Water Flow Through Xylem:

An Investigation of a Fluid Dynamics Principle Applied to Plants

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Botany laboratory exercises provide excellent opportunities to investigate fundamental mathematical and physical principles. For example, the measurement of tree heights and trunk diameters allows the investigation of the mathematical principles of scaling, which are as applicable to animal legs and the columns of buildings as they are to tree trunks (Rice, 1999). Another fundamental physical principle, from fluid dynamics, is the Hagen-Poiseuille equation, which (in simplified form) relates the conductivity (J) of a fluid through a pipe to the *fourth power* of the radius (r) of the pipe:

$$J \propto r^4$$

Conductivity here refers to the volume or mass of fluid that flows through a pipe during a given amount of time (e.g., g/hr). This general principle has been widely used to estimate the flow of water through xylem (Nobel, 1983; Zimmerman, 1983). However, it applies equally well to the flow of blood through arteries (Tortora & Grabowski, 2003), and the flow of liquid or gas through pipelines (Batchelor, 1988). It therefore represents a general physical principle of which water flow through xylem is one special case.

At first glance, this equation seems difficult to believe. From it one must conclude that a large pipe, or a blood or xylem vessel, will conduct *256 times* as much fluid as a pipe or vessel that is four times smaller (Figure 1). This relationship, which applies to laminar fluid flow, occurs because, among other things, the fluid experiences friction against the sides of the pipe.

We incorporated an investigation of the Hagen-Poiseuille equation, using live plants, into a college general botany laboratory. Part of a laboratory period is needed to measure transpiration, one laboratory period for xylem measurements, and part of a lecture period to explain the results from the whole class pool of data. An investigation of the relationship between xylem diameter and conductivity using compressed air and stem segments was previously presented by Hicks (1995), but this investigation did not specifically test the fourth-power relationship contained in the Hagen-Poiseuille equation. Students measure both water flow (J) and vessel diameters (d), and investigate the relationship between the two. The diameter is twice the radius, and diameters are easier to measure. This investigation also allows students to gain experience with statistical procedures, and how to choose the best model from among several statistical analyses.

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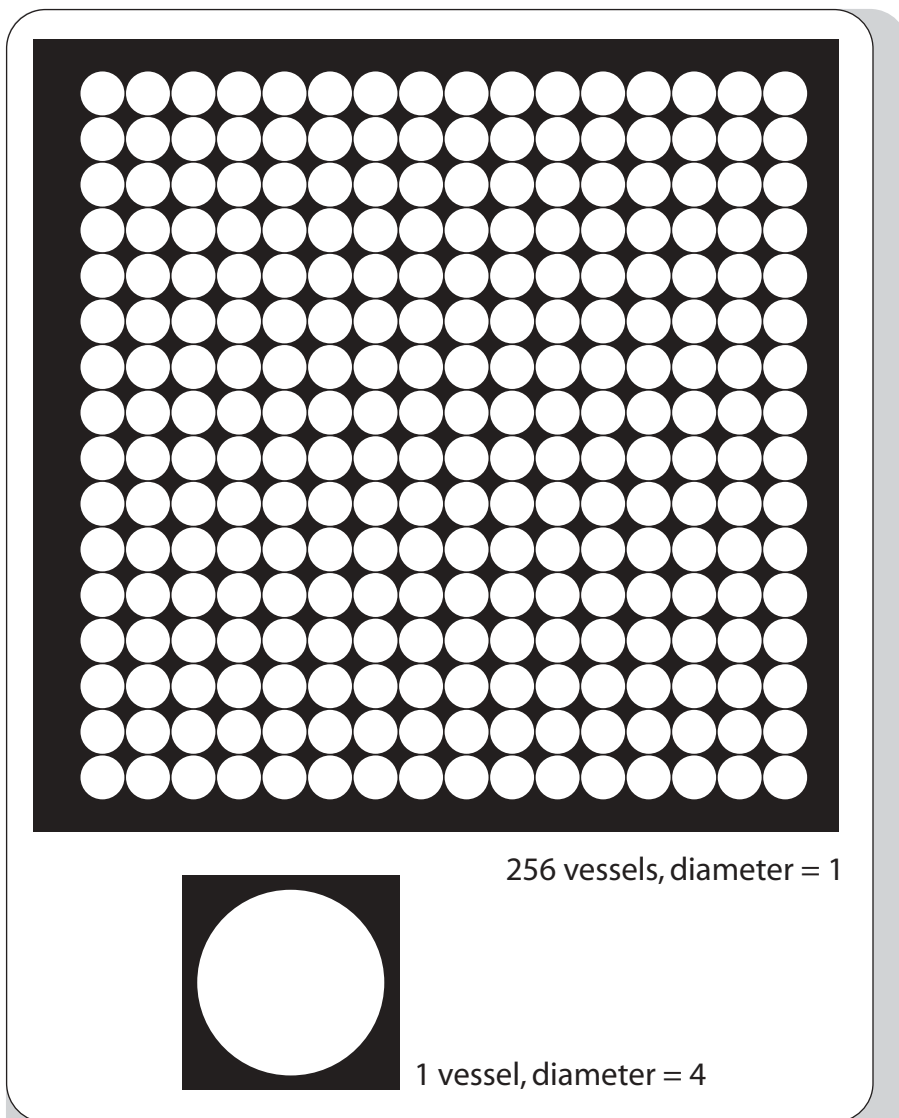
Figure 1.

