Transpiration as a Function of Soil Temperature and Soil Water Stress

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Summary. An apparatus was developed for the measurement of transpiration rates of *Trifolium repens*. The transpiration rates were measured under controlled conditions of soil water stress and soil temperature. Other environmental parameters such as air temperature, relative humidity, light intensity and air speed were held constant. Diffusive resistances were calculated and stomatal aperture changes were recorded for all treatment combinations. A significant interaction between soil water stress and soil temperature was observed for stomatal closures. Stomatal closure was observed even in the so-called wet range of soil water stress. An increase in mesophyll resistance or incipient drying was observed for several treatment combinations. The mesophyll resistance was shown to increase as soil water stress increased.

Practically all the water taken up by plants is ultimately lost to the atmosphere as water vapor. Water moves through the soil from regions of higher to regions of lower potential energy, into the plant root, and through the plant to the leaves. The potential energy continuously decreases until the water reaches the point in the leaves at which evaporation is taking place. The water vapor (4) diffuses from the mesophyll cell walls lining the substomatal cavity to the free air beyond the boundary layer next to the leaf surface. It is usually assumed that the boundary layer next to the mesophyll cell walls is saturated with moisture at the temperature of the leaf, Tp. Assuming transpiration to be a steady-state process and considering only the vapor phase, then the flow of water vapor, \( V \), in g m \(^{-2} \) min \(^{-1} \), from the substomatal cavity through the stomatal opening, through the boundary layer of air adhering to the leaf surface to the free air beyond the leaf may be written:

\[
V = \frac{\rho_v \cdot \Delta h}{R}
\]

where \( R \) is the total resistance of the diffusion pathway expressed in min cm \(^{-1} \), \( \rho_v \) is the vapor density of the stomatal cavity and \( \rho_a \) is the vapor density of the free air.

The total resistance along the flow path is made up of several resistances in series defined as follows: 1) \( r_{i} \) - the resistance encountered by water molecules passing from inside the leaf through the intercellular spaces and the stomatal openings (or the cuticle). 2) \( r_{b} \) - the resistance encountered by water molecules diffusing across the boundary layer which sheaths the leaf surface. 3) \( r_{m} \) - the resistance encountered by water molecules diffusing across the cell walls of the mesophyll cells to the evaporating surface.

The resistance of the mesophyll, \( r_{m} \), is considered to be negligible when adequate water is available to the plant. However, it has been postulated that as the soil dries out and the soil moisture stress increases the resistance of the mesophyll increases to a point where it can no longer be ignored. This process has been termed incipient drying and has been held responsible for reductions in transpiration independent of stomatal closures.

The specific objectives of the study were to quantitatively evaluate the magnitude of the 3 components of total resistance, \( r_{i} \), \( r_{b} \), and \( r_{m} \) at specified values of soil water suction and to make this evaluation at different soil temperatures, with the air temperature maintained constant.

Materials and Methods

Control of Soil Moisture and Soil Temperature. An apparatus was developed in which the soil moisture content of several soil cells in which white Dutch clover plants were growing could be maintained at a constant level by various osmotic solutions (5, 11) at selected temperatures (fig 1). It consisted of 2 banks of 4 independent osmotic chambers, immersed in a water bath. Water was pumped into the bath, circulated through the chamber and recycled to a temperature-controlled water source. This gave a positive temperature control for the osmotic solutions, thus eliminating any temperature variation. Measurements were made at temperatures of 10°, 15.6°, 21.1°, and 26.7°.

The osmotic solutions were continuously stirred by a propeller, connected to a shaft which protruded through the wall of the osmotic chamber. Leakage around the shaft was prevented with oil seals and sealed bearings imbedded in the chamber walls. The
shafts were driven by a geared-down electric motor through a chain drive. Heat developed by the propellers was not a problem.

Soil cells constructed of clear plastic sheets with removable sides are shown in figure 2. The thickness of the soil cells was 8.0 mm. The soil used was a Willamette silt loam (41.8, 41.6, and 16.6 % sand, silt and clay, respectively) passed through a 2 mm sieve.

Plants were grown in these cells for about 6 weeks, at which time the removable sides of the cells were replaced by Visking membranes held in place by specially built frames (2). Leakage was prevented by sealing the membranes and frames to the cell using an O-ring molded from a silicone rubber adhesive.

The cells were then put in the osmotic chambers and sealed in place. Each osmotic chamber was brought up to volume with a solution of polyethylene glycol 6000 (carbowax). A constant-water-level device operating on the same principle as a mariotte bottle was connected to each osmotic device. These devices were graduated, thus making it possible to accurately measure the volume of water used by the plants over a period of time.

