TRANSLOCATION OF ORGANIC SUBSTANCES IN TREES. III. THE REMOVAL OF SUGARS FROM THE SIEVE TUBES IN THE WHITE ASH (FRAXINUS AMERICANA L.) [1]

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It has been shown by various investigators that the substances in the sieve tubes are qualitatively the same throughout the plant. This, together with a number of physiological observations, seems to suggest that substances move through the sieve tubes without their being metabolized (2, 8, 23). There is good evidence, however, that the movement over the short distance from the leaf parenchyma to the sieve tubes is an active process. It has been shown by Phillis and Mason (13) that the sugar concentration of sap expressed from leaf tissue containing mainly mesophyll is much lower than that of sap expressed from the inner part of the bark or of leaf tissue containing mainly veins. This was later confirmed by a number of other workers using a wide variety of plants and different methods. Most important—since concentrations of expressed saps are not directly comparable—is the fact that leaf parenchyma cells have been plasmolyzed by the sieve tube exudate of the same plant (7, 14). Transfer of substances into the sieve tubes must therefore occur metabolically. This process has been called "loading" by Barrier and Loomis (1). Huber and Bauer refer to it as "secretion" (9) because of the striking physiological similarity to the secretion of sugars in nectaries (10, 20, 21). There are a few reports on this movement dealing with both the chemistry of the transferred material and the physiology of the tissues involved (2, 18, 21), but the details are far from known.

A parallel problem to that of the introduction of assimilates into the sieve tubes is that of how the assimilates are removed from these translocation channels. The mere fact that they do leave the sieve tubes has led some investigators to believe that the sieve tubes are "leaking." That this is not the case is indicated by the fact that they are, even at great distance from the leaves, under a high turgor pressure most of the time (see (5)). It will be shown in this paper that the turgor remains high for many days after the leaves have formed abscission layers in the fall, and also after artificial defoliation.

The presence of large amounts of stachyose, raffinose and sucrose make white ash a very interesting object for phloem translocation studies. It is significant that these sugars contain the sucrose unit within their molecules. In order to explain our previous observations it was proposed that there is an enzymatic removal of D-galactose units which results in a gradual break-down of the higher oligosaccharides, stachyose and raffinose, as they move down the tree (24). In the present investigation three ash trees have been defoliated, thus cutting off the supply of photosynthates to the phloem, and the changes that took place in the sieve tubes after this treatment have been followed. A similar study has been made of the changes in the phloem during the natural leaf-fall in autumn.

EXPERIMENTAL

The defoliation experiments were carried out in Tom Swamp Tract I of the Harvard Forest. Three ash trees were chosen which had over 9 meters clear trunk length and a diameter at breast height of about 15 cm. On July 13, 1957, they were completely defoliated with the aid of a "sky-worker." Defoliation of one tree required about 40 minutes. Samples of

