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CURRENT PROBLEMS IN RESEARCH

Movement of Organic Substances in Trees

Photosynthates are translocated in a layer of bark only a fraction of a millimeter thick.

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The development of land plants that began some 400 million years ago involved not only the problem of mechanical support of the plant body but also the problem of distribution of water, mineral nutrients, and photosynthetic products. Trees are among the most characteristic forms of land plants in this respect. The two centers of supply —namely, the roots that take up moisture and minerals from the soil and the leaves that carry out photosynthesis—are located at a distance from each other but are connected by the vascular system which consists of two "channels," the xylem and the phloem. In palms, as in other monocotyledonous plants, and in herbs, strands of xylem and phloem are joined into conducting bundles which are distributed in a ring or throughout the transverse-sectional area of the stem. In trees with secondary growth (increase in stem diameter), the two channels are in the form of cylinders, the wood (xylem) and the inner bark (phloem). The two systems conduct in essentially opposite directions: water and dissolved soil minerals move upward from roots to leaves in the xylem (transpiration stream), photosynthates move down from leaves to stem and roots in the phloem (assimilate stream). The movement toward growing centers, such as shoot tips, flowers, and young fruits, is unidirectional—that is, both streams move in the same direction.

The two systems enable the plant to distribute nutrients efficiently. Minerals that have been carried up by the transpiration stream into the leaves may be re-exported to other places via the assimilate stream. Metabolic energy is supplied through the phloem to roots in the form of carbohydrates, products of the photosynthetic activity of the leaves. These carbohydrates provide, at the same time, the carbon skeletons for nitrogenous compounds that are formed by nitrate reduction in the roots and that ascend via the xylem into the aerial parts (*1, 2*).

Accumulation of reserve materials such as starch is of considerable importance (*3*). A great percentage of the carbohydrates, exported from the leaves via the phloem, is deposited along the whole stem in bark and wood. These reserves are not only drawn upon for seed production during a seed year but are also utilized during the flush of growth in the spring. Some trees depend for their annual growth almost entirely upon the previous year's reserves (*4*). Hartig, one of the first forest botanists to give a detailed description of these matters, compared the awakening of a tree's activities after winter dormancy with the germination of a seed. All vital parts of the tree—leaves, shoots, root tips, and the conducting tissues interconnecting these organs—are renewed in the spring (*5*).

How substances move in the phloem is one of the oldest of botanical questions. Trees are very suitable objects for studies of this movement because they offer the unique advantage of great lengths of uniform translocation conditions. However, working with trees has its difficulties. Large trees can hardly be grown under controlled conditions, and while most plant physiologists can transfer their material to a greenhouse or growth chamber, the tree physiologist has to take his experimental procedures to the trees outdoors.

Structural Organization of Bark

If we look at a transverse section of bark we can distinguish three structurally distinct parts that represent functionally different phases of bark tissue. The innermost and youngest layer, immediately adjacent to the vascular cambium, is the conducting phloem. As this layer is renewed by the cambium, the sieve tubes (the cell series in which conduction takes place) ordinarily collapse and the tissue enters its second phase, with the functional emphasis on storage. Still further out we can see the periderm, with the cork cambium producing cork cells—the outermost, dead bark which protects the living bark from desiccation and mechanical injury (*6, 7*).

In temperate regions, where distinct growth rings are present in the wood, growth rings can usually be distinguished in the phloem as well, although not as clearly as in the wood (*8*). Figure 1 shows a transverse section through the inner bark of white ash (*Fraxinus americana* L.). The innermost layer, adjacent to the cambium, is the 1960 phloem; successively older growth rings are outside (the 1959 and 1958 rings and a small portion of the 1957 ring are visible in Fig. 1). The most striking feature is that conduction usually takes place only through the latest growth ring of the bark, a layer some 0.2 millimeter thick.

In tropical trees there are usually no distinct annual growth rings. Renewal of phloem tissue and collapse of older sieve tubes, nevertheless, take place in a similar fashion. Figure 2 shows a transverse section through the inner bark of teak (*Tectona grandis* L.). The

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conducting layer is about 0.35 millimeter thick; the collapse of the sieve tubes in the older tissue is very conspicuous.

Significance of Callose

The sieve elements are long cells with end walls of a sievelike structure. In anatomical preparations the pores are often restricted with a substance, called callose, that stains in a characteristic way (*9*). Until recently, callose cylinders in the sieve pores have been regarded as standard features of conducting sieve tubes. It seems, however, that callose formation can occur very rapidly upon external mechanical or chemical stimulation (*10*). It was reported as early as 1886 that chemically fixed phloem tissue contains an abundance of callose, while pieces of bark that had been immediately killed by being dropped into boiling hot water contain only very small amounts (*11*). This has been confirmed in our own laboratory. Even fresh sections may contain some callose, since the time that elapses between collecting the piece and staining the fresh section is often sufficient to allow callose formation. Whenever the phloem is injured—for instance, when a branch is cut from a tree or a piece of stem—great masses of callose are formed. This is an important sealing reaction of the plant (supplementing the instant sealing with "slime plugs" that takes place in many plants) that prevents excessive loss of translocated material upon injury. Just how much callose, if any, is present in normal, conducting sieve tubes remains to be investigated.

