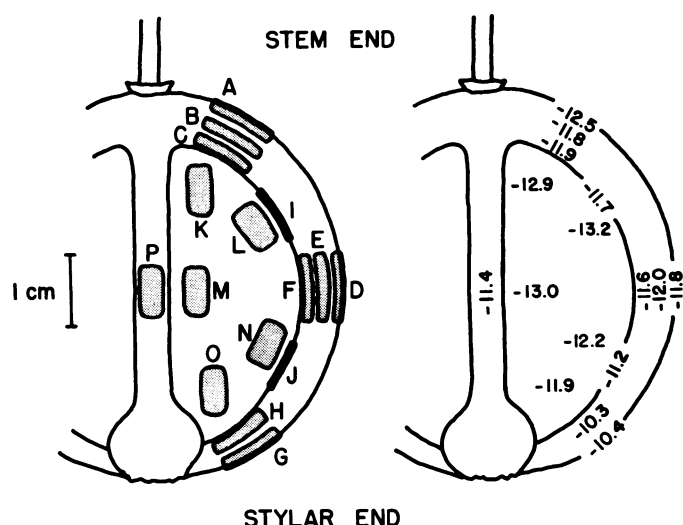


FIG. 1. Longitudinal sections of a navel orange 5.4 to 5.7 cm in diameter. Left: Tissue sampling sites for measurement of osmotic potential. See text for description of tissues. Right: Mean osmotic potentials from each sampling site of five fruits collected at 8:00 AM. Exocarp water potential was -5.5 ± 0.1 bars.

Table I. Diurnal Effects on Fruit Water Potential

Measurements were made on exocarp tissue from five exposed fruits collected from the south side of separate trees. Standard error of the mean water potential is indicated.

Time	Water Potential
	<i>bars</i>
1:30 PM	-10.7 ± 0.4
8:00 PM	-7.7 ± 0.3
7:30 AM	-5.1 ± 0.3

Osmotic potential in the central axis was about equal to that in the pericarp near the fruit equator. The gradients of osmotic potential among tissues were found to be statistically significant ($P = 0.05$) by both an analysis of variance and Duncan's multiple range test.

Another series of measurements was made on fruits collected at 4:00 PM and placed in a humid chamber overnight with stems in water. Results were qualitatively similar to those for fresh fruits with the exception that osmotic potential in the vesicles near the stem end was not significantly lower than that of the pericarp or of the vesicles near the styler end.

Diurnal Change in Osmotic Potential. Diurnal effects on water and osmotic potentials are given in Table I and Figure 2. Water potential was lowest at 1:30 PM (-10.7 bars) and increased to -5.1 bars by the following morning. The measurement of diurnal pattern of water potential was not repeated; other measurements of diurnal changes in water status of citrus leaves (7) indicate a similar response, as do numerous studies on leaves and other tissue reported in the literature.

Osmotic potential in the mesocarp (Fig. 2) also changed throughout the day. Osmotic potential was significantly lower ($P = 0.05$) in the afternoon (-14.5 bars) than in the evening (-13.0 bars) or early morning (-12.5 bars). In the vesicles, no significant difference in osmotic potential was observed for the times sampled or between the two positions; mean values ranged from -12.9 to -13.7 bars.

Relationship of Water Potential, Osmotic Potential, and

Turgor Pressure. The decrease in early morning water potential of the exocarp during a drying cycle between irrigations is given in Table II. Mean water potential during this particular cycle decreased only about 3 bars during a 4-week period. A greater decrease would be expected during a similar period in the hotter summer months.

The effect of a reduction in water potential of the exocarp on osmotic potential in the vesicles and exocarp is shown in Figure 3. In both the vesicles and the exocarp, osmotic potential decreased with a decrease in water potential. However, the decrease in osmotic potential in the exocarp was significantly greater ($P = 0.01$) than in the vesicles, and the data were more scattered. The greater variability of data in the exocarp may be caused by irregular concentrations of pectin, which has a colloidal or matrix effect on water in the tissue (15). Pectin concentration is generally high in all portions of the pericarp and low by comparison in the vesicles.