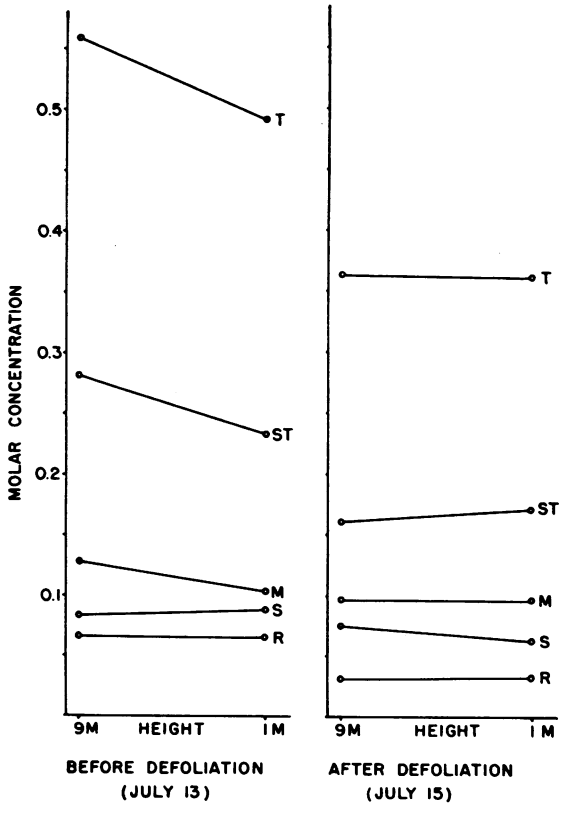

FIG. 1. Gradients along the tree trunk before and after defoliation. The four substances shown represent about 90 % of the exudate's dry weight. As soon as the leaves are removed, the total concentration drops, the total molar concentration gradient disappears and some of the individual gradients (stachyose and to a slight extent raffinose) become negative. M = mannitol, S = sucrose, R = raffinose, ST = stachyose, T = total conc.

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sieve tube exudate were taken on the day before and on several successive days after defoliation. Four tapping cuts were applied each time at 1 and 9 meters height from the ground. From each cut two 5-μl pipettes (for sugar analysis), three 1-μl and three 2-μl pipettes (for mannitol analysis) were filled. Each point on figure 1 represents therefore the average of 8 sugar or 24 mannitol determinations. During the natural leaf-fall, samples were collected from two further trees in much the same way.

The samples were brought to the laboratory, transferred onto the paper chromatograms and dried. The whole process was completed within about one hour from the time of collection. The chromatograms were then stored in a freezer at - 20° C, wrapped in polyethylene foil in packages containing the analytical work of one day (64 sugar analyses including 16 standard values). The analysis of the samples was carried out as previously described (22, 23).

There were no significant enzymatic interconversions after collection. Several days elapsed before a sample, stored at room temperature, showed the first fermentation products (reducing sugars). Such products were not detected during chromatographic analysis following proper storage.

Results and Discussion

It was expected, according to observations that had been made in the previous autumn during leaf-fall, that the sap-flow would last for only a few days after defoliation. Surprisingly, however, sieve tube exudate could always be obtained, and samples were taken for about a month until the trees returned to normal activity with their newly formed leaves. The results from all three trees were the same.

The quantitatively most important substances in the sieve tube exudate of white ash are stachyose, raffinose, sucrose, and D-mannitol (23). They constitute about 90 % of the dry weight of the exudate. There is therefore little error in calculating osmotic pressures from the molar concentrations of these four substances. The gradients before and after defoliation are shown in figure 1. The total concentration drops in the whole sieve tube system immediately after the assimilate supply is cut off. The total molar concentration gradient, which is positive in the downward direction of the trunk throughout the whole summer, disappears. At the same time, and probably as a result of this, some of the gradients of individual substances become negative. This confirms the previously reported observation made after the leaf-fall in autumn (23). It supports the supposition that the turgor gradient is the driving force of translocation through the sieve tubes.

One way of studying the exit of sugars from the sieve tubes would be to isolate a short piece of trunk and to analyze its sieve tube content continuously. Instead of doing this, the samples can just as well be taken from a defoliated tree since defoliation stops the supply of photosynthates to the phloem. Samples taken from the middle of the entire phloem

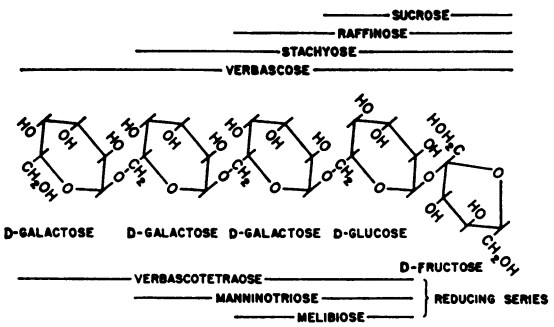

Fig. 2. The raffinose family of oligosaccharides. The sugars of the sieve tube exudate of white ash are sucrose, raffinose, stachyose and verbascose. Verbascose is present in traces only and therefore disregarded in this quantitative study. The reducing series are not native sugars in the exudate, but occur only as fermentation products. (From Zimmermann (24).)

length will yield average values, since gradients are fairly linear. However, in order to obtain gradients from the same experiments samples were taken at 1 and 9 meters height, and the values corresponding to the 5 meter level were calculated by averaging the 1 and 9 meter values. Figure 3 shows the change in sugar concentrations after defoliation. During the 1st 8 days stachyose rapidly decreases. Sucrose increases, obviously at the expense of stachyose. It will be seen from the relationship of the sugars, which is shown in figure 2, that the enzyme capable of such a transformation is an α-D-galactosidase. Since the galactose units never appear in the exudate, it follows that they must be transferred directly out of the sieve tube vacuoles.

