Leaf Diffusion Resistance, Illuminance, and Transpiration

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Abstract. Stepwise increases in fluorescent illuminance, imposed as a single variable in a controlled environment, induced progressive stomatal opening in 8 plant species, as evidenced by a consistent decrease in leaf diffusion resistance (R_L), ranging from 15 to 70 sec cm^{-1} in darkness to about 1 sec cm^{-1} at approximately 40 kilolux. The minimum R_L values were the same for the upper and the lower epidermis, provided that stomatal density was adequate. Saturation illuminance was not achieved in any species; extrapolation indicates that 50 kilolux would bring about full stomatal opening (R_L \leq 0.1 \text{ sec cm}^{-1}).

In 4 species, reasonable agreement was obtained in a controlled environment between transpiration as measured by weight loss and that calculated from determination of (a) the difference in water vapor density from leaf to air, (b) the boundary layer resistance, and (c) the leaf diffusion resistance. This result confirms the physical validity of the resistance measurement procedure.

Transpiration from leaves can be written as

\[ E = \frac{\triangle d_v}{R_A + R_L} \]

where \( E \) is the evaporation (transpiration) rate, in \( \mu\text{g cm}^{-2} \text{ sec}^{-1} \), \( \triangle d_v \) the difference in water vapor density between the leaf interior and the air, in \( \mu\text{g cm}^{-2} \), \( R_A \) the boundary layer resistance in sec cm^{-1}, and \( R_L \) the leaf diffusion resistance, also in sec cm^{-1}. A recently developed leaf resistance meter permits a convenient and rapid measurement of \( R_L \) (8, 9). Provided cuticular water loss is low, \( R_L \) is determined primarily by stomatal width and density, but also by their length and depth (1, 2, 5, 6). For turgid plants exposed to normal levels of \( \text{CO}_2 \), the main regulator of the aperture and, therefore, of \( R_L \), is illuminance of the leaf (3, 7). The first part of this report deals with the effect of illuminance on \( R_L \) for 8 plant species: the second part tests the adequacy of the determination of \( R_L \) by applying 1, assuming that \( R_A \) can be determined unequivocally by the method used here.

The difference in water vapor concentration from leaf to air (\( \triangle d_v \)) was found by measuring leaf temperature (assuming saturation vapor pressure in the substomatal cavities) while controlling the ambient vapor density. Then, at a given illumination, which induced a specific value of \( R_L \), an independent measurement of \( R_A \) permitted \( E \) of equation 1 to be calculated. A comparison of the calculated with the measured value of transpiration, found from the weight loss, gave a test for the validity of the transpiration resistance measurement and its use in equation 1. This comparison was made for 4 of the 8 species already studied, first in complete or virtual darkness, and then at sufficiently high illuminance to induce wide stomatal opening. These contrasting conditions were designed to bring about a wide range in \( R_L \) values, thereby ensuring general applicability of the results.

Materials and Methods

Illuminance Experiments. Eight species were studied: alfalfa, (Medicago sativa, var. Moapa), snap bean (Phaseolus vulgaris, var. 6-Week Bountiful, fava bean (Vicia faba), cotton (Gossypium barbadense), var. Pima S-2, sunflower (Helianthus annuus, var. Greystripe), lemon (Citrus hybrid), var. Rough Lemon), corn (Zea mays, var. Mexican June), and sorghum (Sorghum vulgare, var. RS-610). Plants were grown in greenhouse water cultures (aerated Hoagland solution, 10 meq/l total concentration) for 8 weeks or less, depending on the species. Then they were preconditioned for 24 hours in the controlled environment room, being exposed to 12 hours of fluorescent light (32 kilolux, 0.3 ly min^{-1} at the top of the plant), followed by darkness.

During preconditioning and the experiment proper the other environmental conditions were: air temperature 30.0 \pm 0.3^\circ \text{C}, vapor pressure 15.0 \pm 0.2 \text{ mb}, \text{CO}_2 content of the air 375 \pm 10 ppm by volume. The sensors for both air tempera-
ture and vapor pressure were resistance thermometers located in a ventilated, radiation-shielded housing in the controlled environment room. Vapor pressure measurements consisted of monitoring the cavity temperature of a dew probe with the resistance thermometer. Both air temperature and vapor pressure were recorded continuously. Air temperatures were verified with a thermocouple and vapor pressures with occasional psychrometer readings.

