THE ASKENASY DEMONSTRATION

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In the third edition of Palladin’s Plant Physiology (pp. 147–150, 1926), Livingston calls attention to the fundamental and far-reaching importance of liquid tension in plants, and laments the fact that it is rarely demonstrated in university or college courses in either physics or plant physiology. There is contained in this textbook a brief account of the precautions that seem to be necessary in order to assure the success of the Askenasy demonstration. This was followed by a paper giving additional information on a simplified technique, together with a request that other experimenters record their successes or failures with the demonstration.

The writer has experimented off and on with the Askenasy demonstration during several years. It is not only fascinating to try to better former traction records, but trial after trial leads one to better comprehension of the various forces operating and to appreciate more fully the phenomena existing in the living plant. Various procedures have been tried out and their effects have been carefully noted. Different membranes have been used, in the attempt to find one more nearly comparable with the natural plant membrane. While the experiments leave much to be desired, they have been attended with sufficient success and with such regularity that a traction experiment has been made a regular demonstration in the course in plant physiology at this university, and the apparatus is kept set up so that it may be used from year to year.

The first apparatus was set up in the manner illustrated in Palladin, using a single five-foot length of glass tubing with a one-millimeter capillary, a standard thick-walled atometer cup, and a filter pump of twenty-one pounds capacity for suction. As typical of results one is likely to obtain at first, eleven trials gave the following in centimeters of rise of mercury column above mercury level in lower reservoir: 30.5, 81.3, 80.0, 63.8, failure, 100.3, 100.0, 107.5, 99.7, 103.6, failure. Uncorrected barometric pressure for this series varied between 72.9 cm. and 74.1 cm. It should be further noted that in the sixth trial the meniscus in the cup apparently broke at 95.2 cm., the level fell about 2 mm., and then started back, giving a total rise of 100.3 cm.; and again, in the eighth trial the column fell over 1 cm. at 103.5 cm., followed by recovery, and making a final height of 107.5 cm. In the many experiments subsequently performed, this phenomenon was observed on only one other occasion. The cause has been referred to as a breaking of one or more menisci, but since the cup is opaque and anything

taking place in it is invisible, the phenomenon is a matter for conjecture; and some slight readjustment of parts, magnified by the small bore of the capillary, may be the explanation. The dropping of the mercury column was sudden, and if it was due to breaking of the meniscus, the method of recovery is a matter of considerable importance; for, as pointed out by Livingston, the water system usually breaks, and the mercury column then falls quickly to a height corresponding with the current reading of the barometer. Livingston further states that the system breaks, sometimes in the water in capillary or cup, and sometimes in the mercury. In our experiments, always using distilled water, but often exposing it in the boiling cup to the atmosphere for days before a trial was made, breaking of the system was never observed to take place (in the trials which were at all successful) save in the porous cup.

As the desirability of a longer capillary tube became apparent, the idea of joining two lengths of tubing together was suggested. Various kinds of bonds were tried, all without success. Finally, a method was perfected for fusing two glass tubes together, using a blow pipe and infinite patience, since it is easy to draw the capillary. The operation has been successfully applied on several subsequent occasions, but it is not recommended to the average glass blower. Later, it has been learned that glass tubing may be obtained in longer lengths on special order from the manufacturer. This would be more satisfactory, but might entail some months of waiting. Hard glass would probably be better than soda-lime glass. One set-up, made from soft glass, gave a great deal of trouble due to numerous minute holes developing successively in the upper bend of the tube after a short period of use.

The construction of a suitable evaporation membrane has been the subject of much thought and experimentation. The porous cup constitutes an artificial leaf, just as the capillary tube takes the place of a single vessel. In a living leaf the place where water loss takes place is not essentially through the stomata, but internally from the moist surfaces of the cells which abut upon the intercellular spaces back of the stomata. The walls of these cells and the plasma membranes within are not the porous structures that clay cups are. Ideally, the nearer the porous cup approximates the physical conditions of the evaporating mechanism of the living leaf, the more successful should be the demonstration as measured in lifting-power. The difference in behavior of a larger-pored cup as contrasted with a finer-pored condition (meaning large menisci as against small menisci) was demonstrated, as follows. A thin-walled unglazed porcelain cup was selected, which was boiled in sodium hydroxide and carefully washed; the rubber stopper was new and also properly treated to remove air and sulphur. Five separate trials with this cup gave three failures and the maximum rise ob-
tained was 29.2 cm. of mercury. The cup was then removed and a copper-ferrocyanide membrane was deposited in the walls by the ordinary procedure. Seven trials with the treated cup gave uncorrected results in centimeters as follows: 91.7, 96.0, 91.7, 91.0, 80.0, 88.2, and 58.0, the barometric pressure varying from 72.5 cm. to 75.0 cm. Similar thin-walled cups were used, but impregnated with a very dilute solution of cellulose acetate (Cellon, Paris) in acetone. Results were as follows, in centimeters of mercury column: 57.0, 62.0, 79.6, 63.5, 75.0, 81.0, 54.0, 57.0, 57.5, 57.7, and 82.2. A cellulose acetate membrane presents at least two difficulties; water evaporates very slowly from the treated cup; but more important, it is very difficult to free the system from imprisoned air, and perhaps impossible entirely to rid the system of the smaller bubbles.

