Botany. — *Cambial Activity as Dependent on the Presence of Growth Hormone and the Non-Resting Condition of Stems.* By CORNELIA A. GOUWENTAK. (Communicated by Prof. A. H. BLAAUW.)

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Introduction.

Under natural conditions in springtime a "stimulus" emanating from the buds is required for the growth resumption of the cambium. In diffuse porous wood this occurs when the buds are sprouting (JOST 1891, 1893). In ringporous wood, as e.g. in Fraxinus, the "stimulus" already travels downward before the buds are visibly swollen (COSTER 1927).

Since these publications it was suggested many times (vide SNOW 1933 and COSTER 1927) that the activation of the cambium is due to hormones. Direct evidence for this view was brought forward by attacking the problem along two different lines of investigation. In the first place it was tried to extract a hormone from buds and cambium and the effect of this extract on cambial activity was tested and in the second place the effect of applying pure growth hormone to the cambium was studied. Both questions have been investigated in the last five years.

AVERY, BURKHOLDER and CREIGHTON (1937) diffused buds and shoots of Aesculus and Malus on agar plates and afterwards found the agar to contain a growth hormone, the amount being proportional to the amount of xylem formed. ZIMMERMANN (1936), also with the Avena technique, showed a growth hormone to be present in growing shoots of Aesculus. SÖDING (1937, 1940) found growth hormone in strips from cambium during spring and early summer. That the hormone present in buds is able to initiate the production of new xylem was shown by the present author (1936); an extract from artificially awakened buds of Fraxinus Ornus obtained by extraction with chloroform after THIMANN (1934), caused the production of new xylem in shoots of the same tree. The amount present in one bud under favourable conditions is sufficient. SÖDING (1940) reports that even a smaller quantity may be effective (extraction by diffusion in agar). Nevertheless in the present state of research an applying of larger amounts is to be preferred, as they are more sure to act.

Artificially applied heteroauxin or its sodium salt proved to be able to activate the cambium into production of new xylem in herbaceous plants (SNOW 1935) and in trees and shrubs (GOUWENTAK 1935, 1936, SÖDING 1936, BROWN and CORMACK 1937), but only if the hormone is applied to the cut top surface of a decapitated shoot; an uptake from base to top does not take place (GOUWENTAK and MAAS 1940).

Whereas all workers in this field only obtained a local effect of the growth hormone, since the production of new xylem never lasted more than about two inches down below the terminal application, GOUWENTAK and MAAS (1940) report to have seen in early spring 1939 new xylem to develop down the whole length of shoots of all trees tested: Fraxinus Ornus L., Populus nigra L. and Salix fragilis L., shoots of which were treated apically with an amount of 100 γ heteroauxin or its sodium salt after the Laibach lanolin-paste method. Yet the time of application and the concentration of growth hormone were the same as in previous similar work from the same laboratory and from workers in Canada and in Germany; the experiments lasted as long as before. So there must be a general cause for so different a result.

As the whole treatise of the shoots had been the same in 1939 and in our previous work, this cause had to be found in the shoots. Thus we supposed that growth hormone can only induce new xylem when the cambium is not dormant. The extremely cold winter 1939/1940 might have favoured an early rest breaking, hence the shoots might have emerged from rest very early, whereas in previous experiments of GOUWENTAK, SÖDING, and BROWN and CORMACK the shoots were likely to have been still at rest. The fact that also some new xylem is formed in the neighbourhood of the cut surfaces of resting shoots must be ascribed to the action of wounds, a wound evidently having locally a rest breaking effect. A strong evidence of the hypothesis that a wound locally shortens the rest period may be seen in the experiments of BROWN and CORMACK (1937). These authors found new xylem not only near the treated upper surface, but also "in relation to a bridged ring some considerable distance below the point of application of the heteroauxin. The response at the wound was distinct and separate from the response in the region of application of the heteroauxin, since in the intervening distance no cambial activity had occurred".

Thus experiments of our own and historical data readily corresponded to our supposition, that a non-resting condition of the cambium cells is required, before wood can be produced. As the results of our previous investigation were not known before the season was too much advanced to test the supposition experimentally, the work had to be resumed in winter 1940/1941. The hypothesis was tested by applying growth hormone on resting shoots and on shoots that were first treated with a rest breaking substance. At the end of the experiments the cambium of these latter shoots only, showed to have produced wood throughout their whole length.