In addition to this formation of callose in the conducting layer of sieve tubes upon external stimulation, callose is formed naturally and regularly in autumn. In white ash this happens some two weeks after leaf abscission. The phloem remains essentially unchanged during the winter. About 1 May (in Petersham, Mass.) the buds begin to swell, the first early-wood vessels appear in the wood, and the first sieve tubes are formed in the bark. Shoot extension as well as growth of xylem and phloem is essentially completed within the short period between 1 May and mid-June, at the expense of stored material. The callose in the previous year's phloem that had been formed in autumn disappears only slowly and incompletely (*12*). Occasional callose masses can be found in older phloem tissue. What causes these irregularities we do not yet know, but it is clear that the previous year's phloem can hardly be of any value for transport.

Methods for Physiological Experimentation

For physiological experimentation, trees, or parts thereof, are subjected to experimental conditions such as defoliation, girdling, and local chilling, and the effect of the treatment on translocation is studied. This can be done by paper-chromatographic analysis of sieve-

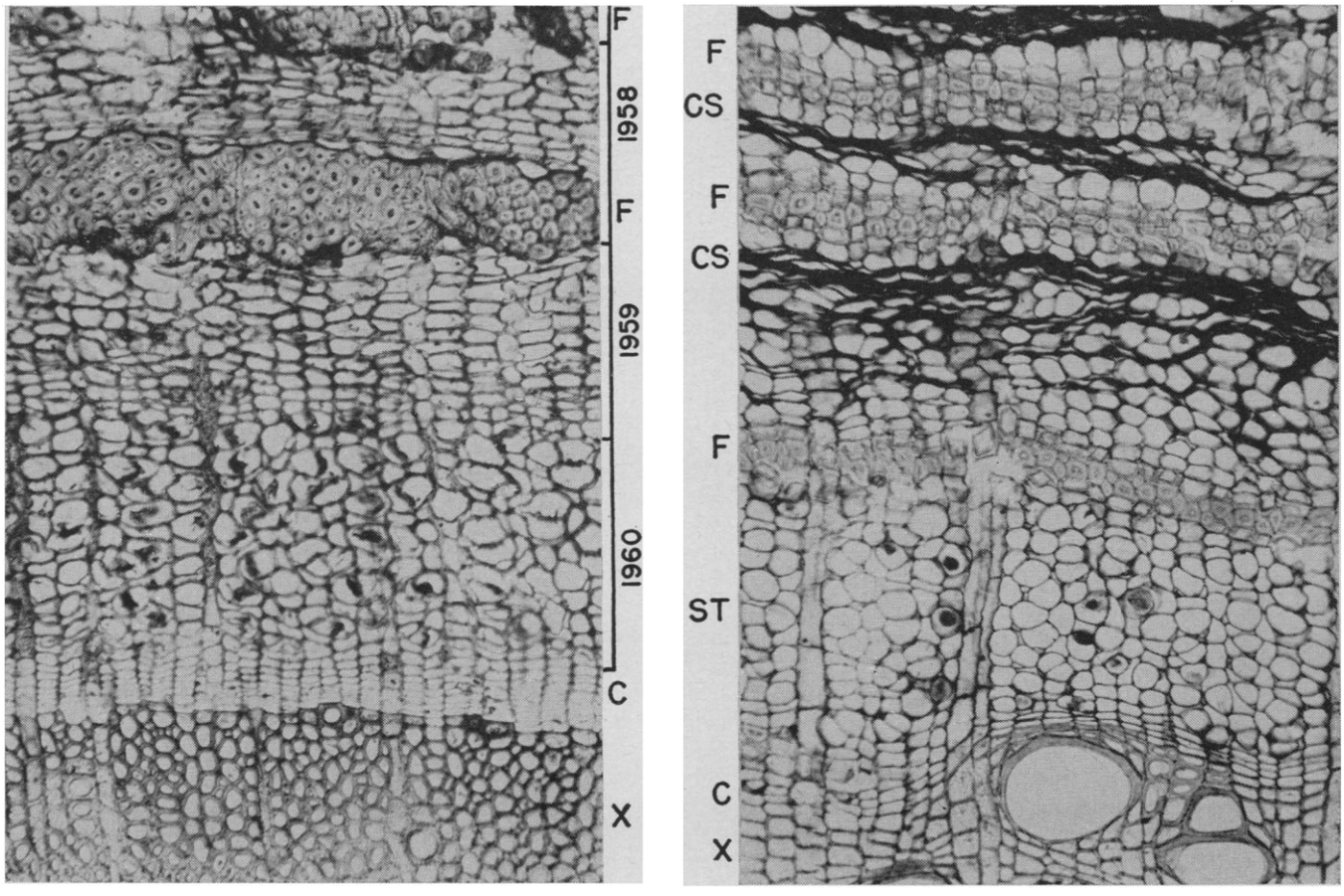

Fig. 1 (left). Transverse section through the inner bark of white ash (*Fraxinus americana* L.), showing growth rings. *X*, wood; *C*, vascular cambium; *F*, fibers ("sclerotic parenchyma cells" is technically more correct; they are formed in late June in 3-year-old phloem). Sectioned on 16 Aug. 1960 (× 165). Fig. 2 (right). Transverse section through the inner bark of teak (*Tectona grandis* L.), showing conducting (*ST*) and collapsed (*CS*) sieve tubes. *X*, wood; *C*, vascular cambium, *F*, fibers ("true bark fibers," directly derived from cambium) (× 165).

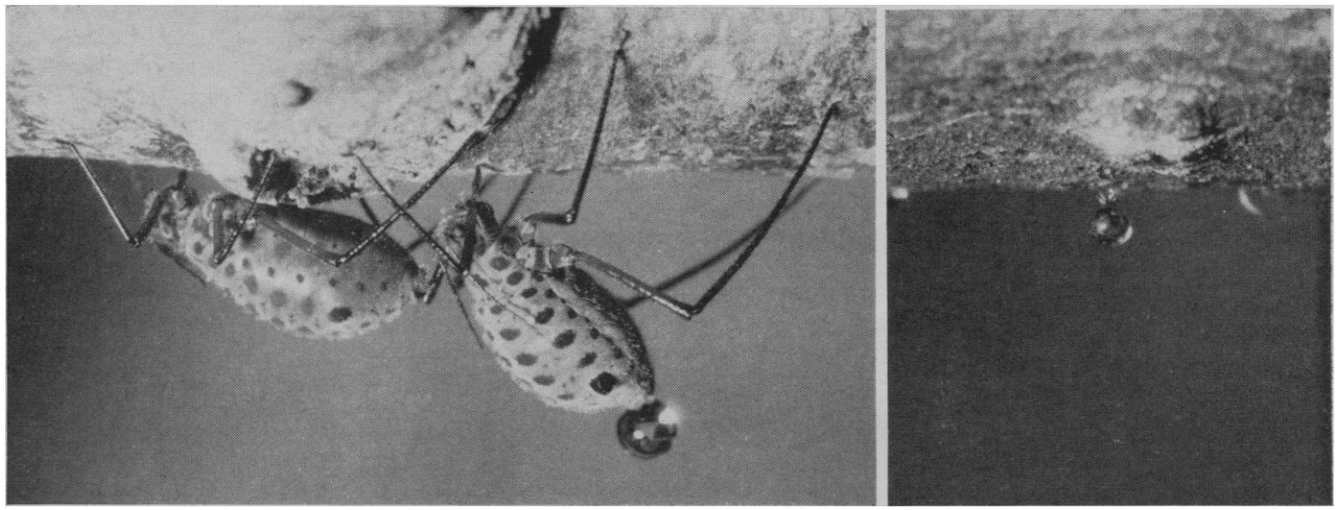