So much success attended the trials with a copper-ferrocyanide precipitation membrane that the idea of making a hot-precipitation membrane was conceived. It is obvious that when heated a porous cup is expanded and the pores are larger than in the cool state. If a cup is then boiled in distilled water with suction to remove as much air as possible and is then filled with hot copper sulphate (2.5 gm. per liter) and suspended in a beaker of hot potassium ferrocyanide (2.1 gm. per liter), the whole being maintained in a hot-water bath for several hours, upon cooling the precipitated membrane must undergo a reorganization which would seemingly tend to make it more compact. Many trials with such a membrane seem to indicate that such is the case. Such a membrane is quite permeable with suction during the preliminary boiling to free the system from air, and water comes down the tube freely. On cooling, however, and making a trial test, such a membrane may be found to be quite impermeable. Enough experimentation has been done to indicate that there is a happy medium as to the amount of heat to apply during impregnation, and good results have attended a moderate application of heat during the process of precipitation. The method of hot-precipitation is worthy of further investigation.

Much time has been spent in trying to arrive at a more effective method of removing air from the system than is possible by the simple expedient of boiling and suction, especially in the case of using rather impermeable porous cups or membranes. Two-holed rubber stoppers were tried, a stop-cock, with and without funnel attached, being inserted in the second hole. Filling the funnel and tube with boiling water, it was thought that the system might thus be flushed out, the gas bubbles being forced out at the lower end of the capillary. This idea was soon abandoned. More trouble was experienced from gas bubbles than before. Solid glass rods were inserted in the hole. These could be withdrawn under water and reinserted. No great success was attained. Either the inrushing water column was sheared off at the instant of closing, thus creating a vapor
tension area which does not "heal," or the forcing in of the rod disengaged gas bubbles from the interior of the stopper, which were forced out by the compression. Metal pistons were also similarly inserted, by which means a pumping action could be set up, combined with agitation. Their chief effect was to loosen the stopper. On the other hand, several devices, mentioned presently, were more successful.

In all, about a hundred and twenty trials have been conducted, many with considerable success, and some were frankly failures. The best record obtained was made with a Livingston calibrated atometer cup (no. 5-548, fifteen x), boiled in weak NaOH and washed well, but otherwise untreated. The capillary tube was constructed from two fused, five-foot lengths of tubing, the upper end of which was bent into a U-shape, three and one-half inches high and four inches across, since it has been found that gas bubbles are more easily removed from gradual bends than from sharp angles. Preliminary to the fourth trial, the apparatus was boiled up for a half hour, with several periods during which the suction was released in the manner suggested by Livingston. The apparatus was allowed to cool, and on the next day full suction was applied, which was successfully withstood. The boiling pot was then heated to 60° C., and then quickly removed. Seventeen minutes afterward the column fell after reaching an uncorrected height of 150.0 cm., the barometer reading at the time being 73.9 cm. This indicates a pressure of more than two atmospheres. This same cup also gave other good records.

For maximum success with the Askenasy demonstration, in addition to the precautions already suggested by Livingston, the following suggestions are made:

a. The mercury used in the lower reservoir should be pure and clean. As soon as it becomes glazed on top, it should be removed and washed before further use.

b. It is an advantage to have the end of the capillary tube relatively close to the surface of the mercury. If, in addition, the end of the tube is beveled up to the capillary, using a file and water lubricant, the removal of air is facilitated, especially in the case of less permeable porous cups and membranes.

c. The rubber stopper must fit tightly in the cup. This is best accomplished by first twisting it in, all parts being bathed with water. After this the stopper may be further inserted by a combined kneading in of the edges at the same time that a pushing thrust is used. When the stopper is well-seated, it may be slipped onto the upper end of the capillary tube, which should have been previously marked with a thread gauge to bring the end of the tube flush with the under side of the stopper. It is perhaps an
advantage previously to prepare the lower end of the stopper by cupping it with a sharp safety razor blade into a low cone, which gives little chance for air bubbles to lodge under the stopper.

d. There is no advantage, when using the ordinary porous cup, in long continued boiling, although this may not be true with treated cups which are difficult to free from confined air.

e. In making class-room demonstrations, it is best to try out the apparatus first by application of full suction, gradually applied and as gradually released. If the system does not break, the experiment is likely to be successful. Preliminary heating of the boiling pot to 60°–65° C. before beginning a trial allows the teacher to finish the demonstration in a few minutes’ time, and the rise of the mercury column is so rapid that it is eagerly followed by the student. A convenient marker to record the progress made by the mercury is made from a short piece of rubber tubing, split, and slipped over the capillary tube. A little water between the surfaces in contact will furnish lubrication so that it may easily be moved. Jarring of the tube should be avoided, and may be largely prevented with proper rigid supports and ordinary care, not neglecting provision for expansion and contraction of parts.

This is a brief record of progress and difficulties. However, no teacher should be easily discouraged. With a suitable porous cup and ordinary laboratory facilities, the AskENASY demonstration is not at all difficult to make; and it is hoped that it may be more generally employed in course work.

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