ON THE FORMATION OF ADVENTITIOUS ROOTS IN CUTTINGS OF COLEUS IN RELATION TO THE EFFECT OF INDOLEACETIC ACID ON THE EPINASTIC CURVATURE OF ISOLATED PETIOLES

R. SOEKARJO

(*Botanical Laboratory, Utrecht*)

CONTENTS

ABSTRACT

The epinastic curvature of isolated petioles of *Coleus* shows a dependence upon the concentration of indoleacetic acid in the ambient medium. In low concentrations (between 10^{-9} and 10^{-7}) of indoleacetic acid, a small maximum followed by a small minimum in the curvature *vs.* concentration curve can be seen. Between 10^{-7} and 10^{-5} indoleacetic acid there is a concentration dependent increase of the curvature. In higher concentrations the curvature diminishes, and a discolouration of part of the petiole occurs. These phenomena occur in concentrations that promote the formation of adventitious roots.

The possibility of a correlation of injurious conditions with the formation of adventitious roots has been investigated, using stem cuttings of *Coleus*.

Exuberant formation of adventitious roots after a treatment with high concentrations of indoleacetic acid was accompanied by necrosis in the lowest part of the cutting.

Sulphuric acid, potassium hydroxide and mercuric chloride alone, were shown to cause a promotion of the formation of adventitious roots when applied in concentrations that cause necrosis in the lowest part of the cutting without being lethal to the whole cutting.

CHAPTER I

INTRODUCTION

The epinastic curvature of petioles and of branches has attracted the attention of many investigators. The problem of the mechanism itself and the role of this process in the orientation of organs plagio-tropic towards the stimulus of gravity have been the subject of extens-ive experiments and speculations.

Although the mechanism of epinastic curving is not the subject of the present paper, some views on this mechanism will be put forward.

Among the earlier workers should be mentioned DE VRIES (1872), who regarded the epinastic curvature as caused by a differential growth of the dorsal and ventral side, based on anatomical differences.

KNIEP (1910), reviewing older work, considered the epinastic curvature as a process by itself, apart from geotropism.

LUNDEGÅRDH (1918) regarded the epinastic curvature as an expression of the influence of gravity.

Whatever the nature of the epinastic curvature may be, a response to added growth substance is evident.

The investigations on the effect of growth substances on the epinastic curvature were started by MAI (1934), who worked with a variety of *Coleus blumei* Benth. When orchid pollinia had been substituted for the lamina as a source of growth substance, strong epinastic curvatures of the petioles could be observed.

FISCHNICH (1935) and LAIBACH & FISCHNICH (1936) used indoleace-tic acid in their experiments with the same *Coleus* variety as that used by Mai, and also with other varieties. Intact plants and plants from which the apical bud had been removed showed epinastic curvature of the petioles, when indoleacetic acid in lanoline paste had been applied to the laminae, the petioles or the main shoot.

BOTTELIER (1954, 1956b) developed a bio-assay for indoleacetic acid in aqueous medium, based on the epinastic curvature of isolated petioles of *Ageratum houstonianum* Mill. Preliminary work by BOTTELIER (1958) showed that *Coleus scutellarioides* Benth. is also suitable for this bio-assay.

Some experiments on the epinastic curvature of isolated petioles of *Coleus* are reported in the present paper. The range of indoleacetic acid concentrations suitable for a bio-assay based on the epinastic response has been determined.

The major part of this paper, however, deals with the nature of the effect of indoleacetic acid in concentrations that are supra-optimal for the epinastic curvature, as related to the formation of adventitious roots.

These experiments confirm older investigations (BOTTELIER, 1959), in which was found that the optimum indoleacetic acid concentration for the epinastic curvature differs from that at which the maximal

number of adventitious roots was formed. This brings us upon the problem of the cause of the optimum curve for the response to indole-acetic acid.

The optimum curve for the response to growth substance concentrations is generally explained as a competition effect. SKOOG, SCHNEI-DER & MALAN (1942) supposed that the basic mechanism of growth substance activity is the association of a growth substance molecule with both an enzyme molecule and a substrate molecule of another kind. In higher concentrations of the growth substance, it is likely that the enzyme molecule and the substrate molecule do not associate with the same growth substance molecule, but each with a different one. So, with increasing growth substance concentration the enzyme molecules and the substrate molecules tend to be kept apart by the growth substance molecules, instead of being combined by them.

THIMANN (1956) too, regarded the optimum curve as an expression of a competition. However, in his opinion the growth substance activity is only exerted by a modified growth substance molecule. When there is an excess of growth substance molecules, the primary converting enzyme system is saturated. Now the competition arises between unmodified, and therefore inactive growth substance molecules, and modified growth substance molecules.

These and other similar explanations are very attractive and, indeed, give a clear picture of a possible mechanism by which, in general, an optimum-type of response could occur. When we consider the growth of shoots and shoot parts, however, the optimal concentration is relatively high, in contrast with the optimal concentration for root growth, for instance. In the case of the growth of shoots, additional effects of the high concentrations have to be taken in consideration.

HOUSLEY, BENTLEY & BICKLE (1954) reported a slackening of the growth rate of oat coleoptile sections in high concentrations of indole-acetic acid, after a rapid growth at the start. At very high concentrations even shrinkage of the coleoptile sections occurred.

HANCOCK (1959) on the other hand, found that wheat coleoptile sections showed good growth after a short stay in high concentrations. The injurious effect of supra-optimal concentrations is, therefore, clearly a result of a long exposure to high concentrations, during which time a great amount of indoleacetic acid is taken up.

BOTTELIER (1959), however, maintained the view that even an exposure to high concentrations for a longer period is not injurious to the tissue. In his experiments with *Ageratum*, BOTTELIER (1954, 1956a) has shown that the maximal number of adventitious roots is formed at concentrations seven to ten times as high as the concentration at which maximal curvature of the petioles occurs. These results are schematically represented in Fig. 1.