It takes 256 conduits (pipes, xylem vessels, or blood vessels) of diameter = 1 unit to conduct as much fluid as one conduit of diameter = 4 units, according to the Hagen-Poiseuille equation.

256 vessels, diameter = 1

1 vessel, diameter = 4

We undertook this investigation as a hypothesis-testing procedure. Possible models that can be contrasted include:

$$J \propto d \text{ (Model 1)}$$
$$J \propto d^2 \text{ (Model 2)}$$
$$J \propto d^3 \text{ (Model 3)}$$
$$J \propto d^4 \text{ (Model 4)}$$

Our expectations were as follows:

- Prediction 1. Transpiration rate would correspond most closely to the conductivity as estimated by Model 4.

- Prediction 2. Leaf area would correspond most closely to the conductivity as estimated by Model 4, since the plants must provide water to all new leaf area produced.

Methods

Preparation of Plant Materials

We undertook two investigations, one in fall 1999, another in spring 2000. We present results for spring 2000 only. We grew about 20 seedlings in small plastic cups of potting soil and kept them continually watered in trays on a classroom windowsill. We used soybean plants because of their relatively large stem diameter. The plastic cups had drainage holes made with a hot dissecting probe. Since the weight of the pots would dominate the weights of the other constituents, we could not use clay pots. In order to provide a range of different plant sizes, we allowed the seedlings to grow in three cohorts, some for three weeks, some for two weeks, and some for one week, before we used them.