Turgor pressure may be calculated as the difference between water and osmotic potentials. For the vesicles, this requires the assumption that the vesicle water potential and mesocarp water potential were equal. Since these measurements were made on fruits collected early in the morning, this assumption is probably valid. Turgor pressure in the vesicles decreased as water potential decreased (Fig. 3). Measurements were not made at

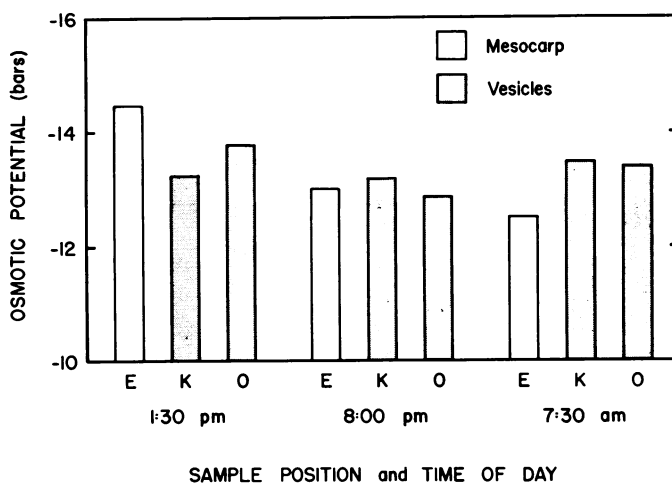


FIG. 2. Diurnal effects on osmotic potential at three sites within the fruit. Outer mesocarp was sampled from site E (see Fig. 1) at the fruit equator. Vesicles were collected from the stem end (site K) and the styler end (site O). Osmotic potentials given are means for nine fruits collected from separate trees. Diurnal changes were significant ($P = 0.05$) in the mesocarp but not in the vesicles.

Table II. Fruit Water Potential during a Drying Cycle

Samples were collected between 7:30 and 8:00 AM. Trees were irrigated 9/30/69. Standard error of the mean water potential is indicated.

Days after Irrigation	No. of Samples	Water Potential
		<i>bars</i>
2	4	-5.5 ± 0.2
7	5	-5.1 ± 0.3
9	10	-6.2 ± 0.2
16	10	-6.1 ± 0.2
21	10	-6.8 ± 0.6
23	10	-6.9 ± 0.6
27	10	-8.2 ± 0.3
29	10	-7.9 ± 0.4