For this investigation it was necessary to have dormant shoots available during winter. Of course, we could have cut them from the tree, as soon as they were wanted, so long as they were dormant out of doors, but as it was apparent from previous work that an extremely cold winter might have a rather shortening effect on the resting condition of the cambium, shoots were cut in autumn and stored indoors. Part of them were stored with buds untouched, another part was fully disbudded before storage, for the following reasons. To store only shoots with buds was not advisable because they might start sprouting during storage and thus become useless for the purpose. To store only disbudded shoots was not advisable either, because it was not known beforehand that dormancy is also localised in the cambium itself and that it can be broken by treating disbudded shoots with a rest breaking substance.

The wood produced in our shoots under different conditions regularly shows differences in structure which obviously correlate with inner factors and therefore induce to distinguish several stages of rest of the cambium-cells (p. 660).

Material and Methods.

The material used in these experiments is Fraxinus Ornus. At the end of the growing season, in 1939 medio October, in 1940 on the 27 September, about 150 shoots were cut before they had been exposed to night frosts. The shoots were one to three years old and measured 15—23 cm. After the removal of the leaves, they were divided into two groups and put into boxes under moist sand, one group with buds untouched, the other one completely disbudded. The boxes were placed in storage in a dark unheated closet, partly situated underground, where for a long time temperature remained much higher than out of doors. When in December 1939 the temperature decreased to + 9° C, the boxes were transferred to a room of constant temperature (+ 16° C).

Emergency of rest was caused by treating the shoots with ethylene chlorhydrin. This was performed either by inclosing the terminal bud and as little as possible of the adjacent shoot in a test tube with one drop of a diluted solution and sealing the openings with modeline (DENNY and STANTON 1928), or by exposing entire shoots to the vapour in a glass container. Since both methods proved to be equally good, the latter was always used in later experiments, as it saves time. After a given period, varying from 24—48 hours, the shoots were removed and either immediately treated with hormone or allowed to stand in the greenhouse for some days. The growth hormone used was heteroauxin or its Na-salt.

The growth hormone paste (or water paste for the checks) was applied apically; the removed tips were fixed as a record of the cambium conditions at the beginning of the experiment. Now all buds were carefully removed and the shoots placed with their bases in water in a warm greenhouse, where they were kept for about a month. Regeneration of buds was not frequent; those buds were picked off as soon as they were recognised. Waterpaste checks which regenerate buds much more readily, showed that in this stage they do not affect the results.

At the end of the experiments the shoots were either immediately examined microscopically or cut into pieces and fixed in a mixture of formalin and alcohol 70 %. Microtome sections were made at different levels, in order to examine to what distance below the treated surface the cambial activity had travelled down.

Experiments and Results.

As in good reason the shoots could be expected to awaken sooner or later without treatment, all experiments were to be finished within as short a time as possible. The experiments began medio Januari 1940; medio February some buds of the non-treated shoots in storage started to develop etiolated shoots and so put a stop to further research. Thus all experiments of early spring 1940 were taken within a month.

Because of the chance that the growth hormone would be only effective if applied after a fixed period after forcing, it was advisable to apply the hormone at different intervals after ethylene chlorhydrin treatment; for these intervals 0, 3, 5 or 7 days were chosen, during which the shoots stood in the same greenhouse where they were placed after the growth hormone treatment, temperature varying between 20—27° C.

The experiments with shoots "stored disbudded" included 46 units. The wounds caused by disbudding had been healed during storage. From one half of the number of shoots the wound at the apical end was opened again before treating with ethylene chlorhydrin, the other half was treated without this operation. In some cases the vapour had a deleterious effect, the number of shoots that died being larger in the operated than in the non-operated shoots, viz. 15 and 8 respectively. So the experiments of this group were carried out with 23 shoots. In each of the experiments one part of the shoots was treated with growth hormone, the other part with waterpaste.

TABLE I.

Forcing treatment from	Time between forcing and growth horm. on	Number of Shoots treated with		Nr. of Exp.	Results of	
		gr. horm.	water		growth hormone	water
19—20 Febr.	0 days	3	3	1 a b	2 shoots: early wood 1 ,, : large cambial layer	no wood
2—3 Febr.	3 days	3	3	2 a b	2 shoots: transition wood 1 ,, : early wood	no wood
16—18 Jan.	5 days	4	3	3	All shoots: early wood	no wood
16—18 Jan.	7 days	3	1	4 a b	2 shoots: no wood 1 ,, early wood	no wood

Table I shows that the growth hormone is able to activate the cambium in producing new early wood. The wood was formed along the whole length of the shoots. In most cases this new xylem was early wood of the same kind as is produced under natural conditions. In some cases the new wood was intermediate between early wood and late wood ("transition wood"). Only one single shoot had formed nothing but a thick cambiumlike layer, and two other shoots had not produced any wood at all. A possible explanation of this fact will be given after the statement of all results.

