

Phyllotaxy and Water Relations in Tobacco

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Summary. The relative effectiveness of vascular connections between adjacent leaves of tobacco is demonstrated. It is shown that water movement between adjacent leaves is more difficult than between phyllotactically related leaves. Total and specific resistance ratios between adjacent and phyllotactically related leaves are calculated. These figures indicate that vertical water potential profiles need to be interpreted with a full knowledge of the vascular structure and phyllotaxy as well as gross structural and environmental parameters.

Introduction

Water flow through plants occurs along gradients of water potential (Slatyer, 1967). General phenomenological considerations dictate that this flow is proportional to the driving force and inversely proportional to the resistance of the pathway. Attempts to determine resistance to flow in plant stems by measuring the flow and the potential gradient along the stem therefore appear to be straightforward. Unfortunately, failure to consider the vascular structure of the stem and the phyllotaxy (see Esau, 1965) can lead, in many cases, to quite surprising and erroneous conclusions.

A number of attempts have been made to characterize the water potential gradients in plants, particularly along the stems, by measuring the water potential of the leaves along the stem (Begg and Turner, 1970; Cary and Fisher, 1971; Hoffman and Splinter, 1968). The assumption generally made when using this methodology is that the leaves all have more or less direct vascular interconnections and that their relationship to each other is determined primarily by their distance from the base of the stem. For this assumption to be correct it is necessary for the resistance to flow between adjacent leaves to be less than the resistance between leaves farther removed from each other on the stem (*i.e.*, the total resistance is some simple function of the distance between the two leaves under consideration). This paper describes a test of that assumption in tobacco.

Consideration of the vascular system of the stem of tobacco shows that there are no direct connections between vascular bundles supplying

adjacent leaves. In a mature tobacco stem the vascular tissue forms a closed ring built around the primary vascular tissue. The question therefore arises as to how much resistance there is to flow around the ring (or through the pith) between adjacent leaves relative to flow along the axis of the stem. Since tobacco has an alternate leaf arrangement there is only one leaf at each node and when we speak of adjacent leaves we refer to proximity in the vertical plane. In this case, flow between adjacent leaves involves a vertical as well as horizontal component. We will refer to the resistance to flow across the stem in such a case as the transvascular resistance and to flow along the axis of the stem as the axial resistance.

Materials and Methods

Tobacco plants (*Nicotiana tabacum* L. cv. Coker 254) were grown in the temperature-controlled greenhouses of the Duke University branch of the Southeastern Plant Environment Laboratory (Kramer *et al.*, 1970). The plants were pot grown in a 1:1:2:4 mixture of peat, Jiffy-mix¹, vermiculite and gravel. Day and night temperatures were 29 and 26°, respectively. The light period was extended to 16 h with the use of supplemental light from incandescent lamps. Plants were kept well watered throughout the growth period with half-strength Hoagland's solution. The plants were approximately 1.2 m tall with at least 12 mature leaves when the experiments were carried out. About 12 h before each experiment the plants were placed in a pan of water in a darkroom to insure minimum water stress at the beginning of an experiment.

The phyllotaxy of tobacco is variable and can change within the plant (Allard, 1942). In this group of plants it was determined by a dye tracer technique and found to be variable and either 2/5 or 3/8.

Working in a darkroom under subdued light, the stem was cut off at the base and some leaf was arbitrarily designated leaf zero (Fig. 1). All of the leaves except numbers 0, 4, and 5 were cut off and the severed petioles sealed with a 1:1 mixture of paraffin and petroleum jelly. Leaves 0 and 5 were chosen because the phyllotaxy indicated a direct vascular connection between them. Leaf 4 was chosen because of its proximity to leaf 5. The stem was cut and sealed several nodes above leaf 5. The excess leaves and stem were cut off and sealed in order to minimize the quantity of air and sap drawn from these sources. A piece of Tygon tubing connected to a vacuum line was attached to the petiole of leaf 5. Petioles numbered 4 and 0 were cut and the ends immersed in 0.1% aqueous solutions of two different dyes. All cutting of leaves, stems, and petioles was done under water to insure that none of the vascular system became blocked with air bubbles. A constant vacuum of 38 cm Hg was applied for the duration of the experiment. Samples of fluid drawn through the plant were taken periodically from the vacuum line with a syringe and needle. The experiments generally lasted about 8 h and samples were taken at 15- to 20-min intervals.

The absorption characteristics of the dyes used were examined on a Zeiss DMR-21 recording spectrophotometer. Percentage extinction coefficients were estimated for each dye at their visible maxima and at any points of spectral overlap

¹ Soil conditioner containing shredded sphagnum, peat, and vermiculite.