Four concentrations of carbowax 6000 were utilized corresponding to the following osmotic pressures: 0.35 bar, 0.70 bar, 1.00 bar, and 1.32 bar. The required concentrations (11) were obtained from figure 3.

**Other Environmental Parameters.** All experiments were performed in a growth room in which the air temperature, relative humidity, and light intensity were maintained constant at 23.9°, 45 % and 2300 ft-c, respectively. Air movement in the growth chamber was maintained constant by drawing air over the plants with a squirrel cage fan. The wind speed was approximately 2 m sec⁻¹.

Leaf temperatures were measured by placing a bead thermistor in intimate contact with the underside of the leaf surface. Air temperatures were measured by placing a bead thermistor directly under the leaf but displaced a short distance below the leaf.

The method of Zelitch (10) was used for observing stomatal apertures. The method was modified in that only cellulose acetate impressions (negatives) were taken of the leaf surfaces for direct microscopic examination. Impressions were made only of the lower surface of the leaf because previous work indicated that practically all leaf stomata in *T. repens* are located on this surface.

Leaf areas were measured using an MK area calculator with a grid of 100 squares per inch.

**Procedure.** White Dutch clover plants (*Trifolium repens*) were grown in the soil cells at an
air temperature and soil temperature of 23.9°. When the plant roots had adequately permeated the soil mass (6-8 weeks), the removable sides of the soil cell were replaced with the Visking membrane. The cells were then immersed in the various osmotic solutions. A waiting period of 60 to 72 hours was observed for the system to come to equilibrium and to check for leaks. Evaporation from the soil surface was prevented.

When the system reached equilibrium, the air temperature, leaf temperature, soil temperature, relative humidity and water level of the graduated constant-water-level devices were recorded. The plants were then allowed to transpire for 8 hours, at the end of which time the above parameters were again recorded. At this time the plants were harvested and all plants from each cell were weighed for the initial weight in the relative turgidity measurement (11).

The above parameters were measured at all possible combinations of the 4 levels of osmotic pressures and 4 soil temperatures.

**Results**

*Transpiration Measurements.* Transpiration was measured by taking the difference in water levels of the graduated constant water level devices at the beginning and end of each test period.

The results of the transpiration measurements are shown graphically in figure 4 in which transpiration in gms of water per square decimeter per

![Graph showing osmotic pressures of Carbowax 6000 solutions.](image1)

**Fig. 3.** Osmotic pressures of Carbowax 6000 solutions.

![Graph showing transpiration as a function of soil temperature at 4 levels of soil water tension.](image2)

**Fig. 4.** Transpiration as a function of soil temperature at 4 levels of soil water tension.

![Photomicrographs of stomatal openings on Trifolium repens.](image3)

**Fig. 5.** Photomicrographs of a cellulose acetate impression of stomatal openings on *Trifolium repens*. Treatment combinations: A) (top) Soil temperature 10° and soil water stress 0.35 bar, and B) (bottom) Soil temperature 10° and soil water stress 1.32 bar. Magnification approximately 1000 X.
hour is plotted versus soil temperature in centigrade for 4 levels of soil water stress in bars. The curves in this figure markedly demonstrate the important effects of soil water stress, even in the so-called wet range, and soil temperature on transpiration.

Stomatal Apertures. Photomicrographs of various replicas of stomatal apertures were taken using a student microscope equipped with a 35 mm camera attachment. The photomicrographs in figure 5 were taken of stomata from plants subjected to a soil temperature of 10°C and soil moisture stress of 0.35 and 1.32 bar, respectively. The marked decrease in the stomatal opening with increased soil water stress is apparent. By inserting an ocular micrometer into the eyepiece of the microscope, it was possible to obtain a measurement of the stomatal width. No attempt was made to calibrate the micrometer to obtain an actual rather than a relative measurement of stomatal width. A graph of relative openings of stomatal apertures as a function of soil water stress at various soil temperatures is shown in figure 6.

Boundary Layer Resistance. The boundary layer resistance $r_b$ may be calculated (3, 6, 8) by determining the rate of water loss from evaporating wet surfaces as similar as possible in surface geometry to the actual leaves. The resistance $r_b$ can be calculated by using equation I. An electrobalance was designed to determine evaporation from saturated blotting paper having the same geometry as the clover leaves.

A boundary layer resistance of $r_b = 0.0055$ min/cm was determined for the environmental conditions used in the transpiration experiments. In this work, leaf size and shape, air temperature, relative humidity, light intensity and wind velocity were maintained constant; therefore, the assumption was made that the boundary resistance did not change throughout the experiment.