Waterpaste had no effect at all, thus proving that ethylene chlorhydrin is not able to induce cambial activity when applied alone, but only in cooperation with growth hormone.

But for shortness of material each set of experiments should also have included a group of shoots treated with growth hormone without having been forced, thus showing if the material was still dormant. Therefore this check experiment was only made at the end of the season, on the 19 February 1940 with 3 shoots. The shoots proved to be dormant.

The experiments show, moreover, that the cambium itself is dormant and that its dormancy can be broken by direct treating of the shoot without any intermediate action of the buds.

As mentioned above, the same experiments were carried out with shoots stored with buds. Between the ethylene chlorhydrin treatment and the growth hormone supply, these shoots stood in the greenhouse with, of course, buds on. Those which remained there for more than 3 days before growth hormone was applied had to be rejected as they started sprouting.

Therefore only the results of the shoots treated immediately after rest breaking or 3 days afterwards, are stated here. Both experiments took place with 3 shoots treated with growth hormone and 3 and 4 shoots treated with waterpaste. The controls with the cap of waterpaste produced no xylem, whereas in the test shoots new early wood was formed down the whole length, as in the experiments with disbudded shoots. These experiments were made on the 19 and on the 31 January 1940 respectively. On the 8 February, which is a rather advanced date, 4 shoots of this group were tested on dormancy. A month afterwards in one shoot no wood was produced at all, but three shoots had formed more xylem than was expected. The shoots were only 10 cm in length, the apical and basal wound thus being nearer to one another than in any other experiment. Longer shoots were no more in store. Whether in this case the cooperation of the apical and basal wounds had shortened the rest period of the whole shoot or whether the shoots had ceased their resting condition because of the advanced date cannot be decided; in any case it is evident from this experiment that in 1940 it would have been precarious to start experiments with shoots stored with buds about this date. Even the conclusion from the experiment of the 31 January could be doubted. Therefore the experiments here reported were repeated in autumn 1940, the results proving the general conclusions here arrived at, to be correct.

The autumn experiments were, moreover, extended by also testing shoots from the tree in the field. To test these shoots and the stored material on dormancy five shoots from either of these groups were treated on the 30 September 1940 with growth hormone without preceding rest breaking treatment. After one month all showed the same result as did the unforced shoots in autumn 1937 (GOUWENTAK and MAAS 1940), viz. a small quantity of wound xylem only in the uppermost 2 or 3 millimeter, with vessels not yet as wide as those of normal summerwood.

So at the moment of storage and a month afterwards the shoots did not produce wood down their whole length and thus proved to be the right material for the experiments mentioned. I did not start earlier, from fear of getting into the stage of deep rest, in which buds and perhaps also the cambium do but poorly react on rest breaking treatment.

In the first experiment (which lasted from November 9 till medio December) from each group 4 or 5 shoots were immediately treated with growth hormone, and 4 shoots after ethylene chlorhydrin treatment. Waterpaste checks were left out, as it was now sure enough that ethylene chlorhydrin alone does not cause wood production.

TABLE II.

Shoots	Nr. Exp.	Forced	Nr. Exp.	Not Forced
stored with buds	1 a b	2 shoots: dead 2 „ : early wood: 9 and 5 cm	4	3 shoots: wound reaction: rest ½ cm 1 „ : late wood 1 cm
stored without buds	2	all shoots dead	5	3 shoots: transition wood: 10 cm 1 „ : late wood 9.5 cm
from field	3 a b	2 shoots: whole length early wood 2 „ : early wood: few cm	6	3 shoots: wound reaction: rest ½ cm 2 „ : late wood: 1 cm

Stored with buds: From the 4 shoots forced two died soon after the rest breaking treatment, the other 2 produced early wood as far as 9 and 5 cm down their length respectively. The cambium of the non-forced shoots formed in 1 unit late wood as far as 1 cm from the paste cap, and had given in 1 case the woundreaction typical for cambium treated at the end of dormancy and described and photographed by GOUWENTAK (1936): This wood is characterized by elements that are often smaller than in late wood, but wider and especially thinner walled than the wound xylem described on top of this page.

Stored without buds. The forced units did not survive after the ethylene chlorhydrin treatment. From non-forced checks 3 shoots formed new xylem intermediate between spring and late wood ("transitionwood"), but not beneath about 10 cm from the upper end of the shoot. One shoot had formed late wood down to 9,5 cm.