Measures taken to make sure that illuminance was the only variable affecting $R_L$ were: A) maintenance of a constant air temperature near the optimum for physiological reactions, B) wearing of gas masks by personnel in the room, which enabled carbon dioxide concentrations to be held near ambient levels, $(375 \pm 10$ ppm by volume) C) promotion of a favorable internal water balance in the plant, by having easily available water from an aerated, warm, dilute nutrient solution, and by preventing transpiration surges through gradual increases in illumination.

Each species was studied in a separate experiment, but under the controlled environment specified above. As illuminance increased, stomatal opening caused a progressive lowering of $R_L$, which was measured with a leaf resistance meter (8).

After 1 hour of measurement in the dark, successively higher values of illuminance were established, every 90 minutes, until the following levels had been in effect: 0, 5.4, 10.7, 32.1, and 42.8 kilolux, measured at the leaf with a Weston$^3$ luxmeter. Simultaneous $R_L$ readings were taken at representative sites on both a young, but fully expanded leaf, and an old leaf of each species at every level of illumination, until steady readings were obtained. Since both leaves responded similarly, the data for only the upper one are shown in the figures.

For leaves of legumes (snap bean, fava bean, alfalfa) a given site on the 3 leaflets was tested. For cotton and sunflower 5 locations along the leaf margin, corresponding to major veins, served as replicate sites. These sites were sufficient for the small lemon leaf. Along the leaf edge of corn and sorghum there were several successive 10-cm long sampling areas for $R_L$ measurements, each of which was monitored alternately with a fixed reference site. The different replicate measurements showed similar, but not identical $R_L$ values.

Therefore, the data points shown in the graphs (figs 1 and 2) represent the average of the several sites. The data also are the mean of several readings taken over the last half of each measuring period. The data for the first half of each period were not included, so as to avoid the momentary upset in $R_L$ caused by a moderate increase in illuminance. The trend of the averages over the last half of the period was the same as that for the whole period, but with smaller deviations.

Continuous measurements of leaf temperatures were obtained on a pair of leaves at the same height as those used for $R_L$ readings, but on a different plant located 50 cm away. Supplementary spot readings of leaf temperature also were taken with a thermistor probe (4 mm × 1 mm) on the leaves used for $R_L$ measurements. The continuous leaf temperature measurements lasted from the start of the preconditioning period until the end of the experiment, the recorded data being used in calculating $R_L$ values. This continuous record also helped to determine when stomatal aperture had reached a steady value characteristic of a given illuminance.

On a third plant, located 50 cm from the others, silicone rubber impressions were taken periodically from the lower epidermis of a leaf at the same height as the upper leaf used for $R_L$ data on the main plant. The impressions were taken at the end of each successive level of illumination except the 32.1 kilolux value. The stomatal frequency, length and width were measured from these replicas.

Transpiration Experiments. Four additional experiments dealt with the calculation of transpiration. Light from fluorescent lamps was used for the lemon plant (E3), as in all previous work, but high pressure mercury vapor lamps were used for sunflower (E4), cotton (E5) and bean (E6). Substitution of mercury vapor lamps permitted higher radiant and luminous flux densities and thereby simulated outdoor conditions more completely. After preconditioning, the experiment started in darkness or virtual darkness (with other environmental factors as specified in the antecedent experiments).

The boundary layer resistance, $R_A$, was found from a synthetic plant, located 30 cm away from the live one, with leaves of green blotter paper shaped and oriented like those of the living plant. The following equation was used:

$$R_A = \frac{\Delta d_v}{E}$$

The units correspond to those of equation I. Again one uses the assumptions that the evaporating surface is saturated with water vapor, and that water loss from a synthetic plant will meet only an external resistance. The evaporation from the wet blotter was measured by weight loss to the nearest 0.1 g, and the temperature of the blotter paper to the nearest 0.25°, by means of embedded thermocouples.

Measurements consisted of readings of $R_L$ with the leaf resistance meter, leaf and synthetic plant temperature measurements, weight losses from both the live and synthetic plants, and accurate determinations of the ambient vapor pressure. When the measurements had been completed in darkness or

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$^3$ Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product listed by the United States Department of Agriculture.
low light under steady initial conditions, a high illumination was established gradually, with the other environmental conditions unchanged. Another set of measurements, taken when steady conditions again prevailed, completed the experiment.