The mode of action of such a galactose removal system may be outlined as follows: the enzyme is attached to the inner surface of the side-wall cytoplasm of the sieve tubes. It acts upon the oligosaccharides while they pass, removing D-galactose units and thus producing the next lower oligosaccharide. So long as the supply continues this break-down only shows as a gradient in the concentrations of the oligosaccharides (cf. fig 1). However, as soon as the oligosaccharide supply from above is cut off by defoliation, the break-down becomes apparent. Evidently, it proceeds almost to completion.

There must be a mechanism for the removal of sucrose but we know little about it. This process is very important, since in many plants sucrose is the only translocatory sugar (11, 16, 19, 21, 22). The sucrose curve of figure 3 does not indicate sucrose removal, since, at the same time, there is a sucrose production through break-down of the higher oligosaccharides. However, the removal of sucrose molecules from the sieve tubes is shown by the decrease of the total molar sugar concentration, because transgalactosidation does not affect the total molarity. In other words, the total sugar molarity actually represents the total sucrose molarity, although part of the sucrose units are masked as raffinose and

stachyose. Thus a measure of the rate at which the sucrose molecules leave the sieve tubes is given by the total sugar molarity curve in figure 3. This curve shows that the sucrose removal is very rapid immediately after defoliation, slows down, and, after the 8th day even becomes reversed, that is, the sucrose content increases somewhat without a corresponding break-down of higher oligosaccharides. This rapidly decreasing sucrose removal rate might be explained in the following way: the sucrose removal is somehow controlled by a hormonal factor that comes from the leaves with the assimilate stream. As soon as the tree is defoliated the hormone is exhausted and the removal process stops. It may even become reversed, that is, sucrose may reenter the sieve tubes (after the 8th day in our experiment). It might be argued that the rapid concentration drop is actually some kind of a dilution effect, due to the sudden increase of the relative pressure within the xylem after defoliation. This is hardly possible, since it has been shown that the concentration does not drop at night when transpiration is low (8, 21, 24). Moreover, the rapid exit of sucrose immediately after defoliation must be the same as in a normal, undisturbed tree if we consider the length of the phloem and the order of magnitude of translocation speeds that have been found by many investigators (3, 4, 6, 12, 15, 17).

It will be seen in figure 3 that both the rate of sucrose disappearance—as pictured by the total sugar molarity curve—and the rate of break-down of stachyose drop after defoliation, although the latter drops slowly. In the experiment shown in figure 3 the break-down rate of stachyose is 1.31 % per hour during the 1st day after defoliation, 0.91 % per hour during the 2nd day, and 0.59 % per hour during the 2nd to 8th day. In other experiments it was: 0 to 1st day = 1.44 %/hr., 1st to 4th day = 1.00 %/hr., 4th to 10th day = 0.47 %/hr.; and 1st day = 1.39 %/hr., and 1st to 5th day = 0.61 %/hr. This decrease in rate of stachyose break-down may be due to a decreased enzyme activity, i.e., may be proportional to the lowered substrate concentration, or it may be due to a decreased mechanical turnover of the vacuole content when the flow slows down and finally stops. In other words, fewer stachyose molecules would make contact with the enzyme on the inner cytoplasm surface of the sieve tubes when the assimilate stream becomes stagnant.

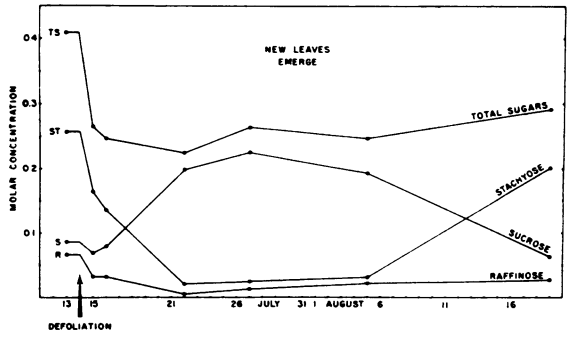

FIG. 3. The effect of defoliation on the sieve tube sugar concentrations at 5 meters height, the approximate middle of the entire phloem length.