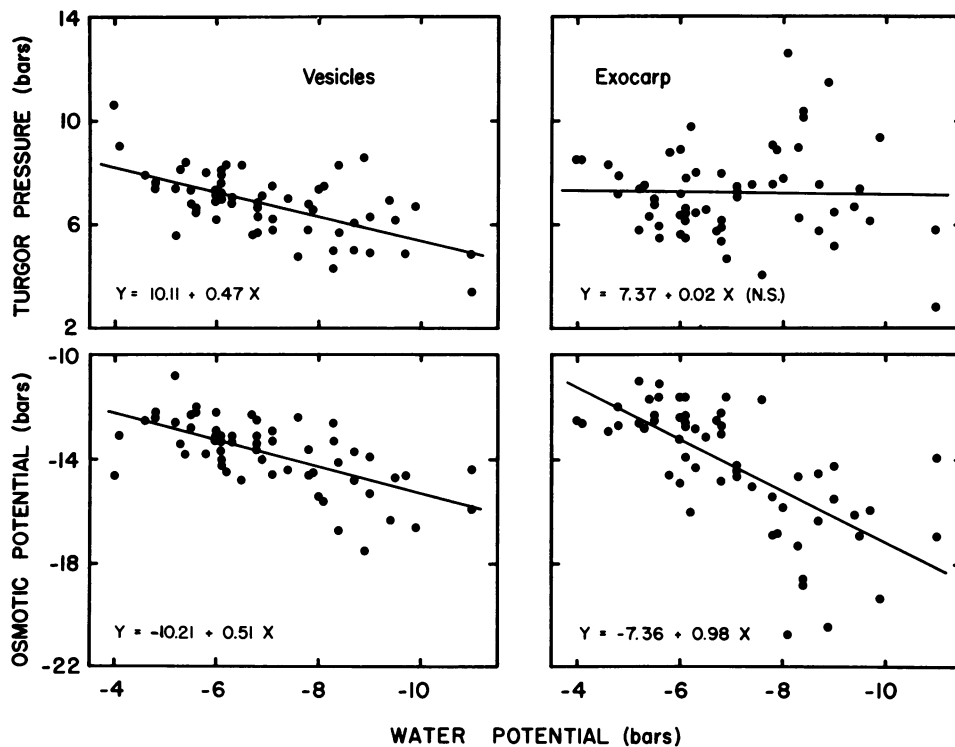


FIG. 3. Effects of decreasing exocarp water potential on osmotic potential and turgor pressure in the vesicles (site M) and exocarp (site D). Turgor pressure was calculated as the difference between osmotic potential and exocarp water potential. All samples were collected between 7:30 and 8:00 AM.

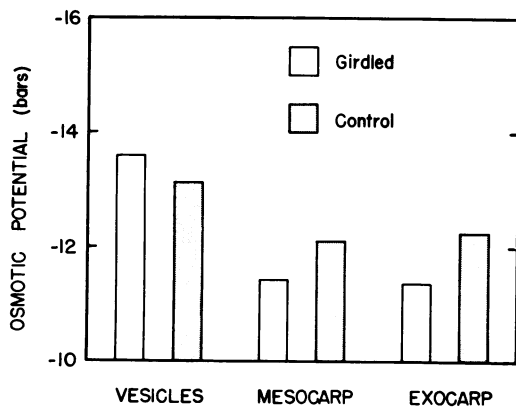


FIG. 4. Effects of girdling on osmotic potential in the vesicles (site M), mesocarp (site F), and exocarp (site D). The stem was girdled 4 to 6 cm above the fruit. Osmotic potentials are the means from six girdled and control fruits taken as pairs from separate trees at 8:00 AM. The statistical interaction between tissue type and girdling treatment was significant ($P = 0.05$).

low enough water potentials to observe a turgor pressure of 0 bar, but if the decrease in turgor is linear it would reach 0 bar at a fruit water potential of about -21 bars. The calculated turgor pressure in the exocarp does not change significantly with water potential. Two possible explanations for this, one involving carbohydrate accumulation and the other an error in osmotic potential measurements caused by pectin, are discussed below.

Effect of Girdling. The effect on osmotic potential of interrupting the supply of carbohydrates to the fruit is shown in Figure 4. Osmotic potential in the mesocarp and exocarp was increased about 1 bar by girdling, but no change or a slight decrease was observed in the vesicles. The interaction between

girdling treatment and position within the fruit is significant ($P = 0.05$), indicating that girdling affected osmotic potential differently in the mesocarp and exocarp than in the vesicles. Girdling had no effect on water potential. The mean water potentials were -6.2 ± 0.4 bars for girdled fruits and -6.3 ± 0.2 bars for adjacent control fruits.

DISCUSSION

The data reported here support the hypothesis that changes in transpiration and translocation have a greater effect on water relations of the pericarp than of the vesicles because the vesicles are anatomically isolated. Osmotic potential of the vesicles remained relatively constant throughout the day and decreased only moderately as fruit water potential decreased after watering. Similarly, the interruption of translocation of carbohydrates to the fruit resulted in no increase of osmotic potential in the vesicles. In contrast, the water and osmotic potentials of the pericarp changed readily in response to the treatments and conditions investigated.