With all of the information specified by the right side of equation I at hand, $E$ was calculated. The reference was the value of $E$ measured by weight loss from the same plant on which the $R_l$ and leaf temperature measurements were made. The weight loss, recorded to the nearest gram, was from 36 leaves for lemon, 10 each for cotton and sunflower, and 9 large leaflets for bean. Small corrections were made for transpiration from stems and petioles by measuring weight loss from a defoliated plant.

The 0.1-mm diameter copper constantan thermocouples were inserted diagonally into the leaf midrib at a site about half way from the leaf tip to the base of the blade. The thermocouple output was referenced against a cold junction; measurement precision was 0.25°. The temperature was recorded on a strip chart, each leaf being read in succession by means of a 10-position stepping switch. The exception to this procedure was the first experiment, E-3, in which the temperature of only 2 pairs of lemon leaves, representing upper and lower halves of the plant, was recorded continually at alternate 1-minute intervals.

Values of $R_l$ were measured separately for upper and lower epidermis, but it was assumed that $\Delta d_i$ and $R_A$ were identical for both sides of the leaf. The loss per leaf was calculated as the sum of the losses for upper and lower epidermis i.e., the result of 2 applications of the transpiration equation. We believe that no previous work has allowed the separation of upper and lower leaf transpiration on intact leaves.

![Graphs showing effect of illuminance on leaf diffusion resistance of cotton, snap bean, rough lemon, and corn.](Image)

*Fig. 1. Effect of illuminance on leaf diffusion resistance of cotton, snap bean, rough lemon, and corn.*
Results and Discussion

Illuminance Experiments. In all 8 species $R_L$ was the highest in the dark and progressively lower (with minor exceptions) with greater illuminance, for either epidermis (figs 1 and 2). The shape of the $R_L$ curves indicates that complete stomatal opening was not achieved for any of the 8 species. Although these curves are not precise, a rough extrapolation indicates that saturation illuminance for the group would be 50 kilolux. This compares favorably with 43 kilolux found by Slatyer and Bierhuizen (7) for cotton. In their experiments the minimal $R_L$ was the same at air temperatures of 30, 35, or 40°, 1.1 sec cm$^{-1}$. Presumably the CO$_2$ level was 300 to 350 ppm, as compared to 375 ppm in the present experiments. In either case experiments were conducted under “water non-limiting” conditions, so as to minimize or exclude the effects of a turgor deficit on $R_L$. These values obtained at saturation illuminance also agree reasonably well with data from field experiments at the United States Water Conservation Laboratory (4) where $R_L$ of recently irrigated sorghum reached a minimum of 0.1 sec cm$^{-1}$ at an illumination of 65 kilolux.

The 8 species can be divided into 2 groups on the basis of epidermal differences in $R_L$ readings at a specific illuminance: (1) species with a similar $R_L$ in upper and lower epidermis, including sunflower, fava bean, sorghum, and alfalfa, and (2) those in which much lower $R_L$ readings occur in the lower than in the upper epidermis, as shown by corn, cotton, snap bean, and lemon.

This finding is easily understood from the fact that in the first group stomatal frequency in the lower epidermis was only slightly greater than in the upper, whereas in the second group the difference was pronounced. For example, in lemon leaves there were 80,000 stomates cm$^{-2}$ in the lower epidermis, but only 4000 cm$^{-2}$ in the upper epidermis; analogous data for bean were 25,000 and 5000 cm$^{-2}$.

Microscopic measurements of stomatal pore width, obtained from silicone rubber impressions.

Table I. Leaf Diffusion Resistance ($R_L$, in sec cm$^{-2}$) and Stomatal Width ($W$, in μ) of the Lower Epidermis of 8 Plant Species, as Affected by Illuminance

<table>
<thead>
<tr>
<th>Species</th>
<th>0</th>
<th>4.0</th>
<th>9.3</th>
<th>36.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton</td>
<td>$R_L$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunflower</td>
<td>$R_L$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lemon</td>
<td>$R_L$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa</td>
<td>$R_L$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fava Bean</td>
<td>$R_L$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snap Bean</td>
<td>$R_L$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorghum</td>
<td>$R_L$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. $R_L$ for another plant exposed to the same environment was 4.2 sec cm$^{-1}$.
2. $R_L$ for another plant exposed to the same environment was 16.2 sec cm$^{-1}$.