Fig. 3. (Left) Two aphids of the species *Longistigma caryae* (Harr.) feeding on the lower side of a linden branch. These fully grown individuals are approximately the size of a house fly. When feeding they release honeydew about once every 30 minutes. The actual stylets that penetrate the bark cannot be seen in the photograph because they are surrounded by the stylet sheath. (Right) Exudation from stylets after severance of the aphid.

tube exudate from various parts of the tree and comparison of the results with those from normal trees. This procedure is based upon the assumption that what we call "sieve-tube exudate" or "stylet exudate" is actually translocated material. Good evidence supports this assumption.

There are two methods for collecting sieve-tube exudate. The "classical" method was the first described by Hartig in 1860 (*13*). An incision is made into the inner bark, and the clear exudate can be collected with a graduated pipet. For quantitative studies, pipets of 5 cubic millimeters are most commonly used; occasionally pipets of smaller size are used, such as 2 or 4 cubic millimeters. Long before its usefulness in botany was discovered, the method was used for commercial production of sugar on a small scale. There are still regions in Sicily where certain *Fraxinus* species are cultivated for the collection of sieve-tube exudate. In the dry climate there, the exudate solidifies into small icicle-like "stalactites" (*14*). The anatomical location of such an incision is important. Exudate is obtained only if the cut reaches to the conducting part of the phloem (*15*). Too shallow a cut does not yield anything. Too deep a cut may cause the loss of the exudate to the xylem (the contents of which are usually under less than atmospheric pressure), especially in early summer; later on, the early-wood vessels are protected by a layer of heavily lignified late-wood fibers. Exudate can be obtained between about 1 July, when leaves have reached maturity, and mid-October, when definitive callose is formed in the sieve tubes. This corresponds to the time when sieve tubes are functional and leaves are exporting photosynthates.

