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FACTORS CONTROLLING CAMBIAL DEVELOPMENT IN THE HYPOCOTYL OF RICINUS COMMUNIS L.*

A. M. SIEBERS and C. A. LADAGE

Botanisch Laboratorium, Leiden

SUMMARY

The development of the cambium in the interfascicular region of young castor bean hypocotyls was investigated under various experimental conditions. The cotyledons were found to be indispensable for the onset of cambial development. Young isolated hypocotyls developed a cambium when cultured in a simple medium lacking phytohormones but fortified with sucrose. Addition of indole-3-acetic acid to the medium did not enhance the rate of cambial development. When 2,3,5-triiodobenzoic acid was added, the typical acropetal extension of cambial development in the hypocotyl was maintained. Gibberellic acid (GA_3) was found to have strong stimulatory properties, and, when combined with sucrose, to be capable of inducing a rate of cambial development comparable to that in the intact plant. Inhibitors of gibberellin biosynthesis applied to a hypocotyl-cotyledon system cultured in vitro or sprayed on the cotyledon of a decapitated seedling in vivo, depressed cambial development in the hypocotyl. Simultaneous application of gibberellic acid in the spray experiments restored the original level of cambial development. The significance of the present findings is discussed in relation to the prevailing concept of cambial stimulation.

1. INTRODUCTION

In the process of cambial development, two steps can be distinguished: (i) an initial phase during which special cells in the shoot and root prepare to become cambial cells, and (ii) a phase of actual cambial development during which the typical serried tangential walls are formed. After the cambium has reached a certain width (in radial cell number), differentiation into xylem and phloem sets in. When the cambium attains its maximal width, all mitotic activity leads to the formation of new xylem and phloem elements. At that moment the process of cambial development can be regarded as completed.

Although cambial activity (i.e. production of cambial derivatives by an existing cambium) and reactivation (i.e. the renewal of cambial activity after a dormancy period) have been widely discussed in the literature (see reviews by REINDERS-GOUWENTAK 1965; TORREY 1966; ROBERTS 1969; PHILLIPSON, WARD & BUTTERFIELD 1971), the preceding processes of initiation and development have received comparatively little attention. Therefore, an earlier study (SIEBERS 1971a,b, 1972) was devoted to the initial phase of cambial development, the results indicating that in the castor bean hypocotyl the determination

* Dedicated to Professor Karstens on the occasion of his retirement.

of the future cambial cells has already been completed in the embryo and that vascular bundle differentiation and cambial development represent two essentially unrelated developmental processes. The present article deals in particular with the factor(s) controlling the second phase of cambial development, i.e. the actual formation of the cambium.

It has long been known that leaves exert a strong influence on cambial development (JOST 1891). Decapitated plants are unable to develop a cambium in shoot parts above the level of the remaining leaves. Grafting experiments have shown that a hormonal stimulus originating from the apical parts is responsible for cambial development, at least in the shoot (SNOW 1933; CARUSO & CUTTER 1968, 1970).

There are a number of reports from which it can be concluded that auxins in particular contribute to this cambial stimulus. Application of indole-3-acetic acid (IAA) to the top of the stump of a decapitated shoot restored cambial development just below the site of the application (SNOW 1935; SNOW & LE FANU 1935; KRAUS, BROWN & HAMNER 1937; SÖDING 1940; KÜNNING 1950). IAA can induce the formation of an extrafascicular cambium in *Iresine lindenii* Van Houtte (HARRISON 1937) and of series of such cambia in *Beta vulgaris* L. (WINTER 1954), and proved capable of inducing an interfascicular cambium in *Cuscuta lupuliformis* Krocker, a species which normally lacks a cambium between the vascular bundles (LIBBERT & URBAN 1967). Furthermore, the inception of cambial development in pea root explants occurred only when IAA was present in the medium (TORREY 1963; see also STREET 1966a,b).

Experimental induction of cambial development is, however, not accomplished exclusively with IAA. In experiments with decapitated plants other substances (ascorbic acid, thiamin) were equally effective (KÜNNING 1950) and in pea epicotyl explants kinetin enhanced cambial development more than did IAA or 2,4-dichlorophenoxyacetic acid (SOROKIN, MATHUR & THIMANN 1962). Cambial development in isolated interfascicular tissue fragments of the castor bean hypocotyl was stimulated by an application of gibberellic acid (GA_3) but even occurred in a medium lacking any phytohormone (SIEBERS 1971b). As far as we know, the role of gibberellins in cambial development has not been further investigated, although their positive influence on cambial activity is very well documented and sometimes exceeds that of the auxins applied (see e.g. DIGBY & WAREING 1966; MOREY & CRONSHAW 1968; SHININGER 1971).

The aim of the present study was to compare auxin and gibberellin as stimulators of cambial development. The castor bean hypocotyl was chosen as object because its distinct and broad interfascicular regions greatly facilitate the observation and quantification of cambial development.