Transpiration Measurements

Prior to the laboratory activity, we measured the transpiration rates of the plants. Because the plants grew indoors, we made the transpiration measurements under conditions that elicited maximum transpiration without imposing stress: We avoided midday on hot days. Since no greenhouse was available, we placed the plants outdoors in the sun and away from the wind: In spring 2000 the maximum temperature was 27°C and the relative humidity was 30%. We allowed the pots to completely drain, then enclosed the pots and soil in aluminum foil to minimize evaporative water loss directly from the soil. We weighed each plant-soil-pot unit before and after transpiration. The Hagen-Poiseuille equation describes conductivity through the xylem, which we measured by total plant transpiration, *not* transpiration rates relative to plant weight or leaf area; therefore we expressed transpiration rates in grams per hour.
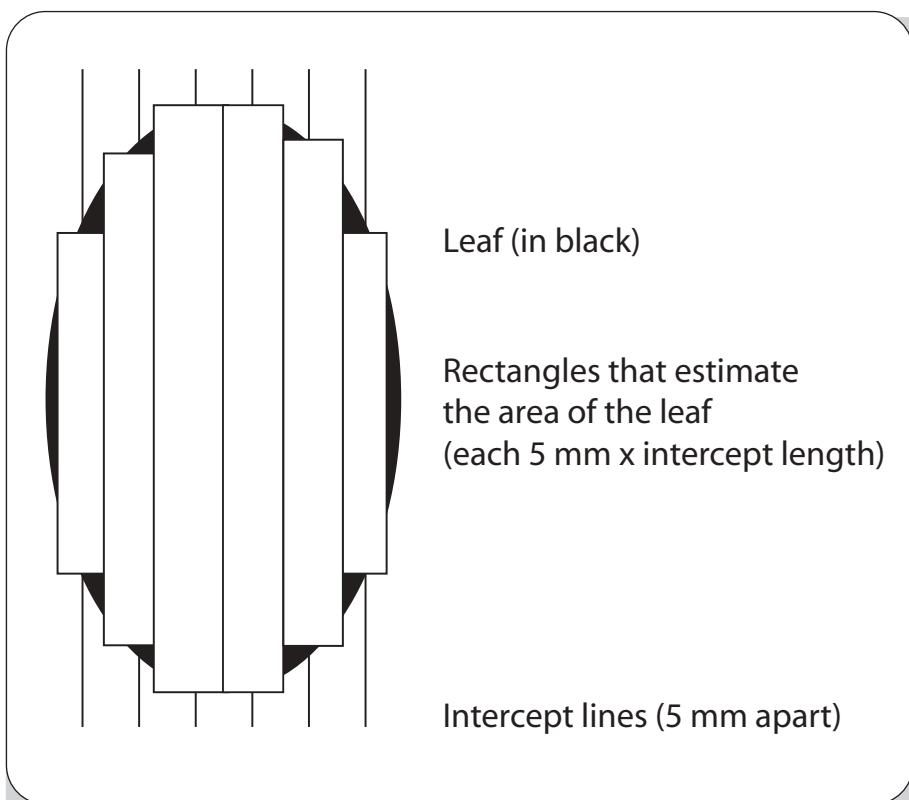
Xylem & Leaf Area Measurements

Students formed groups of three during laboratory sessions, and we assigned two plants to each group. One of the students estimated total leaf area for each of the group's plants as shown in Figure 2. The other two students estimated xylem diameters in the following fashion.

1. **Determination of ocular units.** Four microscopes had ocular micrometers, and on these microscopes one ocular unit approximated 10 μm at 100 x magnification. Students made measurements in ocular units. Because they made comparisons among four models that differ only in their calculation of xylem conductivity, the actual units did not matter because consistent units were used throughout the investigation. However, it was necessary to always use the same magnification (100 x).

2. **Preparation of the stem section.** Students made cross-sections, as thin as possible, of the stem just below the site at which the cotyledons were,

or had been, attached, using new single-edged razor blades. On a microscope slide, they stained the sections with toluidine blue solution prepared from commercially available powder. The stain makes the xylem cell walls show up clearly. After removing the stain, they mounted the section in water under a cover slip.

3. **Xylem vessel measurements.** In a previous laboratory, the students learned how to locate the xylem. We checked to make sure that students were not measuring pith (in the middle) or sclerenchyma (outside the phloem). Even in these young stems, some secondary growth had begun, and the xylem formed a continuous ring rather than discrete bundles. In the larger stems, there was a large enough amount of xylem that the students were able to measure only one-fourth, or perhaps only one-eighth, of the xylem. Accordingly, the students multiplied their final estimates by the appropriate number to produce an estimate of total xylem conductivity.

In each pair, one student measured the xylem vessel diameters, while the other tallied the measurements read to him or her by the first. This allowed the first student to keep his or her eye on the visual field, without getting lost by stopping and restarting. The first student used the ocular micrometer to make a rough estimate of the diameter of each xylem tracheid or vessel ≥ 10 μm that is stained, to the nearest ocular unit. This may sound difficult, but in practice it became easy to "eyeball" the measurements, so that after a few measurements it was no longer necessary for the student to move the microscope slide to line up the vessel with the micrometer. Since small errors are magnified for the larger vessels because of the fourth-power relationship, we recommend that students carefully measure the vessels with large diameter. However, these will be few in number, compared to the numerous tracheids and vessels with one, two, or three ocular unit diameters. We provide a typical

Leaf (in black)

Rectangles that estimate the area of the leaf (each 5 mm x intercept length)

Intercept lines (5 mm apart)

Figure 2.