Table II. Calculation of Transpiration for 4 Plant Species

<table>
<thead>
<tr>
<th>Expt No</th>
<th>Radiative flux density Leaf $d_\lambda$</th>
<th>Leaf Air $R_A$</th>
<th>$R_L$</th>
<th>Lo</th>
<th>Up</th>
<th>Calc</th>
<th>Meas</th>
<th>Calc</th>
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<tbody>
<tr>
<td></td>
<td>Kilolux</td>
<td>μg cm$^{-2}$</td>
<td>μg cm$^{-2}$ sec$^{-1}$</td>
<td>sec cm$^{-2}$</td>
<td>sec cm$^{-2}$</td>
<td>mg cm$^{-2}$ sec$^{-1}$</td>
<td>sec cm$^{-2}$</td>
<td>sec cm$^{-2}$</td>
</tr>
<tr>
<td>E-3 Lemon</td>
<td>0.00</td>
<td>0.00</td>
<td>29.2</td>
<td>30.29</td>
<td>13.66</td>
<td>1.4</td>
<td>13.4</td>
<td>52.5</td>
</tr>
<tr>
<td>E-4 Sunflower</td>
<td>7.0</td>
<td>0.07</td>
<td>(24.9)</td>
<td>(24.56)</td>
<td>11.11</td>
<td>1.1</td>
<td>(3.4)</td>
<td>(2.8)</td>
</tr>
<tr>
<td>E-5 Cotton</td>
<td>2.8</td>
<td>0.03</td>
<td>(29.3)</td>
<td>(29.62)</td>
<td>10.70</td>
<td>0.9</td>
<td>(15.6)</td>
<td>(17.0)</td>
</tr>
<tr>
<td>E-6 Bean</td>
<td>2.2</td>
<td>0.02</td>
<td>(28.6)</td>
<td>(29.30)</td>
<td>11.24</td>
<td>1.0</td>
<td>(17.0)</td>
<td>(113)</td>
</tr>
</tbody>
</table>

1. Vapor density in the leaf.
2. Vapor density of the ambient air.
3. Boundary layer resistance.
4. Leaf diffusion resistance of the lower and upper epidermis.
5. Calculated transpiration, single surface basis.
6. Transpiration measured by weight loss, single surface basis.
7. Values in parentheses are for comparative purposes only; the calculations were not made from the mean data given in the parentheses, but rather from data from individual leaves.
are given in Table I, along with the \( R_l \) values from the same spot on the leaf obtained immediately before the impression. These data show a consistent pattern for all species, a downward trend in \( R_l \) that is associated with a progressive increase in stomatal width with greater illuminance. Noteworthy is the precipitate decline in \( R_l \) from the value in darkness to that at the 4.0-kilolux level, as compared to the gradual decline in \( R_l \) due to further light-induced widening of the pore. Jarvis et al. (5) also found this same relationship between the total diffusive resistance \( (r) \) of a leaf and stomatal width; they obtained good agreement between calculated and measured values of \( r \) over a range of pore widths. The data for slit width given in Table I were used along with actual measurements of stomatal length and an assumed value for pore depth to calculate \( R_l \) by use of an equation from Jarvis et al. The resulting poor agreement between calculated and measured \( R_l \) may be due to misjudgment of the effective pore length. It is significant in this regard that the model used by Jarvis et al. assumed an effective pore length directly proportional to width over the range of actual measurements. The \( R_l \)-pore width data of Table I are also in accord with a response curve presented by Lee and Gates (6) based on earlier research by Brown and Escombe (2), Bange (1) and others.

The foregoing information served as a guide for the second part of the investigation. The illuminance required to induce maximum stomatal opening (low \( R_l \)) could be estimated, and, with mercury vapor lamps as a replacement for fluorescent lamps, established. At the other extreme, darkness or virtual darkness would induce a high \( R_l \) regardless of species. If transpiration could be calculated accurately at the extremes induced by a great range in \( R_l \), it could be assumed that the underlying principles would hold for any inter-

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Fig. 2. Effect of illuminance on leaf diffusion resistance of alfalfa, fava bean, sunflower, and sorghum.
mediate values of $R_L$ and associated transpiration rates.

