Botany. — *On a substance, causing rootformation.* By F. W. WENT. (Communicated by Prof. F. A. F. C. WENT.)

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The following paper will give a preliminary account of investigations, now being made as to the development of roots on cuttings of *Acalypha*.

In former publications (1926, 1927) I proved the existance of a substance, that causes the growth in the coleoptile of *Avena sativa*, by extracting it; with this extract I could induce growth at will. There are several reasons to assume the existance of an allied substance, causing the development of roots. SACHS (1880) was the first to point out, that the phenomena of rootformation in plants could be best explained by the assumption of a specific substance, formed in the sprouts of plants. This substance, normally transported towards the base, caused the appearance of roots in cuttings, as it could not be conveyed further downwards, and was accumulated in their base.

An excellent survey of the literature dealing with rootformation in cuttings is published by VAN DER LEK (1925). But even more important are the description of his own experiments and the theoretical conclusions, drawn from them. Having taken his accurately explained conclusions as a startingpoint for my own expositions, they are summarised here. The rootformation in cuttings of *Salix, Populus, Ribes* and *Vitis* is largely dependent upon the existance of buds, especially on strongly sprouting ones. Roots can be produced by cuttings with all buds removed, but they are less abundant and smaller than in cuttings with their buds present. Thus VAN DER LEK assumes, that one or more hormones are formed in sprouting buds, which are transported in basal direction through the phloem and stimulate the rootformation. In the case of abundant rootformation in cuttings with excised buds, VAN DER LEK was able to demonstrate the existance of rootgerms, which are likely to develop without the above-mentioned hormone. These rootgerms also effect the deviations from the polarity-rules.

I began my investigations on the development of roots on woody cuttings of *Acalypha*, mainly *A. Wilkesiana* f. *triumphans*, based on the above-mentioned results. This decorative shrub, with redspotted leaves is often cultivated in gardens in Java; my material was grown in the Botanic Gardens at Buitenzorg. Cuttings of this plant, with their base placed in water or sand, will form a good many roots, mostly arising near the base.

I began to investigate the effect of removing either the leaves or the buds, or both. For this purpose I placed each cutting in a cylindric glass

of water so that its base was submerged for about 5 mm. In the cuttings with both leaves and buds, roots soon appeared in abundance within 8 days. But also those with only their buds left soon formed a good many roots. The budless but leaved ones produced less roots, and when both buds and leaves were removed, the rootformation was nearly suppressed. And the roots which appeared after all were thin and did not branch. A series of cuttings proved, that even old leaves are able to stimulate rootformation.

As I observed above, even the bud- and leafless cuttings will produce some roots. Before using them as indicators for a rootproducing substance — if at all present — I had to investigate whether the appearance of these roots had to be understood as an aftereffect of the leaves and buds, or as an automatic production of the stem (e.g. rootgerms). To this end I removed all leaves and buds from a forked branch. Half (*a*) of the fork was left on the plant, the other half (*b*) being cut off and placed with its base in water. Five days after both parts of the fork were cut into pieces of 7 cm length and placed with their bases in water. Within 10 days branch (*a*) had produced 0.36 roots per cutting, branch (*b*) 1.63 roots. The factor influencing rootformation had almost disappeared from branch (*a*). Further experiments proved, that in most cases the building of roots is completely suppressed by taking cuttings from branches, that have been left unleaved on the plant for a week. The assumption is obvious, that the rootproducing substance can only be transported within the living tissues. Once a branch has been cut off and placed in water, this substance will not diffuse in the water. So the quantity of the substance present in a cutting will demonstrate itself quantitatively by the number of roots produced.

Of course the question now arises, whether the rootproducing substance can be isolated from the leaves or buds and demonstrated directly by rootproduction on the above-mentioned indication-cuttings.

When using stems, isolation of the substance is impossible, since it does not diffuse outside them. However, considering the fact that *Acalypha*-leaves cut-off never produce any roots, it might be possible that this was caused by diffusion of the substance from the section surface into the surrounding water. To this end I placed a good many leaves with their stalks in a small quantity of water. To prevent evaporation, they were covered with a globe. Every day the water was boiled down at low pressure and replaced by fresh water to repress bacterial development. The concentrated juice was mixed with an agar-solution of 3 % at 60° C. and cast in a thin layer.