Estimation of leaf area. The parallel lines are 5 mm apart. The area of the leaf is approximately the same as the total area of the rectangles. Each rectangle is 5 mm wide, and its length is the distance that one of the parallel lines intercepts the leaf. The student measures the total intercept length of all the lines, and multiplies by 5, for leaf area in square millimeters. Parallel lines drawn on acetate can be superimposed on the leaf for easy measurement.

example of the tally of diameters in Table 1.

4. **Calculation of conductivity estimates.** The students then calculated an estimate for conductivity for each of the four models:

Model 1: $\sum n_i d_i$

Model 2: $\sum n_i d_i^2$

Model 3: $\sum n_i d_i^3$

Model 4: $\sum n_i d_i^4$

where n is the number of xylem elements of diameter d, and i goes from 1 to the ocular unit size of the largest vessel encountered. For ease of calculation, we provided a table of the squares, cubes, and fourth powers of the numbers most likely to be encountered. These estimates were unitless, and only proportional to the actual conductivity that would be expected for each of these models. Note that (for example, with Model 4) *the sum of the fourth powers is not the same as the fourth power of the sums.* For this rea-son the calculations must be done in steps, as indicated previously, rather than simply adding all the diameters together and raising the sum to the appropriate power.

The laboratory work was complete when each group turned in the conductivity estimates and leaf areas, of two plants. We pooled the measurements from all lab sections and discussed the results in the next lecture period.

Analysis of Results

Because this was an introductory class, the instructors performed these regression analyses and made the results for class discussion. These analyses were performed by JMP, the educational version of SAS (SAS Institute Inc., 1995). The expanded version of Microsoft Excel can also perform these analyses.

Scatterplot of results. We provided four scatterplots, with transpiration rate as a function of xylem conductivity, as estimated by each of the four models (to test Prediction 1); and four scatterplots, with leaf area rate as a function of xylem conductivity, as estimated by each of the four models (to test Prediction 2).

Statistical analysis. We provided printouts from computer statistical analysis that estimated r^2 and the F ratio of the analysis of variance of each model. We briefly explained what these numbers indicated, in common-sense terms as follows. The r^2 value indicates the proportion of the variation that is explained by the regression line. Thus $r^2 = 0.75$ (as in Figure 5) means that 75% of the variation was explained by the regression line. This is a better fit than a regression line with $r^2 = 0.50$ (as in Figure 3), which explains only 50% of the variation. The F ratio is the variation explained by the regression divided by the variation not

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Table 1.
A Sample Worksheet for Student Xylem Measurements

Plant # _____   Leaf area: _____ mm²

We measured _____ (what fraction) of the stem area

Tallying up the diameters:

| 1 ocular units	2 ocular units	3 ocular units	4 ocular units	≥ 5 ocular units*
Total: _____	Total: _____	Total: _____	Total: _____	Total for 5: _____
				Total for 6: _____
				Total for 7: _____

*Write the diameter of each xylem element of diameters 5 or greater.

Sum of diameters: _____   Sum of cubes of diameters: _____

Sum of squares of diameters: _____   Sum of fourth powers: _____

You may consult these numbers in your calculations:

