Translocation of Organic Substances in Trees. V. Experimental Double Interruption of Phloem in White Ash (Fraxinus americana L.) [1]

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The student of phloem quickly learns that he is dealing with a tissue that functions normally only in the intact plant. Injury, such as an incision into the phloem, causes a disturbance that extends over great lengths up and down from the point of injury. The disturbance consists of several phases. First of all, there is an immediate and far-reaching turgor release which results in an accelerated shift of the sieve-tube content toward the point of injury (3). This shift may instantly seal the sieve plates with slime plugs (2). Even if no slime plugging occurs, rapid—though not instant—sealing of the sieve plates by callose formation may interrupt the conducting function of the sieve tubes. This can be seen if branches of certain trees, such as hickory, chestnut, etc., are detached and brought to the laboratory, for aphids may then not be able to feed on the phloem because of an interruption of sieve-tube continuity by heavy callose seals. Thus, sieve-tube transport has completely stopped in a branch that may still be alive and apparently healthy for weeks (11).

In ash (*Fraxinus americana* L.) the sieve tubes are not immediately plugged when an incision is made into the conducting phloem. Exudation usually lasts about an hour, sometimes longer. As far as concentration is concerned, only the very first samples can be regarded as true samples of the assimilate stream [except for the possible presence of trace contaminations (9)]. Samples taken a few minutes after incision are osmotically diluted (7). It has also been shown that the concentration in the ash phloem increases above and decreases below an incision, but remains unchanged in tangentially adjacent tissue (10).

In the present experiments two incisions were made, one exactly above the other; exudation occurred from both. Obviously, the upper cut was supplied from phloem tissue above, the lower one from tissue below. Transport toward the lower incision occurred therefore in a direction reverse to the direction of flow under natural conditions. This is a confirmation, on a larger scale, of the results of Weatherley et al. who found that phloem transport toward an aphid-stylet bundle can take place from either the apical or the basal end of a piece of stem (5).

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Materials & Methods

Experiments were made with white ash (*Fraxinus americana* L.) in Prospect Hill Tract I of the Harvard Forest, in Petersham, Mass. Two longitudinally (vertically) adjacent incisions were made, 2 cm apart, both of which interrupted the same band of phloem about three centimeters in width. They were about four centimeters long and placed at an angle of 45° to facilitate exudate collection. Samples were taken with 5-μl pipets, and the total molar sugar concentrations were determined colorimetrically after hydrolysis as described previously (10). The accuracy of the method can be estimated from the scatter of points in figures 1 to 3 in which each circle represents an individual determination.

Rates of phloem exudation (units volume per unit time) cannot be measured easily with the incision method; mass transfer (units weight of solute per unit time) (1) was measured in milligrams of raffinose equivalents per minute by picking up all exudate at given intervals with small pieces of filter paper of suitable shape. 15 μl of invertase were added to each paper and the sugars were eluted from the paper for 3 hours. This proved to be ample time for elution and hydrolysis. Elution was accomplished with the aid of especially designed stainless steel racks that had been developed for paper chromatography. The racks are similar to the polyethylene racks described before (7), but lack the disadvantageous properties of polyethylene (10). Colorimetric determination of these samples was the same as above.

Results & Discussion

Two longitudinally adjacent incisions (one above the other) interrupt the same strands of phloem tissue. This is not only obvious from an anatomical point of view but also is the conclusion based on experimental evidence. Phloem transport in tree trunks seems to take place along extremely well defined longitudinal paths. In the white ash the tangential deviation from the longitudinal axis is less than one degree (10).

Figures 1 to 3 show the concentration values of exudate taken from two longitudinally adjacent incisions. It can be seen, first of all, that osmotic dilution begins immediately when the first incision is made. This phenomenon has been described in earlier papers (7, 10). In the experiment shown in figure 1,

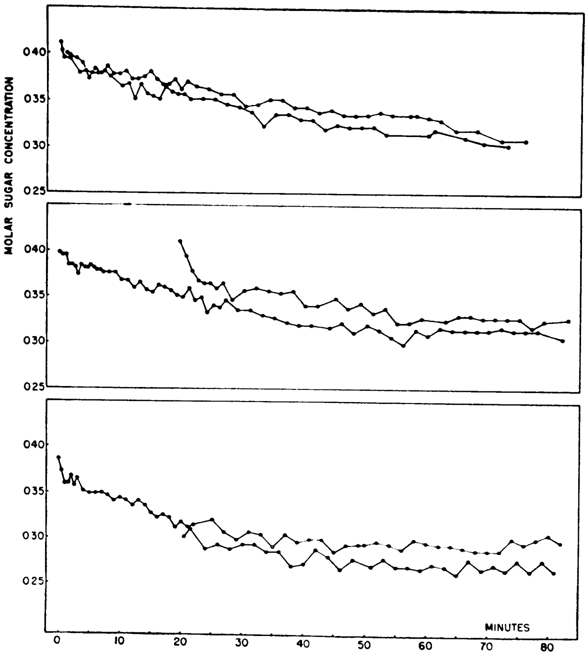

Fig. 1 (*top*). Molar sugar concentrations of exudate samples taken from two incisions interrupting the same phloem strands. Exudate from the upper incision is indicated by open circles (*upper curve*), exudate from lower incision by filled circles (*lower curve*).

