Relationship of Water Potential to Growth of Leaves

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Received January 25, 1968.

Abstract. A thermocouple psychrometer that measures water potentials of intact leaves was used to study the water potentials at which leaves grow. Water potentials and water uptake during recovery from water deficits were measured simultaneously with leaves of sunflower (Helianthus annuus L.), tomato (Lycopersicon esculentum Mill.), papaya (Carica papaya L.), and Abutilon striatum Dickson. Recovery occurred in 2 phases. The first was associated with elimination of water deficits; the second with cell enlargement. The second phase was characterized by a steady rate of water uptake and a relatively constant leaf water potential. Enlargement was 70% irreversible and could be inhibited by puromycin and actinomycin D. During this time, leaves growing with their petioles in contact with pure water remained at a water potential of -1.5 to -2.5 bars regardless of the length of the experiment. It was not possible to obtain growing leaf tissue with a water potential of zero. It was concluded that leaves are not in equilibrium with the potential of the water which is absorbed during growth. The nonequilibrium is brought about by a resistance to water flow which requires a potential difference of 1.5 to 2.5 bars in order to supply water at the rate necessary for maximum growth.

Leaf growth occurred in sunflower only when leaf water potentials were above -3.5 bars. Sunflower leaves therefore require a minimum turgor for enlargement, in this instance equivalent to a turgor of about 6.5 bars. The high water potentials required for growth favored rapid leaf growth at night and reduced growth during the day.

The entry of water into plant tissue is essential for cell enlargement. Since water absorption occurs along gradients of decreasing water potential, the water potential of growing plant tissue must be below that of the water supply. The steepness of the gradient should depend on the resistance of the tissue to water flow.

Efforts to estimate gradients in potential of growing plant tissue have taken 2 main approaches. First, the water potential of the tissue and environment have been determined by transferring the growing tissue to media containing solutes and determining the potential of the solution which results in no net water uptake (1, 2, 9, 11, 13, 14, 16, 25). However, in addition to problems associated with the penetration of solutes into the tissue (29, 31), the interpretation of these experiments is made difficult by the need to use reversible changes in size to identify tissue water potentials while the plant material is growing irreversibly.

In the second approach, measurements of the resistance to water entry have been made by noting the half-time for equilibration of tissue segments in deuterated water (24, 25) or in solutions of various concentrations (29). Potential differences estimated from these measurements have ranged from zero (24, 25) to 5 or 10 bars (29). Ray and Ruesink (29) have pointed out some of the reasons for this variability and suggest that a small gradient, 1 to 1.5 bars, exists between coleoptile tissue and the external environment during rapid growth.

Apart from gradients in potential required for water entry, the water potential of the tissue may also affect growth rates directly because of the role of turgor in cell enlargement. The behavior of tissue varies considerably in this regard. At one end of the range, growth rate may be inversely proportional to the water potential of the tissue, becoming zero at the water potential which corresponds approximately to zero turgor (16, 33). Leaf growth has been reported to behave in this fashion when solute concentrations are high in the root medium (9, 34). In contrast with this type of response, there is evidence that growth rates may be reduced at relatively high water potentials, becoming zero well before tissue turgor drops to zero (14, 16). The latter behavior as well as work on the extensibility of cell walls (17, 28) has supported the concept that a minimum yield stress or minimum turgor is necessary before wall extension occurs (22). The requirement for minimum turgor implies that the lower limit for leaf water potentials associated with growth may be well above the wilting point.

The following experiments were undertaken to determine the water potentials of leaf tissue during growth and the relationship of these potentials to the potential of the water absorbed during growth. Leaf water potentials were measured with a thermocouple psychrometer. Determinations with this equipment
require the transfer of small amounts of water vapor between the thermocouple and leaf tissue. However, the resulting changes in water content of the tissue are negligibly small so that water uptake from the instrument has no influence on the measurement. Determinations also are not affected by solute penetration, since external solutes are not in contact with the tissue being measured. The equipment was designed to permit simultaneous measurement of water entry and leaf water potential and also to allow changes in the potential of water supplied to the tissue during measurements.

Materials and Methods

Leaves were obtained from sunflower plants (Helianthus annuus L.) grown from seed in soil in a controlled environment (4000 ft-c, 14 hour photoperiod; relative humidity, 70 ± 3 %; temperature 27 ± 0.5° day, 22 ± 0.5° night). Leaves of tomato (Lycopersicon esculentum Mill.), papaya (Carica papaya L.), and Abutilon striatum Dickson, were also used and were grown similarly but in a greenhouse with day and night temperatures of 25 ± 3° and 20 ± 3° respectively.