A series of cuttings from one branch was prepared in the usual way. To eliminate the differences in rootformation of the higher and lower part of the branch, the cuttings were used alternately as controllers and indicators. The controllers (*c*) were left untreated, whereas the indicator-cuttings were divided into 2 groups; on one of them (*a*) were grafted young *Acalypha*-sprouts, usually with 2 leaves, on the other group (*b*) I laid a layer of agar with the extract from the leaves lengthways after

removing the cork. After 2 weeks the first roots appeared in groups (*a*) and (*b*) ; and 19 days after the beginning of the experiment the successful grafts of group (*a*) had 5 and 7 roots, the 3 cuttings of (*b*) possessed 1 root each, and group (*c*) (4 cuttings) had not formed any root at all. From this experiment we may conclude that a rootproducing stimulant was added to groups (*a*) and (*b*). In (*a*) it was the presence of the sprout, which led the stimulus across the graft-surface, and in (*b*) it is clear that this stimulus is a substantial one. But we also see that the substance is obtained in very small quantities only in the described way.

As the growth-promoting substance is not specific at all, it was probable that the rootproducing substance was not specific either, therefore I treated leaves of *Carica Papaya* in the same way as I described above for *Acalypha*. The extract was also mixed with agar. A series of cuttings was prepared in the usual manner and divided into 3 portions. One (*c*) was treated with pure agar, a second group (*b*) was provided with the extract of *Papaya*-leaves, and the third one (*a*) with the extract of *Acalypha*-leaves.

Group:	(*a*)	(*b*)	(*c*)
Number of roots within 10 days	0 1 1 1 2 2 2 2 3 6	3 4 5 10	0 0 0 0 1 1 2 3 3
average value	2.0	5.5	1.1
Number of roots within 17 days	0 1 1 2 2 3 3 3 5 6	5 5 8 10	0 0 0 1 1 2 3 3 3
average value	2.6 ± 0.6	7.2 ± 1.4	1.4 ± 0.45

The top figures in each column represent the actual number of roots, produced in each cutting ; the lower ones the average number of roots in one cutting. This proved, the material used for the cuttings was not quite free from rootproducing substance, as even the controls (*c*) had formed some roots. So it is not certain whether the difference 1.2 ± 0.7 between (*a*) and (*c*) is real ; however, it is just the same as in the first experiment (also 1 root). For the group (*b*) it is quite certain that the rootformation is stimulated (difference between (*b*) and (*c*) = 5.8 ± 1.5) ; the root-producing substance from *Papaya*-leaves therefore promotes the root-production in *Acalypha*.

Considering the experiments of Miss SEUBERT (1925), who demonstrated the presence of a growthpromoting substance in diastase, I tried the rootproducing effect of it. The process of extraction of this substance gave rise to the idea that it would have an influence upon the rootformation as well. Of the many experiments, all with positive effect, I will mention only the most striking one.

A new series of cuttings was prepared from a branch, the leaves of which had been taken off 2 weeks before. They were numbered consecutively from 189 up to 211, 189 being the basal one. A week after, the cork was scratched off from the upper half of all cuttings, so that the phloem lay bare. Now every third cutting was used as a controller, and a layer of pure agar was laid on the bare phloem of each (189, 192, 195 etc.). On a second group (190, 193, 196 etc.) a layer of agar, mixed with 5 % diastase at a temperature of 60° C., was laid and on the remaining ones (191, 194, 197 etc.) agar with 5 % diastase, boiled for 20 minutes, was placed. 4 Days after not a single root had developed. But after another 3 days all of a sudden roots appeared on the cuttings with diastase so that the state was : controllers no roots at all, diastase 60° 11 roots, diastase 100° 9 roots. 10 Days after the placing of the agar the controllers still did not develop any roots, the other groups both had 30 roots. The next day a photograph was taken of the series 189 to 201 (see figure 1), as the higher numbers had not formed any roots, nor even those with diastase. In the controllers the first root appeared 13 days, the second one (on another cutting) 23 days after the beginning. After the typical sudden outburst and growth of roots from the 7th till the 10th day, the growth was almost completely inhibited ; the roots hardly reached a length of 30 mm. and did not branch at all, even not after 23 days.

The conclusions to be drawn from this experiment are :

1. A rootproducing substance is present in diastase.
2. It is not the diastase itself that has the influence on the development of roots, as a heating to 100° C. is not injurious at all.
3. The different zones of a branch have a very marked difference in rootformation, due to internal causes.
4. Considering the fact that the water in the cylinders, containing the cuttings 202—211, showed a fairly marked development of algae, we might suppose that the difference in rootformation is due to increased exosmosis in the apical zone of the branch.
5. The action of the rootproducing substance mainly consists in the starting of new roots ; hence there is a considerable inhibition-time between administration of the diastase or any other rootproducing substance and the macroscopical manifestation of the first roots.
6. The placing of the roots, all very near the basal section-surface, shows that they do not arise from rootgerms, which are placed in *Acalypha* similar to those in *Salix amygdalina* (see VAN DER LEK 1925).
7. In this case the rootproducing substance is not specific either, as diastase is prepared out of germinating barley-corns.
8. Not before a considerable time, unbudded and unleaved cuttings will form again a fairly small quantity of the rootproducing substance.