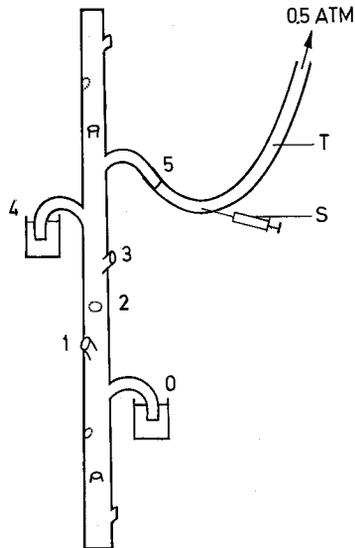


Fig. 1. Designation of petiole numbers and positions of various dyes for experiment

so that the absorbance due to each component could be estimated. The spectrum of each sample was recorded and the ratio of the concentrations of the two dyes determined. After a number of samples had been collected the positions of the two dyes were reversed so that differences in the mobilities of the dyes could be detected. Fig. 2 shows typical data of this type where the dyes used were eosin-Y and patent blue. The data are expressed as fractions of the total amount of both dyes in each sample of the vacuum exudate. The ratio of the concentrations of each dye from petioles 0 and 4 represents the ratio of the resistances to flow from each of these positions to petiole No. 5. This experiments was repeated several times with various combinations of eosin-Y, orange-G, acid fuchsin, and patent blue with essentially similar results.

Results and Discussion

It is readily apparent from Fig. 2 that the resistance to flow between petioles 4 and 5 (the transvascular resistance) was significantly higher than that occurring between petioles 0 and 5 (the axial resistance). Some differences in the mobilities of the two dyes became apparent after their positions were reversed. Reversal of the dyes also indicates that the differences in relative concentration are due to differences in pathway resistance rather than differential accumulation by the tissues. Hand sectioning of the entire stem after each experiment indicated that movement of the dye across the stem was not localized in any particular

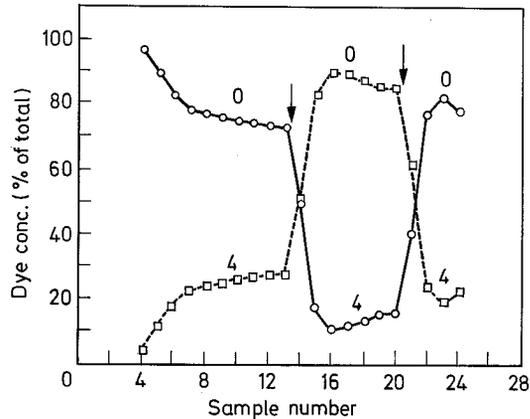


Fig. 2. Relative dye concentrations of sample fluid as a percentage of the total quantity of dye in a sample. Arrows indicate reversal of the dyes between petioles 0 and 4. Numbers on graph identify petioles. Time between samples was approximately 20 min. \square patent blue; \circ eosin-Y

region but occurred along the entire vascular cylinder from one end to the other. In addition, relatively heavy staining at the cut ends of the stems indicated that some leakage may have occurred in those areas, thus forming a partial short circuit in the system.

Since the leakage factor only applies to the transvascular pathway it is felt that this technique underestimates the transvascular resistance. Because of this we cannot assess absolute flow rates.

These two experimental problems restrict us to the determination of the transvascular to axial resistance ratio (r_{45}/r_{05}). We have the additional constraint, imposed by the leakage problem, that the ratio represents a minimum value. To obtain estimates of the total resistance ratios for the two pathways it is necessary only to compare the concentration ratios of the vacuum exudate. It is readily apparent from Fig. 2 that $r_{45}/r_{05} \approx 3-5$. The mean value ($r_{45}/r_{05} = 4$) will be used in further calculations.

A more useful parameter than the total pathway resistance ratio is the specific resistance of each of the pathways. Here again we are constrained to the determination of the specific resistance ratio (R_{45}/R_{05}).

Since the pressure drop along both pathways was the same, it is necessary to know only the concentration ratio in the exudate and the pathway dimensions.

Calculation of the specific resistance ratio may be best understood by reference to Fig. 3. Fig. 3A defines the general transverse dimensions

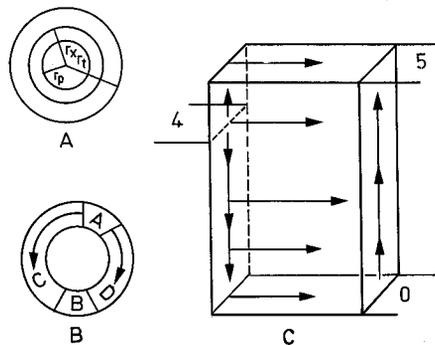


Fig. 3. Model system illustrating parameters used in calculating the specific resistance ratio

of the stem where r_t is the total stem radius, r_x the outside radius of the xylem cylinder, and r_p the pith radius or inside radius of the xylem cylinder. In our subsequent analysis we assume there is no radial gradient within the xylem. We can approximate an average circumferential path length ($C_{\bar{r}}$) such that

$$\bar{r} = r_p + \frac{r_x - r_p}{2} \quad (1)$$

This is the mean radius of the xylem cylinder and will be used in calculating the circumferential path length.