Shoots from field. Two of the forced shoots formed spring wood along the whole length. Another two produced the same kind of wood, but only as far as some few cm from the upper end, whereas a fifth one (not mentioned in the table), damaged by the ethylene chlorhydrin at 2½ cm from top, had formed spring wood only along this distance. In 3 of the non-forced units the cambium was completely resting, while in one of them a small quantity of late wood had been formed in the topmost first cm.

Although the cambium of forced shoots produced early wood throughout the whole length, most shoots were apparently in a stage of rest in which emergency can scarcely be hastened. This is emphasized by the reaction of most shoots in the non-forced group, as the shoots "stored with buds" and "from field" almost made no wood at all. The non-forced units stored without buds made more wood than was expected from non-forced shoots. As, however, in a second experiment, running from the 4 December till the 10 January all shoots — forced and non-forced — made springwood down the whole length, these shoots obviously were already about to emerge from rest before medio December. The early rest breaking must have been caused by too cold storage. Due to war-circumstances the storage room could not be kept at the same high temperature as in previous years.

The fact that the structure and the quantity of wood formed by heteroauxin in dormant shoots differ so much, leads us to distinguish different stages of cambial rest. We have chosen the same names as are used for bud rest, without, however, pretending that cambium rest stages and rest stages of buds must coincide in time.

I. A stage of preliminary rest[1] (Vorruhe JOST, JOHANNSEN 1910) attained during late summer.

II. A stage of deep rest[1] (Hauptruhe JOST, Mittelruhe JOHANNSEN). Woodelements not yet so wide as those of late wood, in only the upper 2 or 3 millimeters (GOUWENTAK and MAAS 1940, GOUWENTAK this paper) or only a layer of undifferentiated cambial cells (GOUWENTAK and HELLINGA 1935, GOUWENTAK 1936).

III. A stage of afterrest

- Phase 1: wound reaction followed by some late wood and rest, or directly by rest (this paper Table II, Exp. 4 and 6.
- Phase 2: early wood or transition wood some few centimeter down the length of the shoot (SÖDING's Exp. 1936, GOUWENTAK's 1936, BROWN and CORMACK's 1937, this paper Table II Exp. 5.
- Phase 3: wood throughout the whole length of the shoot (GOUWENTAK and MAAS' Exp. 1940, this paper Exp. early spring 1941).

[1]) English terminology after MAXIMOV, Plant Physiology ed. by HARVEY and MURNEEK, 1938.

Phase 1 and 2 of stage III are identical with the so-called "Nachruhe" of JOST (in BENECKE–JOST 1923) and JOHANNSEN (1906) in buds. During these phases heteroauxin only activates the cambium after a preceding rest breaking treatment. Phase 3 is identical with the "unfreiwillige Ruhe" of MOLISCH (1909) (enforced rest of HARVEY and MURNEEK, vide note 1, p. 660). JOST and JOHANNSEN call this stage the end of the "Nachruhe", in which only unfavourable external conditions prevent growth. During phase 3 the cambium starts growth if growth hormone is supplied and the environmental conditions are favourable.

As to the different types of xylem produced after a rest breaking treatment, stated in Table I and II, the suggestion is that not all shoots were in the same condition of rest. The shoots of experiment II: 3a and I: 1a, 2b, 3, 4b apparently were in phase 1 or 2 of the afterrest before they were treated with the ethylene chlorhydrin. Those of experiment II: 1b, 3b and I: 1b, 2a, 4a probably were in deep rest. This suggestion remains to be tested next winter.

The sections of the rest stages of the cambium are chosen so, that rest breaking is also possible during the stage of deep rest. Thus I agree with JOHANNSEN in his classification of 1906, where he terms deep rest as the stage in which emergency, though not impossible, is very difficult; afterwards (WARMING–JOHANNSEN 1909) he changed the meaning of the term into the stage from which artificial hastening of emergency is impossible, but according to WEBER (1916) a deep rest in the latter sense does not exist at all.