Number	2	3	4	5	6	7
Square	4	9	16	25	36	49
Cube	8	27	64	125	216	343
Fourth powers	16	81	256	625	1296	2401
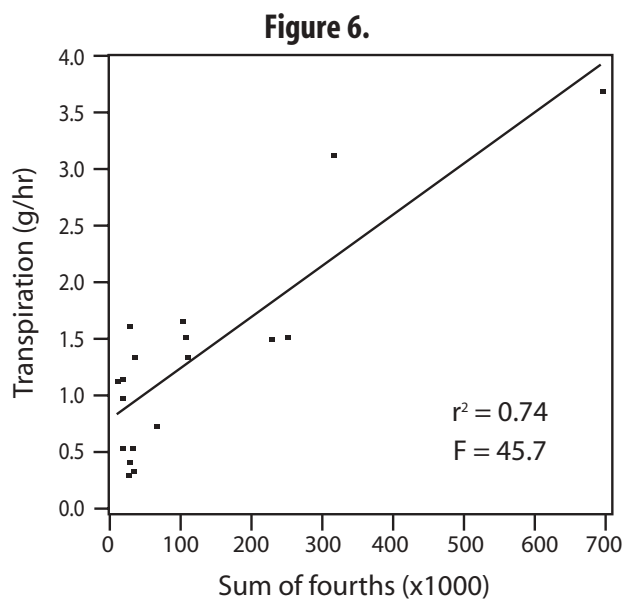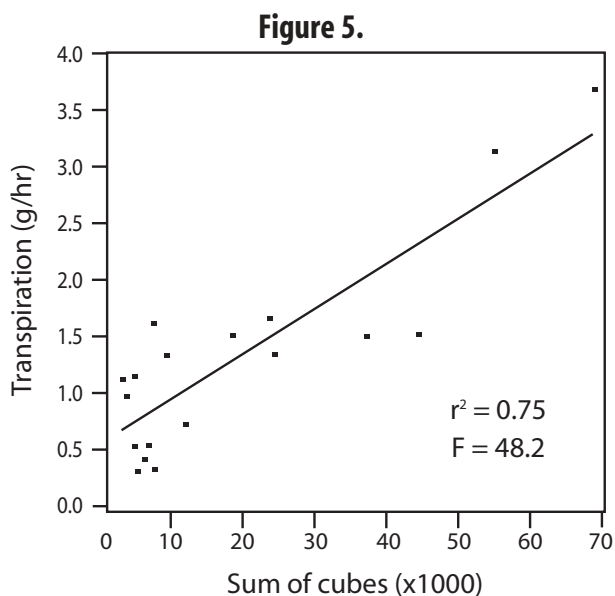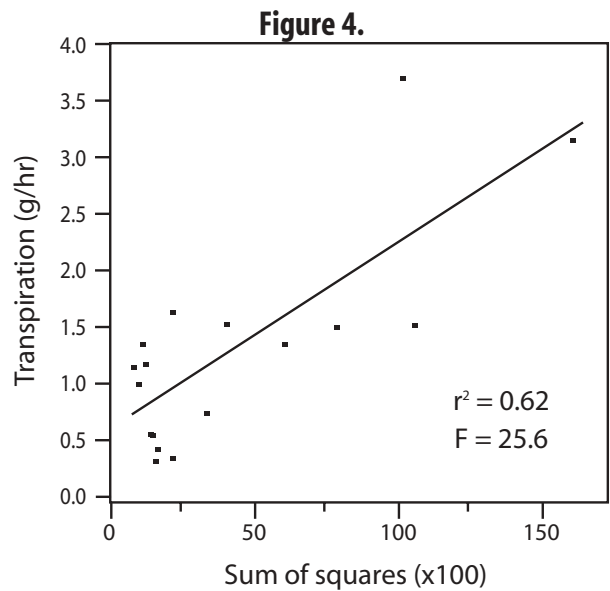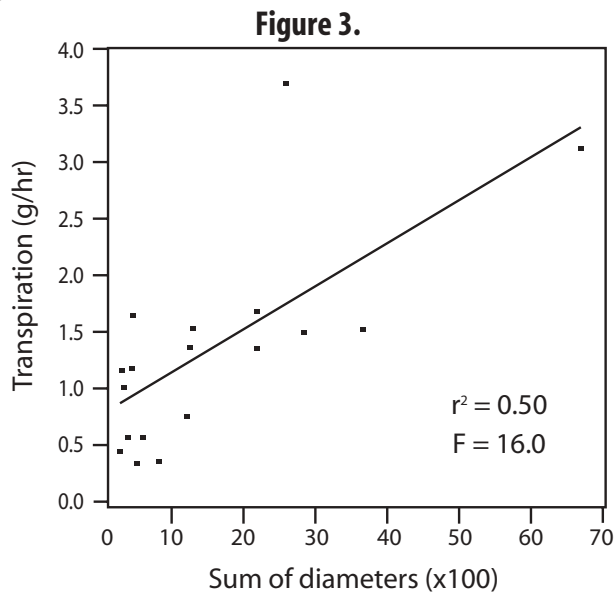
Figures 3-6.

