Botany. — *"Ringing Experiments with variegated branches."* By Prof. TH. WEEVERS. (Communicated by Prof. J. W. MOLL.)

(Communicated at the meeting of September 29, 1923).

For a long time already the transport of carbohydrates and proteins in plants has been considered as a question that seemed fairly set at rest. Of late years, however, the problem has again been brought into prominence.

The well-known ringing experiments, notably the extensive observations made in this field by J. HANSTEIN[1]) had settled the belief that the organic matter was transported along the elements of the phloem. It was left undecided whether the elements of the cribral system (sieve-tubes and companion cells) or those of the parenchymatous phloemsystem (cambiform cells) play the principal part. CZAPEK'S[2]) experiments favoured the first view, however, owing to the diametrically opposite conclusions of DELEANO[3]) a decision was impossible at the time.

The primary and the secondary phloem was generally considered as the passage for the conduction of the organic products, which, being formed in the leaves, have to be conveyed to the growing points and the reserve-organs.

In accordance with TH. HARTIG'S[4]) conception it was, however, generally received that in the early spring, when the woody plants start new shoots, the organic matter finds its way from the reserve-stores to the shooting parts through the xylem. This hypothesis was based partly upon the results of HARTIG'S experiments with ringed plants and partly upon A. FISCHER'S[5]) observations regarding the occurrence of carbohydrates in the wood vessels. Researchers refrained from approaching the question as to how this happens in shooting herbaceous plants.

Now the above theory has latterly been impugned from various quarters.

---

1) J. HANSTEIN, Jahrb. f. wiss. Botanik, 1860.

2) CZAPEK, Jahrb. f. wiss. Botanik, 1897.

3) N. DELEANO, Jahrb. f. wiss. Botanik, 1911.

4) TH. HARTIG, Bot. Ztg., 1858.

5) A. FISCHER, Jahrb. f. wiss. Botanik, 1890.

On the one side OTIS CURTIS[1]) made single and double ringing experiments and arrived at the conclusion that the transport of carbohydrates and proteins to the shooting parts may occur through the secondary phloem just as well as the transport in the opposite direction does, when the surplus of assimilates is removed from the place of formation. In my judgment, however, his view has not been sufficiently reinforced by indispensable quantitative examination.

On the other side it is ATKINS[2]) and DIXON[3]) in England, and LUISE BIRCH HIRSCHFELD[4]) in Germany who deny almost any significance to the phloem for the matter-transport. Their arguments consist in the main of indirect evidence. ATKINS argues that the bleeding saps are more or less rich in carbohydrates not only in spring but also in other seasons. LUISE BIRCH HIRCHFELD and afterwards DIXON base their most cogent arguments upon their belief that an adequate transport of matter along the phloem can hardly be presumed. This difficulty had already been obviated by HUGO DE VRIES[5]), who made a quicker transport than the law of diffusion admits conceivable by assuming protoplasm-streams in the phloem-elements. DIXON, however, considers the impossibility of a transport of adequate capacity along the phloem as conclusive evidence for denying any significance to the phloem in this respect. BIRCH HIRSCHFELD is less positive in her assertion.

That, beside an ascending stream in the wood, there may also be a coinciding transport along it towards the bottom of the stem, may be concluded from various investigations i.a. the above-named by L. BIRCH HIRSCHFELD. Then the rate of transport can be much quicker than in the phloem and the capacity of the conducting channels can likewise be greater, as it is a fact that the phloem-production of cambium is invariably smaller than that of the xylem, while the generated phloem is obliterated much sooner.

This conception of DIXON's, however, does not square with the result of the ringing experiments of HANSTEIN, which result points indubitably to the stream of assimilates being stopped when the ringing wound is made deep enough to reach the cambium. DIXON therefore assumes the transport to pass through the youngest parts of the secondary xylem, which parts being located close to the cambium, are by him believed to be injured and thus rendered inactive by the ringing.

[1]) OTIS F. CURTIS, American Journal of Botany, 1920.
[2]) W. R. G. ATKINS, Some recent researches in Plant Physiology, 1916.
[3]) H. H. DIXON, Pres. Address. Bot. Society, 1922.
[4]) L. BIRCH HIRSCHFELD, Jahrb. f. wiss. Botanik, 1920.
[5]) HUGO DE VRIES, Bot. Ztg., 1885.

To my knowledge this hypothesis has not yet been substantiated by experiments, so that it seems expedient to reconsider the question along what way the carbohydrates and the proteins are transported in plants.

The question can be approached from different sides; in this paper I will confine myself to a discussion of some experiments with ringed branches of variegated plants.

Similar experiments have been made repeatedly with green branches, but then the trouble is that after the buds have opened out, the younger parts above the ring begin to assimilate.