Leaf water potentials measured with thermocouple psychrometers generally require tissue that has been excised from the plant (4,8,19). Because of the difficulties in making repeated growth and water potential measurements on the same tissue with such a system, an instrument was designed for determining the water potential of intact leaves (fig 1). The method is similar to those requiring excised leaf tissue except that the psychrometer chamber has been enlarged to hold an entire leaf blade and the petiole of the leaf is led through a hole in the side of the chamber. The chamber opens into a top and bottom half to allow insertion of the leaf. The petiole is sealed in the chamber wall with petrolatum and the upper surface of the chamber is coated with melted and resolidified petrolatum to reduce adsorption of water vapor (6). The entire instrument is water jacketed for control of chamber temperature by circulating water from a bath kept at 26.0 ± 0.0005°. The instrument requires a similar room temperature controlled to ± 1.5°. Changes in room temperature are buffered by enclosing the chamber and thermocouple in a small Styrofoam container.

Leaves were prepared for measurement of water potential by washing them briefly in distilled water and allowing them to dry for at least one-half hour. Tests indicated that this treatment did not cause detectable change in the water potential of the tissue. During a measurement, an intact leaf was placed in the psychrometer chamber and a thermocouple with water on the junction was inserted. After 45 to 90 minutes, thermocouple output became steady and the water was replaced by a solution on a second thermocouple. The second solution had a water potential close to that of the leaf. The outputs of the first and second thermocouples were plotted versus the potential of the solution on the thermocouple and the plot was extrapolated to zero output. The solution producing zero output was considered to have a potential equal to that of the leaf tissue since its vapor pressure was the same as that of the tissue. This isopiestic value (4) was not affected by such factors as chamber conformation or the resistance of the leaf to vapor transfer.

The above procedure gave a calibration curve with a slope that was the same for a particular leaf at any water potential. After the initial calibration, a thermocouple bearing water was placed back in the chamber and used to give continuous isopiestic values computed from the calibration curve. The determination of the initial isopiestic value was corrected for heat of respiration by inserting a dry thermocouple into the chamber and, since this correction remained the same throughout each experiment, all subsequent values were corrected as well. This type of correction has been shown to be accurate for psychrometers which require excised leaf tissue (1) and has been assumed to be accurate in the intact leaf psychrometer used here. The time constant of the intact leaf instrument was approximately 30 seconds and was short enough to allow rapid changes in leaf water potential to be followed.

Subsequent to initial determinations of leaf water potential, the potential of the water supplied to the leaf was often changed by cutting the petiole under degassed water. Growth was measured both as
increase in leaf area or as water uptake. Water uptake was determined by measuring the water loss by weight from a container into which the cut end of the leaf petiole was immersed. To check whether transpiration was occurring during these experiments, the inside surfaces of the chamber were inspected for evidence of condensation due to water loss by the leaf. No water droplets were detected even after 30 hours. Thus, transpiration was negligible.

Inhibitor studies were conducted by allowing excised leaves to absorb inhibitor solutions (2.5 mM potassium maleate buffer at pH 4.8 bearing 1 of the following inhibitors: puromycin, 1.5 mM; hydroxyproline, 1 mM; actinomycin D, 50 µg ml⁻¹). The total water content of the leaves was known from calculations of water content per unit leaf area of parallel samples. It was assumed that the distribution of inhibitors in the leaf was uniform. The concentration of inhibitor in the leaf blade was determined by calculating the quantity of inhibitor absorbed per unit leaf water content. After absorption of a specific amount of inhibitor, the leaf was placed in buffer without inhibitor to grow. Growth was measured as increase in fresh weight. The experiments were conducted in a humid chamber which reduced transpiration to a negligible amount.

Some growth experiments were conducted in a pressure chamber (6, 30). The leaf petiole was sealed in the chamber top so that, when closed, the blade was inside the chamber and the petiole projected to the outside. The chamber was then inverted and the tip of the petiole placed into a container of water on a recording balance. Growth was measured as water uptake. The pressure around the leaf blade was controlled by allowing variable amounts of compressed air to enter the sealed chamber. Transpiration was reduced to a negligible amount by lining the walls of the chamber with moist filter paper.

Results

The accuracy of the intact leaf psychrometer was checked by comparing determinations with those made with a thermocouple psychrometer which required leaf samples (4) and is thought to give accurate values of leaf water potential (5). Comparisons were made by first measuring the water potential of intact sunflower leaves, then rapidly sampling the leaf blade for the excised leaf psychrometer. Table I indicates that values from the intact and excised leaf psychrometers agreed to within 0.3 bars.