Transpiration rates of bean plants as a function of the sum of xylem vessel diameters (Figure 3), the sum of the squares of xylem vessel diameters (Figure 4), the sum of the cubes of xylem vessel diameters (Figure 5), and the sum of the fourth powers of xylem vessel diameters (Figure 6). Vessel diameters are in ocular units as measured at 100 x.

explained by the regression; a larger F value therefore also indicates a better fit.

We used linear regression, since higher orders were already represented by the calculations that went into the four models.

Results

We here present results for the spring 2000 experiment. Of course, since larger plants transpire more water, and have more leaf area, we obtained a significant regression for both transpiration and leaf area using all four models. However, the models differ in the *relative amount of variation that they explain.* Model 1, which simply adds together the xylem diameters as an estimate of conductivity, explains the least variation in transpiration (50%), and has the lowest F value (Figure 3). Model 2, which uses the sums of squares of diameters, provides a noticeable improvement, explaining 62% of the variation in transpiration and having a higher F value (Figure 4). Model 3 provides an even better fit, as measured by both r^2 (75%) and F (Figure 5). In this investigation, however, Model 4 explained 74% of the variation and did not provide a noticeable improvement over Model 3

Figure 7.
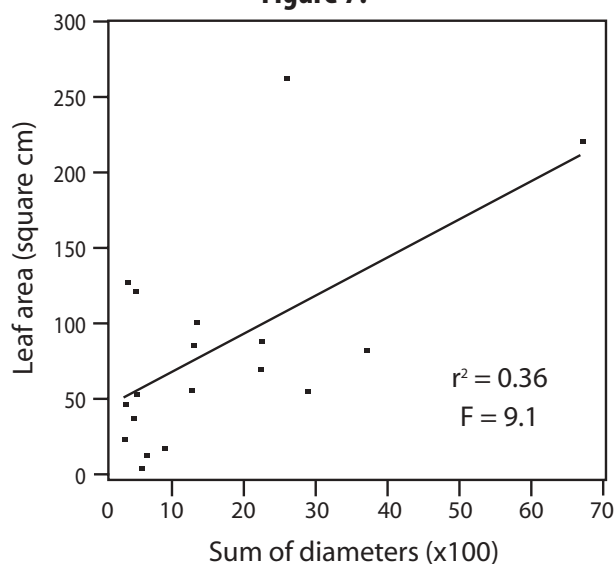
Figure 8.
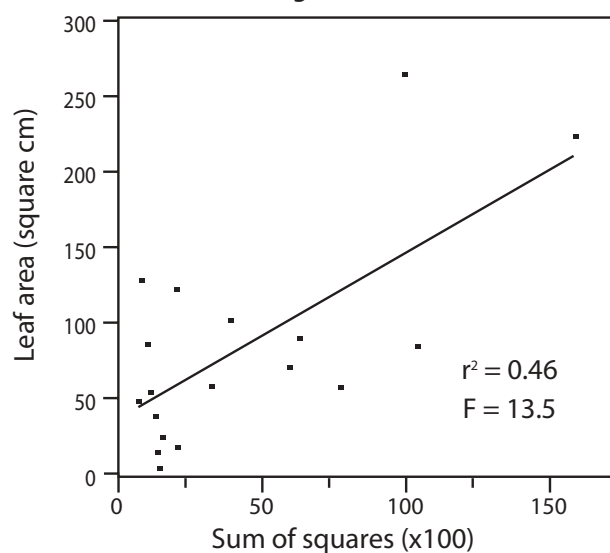
Figure 9.
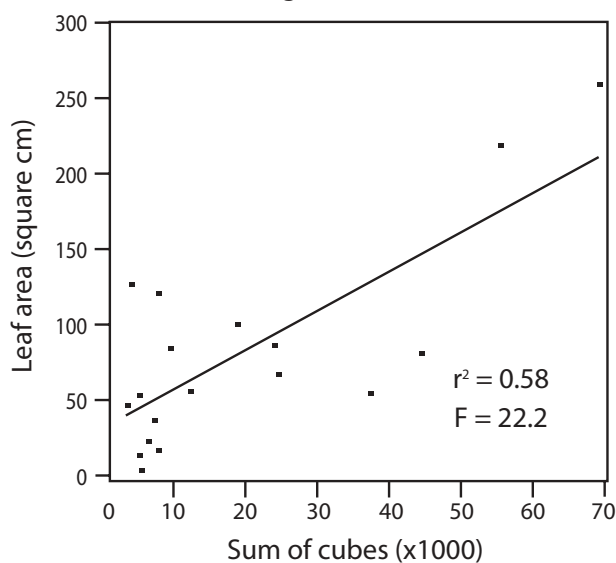
Figure 10.
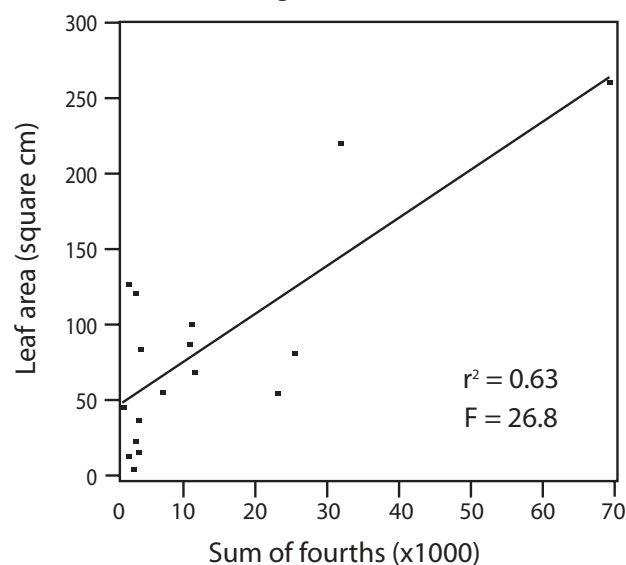
Figures 7-10.