The rate of stachyose break-down is probably nearest to normal immediately after defoliation. This allows us to make an approximate calculation of the rate of translocation of stachyose on the day before defoliation. The stachyose decreased then from 0.281 M at 9 meters to 0.233 M at 1 meter height, which is a decrease of 17 % over a distance of 8 meters. During the first 27.5 hours after defoliation the stachyose decrease at 5 meters height, which represents about the average of the whole tree, was 1.31 % per hour. If we consider this break-down rate normal, the decrease of 17 % would have required 17/1.31 or 13 hours, and must be the time taken to travel 8 meters. This corresponds to a stachyose translocation rate of 62 cm per hour. The same calculation carried out with the data of the two other experiments yields speeds of 73 and 54 cm per hour respectively. These figures are of the order of magnitude of translocation rates that have been found by many other investigators using different methods (for instance 3, 4, 6, 12, 17). It would be interesting to compare the rates of all the different substances that are present in the sieve tubes. Unfortunately, however, stachyose is the only substance that allows the application of such calculations, because we are reasonably sure it is the only one that fulfils the requirements of being synthesized only in the leaves and broken down only in the sieve tubes (cf. discussion in (24)). Raffinose and sucrose are also synthesized in the leaves but they are produced in the sieve tubes by galactose removal from higher oligosaccharides while they move down the trunk, and there are indications that D-mannitol is also produced in the sieve tubes, possibly in connection with the sucrose exit.

Production of sucrose through oligosaccharide break-down would explain why, in a few instances, the sucrose gradient—and only the sucrose gradient—in undisturbed trees was found to be slightly negative in the downward direction during the summer. Figure 1 left, which shows the gradients before defoliation, presents an example of this. The rate of sucrose production by oligosaccharide break-down may sometimes exceed the rate of sucrose removal.

New leaves emerged on all defoliated trees after about two weeks. At this time (July 27, fig 3) the increase in sugar concentration changes to a decrease. This shows that the growing leaves draw upon the sugar supply of the phloem. The final increase after August 8, and particularly the reappearance of stachyose indicate that newly formed leaves are exporting photosynthates again.

The situation during the natural leaf-fall in autumn is quite similar to that of artificial defoliation. Figure 4 shows the data obtained from a tree between September 26 and October 21, 1957. If these curves are compared with those of figure 3 it will be seen

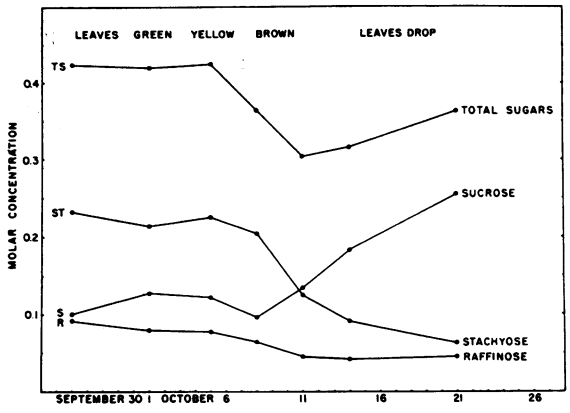

FIG. 4. Sieve tube sugar concentrations during the natural leaf-fall in autumn. A comparison of these curves with those of figure 3 suggest that the abscission layer was formed between October 5 and 8, a few days after the leaves turned yellow.

that the point equivalent to the time of defoliation must fall on about October 5 to 7. Obviously the abscission layer was then formed. If samples are taken around the trunk at one height in late autumn, it may be found that the sugar conversion is farther advanced on one side of the trunk than on another. This observation, which has been previously reported (cf. (24) figure 6), may be due to an earlier formation of the abscission layer on parts of the crown. The changes in sugar concentrations after October 5 suggest that the leaves, although still on the tree, were physiologically disconnected from the phloem of the trunk. The changes in sugar concentrations between October 5 and 21 are the same as those between July 14 and 27 of the defoliation experiments.