Cambial development and differentiation always started at the basal end of the cultured hypocotyls and extended in an acropetal direction. Since this might be a reflection of differences in the concentration of endogenous auxins, which in turn can be the result of the dominating basipetal transport of this phytohormone, we investigated the effect on secondary tissue development of an application of triiodobenzoic acid (TIBA, an inhibitor of the polar auxin trans-

port: NIEDERGANG-KAMIEN & SKOOG 1956; NIEDERGANG-KAMIEN & LEOPOLD 1957; see also SCHNEIDER 1970).

The inhibitory effect of cotyledon removal on cambial development, in combination with the promotive effect of exogenously supplied GA_3 in our experiments, suggests a gibberellin-mediated role for this organ. This possibility was investigated by the application of inhibitors of gibberellin biosynthesis (LANG 1970, review), such as CCC[1] (ROBINSON & WEST 1970), AMO-1618[2] (CLELAND & ZEEVAART 1970, ROSS & BRADBEER 1971), and NETC[3] (VAN HAASTEREN 1969) to the cotyledons.

2. MATERIAL AND METHODS

2.1. In vitro experiments with isolated hypocotyls

Seeds of *Ricinus communis* L. (received under the name *R. sanguineus* from Vilmourin-Andrieux, Paris) were sterilized for 6 minutes with a 0.1 per cent $HgCl_2$ solution. The seedlings were grown under long-day conditions (16 hr light at 25°C; and 8 hr darkness at 20°C) in sterile culture units described elsewhere (SIEBERS 1971b).

After a 9-day culture period during which the hypocotyls reached a height of about 12 cm, each hypocotyl was aseptically detached from the root system, deprived of the plumule and one or both cotyledons, and transferred to a 2-litre Fernbach flask containing 100 ml culture medium. Culturing occurred under constant stirring on a Gyrotary shaker (60 r/min) and under the same light and temperature conditions as were used for the seedlings. The flasks were placed at a slight slant to keep the hypocotyls submerged and in the same position. The basal medium consisting of Heller's mineral solution with 4 per cent (w/v) sucrose (B.D.H. Analar) was adjusted to pH 6.0 before autoclaving. The phytohormones and growth retardants, i.e. IAA (Merck), NAA (B.D.H.), TIBA (Aldrich Chem.), kinetin (N.B.C.), GA_3 (B.D.H.), CCC (B.D.H.), AMO-1618 (Rainbow Color & Chemical Comp.), and NETC (synthesized in the Department of Biochemistry, University of Leiden, The Netherlands), were prepared separately in stock solutions, adjusted to pH 6.0, sterilized by ultrafiltration, and added to the autoclaved basal medium.

2.2. In vivo experiments and spray technique

Nine-day-old seedlings cultured as described above were deprived of one cotyledon and the plumule before transfer to plastic beakers with perforated bottoms (150 ml, 3/4 filled with gravel, one seedling per beaker), where they received about 100 ml Hoagland solution every 12 hours. The substances to be tested (GA_3, CCC, AMO-1618, and NETC) were dissolved in a 0.1 per cent solution of Tween 80 (final pH 5.0) and sprayed to run-off on both sides of the

[1] CCC: 2-chloro-ethyltrimethylammonium chloride

[2] AMO-1618: 2-isopropyl-4-dimethylamino-5-methyl phenyl-1-piperidine carboxylate methyl chloride

[3] NETC: naphtyl-ethyl-trimethylammonium chloride

cotyledons. The controls were sprayed with the Tween solution alone. Spraying was performed daily except during the weekend in the middle and at the end of the two-week experimental period.

2.3. Anatomical observation and determination of cambial development

At the end of the experimental period the hypocotyls were divided into 8 equal segments, after which a transverse section was cut by hand from the apical end of all but the top segment. Per section, cambial development was quantified by averaging the values (in number of tangential walls per radial file) found to be representative for cambial development in each of the eight interfascicular regions within the section.

If xylem and phloem differentiation followed cambial development within the experimental period, only those tangential walls belonging to the zone of morphologically undifferentiated cells were counted as cambial walls. In a number of experiments the total amount of secondary tissues (i.e. the cambium plus its derivatives, in numbers of tangential walls per radial file) and the production of tracheary elements (tracheids as well as vessels, in total number of these elements per interfascicular region) were estimated as well.

The development of the secondary tissues along the hypocotyl (cambium, total amount of secondary tissue, and tracheary elements) can be shown in a histogram by plotting the tissue development data of each section against the location of the section on the axis of the hypocotyl (see e.g. *fig. 1*). The "surface" occupied by a tissue in the histogram can then be calculated and used as a measure for tissue development per hypocotyl, which permits comparison with other hypocotyls if there were no significant differences in hypocotyl growth. Because this condition was not fulfilled in a number of experiments (the GA_3-treated hypocotyls in particular were much longer at the end of the experimental period), the growing part of the hypocotyl had to be excluded from the calculation. Since the lowermost 9 cm of the hypocotyl showed almost no growth during the experimental period, the area used for the surface calculation was taken smaller, the upper limit being taken at the 9-cm level. At the lower side the limit was taken arbitrarily at the 1.5 cm level (1/8 of the mean hypocotyl length at the beginning of the experimental period). For the approximately 400 hypocotyls included in this investigation, cambial development was determined by computer calculation of that part of the cambial "surface" between the 9.0 and 1.5 cm levels (cf. shaded areas in *figs. 1* and *3*).