Leaf areas of bean plants as a function of the sum of xylem vessel diameters (Figure 7), the sum of the squares of xylem vessel diameters (Figure 8), the sum of the cubes of xylem vessel diameters (Figure 9), and the sum of the fourth powers of xylem vessel diameters (Figure 10). Vessel diameters are in ocular units as measured at 100 x.

(Figure 6). The results, while consistent with the Hagen-Poiseuille equation, did not confirm that the fourth-power relationship between pipe diameter and conductivity was better than a cubic relationship, but did show that the relationship between conductivity and xylem diameter was not linear or quadratic.

We obtained similar results for leaf area as a function of conductivity estimate (Figures 7-10), except that in this case, Model 4 was superior to Model 3. Adding the xylem diameters only explained 36% of the variation in leaf area, and had the lowest F value (Figure 7);

adding the squares of xylem diameters explained 46% of the variation in leaf area, with a higher F value (Figure 8); adding the cubes of the diameters explained 58% of the variation (Figure 9), and adding the fourth powers of the diameters explained 63% of the variation in leaf area, with the highest F ratio (Figure 10).

Discussion

In some studies, both leaf area and rate of water loss can be explained by xylem diameter (Singh & Sale,

2000). However, in many other cases, such as the present study and that of Ewers, Fisher and Chiu (1989), the results do not clearly confirm a fourth-power relationship between vessel diameter and transpiration. This problem can be posed for class discussion. The observed transpiration rate was not the maximum possible conductivity because other factors limited transpiration. Among the possible limitations are structural limitations and environmental limitations.