Leaf Resistance. By using equation I, it is possible to calculate the total resistance encountered by water molecules passing from inside the leaf through the intercellular spaces, the stomatal openings (or the cuticle) and the boundary layer of air at the leaf surface into the surrounding air. In these calculations, the assumption is made that the...
vapor pressure of water vapor within the plant leaf is a function of leaf temperature alone.

Subtracting the boundary layer resistance, 0.0055 min/cm, from the total resistance for each treatment gives the corresponding leaf resistance. Results of these calculations, shown in table I under a, illustrate the combined effect that soil moisture stress and soil temperature has on leaf resistance.

Mesophyll Resistance. A decrease in the water content of the leaf can be associated with a partial drying of the mesophyll cell walls, which could cause an increase in the resistance of the walls to water movement. As a result, changes in the transpiration rate would be observed in the absence of measurable stomatal action. To check for the occurrence of this phenomenon, the stomatal aperture measurements were subjected to a statistical analysis. The least significant difference at a 1% level of significance was used to test the means of the measured apertures for changes. This high level of significance was used because of the difficulties involved in determining the actual stomatal boundaries in the replicas. Results of this analysis are shown in figure 7. The unshaded blocks represent the treatment combinations at which no stomatal closure occurred. The values of leaf resistance calculated from the transpiration measurements are shown in figure 7 for each treatment. Any increase in leaf resistance in the absence of stomatal closure indicates a possible increase in the mesophyll resistance. At a soil water suction of 0.35 bar, the mesophyll resistance was considered negligible, and the leaf resistance was 0.041 min cm$^{-1}$ for all temperatures. Subtracting the value of 0.041 min cm$^{-1}$ from all other leaf resistance values for the treatment combinations which did not result in stomatal closure, gives a quantitative value for the mesophyll resistance. Results of these calculations are shown in table II.

Leaf Temperatures. Figure 8 is a graph of leaf temperatures as a function of soil tempera-

<table>
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<th>Soil temp</th>
<th>Mesophyll resistance</th>
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![Fig. 7. Block diagram of leaf resistance as a function of soil temperature and soil water suction. Conditions under which significant stomatal closure was observed are indicated by cross-hatching.](image)

![Fig. 8. Leaf temperatures as a function of soil temperature and soil water suction.](image)

![Table II. Mesophyll Resistance as a Function of Soil Temperature and Soil Moisture Stress.](table)

Discussion

Transpiration Measurements. At a soil temperature of 10°, the transpiration rate is decreased by more than 50% by increasing the soil moisture stress from 0.35 bar to 1.32 bar. At higher soil temperatures, the relative displacements are not as...
marked, but are still apparent. Very little if any, difference in transpiration rate occurs between a soil moisture stress of 0.70 bar and 1.00 bar.

The effect of soil temperature on transpiration also is very clearly demonstrated in figure 4 by the change in slopes of the transpiration versus soil temperature curves. Two distinct regions centered around 15.6°C are evident from these curves. Above a soil temperature of 15.6°C there appears to be a linear relationship between soil temperature and transpiration. Below 15.6°C there is a much more rapid decrease in transpiration as a function of soil temperature which produces a more curvilinear relationship.

The relative displacement of the curves in figure 4 and the change in slopes of all curves with temperature indicate that an interaction effect on stomatal aperture exists between soil water stress and soil temperature. At high soil temperatures, such as 26.7°C, the stomatal openings were not significantly influenced by increases in soil moisture stress over the range tested. Slight decreases in stomatal apertures were indicated over this soil moisture stress range for soil temperatures of 15.0 and 21.1°C. For a soil temperature of 10°C, progressive decreases in stomatal apertures were evident over the entire soil moisture stress range, demonstrating the marked interaction between stomatal openings and soil water stress and soil temperature. Possibly, reduced water uptake at a soil temperature of 10°C is effective in reducing turgor in the guard cells to the point that decided stomatal closures do occur in this particular species. It is interesting to note that the incidence of stomatal closure decreases as soil temperature increases.

Stomatal closure was not observed at a soil temperature of 26.7°C over the entire range of soil moisture stresses studied. It appears that the viscosity effects on water transport within the soil-water-plant system produces an end result very similar to that of increased soil moisture stress.

Since the degree of stomatal closure is more or less directly proportional to the magnitude of increased leaf resistances, it follows that in most cases the increase in leaf resistance can be attributed to an increase in stomatal resistance. The increases in resistances reported here are comparable to those found by Kuiper (6) who showed that the relationship between width of stomatal aperture and stomatal resistance to diffusion was depicted by a hyperbolic curve. In this relationship, the stomatal aperture could change from fully open to almost half closed before any great changes in stomatal resistances could be observed. However, small further decreases in stomatal aperture would bring about much greater increases in stomatal resistance. The above relationship is made assuming that no incipient drying of the mesophyll cell walls occurred.