The obvious conclusion is, that concentrations supra-optimal for the epinastic curvature of the petioles cannot be injurious, as even higher concentrations still give an increase of the number of roots formed on the petioles of the same plant species.
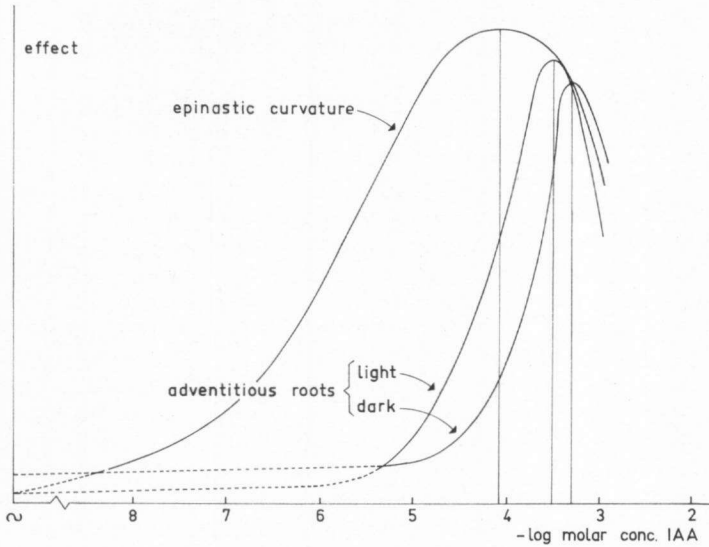
(redrawn after BOTTELIER, 1954, 1956a)

Fig. 1.   Comparison of the effect of indoleacetic acid on the epinastic curvature
with that on the formation of adventitious roots in petioles of *Ageratum*.

The present author did some experiments on the formation of roots
especially under influence of injurious treatments. Shoots, not petioles,
were used, because a greater tolerance to injurious treatment could be
expected, as there is a greater mass of tissue and the higher degree of
lignification makes the shoot more suitable for an experimental
purpose of this kind. Even if the lower part would be severely damaged,
the effect of the injury on the rooting response of higher parts would
still be observable.

DORE (1965) has recently published a critical review of the literature
on the formation of adventitious roots in a contribution to the Ency-
clopedia of Plant Physiology, dealing with regeneration phenomena.
In the present paper, therefore, no survey of the literature will be
given.

CHAPTER II

MATERIAL AND METHODS

PLANT MATERIAL

The clone of *Coleus* used was the same as that used in earlier experi-
ments by the present author (SOEKARJO, 1961 and 1965). This clone
has been used earlier by TERPSTRA (1956) and VENDRIG (1960 and
1961) under the name of *Coleus rhenaltianus*. As there was some uncer-
tainty as to the correctness of the species name, material was sent to

the „Rijksherbarium" at Leiden where it has been re-identified as belonging to the polymorph species *Coleus scutellarioides* Benth.

The plants were propagated from cuttings, and grown in the greenhouse in separate pots. When necessary, day-length was supplemented by illumination with fluorescent light tubes (Philips TL 40 W/33).

EXPERIMENTS ON THE EPINASTIC CURVATURE

The procedure followed is the same as in the experiments reported earlier (SOEKARJO, 1961 and 1965): a slight modification of the method described by BOTTELIER (1954).

The plants were used when they had seven or eight fully grown leaf pairs; the leaf pairs are counted from the apical bud downward. The shoots were harvested and kept in darkness for 24 hours, with their basal ends in tap water. The petioles were then cut off from the shoot and the laminae were discarded. Ten petioles were put in vials containing 100 ml of a test solution. All manipulations were done under green light (emission between $\lambda = 500$ nm and $\lambda = 580$ nm, with an intensity of 0.4 μ watt·çm^{-2}), the time needed for one batch was less than 5 minutes.

The light in the experimental treatment was obtained from two Philips TL 40 W/33 fluorescent light tubes, suspended 60 cm above the working table.

After a 24 hours' stay in the test solution, either in darkness or in light, the petioles were shadowgraphed and the curvatures measured by means of a goniometer.

The values given are the mean values of 10 curvatures together with the standard error of the mean:

$$\bar{x} \pm \sqrt{\frac{\Sigma (x - \bar{x})^2}{n (n-1)}},$$

where: $\bar{x} = \dfrac{\Sigma x}{n}$,

x = measured value, and

n = number of values measured (10 in the epinastic curvature experiments, variable in the rooting experiments). The values are rounded off to the nearest degree.

The concentrations of indoleacetic acid are given in relative weights (g/g).

EXPERIMENTS ON ROOT FORMATION

The plants used had seven or eight fully grown leaves, as was the case in the experiments on the epinastic curvature. The plants were cut off at the first node above the ground and used as indicated in the description of the experiments. The internodes and nodes are counted from the base upward, because the lowest 1 cm *or* a longer part of the

cutting is put in the test solution and injurious effects therefore can be seen most clearly on the lowest part, gradually diminishing in upward direction.

For each treatment a batch of 10 cuttings was used. The values given are the means and the standard errors and when necessary the number of cuttings in brackets.

The light in the experiments under a 12 hours light/ 12 hours darkness regime was obtained from four Philips TL 40 W/33 fluorescent light tubes, suspended 80 cm above the table.

In the preliminary experiments on the influence of light the same setting was used as in the experiments on the epinastic curvature.

VARIABILITY AND EXPERIMENTAL PROCEDURE

Although the plants used for the experiments had all been grown in the same way and were used when they had grown to the same visual standard, great differences in their behaviour were observed. Therefore, equality in type of response, rather than equality in the values obtained, were taken in consideration when evaluating the experiments. When comparing different experiments, the author tried, as far as possible, to use experiments with control values that did not differ too much.

It appeared, that proceeding in this way, even when no absolutely controlled plant growing conditions are available, valuable results can be obtained. On the other hand, when a conventional mathematical treatment of different experiments is carried out, i.e. the calculation of mean values for a group of experiments done with plants of different age and at different moments, responses of a less pronounced nature, as for instance the response to low concentrations of indoleacetic acid as described in Chapter III, may be blurred and pass unacknowledged.

CHAPTER III

THE EPINASTIC CURVATURE OF ISOLATED PETIOLES AS A RESPONSE TO INDOLEACETIC ACID

INTRODUCTION

The experiments reported in this chapter were carried out in order to determine the concentration range of indoleacetic acid, in which the curvature of the isolated petioles shows a distinct dependence on the concentration.

EXPERIMENTAL

The first experiments were done to obtain a general picture of the behaviour of the isolated petioles towards indoleacetic acid. Parallel

experiments were done in darkness and in light. When a series of concentrations of indoleacetic acid was tested, the response was not the same in the different experiments.

Generally, an increase in curvature was observed in concentrations between 10^{-7} and 10^{-5} indoleacetic acid.