Structural Limitations

The vessels of the xylem tissue are not uninterrupted conduits. For example, the Hagen-Poiseuille equation assumes a smooth pipe; vessels may, however, have internal corrugations (Roth, 1996) which may create turbulent flow. The equation also assumes a perfect circle, while xylem vessels are frequently elliptical (Lewis & Boose, 1995). The scalariform plates between vessels can prevent the spread and coalescence of bubbles that result from cavitation, when the column of water actually snaps under conditions of high tension; these same plates, however, slow the movement of water. Suberin and lignin in the endodermis may impart a significant resistance to water movement into the root (Cruz, Jordan & Drew, 1992). In some cases, the leaves offer the greatest resistance to water flow (Yang & Tyree, 1994; Martre, Cochard & Durand, 2001). Constrictions at nodes can reduce hydraulic conductivity (LoGullo et al., 1995). For these reasons, the Hagen-Poiseuille equation may explain differences among individuals but not between cultivars or species (Gartner et al., 1990; Yambao, Ingram & Real, 1992).

Environmental Limitations

Water stress, which may include the formation of embolisms, can cause stem conductivity to not follow the Hagen-Poiseuille equation (Lovisolo & Schubert, 1998). However, all of the plants used in this project had their roots in soil that was near the field capacity, and differences in xylem tension among plants was probably minimal. Furthermore, if the stomata are not completely open, or if the plants experience any water limitations, transpiration rate will not occur at its maximum rate. The duration in which we measured transpiration (about two hours)was not long enough to induce water stress. The credibility of this explanation is enhanced when we consider that leaf area corresponded better to Model 4 than to Model 3 (Figures 8, 9), while this was not true of transpiration (Figures 4, 5). Some plants rely more on stomatal control than on vessel conductivity control of transpiration as an adaptation to resist drought (Harvey & van den Driessche, 1997). Even xylem diameter itself is not constant for a given vessel, as diameters can change diurnally with water stress (Peramaki et al., 2001).

If large vessels can conduct so much much much much more water (to the fourth power) than smaller vessels and tracheids, why do plants have small vessels and tracheids? This is also a good question for class discussion. Most obvious to students is that tracheids function, like fibers, in support. Less obvious might be the safety factor against cavitation: Larger vessels are more vulnerable than smaller ones to cavitation during water stress (LoGullo & Salleo, 1991; Neufeld et al., 1992; Lipp & Nilsen, 1997). However, individuals with greater vulnerability to cavitation are not necessarily those with larger vessels (Hacke & Sauter, 1996; Cochard, Lemoine & Dreyer, 1999).

An alternative procedure, that avoids the difficulties of using an ocular micrometer to estimate xylem diameters, is for the instructor to take photographs of the xylem sections, and provide prints (with scaling indicated) to the students. The students can then use rulers to measure and tally up the diameter estimates.

Our comparison of the four models was, in each case, a simple inspection of the r^2 and F values. While such comparison is not itself statistically valid, we considered it sufficient for classroom instruction purposes. Moreover, some outlier data strongly affected the regressions. Since we could not control the xylem development patterns, we could not prevent this pattern from occurring.

The students wrote discussions of the results, and their grades depended primarily upon them understanding the main point. The average laboratory write-up score was 89.6%, which was not significantly different ($p > 0.05$) from the average laboratory write-up score for the whole semester (91.5%); therefore the students understood this exercise as well as any other. Student scores for this laboratory write-up were correlated ($p < 0.0001$) with their average laboratory write-up scores.

In another semester, we attempted to use cuttings from a large coleus plant for this project. However, none of the correlations was significant, perhaps because sap from the cuttings partially blocked the xylem.

Transpiration measurements are a good educational tool (e.g., Ford, 1998). But for a full educational experience, the students should apply the results of such studies beyond an understanding of transpiration. For example, if arteriosclerosis causes an artery to lose half of its effective diameter, to what extent is blood flow affected? The answer to this question (blood flow is reduced by fifteen-sixteenths) may help induce the students to pursue a life of healthy nutrition and exercise, and to avoid smoking.

Acknowledgments

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