3. RESULTS

3.1. Development of cambium, total secondary tissue, and tracheary elements in the hypocotyl of the intact plant

Reliable interpretation of the data on secondary tissue development in the in vitro experiments requires knowledge of the normal development in the intact seedling. Therefore, hypocotyls deriving from intact seedlings aged 10, 12,

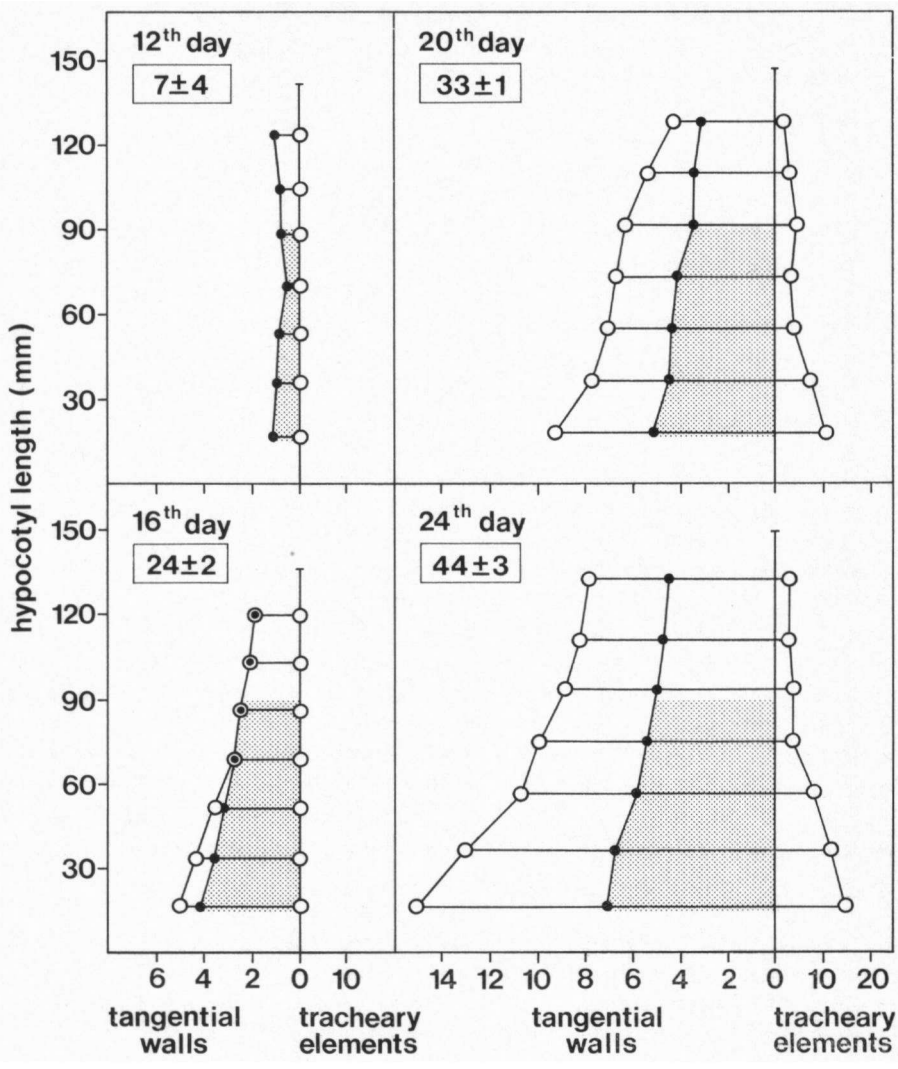

Fig. 1. Development of interfascicular secondary tissues in intact seedlings at different ages. Each of the diagrams is based on measurements in 4 hypocotyls. Values for tangential walls and tracheary elements per section are plotted proportional to the mean hypocotyl length at the different ages.

Development of the cambium (●) and total secondary tissue (○, left), i.e. the cambium plus its derivatives (xylem and phloem), are presented as number of tangential walls per radial file but the differentiation of the tracheary elements (○, right) as the number of elements (vessels and tracheids) per interfascicular region. The boxed figures indicate mean cambial development (in units "cambial surface" calculated for each individual hypocotyl as described in 2.3, and averaged) and the standard deviation.