There are a number of organisms that have discovered the translocation channels as rich feeding grounds. They represent a great variety of both botanical and zoological orders, from fungi to flowering plants (such as *Orobanche* sp.) and from insects to birds (such as sapsuckers, *Sphyrapicus* sp.). Students of translocation have always taken great interest in these organisms (*6*). A most fascinating observation, made not long ago by entomologists, is that aphids do not suck but are being fed by the internal pressure of the sieve tubes: cut-off stylets from aphids which have been feeding continue to exude. This observation has been systematically developed into a method by Kennedy and Mittler, and by Mittler, with the willow aphid *Tuberolachnus salignus* (Gmelin) (*16*).

Figure 3 shows the North American aphid *Longistigma caryae* (Harr.) that feeds on linden, hickory, oak, and other trees (*17*). Colonies of parthenogenetically reproducing females live on the lower side of branches during the summer. They insert their stylets into a sieve element of the conducting phloem. So far we have sectioned some 40 blocks of bark containing stylets and have found that, whenever they exude, they reach the conducting phloem. The stylets tips are often disturbed or cut away by the microtome knife (the thinner the sections the greater the chance of such disturbance), but in about one out of five cases the stylet tips can be found undisturbed within the section. One of these successful sections is illustrated in Fig. 4.

While aphids are feeding they eliminate surplus sugar by releasing honeydew (Fig. 3), which in nature is collected by ants and honeybees (*18*). When the stylet tips are properly placed in the phloem, as indicated by honeydew production, the insect can be cut from its mouth parts under anesthesia. If this is carefully done, exudation from the stylets continues, often for hours or even days (Fig. 3, right), and the exudate can be collected with a pipet. Exudation rates from *Longistigma* stylets are around 5 cubic millimeters per hour (*19*).

The stylet method does not replace Hartig's method; it is, rather, a valuable addition, having the advantage of operating with a minimum of disturbance and on a small scale. The exudate is the purest we can get, particularly suitable for the analysis of substances in low concentration (substances other than sugars). Furthermore, the stylet method can be used at times or with plants where Hartig's method does not yield sufficient material for analysis (*20*). Last but not least, it is in itself an interesting piece of evidence that the contents of sieve tubes are under pressure. The classical method, on the other hand, is most effective in experiments with large trees, such as those described below. A great number of samples can easily be taken within a short time.

Nature of Sieve-Tube Substances

Exudates of over 250 species, from 55 plant families, are known (*2*, *21*). They contain 10 to 25 percent dry matter of which 90 percent or more is sugar, occasionally accompanied by a sugar alcohol. Classified on the basis of sugar composition, three groups may be discerned, two extreme groups and a third with a large number of intermediate types. The first of these groups, represented by 50 known species of the Leguminosae, contains sucrose as the only sugar. Exudates of the other extreme group contain large amounts of oligosaccharides of the raffinose type, which consist of sucrose and one or more D-galactose residues (Fig. 5).

Typical concentrations are, verbascose, 0.01*M*; stachyose, 0.25*M*; raffinose, 0.1*M*; sucrose, 0.1*M*. Families that belong to this group are the Bignoniaceae, Oleaceae, and Verbenaceae. The Combretaceae, Myrtaceae, and Onagraceae are very close to this group. The majority of other plant families are included in the third, intermediate group, with a high sucrose concentration (such as 0.5*M*) and variable, smaller amounts of oligosaccharides.

Hexoses are not present in most exudates and in the few species that do contain them, are present in very small amounts. They seem to play a very minor role in translocation.

Some sieve-tube exudates contain sugar alcohols in addition to sugars. Mannitol occurs in the Oleaceae and