Stripping off the leaves or moving the plant to a dark space involves other difficulties; with variegated shoots it is much easier to state any supply of organic matter.

In consideration of DIXON's hypothesis due precautions should be used in the ringing and the protection of the injured part. A coating of melted butter of cocoa I deem more effectual than one of paraffin. It was applied to the wound at a temperature of 32°—33° C. and can hardly injure the exposed surface, as it does not penetrate into the intact cells. [1]) Moreover, it soon congeals and then affords sufficient protection against outside influences. The parts were then screened from immediate effect of the sun's rays in order to prevent melting.

We performed our experiments with variegated branches of Aesculus hippocastanum L. and Acer Negundo L. The former were derived from a stout specimen, whose green top provided the trunk with abundant food and from this trunk numerous yellow shoots had developed. In about 20 years these shoots attained a length of 1 M. and a thickness of 7—8 mm. in diameter. The specimen of Acer Negundo was provided at the top with green-white variegated leaves and developed from the main stem and side branches perfectly white shoots. In neither specimen did the yellow-white leaves contain any chlorophyl [2]). An iodine test pointed to the absence of starch.

In the spring experiments the branches were ringed (1—2 cm.) just before the buds began to open out and at a distance of 1—2 dm. below the end-bud.

Three series of experiments were always made at a time.

1st *series*: green shoots ringed all round.

2nd *series* variegated (yellow-white) shoots ringed all round.

3rd *series* variegated (yellow-white) shoots partially ringed, viz. so as to leave as trip of bark as a connecting link, 2—4 mm. in breadth.

---

1) R. H. SCHIMDT, Flora Bd. 74, 1891.

2) Guard-cells of the stomata excepted.

After rather more than a week a contrast was noticeable between the green and the partially ringed variegated shoots on the one side and the completely ringed variegated shoots on the other. The first two (1^{st} and 3^{rd} series) continued growing normally. The third (2^{nd} series) lagged behind and died off after 2 or 3 weeks, the leaves having previously shrivelled and dried up.

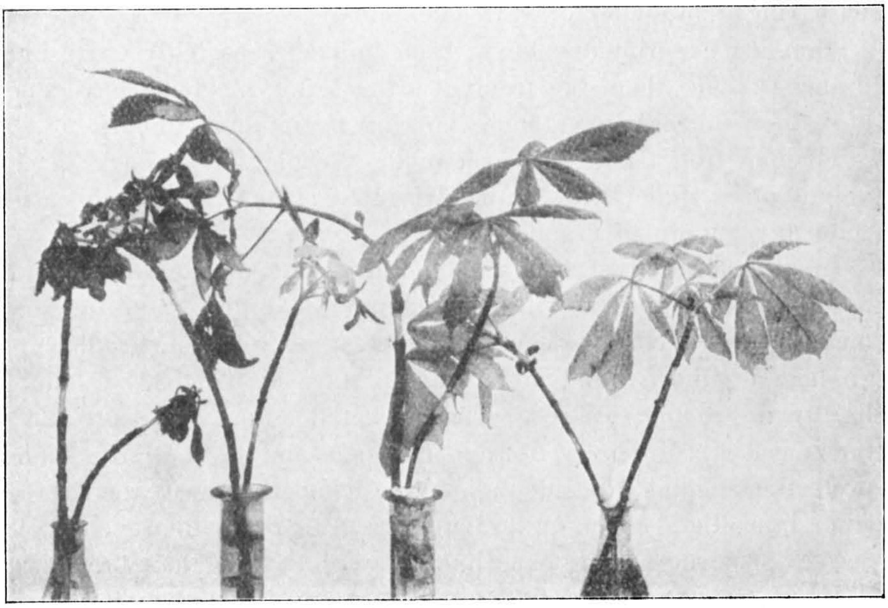

Fig. 1.

That ringing in itself did not injure the plant appeared distinctly from the results of the first and the third series. (See the photos): from left to right we see first 4 completely ringed yellow branches, some brown and dead, others small but still living; the next following are two completely ringed green ones and lastly to the right two partially ringed. The last four have developed normally.

It is clear that with the completely ringed green shoot the supply of water is normal; why then does the completely ringed yellow branch die off under symptoms that point to a deficiency of water?

The reason is obvious. In consequence of too little osmotic pressure the absorptive power of the tissues is too low as compared with that of the other parts.

The researches by DIXON and ATKINS [1]) on the determination of the osmotic pressure by lowering the freezing point of the expressed

[1]) Notes Botanical School. Trinity College Dublin, 1912.

sap, clearly show how the osmotic value of the leaf-cells increases with the possibility of assimilation.

