KATHERINE ESAU

Plants, Viruses, and Insects

Distributed in Great Britain by Oxford University Press, London

Library of Congress Catalog Card Number 61–16692
Printed in the United States of America

Preface

The natural result of continued development of science and accumulation of knowledge is an increasing specialization of scientists. Specialization, however, limits one's outlook and sooner or later an investigator, in his effort to obtain a complete picture of the object of his study, finds it necessary to turn for assistance to branches of science other than that of his specialty. The need for utilizing methods and concepts from many sciences is particularly keenly felt by the biologist because he deals with life, the most complex object of study.

The history of research on movement of organic materials in plants provides an excellent example of integration of many aspects of scientific inquiry and of the benefits of such integration. In the following pages this history is reviewed, not in its entirety, to be sure, but with emphasis on the relation between the structure and function of the tissue—the phloem—concerned with this movement, and with reference to the behavior of the insects that feed on the contents of this tissue and of the viruses that cause its degeneration.

My interest in the phloem tissue has developed through studies of anatomic effects of plant viruses. These studies revealed the remarkable specialization of certain viruses in their relation to plant tissues and their apparent ability to utilize the food conduits of the host plants for their spread through the plant body. Plant viruses also utilize insects as hosts and these, in turn, have evolved into efficient carriers of viruses capable of placing them in those tissues of the plant to which the viruses are best adapted. These complex and fascinating relations constitute the theme of this book.

It was a pleasant task and a great privilege to have been given the opportunity to discuss Plants, Viruses, and Insects in the 1960 John M. Prather Lectures in Biology at Harvard University. The present published version of this theme includes the material given in the Prather Lectures and also that discussed in a colloquium and seminars held during my stay at Harvard. The pertinent literature that appeared after the Lectures were given has been reviewed.

I wish to thank the Committee on the Prather Lectures of Harvard University for inviting me to give the Lectures, and Professor Reed C. Rollins, Chairman of the Committee, for the warm reception he prepared for me at Harvard. The hospitality extended by the President and Fellows of Harvard College and by the officers of the Biological Laboratories is deeply appreciated. The privilege of having the present account published by the Harvard University Press is also gratefuly acknowledged.

K. E.

Davis, California
November 1960

Contents

Illustrations

Figures

Plates

Tables

1

The Early Explorations of Conducting Tissues in Plants

Among the various live activities in plants, the conduction of water and food has been the object of study by man since the beginning of biological research. As Münch (1930) has pointed out, the sustained attention given to questions of translocation in plants by botanists is not surprising: the ability of such a rigid organism as the plant to transport water and organic materials without any movement or any obvious mechanical device must have challenged generations of investigators in the past; and as we shall see in the subsequent pages the interest in the phenomenon of translocation has not abated to this day.

The research in translocation, like that in many other areas of plant study, was first stimulated and directed by the preceding research on the animal body. After the circulation of blood in animals came to be understood in the first half of the 17th century, particularly through the work of William Harvey, the idea arose that plants had a similar circulatory system, and the subsequent study of conduction of plant sap was long colored by this idea.

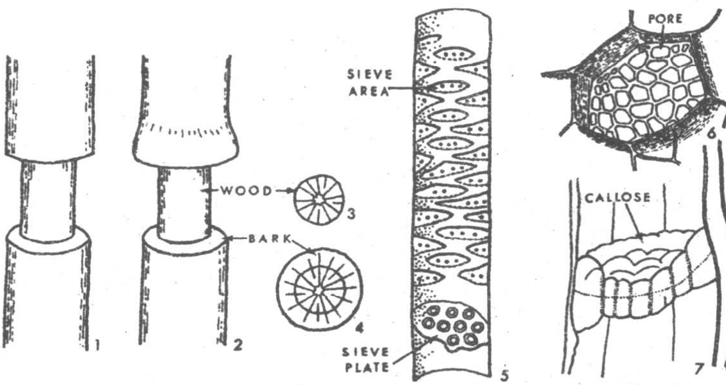
The chief contributors to the study of sap movement in plants and of the tissues concerned were, in the 17th century, two physicians, Marcello Malpighi (1686) of Italy and Nehemiah Grew (1682) of England. Working independently, they produced monumental works on the structure of plants. Indeed, Grew and Malpighi are often cited as founders of plant anatomy; but they also attempted to relate structure to function and, Malpighi especially, searched for similarities between plants and animals.