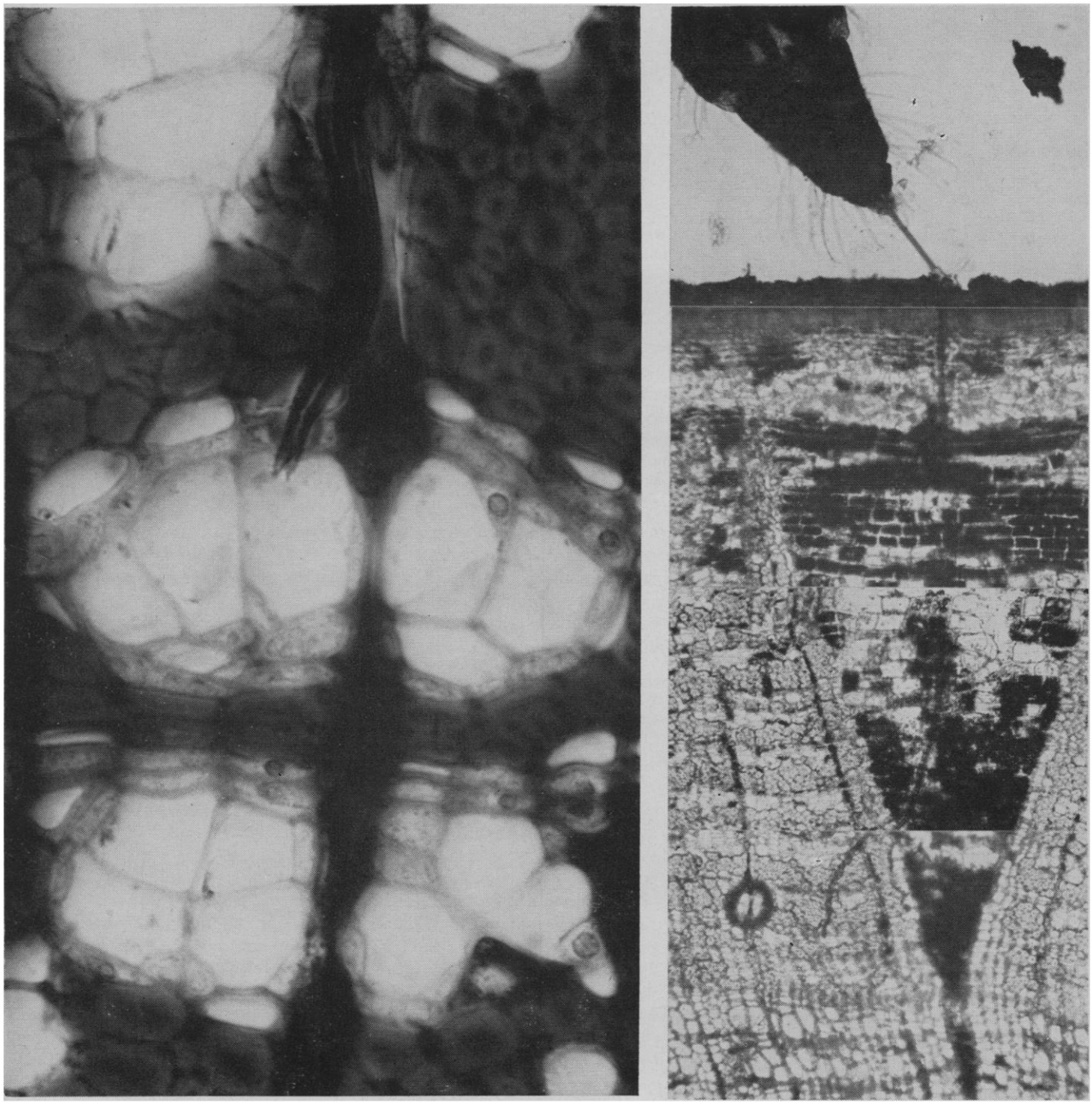

Fig. 4. (Left) Stylet tips in an individual sieve element. Two of the three tips are in focus (× 950). (Right) The path of the stylets through the bark of linden (*Tilia americana* L.), shown in four subsequent transverse sections mounted to form the correct sequence. Photograph at top shows the stylet sheath, which is also shown in Fig. 3 (× 108). The sections were made by Gerda Aerni.

possibly in some *Terminalia* species of the Combretaceae. Another sugar alcohol, tentatively identified as sorbitol, is found in some members of the Rosaceae, as well as sucrose. Typical concentrations are those in the sieve-tube exudate of *Prunus serotina* (Ehrh.): sucrose, 0.2*M*; sorbitol, 0.3*M*.

The concentrations of other substances (amino acids and so on) are only a small fraction of those of the carbohydrates; details of what is known about these have been reported in a recent review (*2*). The aphid stylet method will undoubtedly yield additional information in the future.

The concentration of samples, taken from a single incision, decreases continuously with time, so that the last sample, taken 30 minutes to 1 hour after the incision had been made, shows a concentration only 60 to 80 percent of that of the first one (*22*). This change in concentration, which becomes apparent in the exudate at the point of opening of the sieve tubes, extends within a short time over great longitudinal distances, but it cannot be detected in tangentially adjacent phloem (*23*).

The ease of longitudinal transport and the lack of tangential movement has been quantitatively demonstrated in still another way. Defoliation causes characteristic changes of the sieve-tube exudate, as described below. Defoliation of one half of a Y-shaped tree shows these changes to be very sharply defined all the way down the trunk (Fig. 6). There is a slight fanlike spreading of translocated material from the leafy side, but it follows an angle of less than 1 degree (*23*).