Now I endeavoured to determine the suction force by URSPRUNG's [1]) method but the subject appeared to be difficult to experiment on.

A quantitative determination gave in the green leaves of Aesculus an amount of reducing sugars of 3 % [2]), in the variegated (yellow) leaves 1 %, in the ringed variegated (yellow) branches only traces. In general also the amount of extractable salts is trifling; in green and variegated leaves 0,9 % of the fresh weight [3]). SPRECHER finds in yellow varieties lower osmotic values for the cell sap than in the green specimens [4]).

True, the variegated leaves of the ringed branches of Aesculus contain from 18 to 20 % protein and 5 % dextrin (calculated at dryweight) but the influence of these amounts on the osmotic pressure is nothing to speak of. Yet this does not explain all, for in the variegated completely ringed shoots wood and bark above the ringing appeared to contain still a fair amount of starch (6 % of the dryweight, against 9 % in the partially ringed branch), while the leaves were already shrivelling.

Why this starch is not converted into sugar and why, when transported to the leaves, it does not raise the osmotic pressure has not yet been explained.

However this may be, the partially ringed variegated branches do not die off. It appears, then, that there the supply is not cut off and that consequently the young parts are provided with the nutriment that in the green ringed branches is produced by assimilation.

According to HANSTEIN the organic products are conveyed along the bridge of bark, but if this is the case, we must relinquish HARTIG's hypothesis that the transport is effected along the xylem while the branches are budding.

OTIS CURTIS (l.c.) does so and was led by his ringing experiments to regard the phloem exclusively as the path, along which the saps

---

[1]) URSPRUNG, Ber. d. d. bot. Ges., 1918.

[2]) Strictly speaking 2 % and 1 % reducing sugars derived from glucosids (calculated at dry-weight).

[3]) The starch determinations were performed by putting the pulverized material immersed in water for 3 hours into an autoclave at 4 atm., and by subsequently boiling the aequeous extract with diluted hydrochloric acid during 60 minutes.

Plasmolytic experiments are objectionable on account of the osmotic pressure in the various cells being unequal. Still, a 10 % saccharose solution plasmolyzes the variegated Aesculus-leaves, not however the green ones.

[4]) A. SPRECHER, Rev. Gen. Bot. 1921.

are transported. From DIXON's point of view, however, it might be objected that in CURTIS's experiments the peripheral woodlayers were injured and thereby the transport along the peripheral xyleem had been suspended indirectly.

This objection can hardly be raised against the above experiments, in which a coating of butter of cocoa was spread on the injured part.

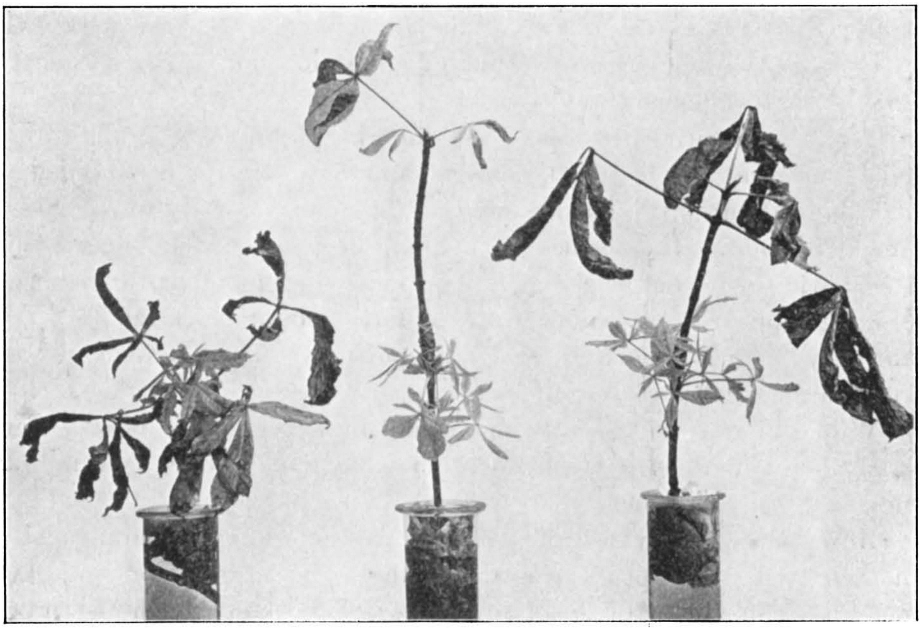

Fig. 2.

Moreover, another series of ringing experiments was carried out. In these experiments the ringing was performed as much as possible aseptically by previously washing the branch bark with 96 °/₀ alcohol and then peeling it off aseptically down to the cambium. Subsequently the decorticated surface was covered with sterilized absorbent cotton wool saturated with water; finally the whole was wrapped up with wax taffeta.