Malpighi and Grew did not conceive of the cell as a structural unit of the plant, but they distinguished between elongated structures, such as fibers, vessels, resin ducts, and laticifers (cells or vessels containing latex), and the small utricles, or cells, surrounding the long structures (Plate 1, *A*, *B*). Logically, Malpighi and Grew suggested that the sap was moving through long elements. They visualized a stream carrying the "raw sap" taken up by roots from the soil to the upper parts of the plant, that is, an ascending stream. In the leaves, bark, and wood rays the raw sap assumed a new composition as it became mixed with other cell substances, and in its long flow through the network of veins in the leaves it was reconstructed and refined through exposure to sunlight. The refined, or elaborated, sap served as a nutrient substance and was conducted downward, that is, it moved in a descending stream. The idea of the elaboration of sap in the leaves did not include the concept of photosynthesis; the role of air in plant nutrition had not been discovered at that time.

Malpighi and Grew carefully recorded their observations and conclusions so that it is possible to recognize what phenomena had led them to thoughts about the ascending and descending streams. Grew discussed the so-called bleeding of plants, that is, exudation from a cut in a plant of liquid derived from the wood. This phenomenon may be sometimes observed, especially in vines, when a strong positive pressure develops in the root. As mentioned, bleeding is revealed when a plant is cut, but the sap may exude through natural openings also. Such natural exudation is exemplified by guttation, a release of water from openings in the leaves, so common among herbaceous plants. Grew related bleeding to the wet condition of the wood in living plants and concluded that the sap moved upward in the wood.

The occurrence of a descending stream was demonstrated by Malpighi's ringing experiments. In these experiments two parallel cuts encircling the stem are made in the bark and the ringlike piece of bark formed by the cuts is removed from the stem (Figs. 1, 3, and 4). Malpighi noted the swelling of tissues above the ring (Fig. 2) and interpreted the phenomenon as new growth of wood and bark tissues stimulated by an accumulation of food coming down from the leaves and intercepted at the ring. It is instructive to read Malpighi's own description of his experimentation and reasoning. As freely translated, he said (Malpighi, 1686, vol. 2, p. 60):

Whether the sap flows from the furthest apices back to the lowest parts of the plant . . . is still not clear. Roots breaking forth from the extreme tips of their furthest branches bring the sap under a strain in an inverse direction [that is, inverse from that of the ascending stream] and prescribe a new route, for there are no valves inserted that would impose a determined motion. The things that I have tried on various trees shed some light, however. In various stems and branches I made horizontal sections in the cortex taking away an annular portion of the same and of the bark so that the wood underneath lay exposed. On the branches of *Prunus*, quince, *Quercus, Salix* [and others], after a circular section is made, the upper part of the stem soon grows above the section so that it is rendered much swollen, for the cortex . . . extends the horizontal rows of its utriculae [this apparently means ray cells] to such an



Figs. 1–7. (1–4) Diagrams illustrating ringing of a stem (removal of bark) and its effect upon the tissues above the ring; these undergo growth and thus cause an enlargement to appear above the ring (2). Malpighi (1686) was the first to employ this type of experimentation in the study of movement of sap and deduced that nutritive sap moved downward in the bark. (5) Copy of a drawing by Hartig (1837) showing a part of a sieve-tube member of *Acer.* Hartig used this drawing and others to document his discovery of the sieve element. (6 and 7) Copies of drawings by Hanstein (1864) of sieve plates of *Cucurbita pepo* without callose (6) and with a thick deposit of callose (7). Hanstein referred to the plate covered with callose as the "calloused plate" (*callöse Platte*) and thus introduced the term "callus" into phloem anatomy. He recognized the vertical lines traversing the callose (7) as canals corresponding with the original pores (6) in the sieve plate.

extent that frequently appendages are thrust forth with which the denuded portion of the wood is covered and, after mutual anastomoses are again made with the lower lip of the cut cortex, the cortex is rendered whole . . . But the exposed wood portion still stays slender, with no flourishing increase . . . The growth above the section makes it unbelievable that the nutritive sap is moving to the lower part: because the vessels of the cortex and bark are cut the nutrient cannot continue to move from above to the inferior parts.

Malpighi also studied the effect of ringing at different times of the year and found that no overgrowth was produced during the months of December and January.

Ringing experiments were resorted to by many subsequent workers and yielded much information on movement of materials in the bark (see Chapter 3). Malpighi's ringing experiments mark the beginning of study of translocation of organic solutes in plants.

Malpighi went beyond the recognition of a simply descending stream of food materials. He also visualized a movement of nutritive sap to the young branches and ultimately into flowers and fruits. Moreover, he suggested that lateral connections existed among the "nutritive-sap vessels," so that the sap did not have to flow always in one direction through a straight tube. He severed some veins in leaves of squash, lemon, and other plants and found that the tissue beyond the cut remained alive. From this result he concluded that nutritive material was supplied beyond the cut by lateral transfer.