**Diffusive Resistances.** Leaf resistance values shown in table I were calculated with equation I assuming a relative humidity of 100% in the stomatal cavity. Since incipient drying occurred at the surface of the mesophyll cells, the assumption that the relative humidity in the sub-stomatal cavity is 100% might have been invalid, and calculations using this assumption would be erroneous. To determine the degree to which the values of leaf resistance shown in table I might be in error, the vapor concentrations $c_{st}$ of stomatal cavities were calculated with the equation

$$c_{st} = c_s \cdot \exp \left( \frac{v_w \cdot \psi_s}{RT} \right)$$

where $\psi_s$ is the water potential of the leaf in bars; $c_s$ is the vapor pressure in the stomatal cavity and $c_s$ is the saturation vapor pressure at absolute temperature $T$ expressed in terms of the vapor concentration; $R$ is the gas constant for water vapor in erg per gm per degree; and $v_w$ is the molar volume of water.

The water potential ($\psi$) of the plant leaves was determined by floating samples of plant leaves on sucrose solutions of various concentrations. Relative turgidity of parallel leaf samples was determined by floating the previously weighed samples on distilled water for 5 hours at a light intensity of 65 ft-c. The samples were reweighed and then oven dried for 1 hour at 90°C (Barrs and Weatherley, 1). Figure 9 shows the calibration curve obtained for T. repens. Results of the relative turgidity determinations are given in table I.

Leaf resistances are shown in table I under b and were calculated by using the relative turgidity values to determine the water potential of the plant leaves from figure 9. Then the vapor concentration of the stomatal cavity was determined using equation II. Leaf resistances were determined as before by subtracting the boundary resistance from the total resistance as calculated from equation I. Leaf resistances calculated by the 2 methods are identical for almost all treatments indicating that the degree of incipient drying which occurred under these experimental conditions was not sufficient.
to result in reduction in water vapor concentration large enough to cause an error in the calculations of leaf resistance.

In using osmotic solutions to control soil water suction it should be recognized that since water moves along a potential gradient, the soil water will be at a lower potential than the osmotic solutions when water is moving through the system. Therefore, the osmotic potential of the solution gives only a maximum value for soil water potential and not the actual values when the plants are transpiring. Similarly the cell walls will be at a lower potential than the leaf-water potential measured at zero transpiration.

When using equation (II) to calculate vapor pressure in the stomatal cavity from the leaf-water potentials, the assumption is made that there is no water movement and zero mesophyll resistance. The data at hand do not allow a quantitative evaluation of the error involved in making this assumption.

At a soil moisture stress of 0.35 bar, the mesophyll resistance was considered negligible and the total resistance as determined by equation (I) was mainly a function of the leaf resistance and the boundary layer resistance. Therefore, it is assumed that the leaf resistances at 0.35 bar and soil temperatures greater than 15.6° are constant for a given stomatal opening. As shown in table I, these leaf resistances are maintained constant at about 0.041 min cm⁻¹. Therefore, any increase in leaf resistance in the absence of stomatal closure would indicate a possible increase in the mesophyll resistance. Subtracting this value of 0.041 min cm⁻¹ from all other leaf resistance values in which stomatal closure has not been observed gives a quantitative value for the mesophyll resistance. These values are shown in table II. The values of leaf resistance calculated for all other treatment combinations are the result of stomatal closure and increased mesophyll resistance. With the data at hand, it is not possible to separate these 2 effects.

Leaf Temperature. Leaf temperatures for the various treatment combinations are shown in figure 8. The graph indicates that leaf temperatures increased with increasing soil water suction and with increasing soil temperatures. Leaf temperatures are determined by the energy balance at the leaf surface, of which transpiration is an important component. Raschke (9) provided a quantitative analysis of the relationship of the temperature of leaves to their physical environment. The prediction equations developed by Raschke for leaf temperatures do not apply in this situation. Firstly, the equations do not provide for taking into account changes in transpiration rates mediated by changes in soil water suction. These changes do, however, alter the energy budget at the leaf surface substantially as can be seen in figure 8. Secondly, the increase in leaf temperature with increasing soil temperature is seemingly contradictory to the observation that transpiration rates increased with increasing soil temperatures. This response of leaf temperatures to changes in soil temperature is mediated by the geometry of the experimental arrangement. The path length of the water from the soil to the leaf is quite short. The leaf temperatures are probably directly affected by the temperature of the water in the soil. A quantitative analysis of the effect of changes in transpiration rates and changes in soil temperature on the leaf temperatures was not attempted.

Literature Cited