Fig. 3 B defines the transverse sections of the axial (A, B) and transvascular (C, D) conduction areas for the pathways involved. Staining patterns indicate that for a $2/5$ phyllotaxy the vascular cylinder may be divided into 5 more or less discrete conduction areas which are approximately evenly distributed around the xylem cylinder. A and B represent two of these conduction areas.

Finally, Fig. 3 C is an unfolded longitudinal section of segment C or D between axial conduction areas A and B. Horizontal flow occurs in both areas C and D. The relative positions of the petioles (0, 4, 5) are also indicated.

Staining patterns indicate that movement between petioles 4 and 5 occurred through segments C and D throughout the entire length of the stem. Solution moved from petiole 4 up and down the stem through axial pathway A and perfused through areas C and D and then moved through axial pathway B to petiole 5. However, movement from petiole 0 occurred only through conduction area B.

We can now assign the values of the appendix and calculate the specific resistance ratio. From the appendix we have $A_x = 1.04 \text{ cm}^2$. For the area accessible to pathway 0-5 we have $A_x/5 = 0.208 \text{ cm}^2$.

Referring to Fig. 3 we can now approximate the dimensions of pathway 4-5. Since dye moved up and down the stem from petiole 4 and thence around the vascular cylinder where it followed the axial pathway to petiole 5, we can estimate the average path length as one-half the length of segment A (Fig. 3 B) plus one-half the length of segment B plus C_r of segment C or D. This yields an average path length between petioles 4 and 5 of approximately 51.04 cm, not very different from l_{0-5} . The pathway area is based primarily on the thickness of the xylem cylinder ($r_x - r_p$) and is found to be $l_{4-5} (r_x - r_p) = 15 \text{ cm}^2$, and since there are two pathways available (segments C and D), $A = 30 \text{ cm}^2$.

The relative specific resistance may be defined as

$$R = \frac{rA}{L} \quad (2)$$

where r is the relative total resistance for a pathway, A is the area, and L the length of the pathway. We have then for our specific resistance ratio

$$\frac{R_{45} = L_{05} r_{45} A_{45}}{R_{05} = L_{45} r_{05} A_{05}} \quad (3)$$

Substituting the calculated dimensions yields $R_{45}/R_{05} = 570$. That is, the specific transvascular resistance is some 570 greater than the specific axial resistance. This value should be regarded as a minimum for these conditions since we have maximized A_{05} in our calculations and because of the leakage problem mentioned previously.

We therefore conclude that in tobacco the effective transvascular resistance is significant relative to the axial resistance and cannot be ignored when considering the relationships between various leaves.

A particular case in point is a report by Begg and Turner (1970). The authors believe to have demonstrated a surprisingly large resistance to water flow in the petioles of tobacco plants. They used a straightforward phenomenological approach which consisted of measuring the leaf water-potential, estimating flux, and inferring the stem water-potential at that height. Resistance in the petiole was then calculated from these values. The stem water-potential was inferred by enclosing an adjacent leaf in plastic and aluminum foil and measuring the water potential of that leaf after an equilibration period. It was assumed that this enclosed leaf was in equilibrium with and of the same potential as the stem at that height. It was further assumed that the enclosed leaf was directly connected through the node to the adjacent leaves (*i.e.* the

transvascular resistance was negligible). The resistances calculated from these data were large and changed inexplicably with time. In view of the evidence presented in this paper, we think it likely that they actually obtained some measure of the transvascular resistance of their tobacco plants and not the petiole resistance as they claimed.

Quite aside from the foregoing example the vascular structure of the stem and the phyllotaxy must be accounted for in any sound attempt to model water flow through plants. Clearly the stem cannot be treated as a hollow pipe where all leaves have equal access to the contents at any given level. We must remain cognizant of the fact that plant stems have definite organizational patterns and that these patterns determine which leaves should be most intimately related in their water economies.

Plant anatomy texts contain a wealth of information concerning vascular structure and phyllotaxy so it should not be difficult in many cases to determine by inspection in which plants the transvascular resistance is important.

In addition, it should be recalled that branch development in woody species often follows the patterns of leaf development on the main stem. Therefore what we have shown for the leaves of tobacco may also be true for the branches of many woody species. We may generalize by saying that measured vertical water potential profiles in many vascular plants may appear anomalous if care is not taken to account for the vascular organization of the plants under consideration.

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Appendix

A. Estimated Parameters

l_{05} = 50 cm: vertical distance between petioles 0 and 5.

l_{45} = 10 cm: vertical distance between petioles 4 and 5.

L_n = X: average path length.

r_t = 0.8 cm: total stem radius.

r_x = 0.7 cm: outer radius of xylem cylinder.

r_p = 0.4 cm: inner radius of xylem cylinder.

B. Parameters Calculated from A

$\bar{r} = r_p + \frac{r_x - r_p}{2} = 0.55$ cm: midpoint radius of the xylem cylinder.

$C_{\bar{r}}$ = 3.45 cm: circumference at \bar{r} .

$C_{\bar{r}c} = 0.3 C_{\bar{r}} = 1.04$ cm: Average circumferential length of segment C .

$A_x = 1.04$ cm²: Cross sectional area of xylem cylinder.

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