Using the intact leaf equipment and a recording balance, leaf water potentials and water uptake were followed simultaneously with a sunflower leaf that had its petiole cut under degassed water and was recovering from a water deficit. Both water uptake and water potential recovery curves were 2-phase (Fig. 2). The first phase consisted of a rapid water uptake and rise in water potential usually lasting 16 to 45 minutes. The second phase was characterized by a steady rate of water uptake and a relatively constant water potential. In all but 1 leaf, the water potential was −1.5 to −2.5 bars during the second phase. The aberrant leaf had a water potential of −4.5 bars. The recovery curves for tomato, papaya and Abutilon were similar except that the first phase required 90, 190, and 80 minutes, respectively. Water potentials in these species during the second phase were also −1.5 to −2.5 bars.

The solute concentration of the xylem sap in the leaves was negligible as measured by a thermocouple psychrometer (3, 6), probably due to prior uptake of water. Therefore, xylem solutes could not account for the low potential of the leaves during the second phase. The data suggest that the second phase of recovery represents a water potential and water uptake characteristic of leaf enlargement. The following evidence supports this idea: First, leaf enlargement, measured as increase in leaf area, occurred during the second phase in the psychrometer chamber. The enlargement corresponded to the expected volume of water absorbed and was about 70% irreversible. Irreversibility was measured after allowing the leaf to dry to its original weight following a period of enlargement. Second,
leaf water uptake continued approximately at a steady rate for the experimental period, up to 30 hours. Third, leaf water potentials remained constant or changed only slightly and were below zero for the entire experiment. Fourth, inhibitors of coleoptile growth (15, 18, 21) inhibited water uptake associated with growth in sunflower leaves (table II). Hydroxyproline was without effect but both actinomycin D and puromycin were inhibitory. Under these conditions, water potentials should have risen and approached zero. This was not found with any of the inhibitors. The effect was tested further with puromycin (table II). Water potentials became more negative with higher concentrations of inhibitor. At the highest concentration of puromycin, leaf water potential was −7.3 bars but water absorption by the leaf was negligible. Since water uptake should have been faster rather than slower when leaf water potentials were lowered, it appeared that puromycin altered the absorbing mechanism in some way, possibly by affecting cell membranes. This effect of puromycin has been described for other plant tissues (23).

It might be argued that leaf water potentials measured with the psychrometer did not represent those in leaf cells during growth because of the possibility of water adsorption on leaf surfaces. Thus, the psychrometer would indicate the potential of the leaf surface rather than that of the cells. This possibility was tested by freezing and thawing a growing leaf blade at liquid nitrogen temperatures. Since turgor is zero after this treatment, water in the tissue is subjected only to osmotic and matric forces (7, 19). The blade was subsampled for the excised leaf psychrometer and sap was expressed from the remaining leaf tissue. The sap was placed on a thermocouple junction and inserted into the psychrometer chamber. Under these conditions, osmotic forces at the thermocouple balanced those in the tissue and output represented only matric or surface adsorptive forces associated with the tissue. Tests indicated that epidermal resistance to diffusion of water vapor was not altered by the freezing and thawing treatment and that there was no detectable adsorption on chamber surfaces (6). Thermocouple output was equivalent to approximately −0.1 bar and indicated that adsorptive forces were small enough to be ignored in sunflower.

In all 4 species the characteristics of the second phase of recovery showed that growth occurred when a potential difference of 1.5 to 2.5 bars was present between the leaf and pure water. Except for a starvation experiment, in which a sunflower plant was kept in the dark for 48 hours and the leaf grew slowly at −1 bar, it has not been possible to obtain leaves with potentials higher than −1.5 bars. The osmotic potential of cell sap expressed from a sunflower leaf was measured with the psychrometer (3). Values of approximately −10 bars were obtained, indicating that turgor was about 7.5 to 8.5 bars during growth in the second phase.