Malpighi and Grew developed rather definite notions about the plant-tissue constituents concerned with sap conduction. As was mentioned before, they assumed that elongated structures were the conduits; but, in their opinion, not necessarily all such structures were primarily concerned with conduction. When Grew sectioned the wood he found air in the vessels, except at the times when bleeding occurred. Therefore, he concluded that the vessels conducted sap only when the latter was in excess, that is, when, in modern terms, root pressure was high and transpiration low; at other times the vessels carried air. The principal conduits of sap were thought to be the fibers.

To prove that vessels contain air Grew thus described what he saw:

> The Content of these Vessels, is, as hath already been intimated, more Aery. The Arguments for which, are, That upon a transverse Cut of the Root, the Sap ascendeth not there, where These stand. Being also viewed through a Microscope, they are never observed to be filled with Liquor. Besides a Root cut and immersed in Water, till the Water is in some part got into these Vessels, and then the Root taken out and crushed; the other Parts will yield Liquor, but These, only Bubbles: which Bubbles are made, by some small quantity of Liquor mixed with Aer, before contained in the said Vessels.

The idea that vessels were containers for air was reinforced by Malpighi's discovery of spiral wall thickenings in the vessels. When he observed the discharge of gases from plants cut under water (probably from intercellular spaces in parenchyma tissue), he associated this phenomenon with the occurrence of the spirally thickened wood elements, which he compared with the similarly sculptured tubes serving for gaseous exchange in insects and other animals (Plate 2). Malpighi named these tubes tracheae and later applied the term also to vessels in plants. We still use this term in referring to the water-conducting elements of the xylem as tracheary elements, but we teach that they are concerned with conduction of water and not with aeration.

Malpighi raised the question of the origin of the air "for breathing" contained in the vessels and argued that it must come from the soil and be taken up by the roots because the tracheae of roots are particularly large and constitute a substantial part of the root tissues; moreover, he remarked, the air moves more easily upward.

The development of the idea that conducting vessels are normally filled with air is easily explained. If the root pressure is low, the transpiration pull holds the water in the vessels under tension. This tension causes the water columns to break when the plant is cut and air to enter the vessels. Moreover, the early botanists did not appreciate that only the younger wood, the sapwood, conducts water. In the heartwood the vessels are normally filled with gases when they are not plugged with tyloses or various organic substances.

Many investigators continued to associate vessels with aeration throughout the 18th and part of the 19th century. Under the influence of Strasburger, however, the concept of vessels as water conduits became dominant toward the end of the 19th century. Strasburger (1891) repeated the experiments done by some earlier workers on injecting vessels at the base of a cut shoot with gelatin and similar substances and confirmed that the plugging of vessels was followed by a wilting of leaves. He also introduced dyes (eosin, methylene blue) into plants through the roots and saw them enter vessels and tracheids of the xylem; he found that the fibers were not involved in this transport.

Although generally adopted after Strasburger, the view that sapwood vessels are filled with water in an undisturbed plant has not remained unchallenged, even in our century. As recently as 1957, Lundegardh proposed that the medium-sized tracheids primarily carry the mobile water, whereas the wide vessels and tracheids have a double function, aeration and transitory storage of water. The prevailing view, however, is that the appearance of air in the sapwood vessels is an indication of an approaching transformation of sapwood into heartwood (see Huber, 1956*b*).

In Malpighi and Grew's time the descending stream of elaborated sap was thought to be moving in fibers, laticifers, and resin and gum ducts of the bark, although Malpighi must be credited with the notion that laticifers were not actually conducting but serving as temporary reservoirs. Most modern workers reject the thought that laticifers might be conducting elements.

The early 18th century witnessed a great advance in the understanding of movement of water and substances dissolved in it, that is, the ascending sap movement, and credit for much of this advance is due to Stephen Hales (see Reed, 1942). Hales attacked the problem by a combination of experiments, observations, measurements, and calculations. He attempted to relate mechanicophysical phenomena, as they were then understood, to the phenomena observed in plants. He designed experiments that demonstrated the force of suction in wood and roots and the presence of root pressure in a bleeding vine. He measured the transpiration and was the first to use the balance as an experimental tool. Hales, however, did not advance the understanding of movement in the phloem; in fact, he denied the existence of a descending movement of sap. He admitted only that the sap in the wood might possibly sink during the night because of the lowering of temperature, like mercury in a thermometer.