16, 20, and 24-days were investigated with respect to cambial development, total secondary tissue production, and tracheary element differentiation. The results, except for the 10-day-old material in which no cambium formation could be observed, are shown in *fig. 1*. Cambial development started almost simultaneously along the whole hypocotyl (on the 11th or 12th day) but soon became more pronounced toward the base. Tracheary element differentiation (vessels and tracheids taken together) started on about the 20th day at the base and extended in an acropetal direction.

3.2. Influence of organ removal on cambial development

In one experiment 9-day-old seedlings were subjected to excision of the plumule (including the lateral buds), the cotyledons, or both, and the effect on cambial development in the interfascicular region was investigated two weeks later (*fig. 2*).

Removal of the plumule did not depress the rate of cambial development as compared with the development in the intact seedling, but removal of the cotyledons totally arrested cambial development. Evidently, the growing leaves of the plumule are unable to compensate for the excised cotyledons within the experimental period. Complete decapitation had the same effect as decotylization. No discrepancy between cambial development in the interfascicular and fascicular region was observed in these and subsequent experiments.

It is not clear from these experiments how the cotyledons exert their decisive influence on cambial development. This could occur directly, by the production by the cotyledons of stimulatory substances reaching the hypocotyl by active or passive transport, but also indirectly, the cotyledons maintaining the trans-

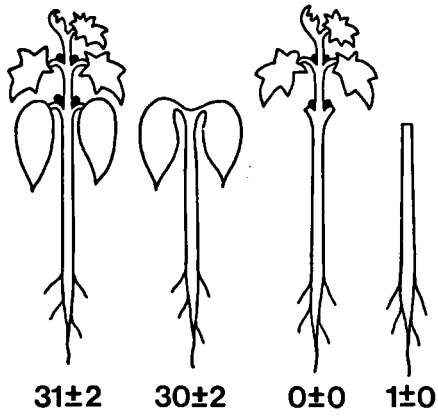

Fig. 2. Influence of organ removal on cambial development. Excision performed on 9-day-old seedlings; cambial development evaluated two weeks later. Data indicate mean cambial development (in units "cambial surface") and standard deviation, and are based on 4 hypocotyls per treatment. Drawings show the appearance of cotyledons and leaves at the end of the experimental period.

piration stream and thus facilitating the input of possible stimulants originating from the root.

3.3. The influence of TIBA on the acropetal extension of secondary tissue development in hypocotyls cultured in vitro

The influence of TIBA on the acropetal extension of cambial development, the production of cambial derivatives, and xylem differentiation, was investigated at concentrations of 10 and 50 ppm and also at 10 and 20 ppm with 0.5 ppm GA_3 in the basal medium (GA_3 was chosen for addition because in preliminary