In recent literature heteroauxin is sometimes considered a rest breaking substance (AMLONG and NAUNDORF 1938, BENNETT and SKOOG 1938, VEGIS 1937). A review, however, of the results of AMLONG and NAUNDORF demonstrates that although the one control plant present started flowering much later than the hormone-treated plants, its leaf buds were unfolded at about the same time as those of the treated plants. So not a premature unfolding but at the utmost a premature opening of the flowers took place. The preliminary experiments of BENNETT and SKOOG were not made on a large scale and the effect of heteroauxin was only small. In the experiments of VEGIS many turions of Stratiotes were used and the author obtained the striking effect that the turions began to sprout within 24 hours when put into a diluted heteroauxin solution, whereas the controls in water remained dormant. Now, perhaps, VEGIS experimented during the period of enforced rest. If so, the effect of the heteroauxin may have been due to a want of growth hormone and thus the turions could start growth as soon as the hormone was available. But even if heteroauxin should prove to be a rest breaking substance for buds and turions, it does not effect the conclusions arrived at in this paper. In cambium cells in different stages of rest the effect of ethylene chlorhydrin or heteroauxin alone and of the cooperation of both is so strikingly different, that the action of the heteroauxin on cambial cells is not that of a rest breaking treatment.

As to the nature of the effect of ethylene chlorhydrin and other rest breaking means on dormant protoplasts little is known. KESSLER (1935) and KESSLER and RUHLAND (1938) ascribe the rest breaking effect to a decreased hydration of the protoplasts as in active protoplasts the viscosity proved to be lower than in resting cells. If so, heteroauxin and, perhaps, natural growth hormones can only induce cambial activity in the dehydrated condition of cambium cells.

Summary.

Heteroauxin, or its Na salt, can only activate the cambium to produce new early wood throughout the whole length of shoots if these shoots are in a non-resting condition. This condition is artificially hastened by an ethylene chlorhydrin treatment. The growth hormone may be supplied directly of after some days. Ethylene chlorhydrin treatment alone does not activate the cambium. The resting cambial cells can be roused into renewed growth without the interference of buds.

Heteroauxin may also activate resting cambium to produce wood, but only in the direct neighbourhood of wounds, the wound having locally a rest breaking effect. In this case the kind of wood produced varies from undifferentiated cells to real springwood. This is explained by distinguishing different stages of rest in the cambium cells (scheme on p. 660).

LITERATURE.

AMLONG, H. U. und G. NAUNDORF, Planta 12, 116—120 (1938).
AVERY, G. S., P. R. BURKHOLDER and H. B. CREIGHTON, Am. J. of Bot. 24, 51—58 (1937).
BENECKE–JOST, Vorlesungen ü. Pfl. Physiologie, Bd. II (1923).
BENNETT, J. P. and F. SKOOG, Plant Physiology 13, 219—226 (1938).
BROWN, A. B. and R. G. H. CORMACK, Can. J. of Res. C 15, 433—441 (1937).
COSTER, C., Ann. d. Jard. bot. de Buitenzorg 37, 49—160; 38, 1—114 (1927).
DENNY, F. E. and N. E. STANTON, Am. J. of Bot. 15, 337—344 (1928).
GOUWENTAK, C. A. und G. HELLINGA, Meded. Landbouwhoogeschool Wageningen, 39 (Verh. 6), 3—12 (1935).
GOUWENTAK, C. A., Meded. Landbouwhoogeschool Wageningen, 40 (Verh. 3), 3—23 (1936).
GOUWENTAK, C. A. und A. L. MAAS, Meded. Landbouwhoogeschool Wageningen, 44 (Verh. 1), 3—16 (1940).
JOHANNSEN, W., Das Aetherverfahren beim Frühtreiben (1906).
———— in Warming–Johannsen, Lehrb. d. allgem. Botanik (1909).
JOST, L., Bot. Ztg. 49, 485 ff. (1891).
————, Bot. Ztg. 51, 89—138 (1893).
KESSLER, W., Planta 24, 312—352 (1935).
KESSLER, W. und W. RUHLAND, Planta, 28, 159—204 (1938).
MOLISCH, H., Das Warmbad als Mittel zum Treiben der Pflanzen (1909).
SNOW, R., The New Phytologist, 32, 288—295 (1933).
————, The New Phytologist, 34, 347—360 (1935).

SÖDING, H., Ber. d. d. bot. Ges., **54**, 291—304 (1936).
——————, Jahrb. f. wiss. Bot., **84**, 639—670 (1937).
——————, Ztschr. f. Bot., **36**, 113—141 (1940).
THIMANN, K. V., Journ. of Gen. Physiol. **18**, 23—34 (1934).
VEGIS, A., Bull. d. l. Soc. de Biologie de Lettonie, **7**, 87—102 (1937).
WEBER, F., Ber. d. d. bot. Ges., **34**, 7—13 (1916).
ZIMMERMANN, W. A., Ztschr. f. Bot. **30**, 209—252 (1936).

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