These experiments were carried out mid-June in the same way as the others described above, and yielded after four weeks an unequivocal result in connection with the midsummer growth which was very abundant, especially in Aesculus.

With the normal yellow variegated shoots the formation of midsummer growth occurred at the top of the branch and the yellow young leaves contrasted sharply with the others, which had been damaged by the high wind and browned by the sun. (See photo).

It appears then, that here also the yellow leaves suffer under a deficiency of suction force, and under circumstances brought about by stronger evaporation are sooner destroyed than the green ones, although the latter evaporate comparatively more intensely.

With partially ringed variegated shoots the midsummer growth occurred also at the top. With completely ringed specimens, however, it appeared *below* the surface of the wound from lateral or dormant buds. (See photo). This occurred as well when the surface of the wound was covered with butter of cocoa, as when it was dressed with a water-bandage.

The check to the food-supply is apparently as great with Aesculus as with Acer Negundo, in spite of the greatest precaution used in cutting the ring. It follows, then, that the experiments do not yield any evidence whatever, to lend support to DIXON's theory. They rather go against it.

Still conclusive evidence to disprove DIXON's theory cannot be brought forward by this procedure, since in spite of all due precaution the peripheral wood may be prevented by the ringing from performing its function, as far as the transport of the organic products is concerned.

With regard to other inquiries, whose results tell strongly against DIXON's theory, we first of all have to think of HANSTEIN's experiments (l.c.) on the root-growth of ringed branches in water culture.

HANSTEIN finds that detached branches placed in water send out roots chiefly at the basal extremity of the stem, which VÖCHTING ascribes to the polarity of the parts. Leafless branches when ringed develop a large number of roots just above the wound; whether and to what number they will grow at the bottom of the branch, depends on the distance between that extremity and the ringing.

HANSTEIN ascribed this to the check to the transport of nutriment consequent on the removal of the phloem, and established, indeed, in such circumstances a distinct difference in the root-growth, between dicotyledonous plants with an anomalous stem-structure and those with a normal stem-structure, in which the stem derived its thickness form a ring of collateral vascular bundles. With the former the transport of carbohydrates and proteins is believed to be only partially checked. This is ascribed to the fact that the vascular bundles are contained within the xylem (as with Piperaceae and Nyctaginacea) or (as in the case of Apocynacea and some Solanacea) to the fact that there are originally bicollateral vascular bundles or rather medullary phloem strands and consequently phloem remains also within the secondary xylem. Owing to this HANSTEIN stated in this case only a very slight influence of the ringing upon the root-growth.

This evidently does not fit in with DIXON's view; if the transport is effected along the peripheral parts of the xylem, ringing must in these plants have the same effect. It struck me, therefore, that it would be worth while to repeat some of HANSTEIN's experiments. The Solanacea Cestrum aurantiacum proved to be an unsuitable subject since detached branches sent out roots very sparingly in water culture, but Nerium Oleander yielded quite satisfactory results: all the twelve cuttings presented an aspect, quite in harmony with HANSTEIN's description. The root-growth may be somewhat more abundant above the wound, but the behaviour is quite different from e.g. that with Salix and Cornus spec. In these the roots appear almost exclusively above the wound, unless the stem-piece below it be very long, and the once formed roots are even destroyed when the bark above them is stripped off.

Provisionally all this tells very strongly against the validity of DIXON's conception of a transport of the carbohydrates and the proteins along the peripheral xylem.

If the above-discussed experiments with variegated shoots could also be made with variegated Oleanders, the medullary phloem of these plants would probably cause a quite different result from that yielded by Aesculus and Acer. But unfortunately variegated Oleanders I had not at my disposal, so that now I made a trial with ringed, normal shoots, which, while still attached to the plant, were wrapped up in black paper. The result was rather conclusive. Although some leaves had fallen off, the shoots themselves were still alive ten weeks after the ringing and had increased in length.

We see, therefore, that not only in the formation of the roots of branches in water-culture but also in the budding and the growth of Oleander Aesculus and Acer Negundo in spring, the results of our experiments with ringed branches imply a transport along the phloem.

In a subsequent publication I intend to discuss the question whether the capacity of these paths is sufficient.

For the present the above observations on Aesculus and Acer Negundo, where the detached branches did not bleed, are not applicable to the cases in which this bleeding is so copious, and as with Betula alba the highly sacchariferous sap is exuding directly after the ringing[1]).

[1]) The cases described by MOLISCH, (Bot. Ztg. 1902) as wound-reaction with local bleeding pressure, are of quite a different nature; then the bleeding pressure manifests itself only after days or weeks.