These results agree very nicely with phloem anatomy. The evolutionary trend is toward larger pores in the end walls (sieve plates), which may indicate increased conductibility (*7*). This emphasis on longitudinal translocation brings about a relative independence of the various sides of a tree. One side may die while the other side may survive for decades or even centuries. Or, in a tropical rain forest, one side of a tree may be dormant while another side is active.

The Mechanism of Translocation

The rate of phloem translocation is often extraordinarily high. According to Mason and Maskell it can be as much as 40,000 times the rate of sugar diffusion in water (*24*). The quest for the mechanism of this remarkable phenomenon has always greatly stimulated research. It appears to be clear that there are, in addition to rapid long-distance translocation in the phloem, other, usually much slower, types of transport in living tissues—movement in parenchyma cells (*25*), polar movement of auxins (*26*), secretion in nectaries (*27*).

Some workers regard long-distance movement in the phloem as a mass flow of solution; others visualize it as an active process involving translocation of solutes without solvent (water). Biddulph and Cory have recently even claimed two different transport mechanisms in the phloem, but their evidence is far from convincing (*28*). Whoever has been able to observe the rapid and consistent exudation from cut-off aphid stylets cannot doubt that we are dealing with a mass movement of a solution.

Theoretically, of course, stylet exu-

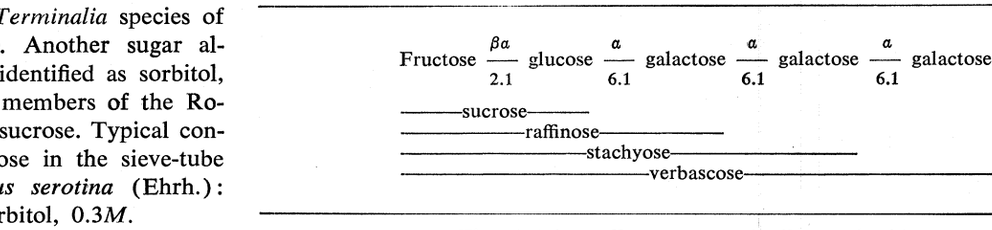

Fig. 5. The raffinose type of oligosaccharides.