The composition of citrus fruits at different stages of development is well known. Many substances are water-soluble and occur in moderate to high concentrations and are important, therefore, in affecting the osmotic potential. However, the data in the literature are not useful for calculating actual potentials. Since the composition and concentration of osmotically active substances vary among different portions of a growing fruit, differences in osmotic potential throughout the fruit are readily understood. According to Sinclair (14), unpublished work of Reed shows that cuticle coating the vesicles makes them quite impermeable, so that movement of water or solutes must involve passage through the vesicle stalks. Thus the vesicles are effectively isolated from each other, and longitudinal gradients of osmotic potential can exist (Fig. 1) because direct exchange of solutes among vesicles is greatly impaired. In these studies os-

otic potential generally increased from the stem to the styler end presumably because the osmotically active substances enter at the stem (fruit photosynthesis is believed to contribute little net carbohydrate). There were several exceptions to this gradient (e.g., Fig. 2), however, which may be caused by sampling from fruit segments around the axis having no longitudinal gradient (4). Materials entering the vesicles from the pericarp apparently move against an osmotic potential gradient (Fig. 1).

Measurements throughout the experimental period indicate that changes in water potential of orange exocarp are similar to those which occur in leaves. In growing oranges a diurnal change in exocarp water potential of 5.6 bars (Table I) corresponds closely to citrus leaf water potential fluctuations in similar trees of 5.5 to 6.2 bars (7). Even though the fruit is a large organ with a high water content, the exocarp of a citrus fruit may not be buffered against sizable diurnal changes in water potential while other tissues such as the phloem in a tree bole are buffered against large changes (8). The large short term change in exocarp water potential probably results from a relatively low resistance to loss of water vapor by transpiration and the movement of water from fruits to leaves during the day.

Fruit exocarp water potential is not affected by girdling the phloem since the pathway for water movement is not disturbed. However, measurements indicate that it is difficult to increase water potential of fruits substantially by placing them in a humid chamber with stems in water. Fruits subjected to this treatment overnight had a water potential of -7.0 bars, while many fruits sampled freshly in the morning had water potentials between -4 and -6 bars (Fig. 3). A similar difficulty in raising the water potential of leaves on detached branches has been observed.

Water potential could not be measured in the vesicles, but it is probable that at least moderate diurnal changes in vesicle water potential occur. A sizable change in water potential could occur with no measurable change in osmotic potential if vesicle turgor can change with little change in volume, *i.e.*, if the vesicle walls are not very elastic. Also, if a low amount of water is required for changing turgor in the vesicles, the water potential fluctuations may be rather rapid.

Estimates of turgor pressure in different tissues can be made if it is assumed that the water potential is equal throughout the fruit. Early morning measurements of osmotic potentials given in Figures 1, 2, and 4 and of water potential in the same fruits indicate that turgor pressure differed among various parts of the fruit. For instance, in samples having osmotic potentials given in Figure 1, turgor pressure in the vesicles was higher near the stem end than near the styler end of the fruit. However, the presence of large amounts of pectic substances in the cell walls of the pericarp suggests that the matric component may bias the osmotic potential measurements in pericarp tissues. For a sound prediction of turgor pressure within the cell, the measurement of osmotic potential should reflect only the concentration of solutes in the cell

vacuole. In sampling cell sap from pericarp tissue, however, a certain amount of cell wall pectin always contaminates the sample. The more pronounced decrease of exocarp osmotic potential with decreasing water potential and the greater variability of data in comparison with that of vesicles (Fig. 3) may reflect an increased pectin effect as the tissue is dehydrated. Therefore, it may be argued that the lack of correlation of exocarp turgor pressure with water potential is erroneous, and that turgor within the cell actually does decrease along with water potential. Another explanation for the lack of correlation is that at reduced water potentials carbohydrates may accumulate in the pericarp rather than move to the vesicles or be utilized in growth and metabolism. Carbohydrate translocation is known to be reduced by water stress (10). Whether the lack of a decrease in turgor pressure in the exocarp with a decrease in water potential results from a measurement error or from accumulation of osmotically active solutes is not yet clear.

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