At concentrations between 10^{-9} and 10^{-7} indoleacetic acid there was a great dissimilarity in the results of the different experiments: the response either increased, showed a small maximum, or a small minimum. These three types of response occurred in darkness as well as in light. The curvature was enhanced by light at concentrations of indoleacetic acid between 10^{-7} and 10^{-5}.

TABLE I

The epinastic curvature of isolated petioles of the third leaf pair, after 24 hours in solutions of indoleacetic acid, in light and darkness. Mean values of the curvature of 10 petioles and the standard error of the mean, in degrees

IAA concen-tration	exp. 1		exp. 2		exp. 3	
	light	dark	light	dark	light	dark
control	37 ± 6	24 ± 5	26 ± 9	34 ± 5	20 ± 6	22 ± 5
10^{-9}	36 ± 5	24 ± 3	32 ± 3	28 ± 7	22 ± 4	28 ± 5
10^{-8}	39 ± 8	29 ± 4	28 ± 5	20 ± 4	21 ± 3	44 ± 6
10^{-7}	48 ± 10	43 ± 7	27 ± 9	26 ± 6	59 ± 13	47 ± 7
10^{-6}	81 ± 8	58 ± 6	63 ± 8	45 ± 9	100 ± 16	81 ± 9
10^{-5}	129 ± 12	102 ± 11	98 ± 13	97 ± 13	121 ± 14	110 ± 15
10^{-4}	100 ± 17	91 ± 19	87 ± 26	104 ± 20	99 ± 11	97 ± 18

In Table I the results are given of three experiments which show the effect of indoleacetic acid in different concentrations, in darkness as well as in light. Only petioles of the third leaf pair are represented here.

These results are given graphically in Fig. 2.

The region of low concentrations of indoleacetic acid to which the response varied in the different experiments, was termed "labile" and further experiments were done to obtain more information on this phenomenon, as already published in a preliminary note (SOEKARJO, 1961).

These experiments were restricted to the response in darkness only, as light can have an enhancing as well as an inhibiting effect, as has been indicated above.

As can be seen from Table I, the values of the standard errors of the mean reflect a great variability of the plant material. Therefore, it has been tried to repress the variability by extreme care in the selection of plant material for further experiments.

For these experiments on the response to low concentrations of indoleacetic acid, petioles of the third and the fifth leaf pairs were used, so that not only the underlying process might become clear, but also the response of petioles of different age could be compared.
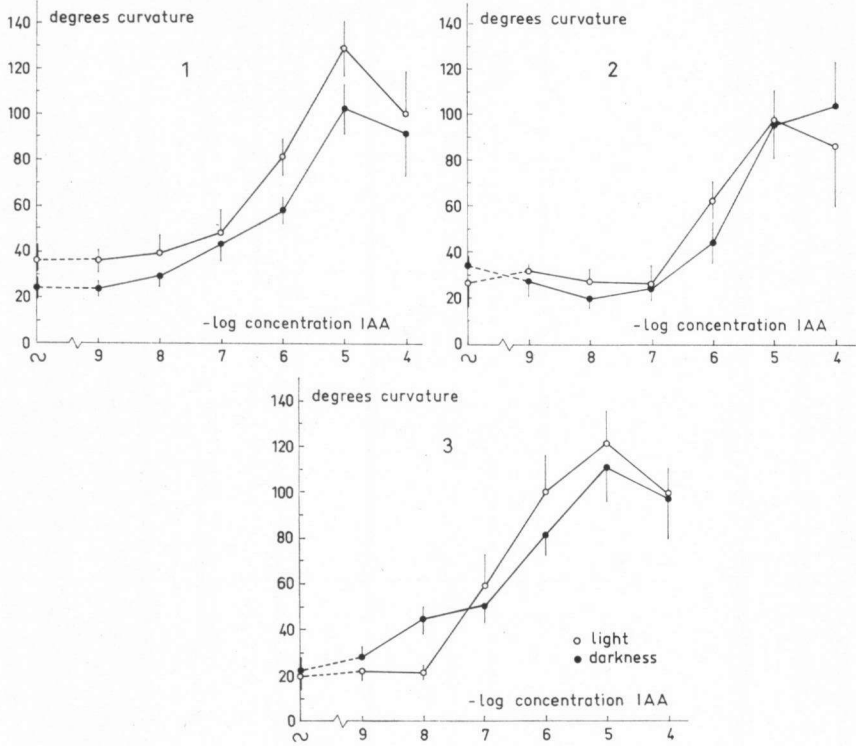
Fig. 2. The epinastic curvature of isolated petioles of the third leaf pair, after 24 hours in solutions of indoleacetic acid, in light and in darkness respectively. Mean values of the curvature of 10 petioles and the standard error of the mean.

TABLE II

The epinastic curvature of petioles of the third and the fifth leaf pair, in darkness. Mean values of the curvature of 10 petioles and the standard error of the mean, in degrees

IAA concen-tration	exp. 1		exp. 2	
	3rd pair	5th pair	3rd pair	5th pair
control	42 ± 4	29 ± 4	39 ± 5	31 ± 4
1 × 10	45 ± 4	32 ± 2	45 ± 3	21 ± 4
1 × 10	52 ± 5	35 ± 4	47 ± 3	19 ± 2
2.5 × 10	54 ± 4	38 ± 3	36 ± 3	22 ± 4
5 × 10	48 ± 2	29 ± 3	38 ± 3	25 ± 4
7.5 × 10	44 ± 4	24 ± 3	33 ± 3	20 ± 2
1 × 10	41 ± 6	21 ± 2	47 ± 4	21 ± 2
2.5 × 10	60 ± 6	36 ± 3	55 ± 5	23 ± 3

As can be seen in Fig. 3 and from Table II, the petioles of the third as well as those of the fifth leaf pair show the same type of response.
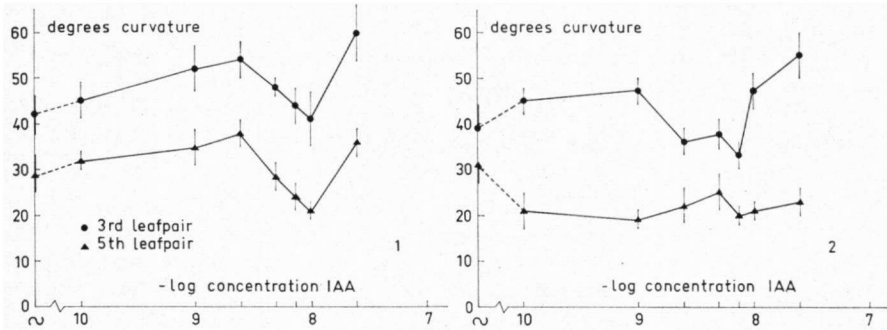

Fig. 3.   The epinastic curvature of isolated petioles of the third and the fifth leaf pair, after 24 hours in solutions of indoleacetic acid, in darkness. Mean values of the curvature of 10 petioles and the standard error of the mean.