The results indicate that growth was occurring after the leaves had recovered from water deficits. Does it occur at any other water potentials? To answer this question, leaf enlargement was measured on sunflower plants over a 24-hour period during growth in soil of various moisture contents. Transpiration was made negligible by sealing the plants in polyethylene bags and placing the plants in the dark. The water potentials of the intact leaves were measured before and after the growth period and did not change during that time. The plant and soil were weighed before and after the experiment to detect possible water loss. Weight remained essen-

![Fig. 3 Enlargement of sunflower leaves on plants growing in soil with various moisture contents. The leaves had an initial area of 60 to 65 cm². Growth is expressed as a percentage of the initial area.](image)

Table II. Effect of Growth Inhibitors on Cell Enlargement and Water Potential of Sunflower Leaves

Leaves were allowed to absorb water through the petiole for 1 hour before treatment, then inhibitor was absorbed to the concentrations noted. Growth in buffer was measured during the subsequent 4 hours after which the leaf was subsampled for determination of water potential with an excised leaf psychrometer.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Conc in leaf</th>
<th>Fr wt gain mg·cm⁻¹</th>
<th>Water potential Bars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>...</td>
<td>38</td>
<td>−1.9</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>0.1 mM</td>
<td>37</td>
<td>−1.7</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>50 μg·ml⁻¹</td>
<td>33</td>
<td>−1.8</td>
</tr>
<tr>
<td>Puromycin</td>
<td>0.1 mM</td>
<td>27</td>
<td>−2.0</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>14</td>
<td>−2.9</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>11</td>
<td>−3.0</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>0.4</td>
<td>−7.3</td>
</tr>
</tbody>
</table>

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Initially constant. The water potential of the soil was not measured. Figure 3 shows that the leaves grew at leaf water potentials as low as −3.5 bars but that no growth occurred below this potential.

To check these data, growth was measured in an experiment of different design. A leaf was placed in a pressure chamber with the leaf petiole projecting from the top. Steady state water uptake due to growth was measured as a function of the air pressure around the leaf. This experiment differs from the soil experiment in that the leaf water potential was varied relative to that of the water source rather than the reverse, where soil water potential was varied relative to that of the leaf. The experiment has the advantage that both the potential of the leaf and the potential of the water source are known.

Figure 4 shows that growth of a sunflower leaf became zero at 1.8 bars of pressure. Solutes could not be detected in the xylem sap due to prior uptake of water by the leaf. Therefore, the 1.8 bar pressure represented a leaf water potential of −1.8 bars (6). The water potential of this leaf during the second phase of recovery had been measured previously in the psychrometer and was −1.9 bars. The correspondence between the water potentials measured with the pressure chamber and the psychrometer lends further support to the idea that water adsorption errors were negligible in the psychrometer. Table III shows the results of a similar experiment with sunflower leaves of varying area. In all cases, growth was zero at pressures greater than 3 bars.

The data from both the soil and pressure experiments indicate that sunflower leaf growth becomes zero at leaf water potentials below −3 to −3.5 bars. Furthermore, the pressure experiment suggests that there is little change in growth characteristics of sunflower leaves of increasing size. It is probable, however, that the pressure required to stop growth represents a slightly more negative water potential than indicated by figure 4 and table III. since it is known that water potentials measured with the pressure chamber are usually too high in well-hydrated leaf tissue (6).

The −3.5 bar leaf water potential required for leaf enlargement in sunflower indicates that little leaf expansion should occur during the day, since sunflower often has leaf water potentials below this value on sunny days. To test this idea, enlargement of a leaf was measured diurnally on a well-watered sunflower plant growing in soil. Figure 5 indicates that leaf growth was 5 to 6 times higher at night than during the day. The temperature and relative humidity during the day probably resulted in leaf water potentials that were high enough to account for the small amount of growth which was observed in the light. The −3.5 bar leaf water potential measured during the last day tends to support this idea.

**Discussion**

The results indicate that leaves were not in equilibrium with the potential of the water supply during growth and, at least in sunflower, that growth occurred only at high water potentials. The gradient in water potential required for rapid cell expansion was −1.5 to −2.5 bars and remained at that level regardless of the length of time for water uptake. The gradient is similar to that calculated for oat coleoptiles from kinetic experiments (29). A gradient of this magnitude indicates that there is a significant resistance to water flow in leaf tissue and implies that a potential difference must also be present when leaves grow in the presence of water having a potential lower than zero.