As I concluded in 5, only the first stages in the development of roots are stimulated by the substance, just now investigated. In the normal plant there must be an other stimulus, causing the elongation of the roots.

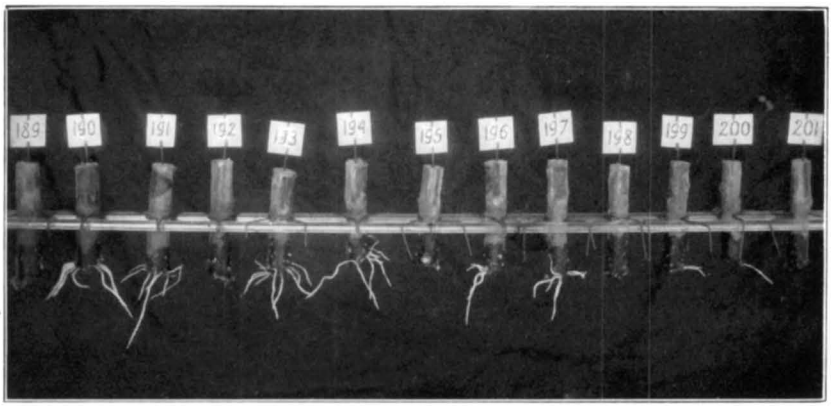

Fig. 1. Cuttings from one branch; 190 fits to the top of 189 etc. 189, 192, 195, 198 and 201 are treated with pure agar; on 190, 193, 196 and 199 agar with diastase heated to 60° is placed, and on 191, 194, 197 and 200 agar with diastase heated io 100°.

Photograph taken 11 days after the treatment.

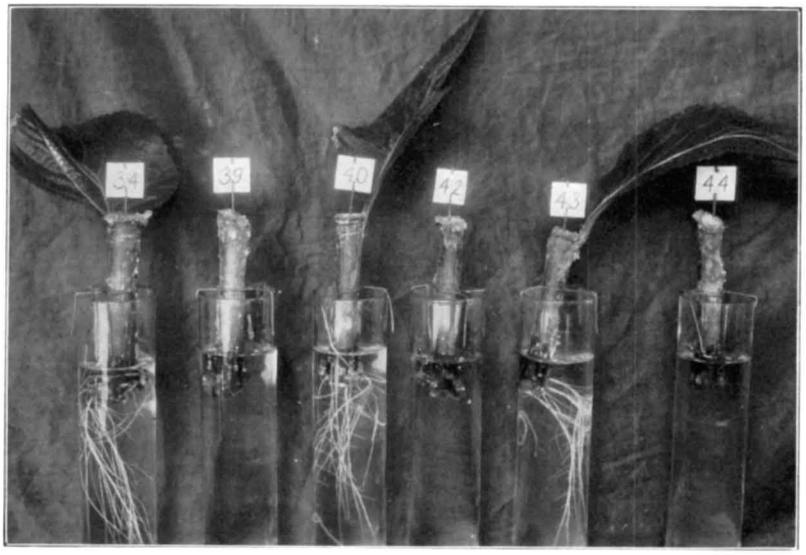

Fig. 2. Cuttings from one branch; 39, 42 and 44 controllers left untreated; 34, 40 and 43 grafted with one leaf each.

Photograph taken 24 days after grafting.

Experiments of ROBBINS (1922*a* and *b*) have shown, that this stimulus is probably a special substance, present in a considerable amount in the tips of germroots of different plants, and that it is used up whilst growing. If he autolized yeast or peptone added to his cultures of excised roottips, the growth was continued for a considerable time longer than without the addition of yeast.

In my experiments this stimulus was exercised by leaves. In a series of cuttings (31—44) the controllers formed no roots or only some very poor ones, which did not develop any further. On some cuttings a leaf of an allied variety of *Acalypha* was grafted. After 12 days the first roots appeared in these cuttings and after another 12 days a photograph was taken (figure 2). At this time the grafted cuttings had many strong well-branched roots, mostly arising from the side of the base that was just under the leaf. The thickening of the bases of the cuttings is due to excessive development of the lenticels, which is quite apart from any rootformation.

The results described in this paper can be summarized as follows.

A special rootproducing substance (hormone according to VAN DER LEK), not specific, and heat-resisting, is shown to be extractable from leaves and germinating barley, and to have the effect of starting the development of new roots. It seems to be transported by the phloem and is formed in leaves and sprouting buds, and occurs in considerable quantities in the branches. If no new substance is formed, it is removed from the branch within a week when attached to the plant. However, it does not disappear from cut branches, nor from cuttings from the basal part of them, on account of which the latter are useful as indicators of the rootproducing substance.

A full account of these experiments will follow later.

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