In the curvature *vs.* concentration curves of Fig. 3 the response of both the third and the fifth leaf pair shows a small maximum followed by a small minimum. Generally the response of the fifth leaf pair is considerably weaker than that of the third leaf pair, though showing the same pattern. So we see that the "lability" of this concentration-region is caused by a small maximum in the response followed by a small minimum, which occur at slightly different concentrations of indoleacetic acid in the different experiments.

Apart from this effect of low concentrations, there is another interesting point. In some cases a discolouration of the tissue was observed in high concentrations of indoleacetic acid. This was very clear in concentrations above 10^{-5} indoleacetic acid, but sometimes even in lower concentrations. When occurring in lower concentrations, this discolouration was only seen at the foliar end of the petiole. In higher concentrations both ends were discoloured and sometimes even a larger part of the petiole was brown after 24 hours in the solution.

CHAPTER IV

THE ROOTING RESPONSE OF THE CUTTINGS

INTRODUCTION

Coleus is known to be easily propagated from cuttings and is, therefore, a suitable plant for experimental purposes in general and in particular for investigations on root formation. LUNDEGÅRDH (1915) conducted experiments on polarity and root formation in a *Coleus blumei* hybride. When the leaves are laid flat on the ground and the midrib is severed, roots will develop from the cuts (SMITH, 1925). FISCHNICH (1935), while analyzing the effect of indoleacetic acid on leaf move-

ments in four species of *Coleus*, observed the emergence of adventitious roots along the shoot and even on the petioles. BOTTELIER (1958), working with the same clone of *Coleus* used in the experiments reported in this paper, found that isolated leaves with the petioles still attached, could be used for the root formation test as described by VAN RAALTE (1950) for *Ageratum houstonianum* Mill.

Some introductory experiments on the root formation by the cuttings of *Coleus* are reported in this chapter.

EXPERIMENTAL

The shoots to be used were cut off at the first node above the ground and put in vials containing tap water, after removal of the lowest two leaf pairs together with their petioles.

Light promotes the formation of roots, as can be seen in Table III. Apart from this result, it was noted that in darkness the roots were formed at the lowest node only.

TABLE III

Total number of roots per cutting, counted after 7 days, in continuous light and in darkness. Mean values of 10 cuttings and the standard error of the mean

treatment	exp. 1	exp. 2
continuous light	28.6 ± 2.2	30.4 ± 5.5
darkness	3.4 ± 0.8	2.6 ± 0.5

In light roots were also formed on the internodes. The roots at the nodes appeared within two or three days, the roots on the internodes after a longer period.

This fact suggests the existence of two types of roots which indeed appeared to be true. In older parts of the shoots, just above and below the "hair line" (a circumferential growth of dermal appendages) at a node, root initials are often present. In some cases these root initials are visible as small knobs at the node. The rapid appearance of roots at the nodes is due to these initials. In short-time experiments never more than 8 roots were noticed at a node. In experiments lasting more than two weeks, 12 or more roots could sometimes be seen at one node. In these experiments there is no certainty about the final number of adventitious roots and the number of roots developed from initials already present. The roots on the internodes, however, were all newly formed during the experiment. Sometimes the number of roots at the node was higher than twelve, which was due to the formation of twin roots. The number of places of emergence was not more than twelve, corresponding with the vascular bundles in the shoot.

As the results of the experiments given in Table III suggest no *de novo* formation of adventitious roots in darkness, this was further tested. The lowest 5 mm of the cuttings, measured from the "hair line",

was removed, so as to leave no root initials in the lowest part of the cuttings. These cuttings were kept in darkness. The results are given in Table IV.

TABLE IV

The influence of removal of the lowest 5 mm of the cutting on the total number of roots per cutting, counted after 7 days in darkness. Mean values of 10 cuttings and the standard error of the mean

treatment	exp. 1	exp. 2
lowest 5 mm removed	11.4 ± 4.2	13.0 ± 1.5
control	3.8 ± 0.1	4.6 ± 0.1

It is clear, that removal of the zone containing root initials enhances the number of roots formed on the internodes in darkness.

After these experiments, the rooting response under a 12 hours light/ 12 hours darkness regime was studied. In the experiments given in Table V, the effect of the removal of the lowest 5 mm of the shoot is compared with the effect of the removal of half of the lowest internode.

TABLE V

The number of roots formed after removal of the lowest 5 mm and after removal of the lower half of the first internode, counted after 17 days (alternating light and dark periods of 12 hours). Mean values of 10 cuttings and the standard error of the mean

localization of roots	treatment		
	control	lowest 5 mm removed	lower half of lowest internode removed
lowest node	9.2 ± 0.6 9.9 ± 1.2		
lowest internode	12.6 ± 1.7 15.3 ± 3.0	32.2 ± 4.0 31.3 ± 3.4	35.9 ± 3.7 28.4 ± 2.5
second node	1.2 ± 0.3 1.3 ± 0.3	2.0 ± 0.4 1.2 ± 0.3	0.4 ± 0.3 0.6 ± 0.9
second internode	2.4 ± 0.6 2.8 ± 0.7	2.6 ± 0.8 1.9 ± 0.9	5.1 ± 1.0 4.6 ± 1.0
third node	1.6 ± 0.4 1.2 ± 0.3	1.1 ± 0.5 0.8 ± 0.3	1.0 ± 0.4 1.5 ± 0.4

The effect of the removal of the lowest 5 mm of the cuttings, containing the root initials, is evident. The removal of an additional part of the lowest internode does not, however, increase the number of newly formed roots.

DISCUSSION

A point for consideration is the promotion of the formation of roots, in darkness as well as in light (Tables IV and V), after the removal of the zone containing root initials.

This phenomenon might be described as a "basal dominance". There is an obvious priority of the development of nodal roots over the development of internodal roots. There is no formation of internodal roots at all when the nodal roots have not yet developed; when the nodal roots do emerge, there is a possibility of roots developing on the suprajacent internode. When the nodal zone has been removed, the internodal roots develop readily.