Leaves apparently have a maximum turgor during cell expansion which is determined by the extensibility of the cell walls, and they expand at a rate which does not allow turgor to completely compensate for cell osmotic and matric forces. Thus, it

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Table III. *Water Potentials During the Second Phase of Recovery and Pressure Required to Prevent Growth of Sunflower Leaves of Different Sizes*

<table>
<thead>
<tr>
<th>Leaf area cm²</th>
<th>Leaf water potential Bars</th>
<th>Pressure required to prevent growth Bars</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>−1.3</td>
<td>...</td>
</tr>
<tr>
<td>60</td>
<td>−2.4</td>
<td>2.3</td>
</tr>
<tr>
<td>81</td>
<td>−2.2</td>
<td>2.1</td>
</tr>
<tr>
<td>135</td>
<td>−1.9</td>
<td>1.8</td>
</tr>
<tr>
<td>235</td>
<td>−1.6</td>
<td>1.1</td>
</tr>
</tbody>
</table>

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![Figure 4](https://example.com/figure4.png) Enlargement of a sunflower leaf at various external pressures. Growth was measured as water uptake during the second phase of recovery from water deficits. Leaf water potential during the second phase was −1.9 bars. Leaf area was 135 cm².
LEAF ENLARGEMENT (cm² hr⁻¹) vs TIME (days)

FIG. 5. Diurnal growth of sunflower leaves on plants growing in soil in a controlled environment (temp: 29°C day, 22°C night; relative humidity: 45% day, 80% night; light intensity: 4000 ft-c; photoperiod: 14 hr). At the end of both the last night and the last day of the experiment, a leaf was harvested and its water potential was measured with the excised leaf psychrometer.

was not possible to obtain growing leaf tissue which was fully turgid in the usual sense. This idea is supported by the water potentials which were recorded for a sunflower leaf after a 48-hour dark period. The highest water potential was −1.0 bar when water was being absorbed, higher than any previously observed. The result is consistent with the view that metabolic intermediates were in short supply after the dark period, the capacity for cell wall extension was reduced, and water potentials therefore became higher.

The high water potentials which were necessary for cell enlargement suggest that sunflower leaves require a high turgor for cell enlargement. This conclusion does not agree with the recent suggestion that turgor is not involved in cell expansion (12, 13). The range of water potentials at which cell enlargement occurred in sunflower leaves is most accurately represented by the measurements of leaf enlargement on plants growing in soil at various moisture contents. Enlargement was maximum at water potentials of −1.5 bars but was negligible at about −3.5 bars so that a 2 bar change in turgor spanned the entire range of growth rates from maximum to zero. Since leaf osmotic potentials were about −10 bars, the minimum turgor required for cell enlargement was 6.5 bars and is similar to that required in coleoptiles (14). Turgor of this magnitude indicates that low water availability may inhibit leaf enlargement before it affects photosynthetic rates, since stomata generally remain open at turgor pressures below 6.5 bars in sunflower. Reduction of growth rates before reduction in photosynthetic rates has been observed in sugar beets during drought conditions (26).

The data presented here indicate that increased growth during the dark can be accounted for on a physical basis in sunflower leaves. It has been known for some time that growth of some plant tissues may be faster at night than during the day (10, 27). In addition to effects due to water, however, endogenous rhythms have been described in growing soybeans and Hyoscyamus leaves with the major portion of growth occurring at night (10).

The presence of a resistance to water flow in sunflower leaves and the necessity for high turgor during growth suggest that these factors affect the rate at which growth occurs. Growth over the long term takes place as a series of nightly pulses and the upper limit of leaf water potential reached each night is a function of the capacity of the cells for growth. This, in turn, determines both the turgor and one end of the gradient for water entry. The steepness of the resulting gradient determines the rate of growth which occurs. The most rapid rate of growth takes place when there is an optimum balance between a high turgor and a steep gradient for water entry.

The availability of soil moisture would affect growth rate by altering the supply end of the water potential gradient and by altering the water potential of the leaf. The effect of water status on leaf enlargement in sunflower may be represented for 1 soil drying cycle as shown in figure 6. When soil moisture availability is high, leaf water potentials return each night to a high level which is limited by the extensibility of the cell walls. Growth occurs whenever leaf water potential rises above that which supplies minimum turgor (represented by ψₑgrowth in fig 6). As soil dries, the recovery of leaves from water deficits incurred during the day takes a longer time (20, 32). The nightly growth periods become shorter in the drier soil and leaf water potentials do not rise as high. The potential gradient for water entry becomes less at night due to a drop in the soil water potential and turgor ultimately does

FIG. 6. Schematic representation of changes in soil and leaf water potentials during 1 soil drying cycle. The symbol ψₑgrowth represents the water potential above which leaf growth occurs in sunflower.
not rise above the minimum turgor required for growth. Enlargement then ceases. Measurements of leaf growth made once a day would indicate a gradual reduction in growth rate under these conditions. In the 2 instances where the rate of leaf growth has been studied as a function of water availability to roots (9, 34), this type of gradual reduction has been observed.

Literature Cited