Fig. 2 (*center*). Similar to figure 1. Second incision was made 20 minutes after and above the first one.

Fig. 3 (*bottom*). Similar to figure 1. Second incision was made 20 minutes after and below the first one.

both incisions were made at very nearly the same time. The concentration curves of both incisions are similar except for a slightly higher level of concentration in the upper incision. This level difference is by no means surprising. Under normal conditions there is a positive concentration gradient (decreasing concentration) in the sieve tubes in the downward direction of the trunk (7, 10). The difference in concentration in the upper and lower parts of the trunk is even intensified when the sieve-tube turgor is released (10). The upper incision in the present experiment was therefore supplied by phloem tissue that contained solution of higher concentration than the lower incision.

It was reported earlier that the dilution curves of two incisions in tangentially adjacent tissue were independent of each other. A second cut, made beside a first one, and sometime later, gave a dilution curve identical with the first one (10). Exudation, in that case, was from two different channels; the experiments demonstrated lack of tangential connection and relative independence of the various sides of a tree stem. In the present experiments, on the other hand, the two longitudinally adjacent incisions interrupted the same channels. The upper cut was supplied from tissue above, and the lower cut from tissue below. Whenever the two incisions were spaced in time, the concentration from the second one immediately fell into place without a repetition of the beginning of the curve. Figure 3 shows this quite clearly.

Figure 2 shows an experiment in which the second incision was made above and 20 minutes after the first one. Although the curve of the upper incision very quickly falls into place, its first values are higher than the first values of the initial incision. To the superficial observer this might seem to contradict what has just been stated. However, it will be noted that only the very first values are unusually high. This is due to the concentration increase that takes place above an incision. The upper incision in figure 2 was made 20 minutes after the first (lower) one, at a time when a steep concentration increase from the first incision to a maximum about 50 cm above had developed (cf. fig 2 of ref. 10). Initial concentrations are difficult to obtain in this area because exudation is slow and the first sample may already be diluted. But figure 2 shows that while the concentration of exudate decreases *at* an incision, it increases at a distance of as little as two centimeters *above* the incision. This means that the dilution phenomenon is quite a local one. But it may also mean that water moves somewhat faster toward the incision than the solutes, in other words that the sieve plates differentially withhold the larger solute molecules. This suggestion was made earlier (8). The phenomenon may well be an artifact due to the formation of incomplete slime plugs near the incision.

► Experiments on Gravity. Ready exudation from two longitudinally adjacent incisions suggested an attempt to evaluate gravity as a possible factor in phloem transport. The force responsible for the ascent of sap in the xylem of trees has to overcome a total resistance consisting of two components of about equal magnitude: resistance to flow in capillaries, and the effect of gravity. If gravity acts so effectively against the ascent of sap in the xylem, there is no reason to believe that gravity should not assist phloem transport wherever it occurs in the downward direction. It was thought, then, that the rates of exudation from two longitudinally adjacent incisions might be sufficiently different to show the difference in exudation pressure. The rate from the physically upper cut should be greater than that from the lower cut (exudation pressure in upper cut = osmotic pressure + gravitational pressure; exudation pressure in lower cut = osmotic pressure − gravitational pressure). Concentrations shown in figures 1 to 3 suggest a slight difference between the osmotic pressures at the morphologically upper and lower incisions. But we can neglect this, simply because it is impossible to cover precisely the same amount of phloem tissue with the two incisions. Therefore absolute values of exudation rates for comparative purposes can not be obtained. In order to avoid the need for absolute values, whole trees were inverted during the experiment, and it was thought that differences before and after inversion might indicate the effect of gravity.