The mechanism of the "basal dominance" might be explained as follows. Roots are only formed on the internodes, when the concentration of substances promoting their formation is above a certain level. These substances can be expected to be transported downwards from the higher parts of the cutting, as MOUREAU (1940) has reported an increase in root formation on (younger) *Coleus* plants with an increasing number of leaves that were left on plants treated with indoleacetic acid in lanoline paste, below the place of attachment of the leaves.

The concentration level needed for the emergence of the root initials at the node must be lower than that needed for the initiation of adventitious roots in the internode. When the shoots are cut off at the node, the concentration of root promoting substances that arrive at the root initials is sufficient to cause these initials to grow out. During the process of the growth of these roots a certain amount of the root promoting substances is consumed, keeping the concentration level of these substances in the internode relatively low. We see only a formation of internodal roots in light and not in darkness, when the lowest node is present (Tables III and IV).

Therefore we can assume that the substances needed for this process are only produced in light. MOUREAU (1940) has found a strong stimulation of the formation of roots on (younger) *Coleus* plants after a treatment with indoleacetic acid, when the plants had been kept in light, as compared to those that had been kept in darkness.

So, when the cuttings are kept in the dark, only the amount of root promoting substances present in the whole cutting at the beginning of the experiment is available. In this case, the concentration in the internode is below that required for the formation of new roots in the internode, but high enough for the initials present in the node to grow out.

When the zone containing the root initials is removed, there is no consumption by the rapid growth of the nodal roots and therefore the concentration in the internode becomes higher. If this concentration reaches the level required for root formation, adventitious roots are formed on the internode.

The effect of removal of the lowest 5 mm cannot be due to a relative increase of the amount of substances supplied from higher parts. Because then, of course, removal of half of the lowest internode would result in a further promotion of the root formation, as roughly the same

amount of substances would be available for half as much tissue. It has been shown that this is not the case (Table V).

The difference in results with SMITH (1925), who did not find root initials on the nodes, might be due to the difference in age of the material used and the growing procedure.

CHAPTER V
THE EFFECT OF INDOLEACETIC ACID ON THE ROOT FORMATION OF THE CUTTINGS

INTRODUCTION

Before the effect of injurious conditions could be tested, the effect of indoleacetic acid on the root formation of the cuttings had to be studied. Special attention had to be paid to possible injurious effects of higher concentrations of indoleacetic acid.

EXPERIMENTAL

The effect of indoleacetic acid was tested under a 12 hours light/ 12 hours darkness regime, as was done in the experiments of which the results are given in Table V of the preceeding chapter.
The lower 10 cm of the cuttings were immersed in various concentrations of indoleacetic acid. In the first experiments the treatment lasted 24 hours; the level of the solution was kept approximately constant. After a thorough rinse the cuttings were put in vials containing tap water.

Table VI gives the results of an experiment of the type described.

TABLE VI

Total number of roots on 10 cuttings after a 24 hours' treatment of 10 cm of the basal part with indoleacetic acid, counted after 16 days (alternating light and dark periods of 12 hours)

concentration IAA / localization	control	5×10^{-5}	10^{-4}	5×10^{-4}	10^{-3}
lowest node	88	77	0	necrotic	necrotic
lowest internode	66	851	+++	0	necrotic
second internode	3	147	+++	0	0
third internode	10	130	304	+++	0
fourth internode	8	64	413	+++	+++
fifth internode	2	20	377	+++	+++
sixth internode		1	47	397	+++
seventh internode				118	+++
eighth internode				37	+++
ninth internode				41	+++
top internode				1	+++

+++ completely covered with roots

Only the roots developed on the internodes are given, as these are clearly adventitious roots. The roots at the lowest node are given only as a measure of the injurious effect of the concentration of indole-acetic acid used. The promotion of the root formation was clear in all the concentrations of indoleacetic acid used. The effect of the treatment with a concentration of 10^{-3} indoleacetic acid was very striking. The whole shoot was covered with small wartlike structures, which developed into roots when they were under the water level in the vial.

The damaging effect of higher concentrations on the lower part of the cutting is evident.

After a treatment with 5×10^{-4} and 10^{-3} indoleacetic acid, no roots were formed on the lowest three internodes, a larger part of these internodes being necrotic. The exuberant formation of roots occurs immediately above the region where no roots at all were formed.

Under the same conditions the effect of half an hour's immersion of the lowest 1 cm of the shoot was tested. No necrosis was observed in these experiments.

Table VII gives the results for the lowest nodes and internodes in two experiments of this kind. The number of cuttings on which roots were formed is given in brackets; the mean is only calculated for those cuttings which did form roots. In experiment 2 of Table VII, in which experiment the cuttings did not form roots easily, the number of cuttings forming roots clearly increased after treatment with concentrations of indoleacetic acid from 10^{-4} upward.

TABLE VII

The number of roots at the lowest node and the lowest internode after half an hour's treatment of 1 cm of the basal part of the cuttings with indoleacetic acid, counted after 15 days (alternating light and dark periods of 12 hours). In brackets the number of cuttings that did root (out of a total of 10 cuttings); mean values of these cuttings only, and the standard error of the mean

IAA concen-tration	number of roots			
	lowest node		lowest internode	
	exp. 1	exp. 2	exp. 1	exp. 2
control	7.0 ± 1.1 (7)	9.3 ± 0.6 (10)	2.9 ± 0.6 (7)	1.5 ± 0.5 (2)
10^{-6}	9.1 ± 0.4 (10)	8.6 ± 0.7 (10)	5.6 ± 1.4 (10)	3.8 ± 2.1 (4)
5×10^{-6}	8.6 ± 0.7 (10)	7.9 ± 0.4 (10)	3.3 ± 0.7 (8)	2.8 ± 0.3 (6)
10^{-5}	9.5 ± 0.7 (10)	9.8 ± 0.6 (10)	3.1 ± 0.5 (10)	1.5 ± 0.2 (6)
5×10^{-5}	5.8 ± 0.9 (10)	8.5 ± 0.3 (10)	5.3 ± 1.3 (10)	2.0 ± 0.8 (6)
10^{-4}	7.1 ± 0.8 (10)	8.4 ± 0.4 (10)	4.5 ± 1.0 (10)	2.4 ± 0.4 (10)
5×10^{-4}	5.9 ± 1.6 (8)	9.1 ± 0.6 (10)	16.4 ± 4.0 (9)	8.3 ± 1.1 (10)
10^{-3}	6.5 ± 3.5 (2)	9.6 ± 0.6 (10)	23.5 ± 4.9 (8)	28.4 ± 3.1 (10)
2×10^{-3}		6.0 ± 1.5 (7)		65.1 ± 6.7 (10)

DISCUSSION

The results given in Table VI clearly show that the formation of adventitious roots is stimulated by a 24 hours' treatment with indoleacetic acid. No optimal concentration can be indicated. The number of roots increases with higher concentrations of indoleacetic acid, until the tissue is killed.