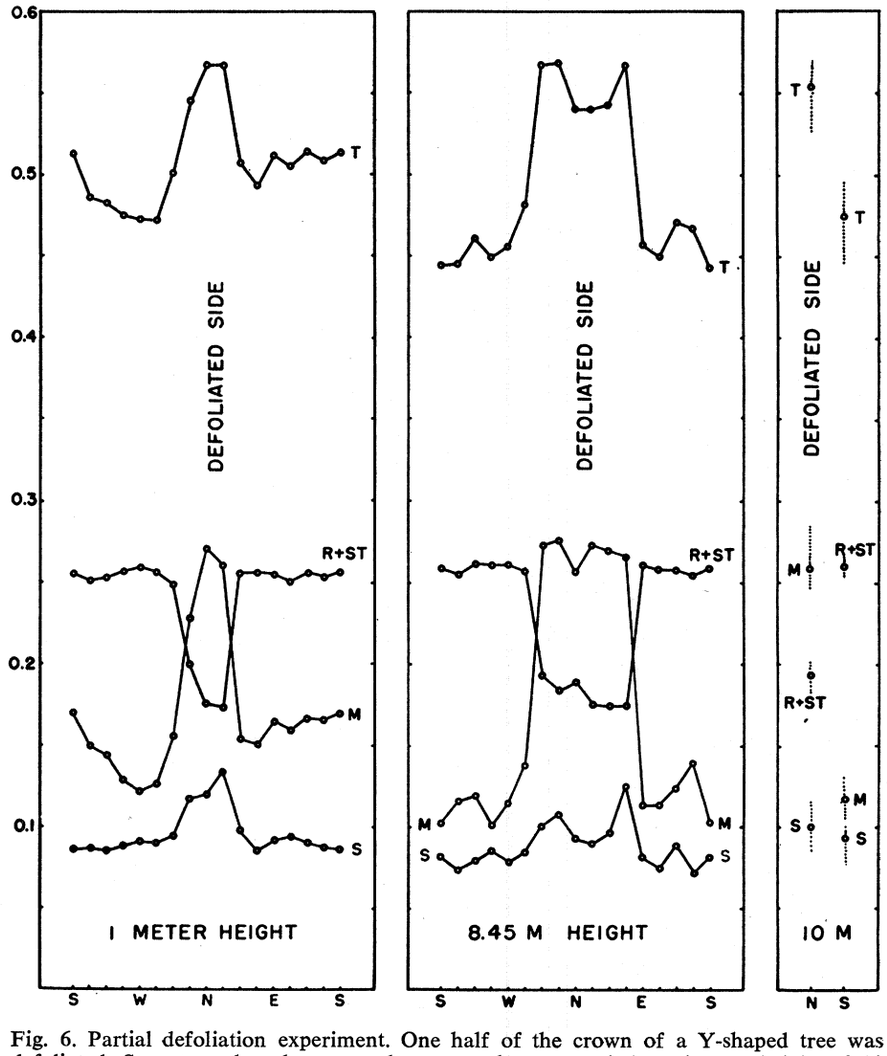

Fig. 6. Partial defoliation experiment. One half of the crown of a Y-shaped tree was defoliated. Seventeen days later samples were taken on each branch at a height of 10 meters, and around the trunk at both 8.45 meters (20 centimeters below the fork) and 1 meter. Molar concentrations are shown on the *y*-axis. *S*, sucrose; *R* + *ST*, raffinose plus stachyose; *M*, D-mannitol; *T*, total molar concentration. (From *23*)

dation per se is not proof of mass flow in the intact plant, because the stylets are artifacts. However, experimental work, by its very nature, involves artifacts, and all our knowledge about translocation is based upon indirect evidence. Rejection of the concept of mass flow raises the difficult question of how a single sieve element 20 to 30 microns in diameter and 0.4 millimeter long can be continuously refilled three to ten times per second with a concentrated sugar solution without any visible injury.

Gage and Aronoff have recently rejected the concept of mass movement because they found it difficult to get tritium water to move out of leaves together with labeled photosynthates (*29*). This experiment is by its very nature a difficult one to carry out, although Biddulph and Cory have been successful (*30*). It must be extremely difficult to introduce water experimentally into the phloem, because in doing so one has to compete with the natural source of water, the xylem, which is very intimately associated with the phloem. Even if tritium water were introduced successfully, a heavy loss must be anticipated because of diffusional exchange with unlabeled water of the surroundings.

The sieve-tube vacuoles seem to represent a metabolic pool which is kept within by the semipermeability of the side-wall cytoplasm. The term *leaking* has often been used to describe how solutes get from sieve tubes to the surrounding tissue. This term is misleading. The sieve tubes do not leak. They remain turgescent for days and weeks after defoliation of a tree (*31*). Entry as well as exit of solute is a metabolic process that appears to be under remote control from the leaves. In the presence of leaves, sugars are removed from the sieve tubes along the stem and roots at a rate equaling that of sugar entry into the phloem in the leaves. When the tree is defoliated the rate of sugar removal from the sieve tubes decreases within a few hours. After about two weeks a net re-entry into the sieve tubes of the whole stem becomes apparent (*31*). Figure 6 shows the situation in a Y-shaped tree 17 days after one half of the crown was defoliated. The two sides of the tree are clearly defined all the way down the stem, and it can be seen that the exudate concentration on the defoliated side is even higher than that on the leafy side.

What is the mechanism of this solute entry into and exit from the sieve tubes? We know very little about it. Defoliation experiments suggest that we are dealing with enzymatic processes. As soon as the source of solutes—the leaves —is missing, we find a rapid increase of sucrose at the expense of oligosaccharides. An α-galactosidase, therefore, must be one of these removal enzymes. This enzyme is not in the sieve-tube vacuole in solution; it is very probably attached to the sieve-tube cytoplasm and is in contact with the vacuole. There are two reasons for this conclusion: (i) the enzyme does not appear in the exudate; (ii) free galactose units do not appear in the exudate. Other enzymes, similarly placed, would be necessary to remove sucrose and mannitol (and all the other translocated substances) from the solution.

If we accept the idea that the sieve-tube vacuole is a metabolic pool, we still have to explain how this pool is moving. Münch postulated in 1930 that differences in turgor pressure are sufficiently great to account for mass flow (*32*). His original hypothesis included all living cells of the plant. Later on his theory had to be restricted to the sieve tubes of the phloem because it was found that substances are often secreted into the sieve tubes against a concentration gradient. Within the sieve tubes, however, concentration gradients have always been found to be positive in the direction of flow (*22, 33*). Trees are particularly suitable for such studies because of the great translocation distances through tissue under uniform conditions. The latter point is of considerable importance if one takes molar concentrations as a measure of probable turgor pressure. Tissues undergoing intense growth, such as shoot tips and young fruits, may well have a lower turgor than the exudate concentration would suggest. For example, if the phloem tissue is interrupted (if the stem is girdled) at one place on the tree trunk, growth along the stem between the live crown and the girdle will not be uniform any more, but instead there will be a very sharp increase of growth above the girdle. Concentration gradients in such a case can hardly be taken as direct indicators of turgor pressure. They are, indeed, difficult to interpret (*23*).