**Transpiration Experiments. A) Boundary Layer Resistance.** In experiment E-3 (lemon) the $R_A$ value was the same in the dark as in high illumination (table II), 1.4 sec cm$^{-1}$. Air turbulence was somewhat less in the controlled environment room during experiment E-3 than for subsequent experiments. Therefore, a comparison of the effect of artificial leaf size on $R_A$ for the small lemon leaf (6 cm × 10 cm) with the considerably larger leaves of cotton (14 cm × 15.0 cm), sunflower (11.0 cm × 15.0 cm), and bean (8 cm × 15 cm for a leaflet) is not entirely valid. When comparisons are limited to the 3 experiments in which the air-stream in the room had the same turbulence (E-4, E-5, E-6) windspeed is seen to be the principal regulator of $R_A$; this follows from the negligible $R_A$ differences among the 3 species, cotton, sunflower, and bean. Species differences incorporate differences in dimension and orientation.

**B) Leaf Diffusion Resistance.** $R_I$ values conformed to the general rule illustrated earlier, in every instance decreasing with increased illumination (table II). However, it is apparent that there are distinct species differences in $R_I$ values in virtual or complete darkness. As an illustration, the $R_I$ of sunflower, 2.8 sec cm$^{-1}$ in the upper epidermis when exposed to 7 kilolux, was not only the lowest value for the 4 species at low illumination, but also was nearly as low as the $R_I$ of the lemon leaf at moderately high illumination (2.5 sec cm$^{-1}$ for the lower epidermis of lemon at 26.1 kilolux).

For lemon (table II, E-3, high illumination) minimal $R_I$ values were not attained in the lower epidermis due to insufficient illumination. However, for the sunflower, cotton, and bean experiments illumination was quite near the level needed for complete stomatal opening. Nevertheless, the minimum was not achieved. This probably was due to a slight leaf water deficit caused by radiant flux densities during the experiments that exceeded those prevailing during earlier growth in a partly shaded greenhouse. To satisfy the primary objective, the calculation of transpiration, it was important for $R_I$ to remain steady at 2 different values, as regulated by illumination. This objective was attained.

**C) Transpiration Rates.** As shown in table II, sunflower had by far the highest transpiration rate at low illumination, despite the lowest vapor density difference from leaf to air. In view of the closely similar boundary layer resistance for the 4 species, sunflower's high rate of water loss is due to its significantly lower $R_L$ values for both epidermises. The large discrepancy between calculated and measured transpiration in lemon can be attributed to greater than normal errors inherent to extremely low weight loss measurements of the reference plant.

The transpiration rates at high illumination (table II) range from 2 to 8 times the rates at low illumination. The increase is due partly to the increased vapor density consequent to the rise in leaf temperature, but mainly to the lowered $R_L$ values brought about by greater illumination.

In an attempt to understand why overall agreement between calculated transpiration and its reference value was not better than about 20%, an analysis was made of the effect of possible errors in measurement on the final value. It was calculated that an error of 1 degree in leaf temperature ($T_L$) measurement could change calculated transpiration by no more than 10%. Since $T_L$ was measured with an accuracy of 0.25° and was the mean of several measurements, it is unlikely that it could account for 20% discrepancies. Similar calculations also excluded slight errors in measurement of the ambient vapor density as the source of 20% discrepancies. Errors in $R_L$ values as such were ruled out, also. However, the method used may not be representative of the leaf boundary layer resistance; the present work allows no verification of our approach. Except for the foregoing consideration, the $R_L$ data are the most likely source of disagreement between calculated and measured transpiration rates. The $R_L$ data depend directly on the method of calibration. The only way to confirm the present calibration would be to devise a new calibration technique. Nevertheless, the overall agreement between calculated transpiration and its reference value indicates that the present calibration cannot be far from correct.

**Conclusions**

Illuminance as the single variable in a controlled environment was shown to be the regulator of stomatal aperture and therefore of $R_L$. Similar responses among 8 species permit a generalized statement: stomatal aperture is small in the dark and progressively greater as illumination increases, attaining a maximum at 50 kilolux (extrapolated). Concurrent $R_L$ values range from 75 to 0.1 sec cm$^{-1}$. Provided that stomatal density is adequate, these results are valid for either epidermises.

Sufficient agreement was achieved between calculated transpiration and its measured value to verify the leaf resistance measurement procedure, not only for 4 quite different plant species, but also over a wide range of transpiration rates. This means that, despite the complexity of transpiration, it can be handled quantitatively in terms of a few relatively simple parameters.

**Literature Cited**