If we assume that with increasing concentrations of indoleacetic acid a greater part of the petiole is killed, the optimum curves for the rooting response of petioles of *Ageratum* as described by BOTTELIER (1956a) might be explained. The petioles namely, will show maximal root formation at the "just sub-lethal" concentration. At higher concentrations the number of roots will be smaller, as the living part of the petiole diminishes, thus suggesting an optimum-type of curve for the response towards increasing concentrations of indoleacetic acid.

In our own experiments, when the cuttings had been treated with indoleacetic acid for 24 hours, we see (Table VI) that for the lowest and second internode 10^{-4} indoleacetic acid might, indeed, be regarded as an optimal concentration for the formation of adventitious roots. However, no roots at all are formed in higher concentrations and the tissue eventually becomes necrotic. The concentration of 10^{-4} indoleacetic acid in this experiment can be regarded as "just sub-lethal".

The treatment with 5×10^{-4} indoleacetic acid must result in a concentration within the tissue that is above the lethal level for the lowest two internodes and perhaps also for the third internode. The concentration in the higher internodes decreases rapidly, as is reflected by the decreasing number of roots formed.

The sensitivity of the younger internodes must be greater in higher (therefore younger) internodes, as a treatment with 10^{-3} indoleacetic acid resulted in an outburst of roots all over the cutting including the very small top internode.

This difference in sensitivity might also be the explanation for the fact that two internodes (the lowest and the second) both showed the reaction to a "just sub-lethal" concentration after a treatment with 10^{-4} indoleacetic acid.

The response of the fourth and higher internodes can be represented by a saturation-type of curve. This, however, indicates the possibility of another complication, namely, that the range of internal concentrations eliciting the response described as the response to "just sub-lethal" concentrations may be rather wide. For the fourth and fifth internode for instance, the results of a treatment with 5×10^{-4} and with 10^{-3} suggest a rather wide range of internal concentrations that should be termed "just sub-lethal". This consideration might, however, be superfluous, as there was no further gradation in the indication "completely covered with roots". So it is possible that there is still an increase in the number of roots formed at the fourth and fifth internode after a treatment with 10^{-3} indoleacetic acid as compared with a treatment with 5×10^{-4}.

The results obtained after a treatment with indoleacetic acid lasting

for half an hour only (Table VII), indicate that in this short period the amount of indoleacetic acid taken up by the cuttings is too small to cause any injurious or "just sub-lethal" effect.

For an indication of the value of the "just sub-lethal" concentration we have to turn to the results of a treatment lasting for 24 hours again (Table VI). For the lowest internode it can be said, with due reserve, that 10^{-4} indoleacetic acid is lethal as an internal concentration: no roots at the lowest node. It can be assumed that the concentration inside the internode must be lower than 10^{-4}. The amount of indoleacetic acid taken up in 24 hours out of the ambient medium results in an internal concentration that is "just sub-lethal". This "just sub-lethal" concentration must therefore be lower than 10^{-4}.

In the experiments of VAN RAALTE (1950) on the formation of adventitious roots on petioles of *Ageratum* there is an indication of the same phenomenon. The adventitious roots formed after a 20–24 hours' treatment with α-naphtaleneacetic acid showed a concentration dependent increase within the range of 10^{-6} to 10^{-4} Mol/l. But above the latter value, so many roots were formed, that accurate counting became impossible. The value of the concentration causing necrosis, however, was not given.

CHAPTER VI

THE EFFECT OF INJURIOUS CONDITIONS ON THE ROOT FORMATION OF THE CUTTINGS

INTRODUCTION

As has been shown in the preceeding chapter, an "outburst" of adventitious roots occurred after a 24 hours' treatment with high concentrations of indoleacetic acid. Furthermore, these concentrations of indoleacetic acid were injurious to the lower parts of the cuttings. Therefore, the concentration in the tissue above the damaged parts was termed "just sub-lethal".

Although indoleacetic acid has a specific activity, this exuberant formation of roots, just above the damaged parts of the cutting, need not be a specific effect of indoleacetic acid.

To investigate this point further, the effect of non-specific injurious substances on the root formation was tested.

In the first place, experiments on the effect of potassium hydroxide and sulphuric acid were done.

Later on, the use of mercuric chloride, an inhibitor of many enzymes and a common poison, was suggested by Dr H. P. Bottelier.

EXPERIMENTAL

A 24 hours' treatment with 0.5% and 1% potassium hydroxide and sulphuric acid respectively, resulted in an irreversible wilting of the cuttings.

When the treatment lasted for half an hour only, the wilted cuttings recovered after two or three days.

The results of experiments carried out in summer are shown in Table VIII and Fig. 4. The total number of internodal, and therefore adventitious roots are given, counted after 15 days.

TABLE VIII

The number of internodal roots on the whole cutting after half an hour's treatment of 1 cm of the basal part of the cuttings with sulphuric acid and potassium hydroxide respectively, counted after 15 days (alternating light and dark periods of 12 hours). Mean values of 10 cuttings and the standard error of the mean. Experiments in summer

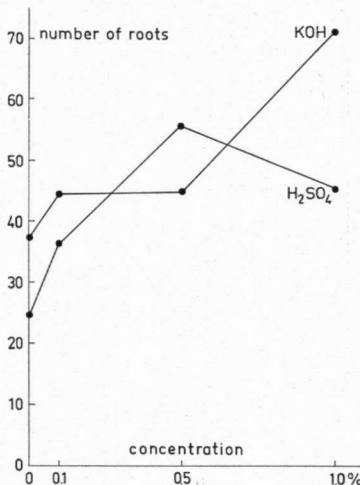| treatment	number of roots
control	24.5 ± 1.7
0.1 % H_2SO_4	36.7 ± 4.9
0.5 % H_2SO_4	55.6 ± 5.9
1 % H_2SO_4	45.5 ± 4.2
control	37.3 ± 3.0
0.1 % KOH	44.7 ± 3.8
0.5 % KOH	45.3 ± 5.2
1 % KOH	71.1 ± 11.9

Fig. 4. The number of internodal roots formed on the whole cutting after half an hour's treatment of 1 cm of the basal part of the cuttings with potassium hydroxide and sulphuric acid, counted after 15 days. Mean values of 10 cuttings. Experiments in summer.