After it had been ascertained that detaching a

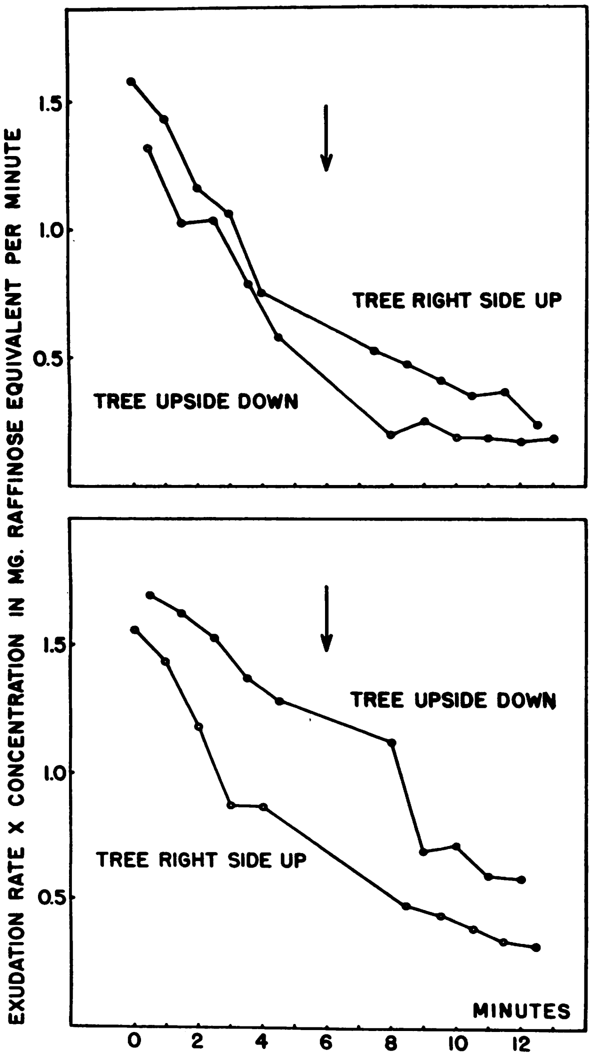

Fig. 4. Gravity experiments. *Open circles* indicate the morphologically upper, *filled circles* the morphologically lower incision. The tree was inverted at the arrow. The fact that the curves are not on the same level is quite incidental. It is impossible to cover precisely the same amount of tissue, and the incision that happens to cover more will exude more. Further explanation in text.

tree from its roots had no effect on exudation during the short duration of the experiment, a tree was cut and brought to the Harvard Forest Headquarters where samples could be taken at about five meters height from a fire escape on the second floor of the building. Two incisions were made, one above the other, similar to those of the previous experiments, and samples were taken from each at 1-minute intervals. The experiment was performed eleven times with four different trees. Figure 4 shows two examples. It was not practical to measure the exudation rate directly. Instead, data were obtained in units weight per unit time, as described in Methods. Canny called the expression (weight/time) mass transfer (1):

$$\begin{matrix}\text{Mass transfer}\\ \text{(wt/time)}\end{matrix} = \begin{matrix}\text{Volume transfer}\\ \text{(exudation rate)}\\ \text{(vol/time)}\end{matrix} \times \begin{matrix}\text{Concentration}\\ \text{(wt/vol)}\end{matrix}$$

It is important to bear this relationship in mind, because the concentration of the exudate does not remain constant during exudation. Our values in figure 4 are exudation rate × concentration, or mass transfer. If they are divided by the corresponding concentrations, exudation rates are obtained. The fact that the slopes of the curves in figure 4 are steeper than those in figures 1 to 3 indicates that not only the concentration decreases during the experiment, but also the rate of exudation. This is a very simple mathematical consideration. If the rate of exudation had not changed during the experiment, the slopes would be exactly identical, and the decrease in mass transfer would entirely represent concentration decrease.

The inversion of the tree was not marked by any significant change in rate difference between the two incisions. Thus the effect of gravity—if there is any—was not obvious.

As previously stated, the exudation pressure consists of the osmotic and the gravitational pressures. The gravitation-pressure difference between the two incisions cannot be much more than one atmosphere in our case, corresponding to a total height of the tree of somewhat in excess of 10 meters. In order to estimate the osmotic portion of the exudation pressure, some cryoscopic determinations of collected exudate were made. The freezing-point depression of the exudate was between 1.48 and 1.52 C, which corresponds to an estimated total solute concentration of 0.7 and 0.75 M. The same sample was chromatographed and the following molar concentrations were found: sucrose 0.132(±0.003), raffinose 0.088 (±0.002), stachyose 0.205(±0.005), mannitol 0.097(±0.003), total carbohydrates 0.522(±0.013). This indicates that there may be a non-carbohydrate component in the exudate of as much as 0.2 molar. The nature of this component is unknown in our case, but various substances have been found in other plants by other workers (4, 6). The total concentration suggests an osmotic pressure of 15 to 20 atmospheres. It can easily be seen that a difference of one atmosphere is not sufficiently great to show up in figures 4 and 5, which have been obtained with the rather inaccurate method of picking up exudate with pieces of filter paper during 1-minute intervals.

The failure of gravity to exert a measurable effect in our experiments does not mean that gravity cannot provide an appreciable part of the force for movement under natural conditions. The osmotic gradient along the tree trunk is of the order of 0.2 atmospheres per meter (9), which is only twice the gravitational pressure gradient. In order to find the effect of gravity on phloem transport, if there is any, one would either have to use a method independent of exudation or greatly improve its accuracy.

Summary

If two incisions are made into the conducting phloem, one above the other, exudation takes place from both. This indicates upward transport toward the lower incision, in other words a reversal of the normal direction of translocation. A series of samples taken from each of these incisions shows a gradual concentration decrease which is ascribed to osmotic dilution of the sieve-tube content. The concentrations of the upper incision are slightly higher than those of the lower incision, because the upper incision is supplied from phloem tissue above, where concentrations are higher, while the lower incision is supplied from tissue below, where concentrations are lower (figs 1–3).

In an attempt to find the effect of gravity, exudation rates (rate × concentration) from two similar incisions (one above the other) were compared in the right-side-up and upside-down position of detached trees. However, the method was not sensitive enough to discern the gravitational portion of the exudation pressure.

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