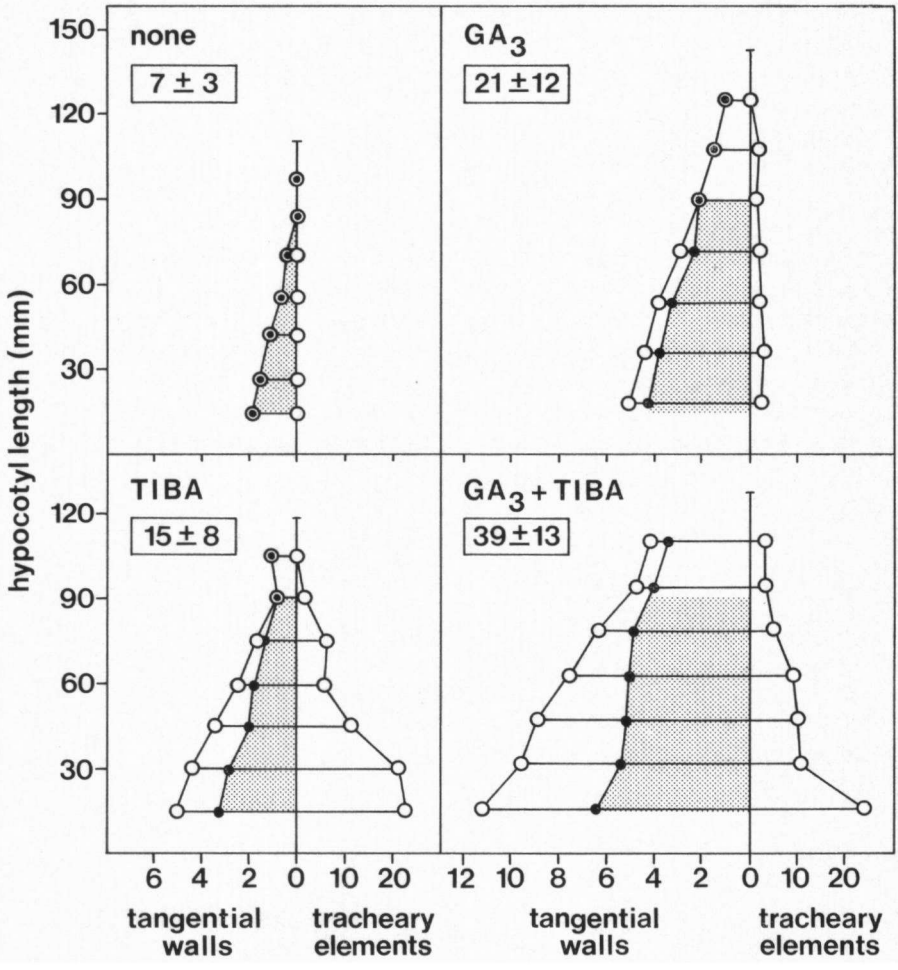

Fig. 3. Influence of TIBA on cambial development, the production of cambial derivatives, and xylem differentiation in isolated hypocotyls. Hypocotyls from 9-day-old seedlings were cultured for two weeks with and without GA_3 (0.5 ppm) in the medium. The diagrams are based on anatomical observations in 6 hypocotyls per treatment (see *fig. 1*).

experiments this phytohormone proved to have strong stimulatory capacities and could therefore be expected to give an approximation of the normal rate of cambial development). After a two-week culture period, 6 hypocotyls were examined for the evaluation of secondary tissue development. The results, which are partially shown in *fig. 3*, give no grounds to assume that TIBA can change the typical acropetal pattern of development into a more evenly distributed type of growth.

Quite unexpected was the stimulatory effect of TIBA on cambial development and differentiation. With or without the presence of GA_3 in the medium, cambial development was doubled and the production of cambial derivatives, particularly of xylem, was markedly enhanced. Higher concentrations of TIBA (50 ppm, not indicated in *fig. 3*) lowered the rate of cambial development and differentiation substantially, which means that there is an optimal level for the action of this compound.

Morphologically, the hypocotyls reacted to the application of TIBA by forming callus at the apical cut surface and by the complete failure to form roots at the basal cut surface (unlike the controls, which frequently showed some small roots but no callus growth). Both of these reactions support the assumption that a reduction of the basipetal transport of endogenous auxins indeed occurred in the cultured hypocotyls under the influence of TIBA.

3.4. Influence of sucrose, IAA (NAA), and GA_3 on cambial development in vitro

The in vitro development of interfascicular cambium in isolated hypocotyls was studied at various sucrose concentrations (*table 1*, control experiments). Without sucrose in the medium, only the basal sections showed some cambial development (1 or 2 tangential walls per radial file), and the value for cambial development per hypocotyl was consequently low. With sucrose in the medium, cambial development was markedly enhanced, maximally with the 4 per cent solution. At this sucrose concentration cambial development was about one-third of that reached by the intact plant of comparable age (see *fig. 1*: 24 days). Differentiation of tracheary elements was limited to a few tracheids in the basal part of the hypocotyl. Higher sucrose concentrations (8 and 16 per cent, not shown in the table) depressed cambial development. Prolongation of the culture period to 4 weeks (applied for the sucrose concentrations of 0 and 4 per cent) did not increase cambial development or affect xylem differentiation.

When IAA was added to the medium a small but non-significant stimulation of cambial development, as compared with the auxin-free medium, was observed at 0 and 4 per cent sucrose but not at 0.25 per cent (*table 1*). Qualitatively, at this relatively low level of cambial development the differentiation of cambial derivatives into xylem was enhanced only slightly or not at all by the presence of IAA in the medium. In one experiment in which IAA was replaced by NAA the results (in 9 hypocotyls distributed over three concentrations: 0.1, 0.5, and 2.5 ppm) give no indication that NAA can stimulate cambial development, in spite of its greater stability.

Table 1. Influence of sucrose, IAA, and GA_3 on in vitro cambial development in isolated hypocotyls deriving from 9-day-old seedlings. Culture period two weeks unless otherwise indicated. The values represent mean cambial development per hypocotyl (in units "cambial surface") and the standard deviation, based on 7 hypocotyls per treatment.

applied phytohormone (ppm)		sucrose		
		0.0%	0.25%	4.0%
none (control)		1 ± 1	3 ± 3	13 ± 6
none (4 weeks)		1 ± 1		12 ± 6
IAA	0.1	3 ± 2	5 ± 3	15 ± 8
	0.5	5 ± 3	5 ± 2	13 ± 7
	2.5	2 ± 2	5 ± 3	18 ± 10
GA_3	0.1	1 ± 2	8 ± 9	34 ± 14*
	0.5	9 ± 6*	20 ± 15*	37 ± 11*
	2.5	19 ± 10*	22 ± 8*	28 ± 17

* Within the 3 sucrose series, differences from the control significantly higher at the 0.05 level (Dunnett's test, after $^{10}\log(x + 1)$ transformation of the data) are marked with an asterisk.

Morphologically, the presence of IAA in the medium gives rise to a number of small adventitious roots along the surface of the hypocotyl and, especially at higher IAA concentrations, some large roots on the basal cut surface. A callus-like outgrowth of the lowermost 2–3 cm part was also frequently observed.