The effect of potassium hydroxide is given in detail in Table IX and Fig. 5. In this table the localization of the roots and the number of cuttings that showed root formation are given.

TABLE IX

The number of roots on five suprajacent internodes (nodal roots excluded) after half an hour's treatment of 1 cm of the basal part of the cuttings with potassium hydroxide, counted after 15 days (alternating light and dark periods of 12 hours). Mean values of 10 cuttings and the standard error of the mean. Experiment in summer

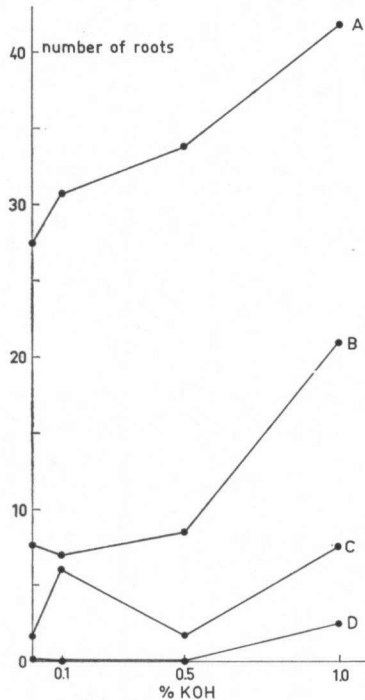| localization treatment	number of roots				
	A lowest internode	**B** second internode	**C** third internode	fourth internode	**D** fifth internode
control	27.8 ± 2.5	7.6 ± 1.6	1.6 ± 0.5	0.6 ± 0.4	0.1 ± 0.1
0.1 % KOH	30.7 ± 3.2	6.7 ± 1.3	6.0 ± 1.4	1.3 ± 0.9	0.0
0.5 % KOH	33.8 ± 4.0	9.4 ± 2.2	1.7 ± 0.5	0.4 ± 0.3	0.0
1 % KOH	41.8 ± 13.2	21.0 ± 8.3	7.6 ± 1.4	6.9 ± 1.9	2.5 ± 1.8

Fig. 5. The number of internodal roots formed on four internodes after half an hour's treatment of 1 cm of the basal part of the cuttings with potassium hydroxide, counted after 15 days. Mean values of 10 cuttings. Experiment in summer.

The promoting effect of 1% potassium hydroxide is evident in five suprajacent internodes.

Experiments carried out in winter did not show this promoting effect in the second and higher internodes after 17 days. The effect on the lowest internode, however, is clear.

TABLE X

The number of roots on lower parts after half an hour's treatment of 1 cm of the basal part of the cuttings with sulphuric acid, counted after 17 days (alternating light and dark periods of 12 hours). In brackets the number of cuttings that did root (out of a total of 10 cuttings); mean values of these cuttings only, and the standard error of the mean. Experiment in winter

concentration of H_2SO_4 localization	0%	0.1%	0.5%	1%
lowest node	8.9 ± 0.9 (10)	9.6 ± 0.8 (10)	2.7 ± 0.9 (3)	0 (0)
lowest internode	2.1 ± 0.4 (8)	1.7 ± 0.6 (6)	8.4 ± 0.7 (10)	14.1 ± 4.1 (10)
second node	2.1 ± 0.5 (10)	2.4 ± 0.4 (10)	2.6 ± 0.5 (8)	2.5 ± 0.2 (10)
second internode	1.7 ± 0.2 (3)	1.5 ± 0.5 (2)	2.0 ± 1.0 (2)	2.0 ± 0.0 (2)
third node	2.3 ± 0.1 (10)	2.7 ± 0.4 (10)	2.3 ± 0.9 (9)	1.9 ± 0.1 (9)

TABLE XI

The number of roots on lower parts after half an hour's treatment of 1 cm of the basal part of the cuttings with potassium hydroxide, counted after 17 days (alternating light and dark periods of 12 hours). In brackets the number of cuttings that did root (out of a total of 10 cuttings); mean values of these cuttings only, and the standard error of the mean. Experiment in winter

concentration of KOH localization	0%	0.1%	0.5%	1%
lowest node	8.8 ± 0.9 (10)	8.0 ± 1.0 (10)	4.9 ± 0.9 (7)	0 (0)
lowest internode	1.4 ± 0.2 (5)	3.3 ± 0.8 (7)	5.6 ± 1.4 (8)	14.8 ± 2.7 (10)
second node	2.1 ± 0.3 (10)	2.2 ± 0.3 (10)	1.9 ± 0.3 (9)	1.9 ± 0.3 (9)
second internode	1.0 ± 0.0 (2)	1.5 ± 0.5 (2)	1.5 ± 0.5 (4)	1.3 ± 0.3 (6)
third node	2.8 ± 0.2 (9)	2.2 ± 0.4 (10)	2.3 ± 0.4 (9)	2.6 ± 0.2 (9)

As it might be possible that this was due to a shortage of substances needed for the formation of roots, causing a delay in their emergence, the roots were counted after 4 weeks in other experiments. The results are given in Tables X, XI, XII and XIII. Even after four weeks, no

TABLE XII

The number of roots on lower parts after half an hour's treatment of 1 cm of the basal part of the cuttings with sulphuric acid, counted after 4 weeks (alternating light and dark periods of 12 hours). In brackets the number of cuttings that did root (out of a total of 10 cuttings); mean values of these cuttings only, and the standard error of the mean. Experiment in winter

concentration of H_2SO_4 / localization	0%	0.1%	0.5%	1%
lowest node	7.9 ± 0.6 (10)	9.7 ± 0.8 (10)	1 (1)	0 (0)
lowest internode	5.2 ± 1.0 (9)	8.1 ± 1.2 (10)	33.8 ± 3.9 (10)	35.0 ± 2.9 (10)
second node	1.9 ± 0.3 (9)	1.9 ± 0.5 (7)	1.7 ± 0.3 (9)	2.3 ± 0.4 (9)
second internode	1.3 ± 0.2 (6)	1.7 ± 0.5 (6)	1.5 ± 0.5 (2)	3.0 ± 2.0 (2)
third node	1.9 ± 0.3 (9)	2.5 ± 0.3 (10)	2.2 ± 0.3 (9)	1.9 ± 0.3 (9)