For further development of concepts of translocation of organic substances (the descending stream) we must turn to the 19th century (see Münch, 1930). New experiments with ringing, grafting, shading of foliage, and removal of leaves definitely singled out the bark as the principal pathway of food materials. At the same time, the understanding of the role of leaves in the nutrition of plants was deepened, especially after the appearance of De Saussure's decisive work on assimilation of CO_2 by plants. Also of great importance were Dutrochet's study of osmosis, which had a bearing on uptake and movement of water in plants, and the development of organic chemistry. The application of the new discoveries and concepts of physics and organic chemistry to plant physiology established beyond any doubt that the matter necessary for the formation of new tissues in roots and shoots, including buds, flowers, and fruits, originated in the leaves and had to be conducted to the growing parts from the leaves. In addition to the transport of assimilates formed in the leaves, the movement of hydrolysates derived from foods, such as starch, stored in various tissues, was recognized; and the main pathway of transport of both kinds of products was the bark.

The identification of the conduit of this movement in the bark at the cellular level also occurred at this time. In 1837 Theodor Hartig discovered the sieve element (Fig. 5) in the secondary phloem of woody species and concluded that not the fibers but the soft-walled elements with the sievelike perforations in their walls were the conducting elements. Thus, he introduced a distinction between the hard bast, the fibers, and the soft bast, the conducting elements and the associated cells of the phloem (see contrast between fibers and sieve elements in Plates 1, *C*, and 3, *A*). His descriptions are remarkably accurate in that they distinguish between elements that we now call sieve cells (relatively unspecialized sieve elements) and those referred to as sieve tubes (see Esau, 1960). In describing the sieve tubes Hartig did not clearly state that they consisted of individual sieve-tube members, as we do now, but he mentioned the sievelike transverse walls located at intervals within a sieve tube (Plate 3, *C*, arrows). These transverse (or oblique) walls occur, of course, where the individual sieve-tube members are joined together.

Hartig (1860) also discovered the remarkable phenomenon of exudation of sap from the bark of trees that occurs when the bark is incised with a sharp knife, deep enough to reach the active phloem (Fig. 12). The cause of this exudation is the high turgor pressure in the sieve elements, which forces the contents out of the tissue when the elements are opened. Hartig immediately suggested that the exudate had to do with the descending stream and by a microscopic study proved that the exudation came from the phloem tissue. He analyzed the exudate and found in it small amounts of ash and nitrogenous substances and up to 33 per cent of sugar.

Hartig's important discovery of exudation from phloem and his postulation of its relation to the movement of assimilates remained largely unnoticed for nearly 70 years. The great master of plant physiology, Julius von Sachs, a contemporary of Hartig, did not find Hartig's discovery in agreement with his diffusion theory of translocation and dismissed it with a few disapproving remarks (see Huber, 1956*a*). In fact, Sachs's adherence to the diffusion theory obstructed all fruitful research on translocation for some 60 years. Only his student De Vries had the courage to question the master's concept; he pointed out that movement by diffusion was 10,000 times too slow for the calculated rates of transport of assimilates and suggested the cytoplasmic streaming as the accelerating principle. Hartig's ideas first received due attention in 1930 when Münch developed his concept of pressure flow in the phloem (see Chapter 3).

In contrast to Hartig's physiological studies, his anatomical observations did not remain in obscurity. His work on phloem was followed by a series of perceptive studies by such well-known anatomists as Nägeli (1861), von Mohl (1855), and Hanstein (1864), so that in less than 30 years after Hartig's discovery of the sieve element the basic information on the structure of this cell became available. Nägeli proposed the use of the term *phloem* (from Theophrastus' *phloios,* bark) for the tissue concerned with conduction. Hanstein related the occurrence of internal phloem (which he discovered) to results of ringing experiments—in the presence of internal phloem ringing proved to be ineffective in interrupting the descending stream—and came to a definite conclusion that the tissue containing the sieve elements was concerned with translocation. He studied the development of callose on the sieve areas (Figs. 6 and 7) and introduced the term callus into phloem histology. He also cleared up the confusion that existed in the minds of investigators regarding the identity of laticifers and sieve elements by showing that the two were distinct structures derived from different meristematic precursors. In the summary to his work of 1864 he gave a remarkably modern picture of the role of sieve elements in the movement of assimilates. He wrote (translation by the writer):

> The sap in the wood rises to the leaves by root pressure. Capillarity and diffusion in the wood cells and the transpiration from the leaves assist the driving force derived from root pressure.
>
> In the leaves the finest spirally thickened xylem elements, the ultimate endings of which occur in parenchyma, deliver the sap to the parenchyma where the nutrients of the sap are combined with those derived from the air.
>
> The sap so changed arrives by osmosis in the sieve tubes, which occupy the lower side of the finest bundles.
>
> Wherever cells are formed, in the cambial cylinder, root tips, and buds, the plastic sap is utilized. The sieve tubes lie next to the cambium [Plate 1, *C*]. They give up their content [to the cambium] and replenish it through their long tubelike combinations [Plate 3, *C*] by new sap from the leaves. Every-