Quite different results were obtained with GA_3 in the medium. Even in the absence of exogenous sucrose, GA_3 can stimulate cambial development very effectively. At the optimal sucrose concentration (4 per cent) even the lowest GA_3 level (0.1 ppm) had a strong stimulative effect on cambial development as compared with the phytohormone-free medium, and higher GA_3 concentrations did not substantially enhance cambial development. It should be mentioned that the striking effect of GA_3 on the development of the cambial tissue is not entirely evident from *table 1*, where development is expressed as the number of tangential walls per radial file within the undifferentiated layer of cambial cells. The increase of this number is, however, gradually restricted by the onset of differentiation of cambial derivatives (cf. *fig. 1*). The number of tangential walls in the cambium reaches a maximum (when about 8 walls have been formed) and, as a result, the value for the cambial development per hypocotyl also climbs to a maximum coinciding with the completion of cambial development at all levels in the hypocotyl part used for observation.

Application of GA_3 led to several morphological changes which are very well known for this phytohormone in other material, such as enhanced hypocotyl growth and inhibition of adventitious root formation.

3.5. Influence of GA_3 and the growth retardants CCC and AMO-1618 on cambial development in vitro

In another series of experiments the effect of sucrose, GA_3, and growth retardants on cambial development was studied in isolated hypocotyls, a number of them bearing one cotyledon.

In the presence of sucrose (4 per cent) and with one cotyledon on the hypocotyl, a high value was obtained for cambial development; this value was not further raised by the addition of GA_3 (10 ppm) and equaled that obtained for the GA_3-stimulated hypocotyls lacking both cotyledons (*table 2*). It may therefore be concluded that if the cotyledon indeed produces gibberellins they must be transported to the hypocotyl in sufficient quantities in vitro to reach approximately the same degree of cambial development as occurs in the intact plant.

Without sucrose in the medium the presence of one cotyledon on the hypocotyl did not give rise to any significant amount of cambial development, and when GA_3 was applied the cotyledon did not additionally enhance cambial development. Under the present culture conditions (cotyledons submerged in the medium) the cotyledons were clearly incapable of producing or exporting enough assimilates to bring cambial development up to the control level. The stimulatory effect of GA_3 observed in this series of experiments was of the same order as that mentioned in section 3.4.

The application of CCC and AMO-1618 in appropriate concentrations markedly decreased cambial development. These retardants were originally supplied in a concentration range of 5000, 2500, 1250, ... 78 ppm. Low concentrations

Table 2. Influence of sucrose, GA_3, and the growth retardants CCC and AMO-1618 on the development of the cambium in vitro in hypocotyls from 9-day-old seedlings. The hypocotyls bore one cotyledon or none. The culture period was two weeks. Values represent the means of 5 hypocotyls per treatment (see *table 1*).

GA_3 or retardant (ppm)	one cotyledon	no cotyledons
With sucrose in the medium (4 per cent)		
GA_3 0 (control)	34 ± 10	14 ± 6
10	32 ± 7	38 ± 10
CCC 2500	21 ± 8	8 ± 5
AMO-1618 78	28 ± 3	
156	25 ± 5	
312	18 ± 4	
GA_3 10 + CCC 2500	5 ± 2	2 ± 1
10 + AMO-1618 312	6 ± 1	
Without sucrose in the medium		
GA_3 0 (control)	4 ± 3	3 ± 1
10	19 ± 3	17 ± 2

without any apparent effect and high concentrations giving morphological aberrations or tissue damage were omitted from *table 2*. The latter was the case with 5000 ppm CCC, which caused yellowing of the cotyledons, and AMO-1618, which gave rise to small black spots on the surface of the cotyledon at 625 ppm and higher concentrations. The use of NETC had to be abandoned because of an aberrant lignification of the cell walls in the inner cortical and cambial cells at all concentrations.

In the control experiments the formation of roots at the base of the hypocotyl was greatly enhanced when one cotyledon was present, which suggests that the cotyledon produced auxins which were transported to the base of the hypocotyl. GA_3 completely inhibited the rooting capacity, as did the growth retardants at the intermediate and high concentrations.

The application of GA_3 in combination with a growth retardant was expected to overcome the inhibitory effect of the latter. Since preliminary experiments with 1.0 and 2.5 ppm GA_3 showed no tendency for cambial development to recover, the GA_3 concentration was raised to 10 ppm (subsequently maintained in the present study), which also gave no recovery. On the contrary, the inhibitory effect of all the three retardants was greatly strengthened. All experiments with GA_3 and CCC were repeated (not shown in the table) and the same results were obtained.

An inhibitory effect of the combination CCC plus GA_3 was also observed when both cotyledons were removed. CCC applied alone again gave a lower level of cambial development than that of the controls.