The sucrose increase between October 11 and 21 (fig 4) and between July 22 and 27 (fig 3) is of interest. This increase occurs during a time when the concentration of the higher oligosaccharides is low and does not change significantly (fig 3). It is therefore not due to oligosaccharide break-down. Nor is it due to a random variation in samples, because it appeared invariably in all experiments and the maximum variation of the individual samples did not exceed $\pm$ 10 % (including a chromatographic error of $\pm$ 2 to 4 %). The increase in sucrose may be nothing more than a mild desiccation of the phloem tissue due to continued removal of outer bark, which is necessary for tapping. The hormonal exit control that has been discussed above may also explain the sucrose increase. Sucrose exit may be a reversible process, going in one direction in the presence of the hormonal factor (sucrose exit) and in the other direction in the absence of it (sucrose re-entry). As soon as the tree is defoliated, sucrose exit slows down, stops, and becomes reversed (sucrose re-entry—due to the exhaustion of the hormone). The translocates in the sieve tubes, instead of being depleted, are thus saved. This hypothesis, if correct, explains how trees that have been defoliated by natural forces such as hail or insects can immediately produce new leaves from reserve material.

The last samples were taken on October 21; no more exudate could be obtained after this time. The sap flow was poor on October 21 in spite of the relatively high concentrations. This suggests that it is not a decreasing sieve tube turgor caused by decreasing concentrations that stops the sap flow in late autumn, but rather something else, possibly callus formation or active sieve tube shrinkage (cf. discussions in (8) and (24)). We made no attempt to check this anatomically.

These experiments are consistent with results reported previously (23, 24), which suggests that there is a passive flow of solutes along a positive turgor gradient which is established and maintained by the metabolic entry of solutes into and removal from the sieve tubes. The secretion of photosynthates into the sieve tubes in the leaves, discussed by a number of workers (1, 2, 21), may be regarded as a metabolic "pressure-pump." It raises the turgor within the sieve tubes of the leaves. The removal mechanisms, which lower the sieve tube turgor at places of solute consumption, would then represent a metabolic "suction-pump," located at the other end of the translocation channel, working under remote control from the leaves.

SUMMARY

The sieve tube sugars of white ash (*Fraxinus americana* L.) were analyzed after artificial defoliation in summer and during the natural leaf-fall in autumn. After artificial defoliation the sieve tubes remained turgescent for an unlimited time. New leaves emerged after approximately two weeks. In autumn the sieve tubes could be tapped until two to three weeks after the abscission layer had been formed. Since sieve tubes retain their turgescence for such a long time after the interruption of the assimilate supply, it is concluded that they are not "leaking." Two metabolic mechanisms are described, both of which may be responsible for the removal of sugars from the sieve tubes.

In the first place there is a rapid interconversion of stachyose into sucrose. This was observed after artificial and natural defoliation as a decrease in stachyose and increase in sucrose. It is suggested that an α-D-galactosidase on the side-wall cytoplasm of the sieve tubes removes D-galactose units while the oligosaccharides pass, thus producing raffinose from stachyose and sucrose from raffinose. In the second place sucrose itself is removed from the sieve tubes. This process seems to be reversible and controlled by a hormonal factor that comes from the leaves (sucrose exit in the presence, sucrose re-entry in the absence of the hormone). The sucrose removal is very rapid immediately after defoliation (hormone still available), but slows down and stops after five days, after which sucrose increases again in the sieve tubes (hormone exhausted).

From the break-down rate of stachyose immediately after defoliation, and the measured stachyose gradient along the trunk before defoliation, it was possible to calculate the rate of stachyose translocation as 62, 73, and 54 cm per hour for the three defoliation experiments.

The total molar gradient of the three sugars and D-mannitol, which constitute about 90 % of the sieve tube substances, is positive in the downward direction of the trunk during the whole summer. After defoliation this gradient disappears and at the same time some of the gradients of the individual substances become negative. This suggests that the turgor pressure gradient is the driving force of translocation through the sieve tubes.

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