Studies of concentration gradients in trees in which several substances are being transported in major amounts are particularly interesting. In white ash the four substances stachyose, raffinose, sucrose, and mannitol make up the bulk of the exudate. During the summer one invariably finds that the total molar concentrations decrease in the downward direction of the trunk. After leaf abscission in autumn or after artificial defoliation at any time during the summer, the concentration of all substances drops, some of the individual gradients (often stachyose and mannitol) becoming negative and the others remaining positive. The result is the complete disappearance of the total molar gradient, indicating cessation of translocation (*22, 31*).

The exudate concentration decreases some 0.01 mole per meter in the downward direction of a normal tree during the summer. According to Poiseulle's equation, the pressure gradient to which this would correspond is fully sufficient to force the solution through capillaries of the dimensions of the sieve-tube lumen and the combined sieve pores at the observed rates. The difficulty is that the sieve pores are not open but are filled with cytoplasm. These so-called connecting strands often appear to be fairly dense in electron micrographs (*34*), but one should not forget that this density is considerably increased by growth of callose cylinders after wounding. Freezing the stem section on the intact plant (without wounding) is absolutely essential if connecting strands are to be studied by electron microscopy. The question of how much resistance the connecting strand represents cannot be answered at present, nor is there any way to calculate it. The suggestion has been made that electro-osmotic forces across the sieve plates may cause the solution to move (*35*). According to this theory the sieve plates would be not a passive resistance but the carrier of the electrical potential. Whatever may cause the solution to pass the sieve plates, we do know that it can pass easily. Mass flow of a solution is the only reasonable explanation for the refilling, three to ten times per second, of a sieve element with a highly concentrated sugar solution during hours and days of stylet exudation.

Evidence that exudate from bark incisions and aphid stylets is translocated material can be summarized as follows. Stylets, as well as incisions, whenever they are successfully placed, reach the conducting layer of the phloem. Living sieve tubes are the only continuous cell series that are unique for this layer. Stylet tips do end in a single sieve element, the cells that were identified as the channels of conduction by Schu-

macher years ago (*36*). Furthermore, the time during which the exudate can be obtained corresponds with the time when sieve tubes are not restricted by callose. When branches of certain species such as hickory (*Carya sp.*) are cut from the tree and brought to the greenhouse, large masses of callose form on the sieve plates. Significantly, aphids are unable to feed on these, although they feed well on the intact plant.

The composition of these exudates corresponds to what has been deduced from tracer studies to be the translocated material (*2*). Lack of tangential transfer, as revealed by exudate analyses (*23, 37*), corresponds with results of tracer studies (*38*) as well as with phloem anatomy. The measurable concentration response to phloem injury over great distances within a short time is also an indication that we are dealing with the transport system (*23*). The sharp drop in exudate concentration after defoliation corresponds with the estimated rate of turnover in the phloem (*31*). Pentoses, applied in agar blocks to the inner bark, are taken up and translocated downward and can be found in exudate there (*39*). During natural as well as artificially induced leaf senescence (yellowing), relatively large amounts of nitrogenous and phosphorus compounds are exported from the leaves (*36*). At such times these substances appear (or if already present, increase) in the exudate from stylets as well as from incisions (*16, 22, 37, 39*). Indeed, the list of evidence could be extended almost indefinitely.

Present and Future Problems

What are the present and future problems in research on translocation in trees? One of the most important aspects is the entry of solutes into, and their removal from, the sieve tubes. How do these mechanisms operate and what are the factors that control them? Of general physiological importance is the finding that sugar alcohols are translocated in certain plant families. Mannitol is often used as a metabolically inert substance for physiological experimentation. One has to be very careful because some plants do utilize sugar alcohols. In addition, the plant's ability to use them may be dependent on the presence or absence of the leaves.

At present, studies are in progress concerning the effect of locally applied temperature on translocation. The problem is complicated by the fact that any applied temperature is carried upward with the transpiration stream. For example, if the tree is chilled at a height of 5 meters, the cooled xylem water lowers the temperature all along the stem above 5 meters; the chilling effect thus remains by no means a local one. To bring about the desired condition, a heating tape above the place of chilling has to warm up the xylem water to normal temperature. Temperatures are measured on a multiple thermistor thermometer with insolated surface probes placed near the conducting phloem, and the voltage of the tape is adjusted accordingly.

From the viewpoint of practical forestry, phloem transport has an importance of which few foresters are aware. Most material that a tree utilizes for its growth is manufactured in the leaves. From there it moves downward through the conducting phloem, a layer only a fraction of a millimeter thick. Leaves as well as the vascular system are rebuilt every spring, beginning with the time of bud burst. For biology as a whole, the study of complete organisms is a necessary complement to cellular physiology, which has become increasingly important during the past decades. There are many problems that can be solved with plant parts or with seedlings in the greenhouse, but there are others that can only be solved with large and intact plants. Trees are ideal for such studies and will undoubtedly yield valuable information in the future.

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