In addition to the remarkable inhibitory effect of the retardant-gibberellin combination on cambial development, it is interesting to mention the stimulatory effect on the elongation growth of the hypocotyl. In the basal medium and whith one cotyledon attached, hypocotyl growth (expressed as percentage of the initial length) amounted to 46 ± 14. Addition of the retardants did not lead to decreased growth, even in the highest concentration indicated in *table 2* (for CCC and AMO-1618 the percentages were 38 ± 10 and 45 ± 15, respectively). GA_3 gave a marked increase of hypocotyl growth when given alone (124 ± 23) but also in combination with the retardants (130 ± 47 and 88 ± 21, respectively) which indicates that as far as hypocotyl growth is concerned GA_3 can indeed overcome the inhibitory effect of the retardants.

3.6. Effect of foliar sprays of GA_3 and the growth retardants CCC, AMO-1618, and NETC on cambial development in vivo

The spray experiments were performed to investigate the effect of application to the cotyledons of retardants alone and in combination with GA_3 on cambial development in the hypocotyl. Because seedlings deprived of the plumule and lateral buds did not show a strong reduction of cambial development when one cotyledon was removed (*table 3*), decapitated seedlings with one cotyledon were used, as in the in vitro experiments.

CCC, AMO-1618, and NETC all gave a marked decrease of the rate of cambial development. Morphological or anatomical aberrations were not

Table 3. Effect of foliar sprays of GA_3 and growth retardants on cambial development in the hypocotyl of seedlings from which the plumule was removed. Data based on 7 hypocotyls per treatment (see *table 1*).

Retardant (ppm)	$-GA_3$	$+GA_3$ (10 ppm)
Both cotyledons left		
none	33 ± 3	
One cotyledon left		
none (control)	27 ± 2	36 ± 3
CCC 2500	14 ± 3	32 ± 2
AMO-1618 312	17 ± 2	35 ± 3
NETC 156	18 ± 3	28 ± 4

observed at the concentrations used. Simultaneous application of GA_3 and retardant almost completely eliminated the inhibitory effect of the latter, the values lying between the control values (with or without GA_3).

The differences in hypocotyl growth found in the various treatments were about the same as described for the in vitro experiments, and thus require no discussion. Under the conditions used in the in vivo experiments, retardant-mediated inhibition of both cambial development and hypocotyl growth was overcome by an application of GA_3.

4. DISCUSSION

The aim of the present study was to investigate the nature of the cambial stimulus, i.e. the factor or complex of factors produced in leaves or buds and transported to the shoot and root that triggers the formation of the cambium. As mentioned in the introduction, a number of authors assign auxin a major role in cambial development (SNOW 1935; SÖDING 1940; TORREY 1963; and others), although other results do not support this conclusion (KÜNNING 1950; SOROKIN, MATHUR & THIMANN 1962; SIEBERS 1971b).

The principal finding in our in vitro experiments was that isolated hypocotyls supplied with only mineral salts and an appropriate concentration of sucrose can develop a cambium. Preliminary experiments have shown that the same holds for the hypocotyls of other herbaceous plants, e.g. *Helianthus annuus* L., *Glycine max* (L.) Merr., and *Phaseolus vulgaris* L. under comparable experimental conditions. In *Ricinus* the addition of IAA to the medium did not markedly enhance cambial development, however. It is evident that at least for the isolated castor bean hypocotyl, the most important factor for the onset of cambial development is sugar and not auxin. In the growing *Ricinus* seedling the relatively large, dark-green cotyledons are obviously the site of intensive photosynthesis enabling them to provide the hypocotyl with sugar. This at least partially explains the dramatic effect of cotyledon removal on cambial development in our in vivo experiments.

The material used for the classic decapitation experiments of Snow and others is comparable with our material in that both concern a system without buds and with a reduced leaf surface. Such a system has a lowered level not only of auxin but also of assimilates as compared with the intact seedling. On the basis of the present results we therefore suggest that in the decapitation experiments, too, a lack of assimilates rather than of auxin was the principal factor in the failure of cambial development. This hypothesis is supported by the fact that in all of the decapitation experiments reported in the literature the growth substance in question promoted cambial development only over a very short distance (a few millimetres) below the point of application, and that the same effect can be achieved as well by other substances besides auxin (KÜNNING 1950). We think that some specific reaction occurring in a limited region below the site of application was involved. It should be kept in mind in this connection that an application of such growth substances as IAA and GA_3 may stimulate the movement of assimilates toward the site of application (SETH & WAREING 1964; ZAERR & MITCHELL 1967; JEFFCOAT & HARRIS 1972). It is therefore possible that the growth substances applied increased the sugar concentration locally and that this in turn was responsible for the observed induction of cambial development.

The emphasis placed on sugar here is not meant to imply that auxin is not involved in cambial development. There are good grounds to suppose that the presence of auxin is obligatory for cell division (cf. work on tissue cultures, GAUTHERET 1955). We may therefore assume that the hypocotyl itself can produce enough auxin to reach the critical level required for cell division. The vascular bundle (SHELDRAKE & NORTHCOTE 1968) and the dividing cambium (SÖDING 1940, 1961; see also SHELDRAKE 1973) are seen as centres of auxin production. Elevation of the critical auxin level, e.g. with an external supply of IAA, may not promote further cambial development, although other processes (in our experiments: xylogenesis, hypocotyl growth, and root formation) can continue to react.

In certain natural and experimental systems auxin production or degradation will be such that the critical level is not reached. This might explain the positive results obtained with IAA in the parasite *Cuscuta lupuliformis* Krocker (LIBBERT & URBAN 1967) and certain root explants (TORREY 1963; DIGBY & WANGERMANN 1965; TORREY & LOOMIS 1967). It is interesting to note that *C. europaea* L. developed a cambium when sugar alone (5 per cent glucose in a lanoline paste) was applied laterally to the parasite-host combination (FRITSCHÉ et al. 1958).

The application of TIBA was expected to provide additional evidence concerning the role of auxin in cambial development. If the predominantly basipetal auxin transport in shoot segments (GOLDSMITH 1969) holds for the isolated hypocotyl as well, it would lead to a gradual increase in auxin concentration toward the base, and if there is indeed a positive correlation between auxin concentration and cambial development, this would account for the observed acropetal extension of cambial development. On this hypothetical basis, an application of TIBA (an inhibitor of the polar auxin transport) was expected to

result in a more equal distribution of auxin along the hypocotyl and therefore in less pronouncedly acropetal development of the cambium. TIBA-treated hypocotyls indeed showed two reactions indicating that the polar auxin transport was at least partly inhibited, i.e. callus formation at the apical end and inhibition of adventitious root formation at the base. The acropetal pattern of cambial development persisted, however. This suggests that the pattern reflected differences in the internal condition of the cells concerned (age) more than differences in auxin concentration.

TIBA appeared to be an effective promotor of cambial development in our explants. This kind of TIBA action, which has already been reported for the soybean epicotyl (see KRAUSE 1971 on increased "procambial activity") seemed to contradict the negative results obtained with IAA. But if the dividing cambium is a site of auxin production, an application of TIBA, which as we have seen immobilizes auxin, will result in an accumulation of IAA in this tissue (this in sharp contrast with an IAA application, which raises the auxin level in all the tissues). As suggested for the decapitation experiments, this can lead to an inflow of assimilates, which in turn favours further cambial development. Since the involvement of auxin in xylogenesis is very well established (ROBERTS 1969, review; ROBERTS & BABA 1970; SHININGER 1971; HESS & SACHS 1972), the enhanced xylem differentiation with TIBA observed in the present study supports the assumed TIBA-mediated auxin accumulation in the cambium.

At optimal levels of sucrose, the isolated hypocotyls showed a rate of cambial development amounting to about one-half of that found in the intact plants. Application of GA_3, with or without sucrose, can bring cambial development up to the level of the intact plant. If it can be demonstrated that gibberellin is produced in the cotyledons (the only organs found to be indispensable for normal cambial development in our material) we will have good grounds to suppose that this phytohormone, possibly in combination with sucrose, forms the cambial stimulus we were looking for. It is indeed known that the cotyledons can play an important role in supplying substances stimulating shoot and root growth (BAIN & MERCER 1966; KATSUMI, CHIBA & FUKUYAMA 1969; BORGER & KOZLOWSKI 1972), substances which in a number of cases proved to be gibberellins (OGAWA 1964; SHININGER 1972).

The inhibitors of gibberellin biosynthesis used in the present study did indeed decrease the rate of cambial development in both the in vitro and in vivo experiments, but the complementary proof, i.e. elimination of the inhibitory effect by a simultaneous supply of GA_3, was only obtained in vivo. There are of course considerable differences between the two series of experiments. The hypocotyls cultured *it vitro* were continuously submerged in the test solution together with their cotyledon. One effect of this treatment is the complete elimination of the transpiration stream. In the seedlings used for the *in vivo* experiments only the cotyledons were sprayed daily for a short time, the root system was present, and the transpiration stream intact. At this moment we can only speculate as to which of these differences would account for the failure of cambial development in vitro in the presence of GA_3 and a retardant. It is conceivable that the direct

and continued contact between the hypocotyl and the inhibitor solution in this series of experiments led to a relatively high and therefore deleterious retardant concentration in the tissues. Examples of such deleterious effects are a reduction of protein synthesis (BERRY & SMITH 1970; KNYPL & CHYLINSKA 1972) and certain changes in fat metabolism (TUNG & RAGHAVAN 1968). It is, however, difficult to explain why the combination of GA_3 and inhibitor would give a more intense inhibition than the inhibitor alone.

Finally, the possibility that cytokinins are involved in cambial development must not be lost sight of. Preliminary experiments have not, however, provided evidence that these phytohormones play an important role in cambial development.

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