TABLE XIII

The number of roots on lower parts after half an hour's treatment of 1 cm of the basal part of the cuttings with potassium hydroxide, counted after 4 weeks (alternating light and dark periods of 12 hours). In brackets the number of cuttings that did root (out of a total of 10 cuttings); mean values of these cuttings only, and the standard error of the mean. Experiment in winter

concentration of KOH / localization	0%	0.1%	0.5%	1%
lowest node	8.8 ± 0.5 (10)	9.4 ± 0.4 (10)	0 (0)	1 (10)
lowest internode	4.6 ± 0.9 (9)	5.7 ± 0.8 (10)	20.2 ± 2.4 (10)	32.2 ± 2.5 (10)
second node	2.1 ± 0.4 (9)	1.6 ± 0.3 (8)	2.1 ± 0.4 (7)	2.0 ± 0.5 (7)
second internode	3.3 ± 0.8 (4)	3.7 ± 0.8 (9)	2.5 ± 1.5 (2)	2.9 ± 0.9 (8)
third node	1.8 ± 0.2 (10)	2.3 ± 0.7 (8)	2.7 ± 0.3 (10)	2.2 ± 0.4 (10)

effect on higher internodes could be observed in these experiments in winter. The number of roots formed was, however, considerably greater than in 17 days, as can be clearly seen in Figs. 6 and 7.

In experiments with mercuric chloride the poisonous effect was only too clear in 1% and 0.5% solutions, even after half an hour's treatment. The shoots were totally wilted and some even did break.

Four days after the treatment the shoots in 1% mercuric chloride showed a brown discolouration, except the two or three highest internodes.

The shoots broke in the fourth or fifth internode. In the 0.1% solution approximately 4 cm of the lowest internode was discoloured.
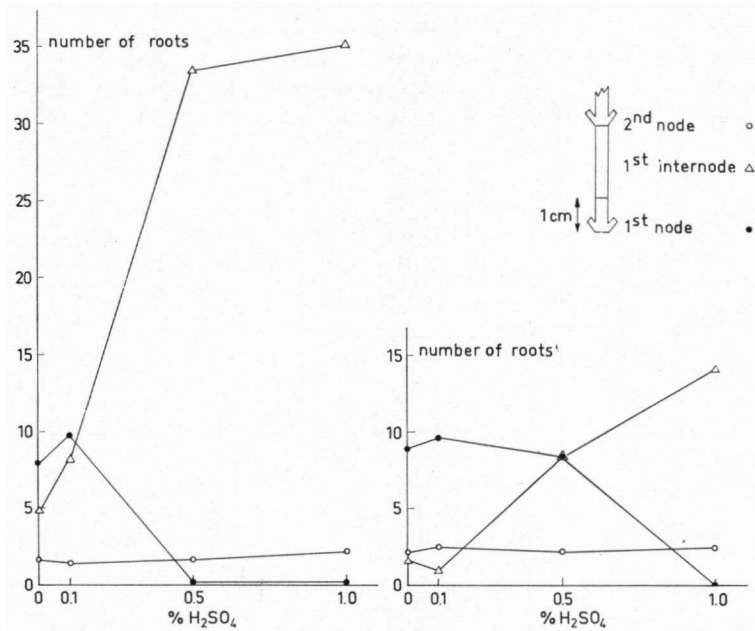
Fig. 6.   The number of roots formed at the lower part of the cuttings after half an hour's treatment of 1 cm of the basal part with sulphuric acid, counted after 17 days (right side) and after 4 weeks (left side). Mean values of 10 cuttings. Experiment in winter.
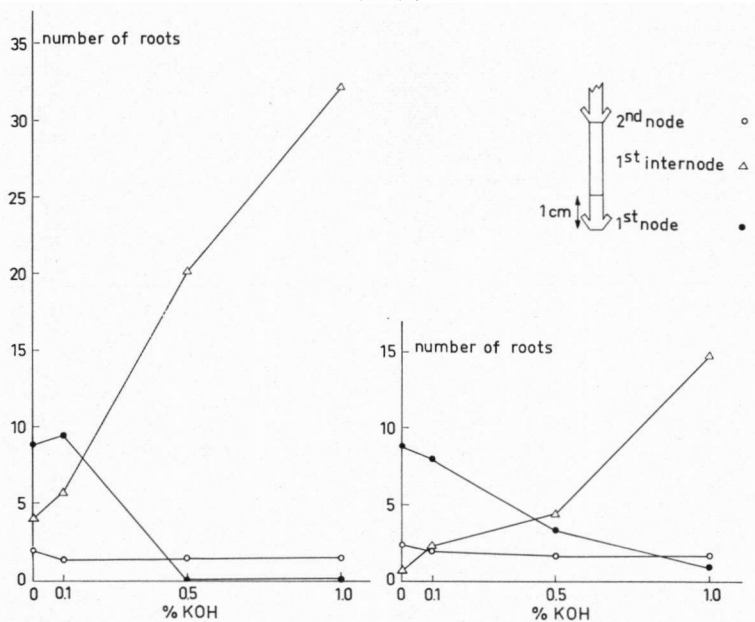

Fig. 7.   The number of roots formed at the lower part of the cutting after half an hour's treatment of 1 cm of the basal part with potassium hydroxide, counted after 17 days (right side) and after 4 weeks (left side). Mean values of 10 cuttings. Experiment in winter.

In lower concentrations no discolouration was observed. In the concentrations causing discolouration in the lowest internode only, the number of roots formed was clearly promoted, as can be seen in Tables XIV and XV, the results of two parallel experiments.

TABLE XIV

The number of roots in exp. 1 after half an hour's treatment of 1 cm of the basal part of the cuttings with mercuric chloride, counted after 18 days (alternating light and dark periods of 12 hours). In brackets the number of cuttings that did root (out of a total of 10 cuttings); mean values of these cuttings only, and the standard error of the mean. Experiment in summer

concentration of HgCl₂ / localization	control	0.01%	0.1%
lowest node	8.2 ± 0.6 (10)	8.1 ± 0.8 (10)	0
lowest internode	19.6 ± 3.0 (10)	25.1 ± 3.2 (10)	8.9 ± 1.7 (8)
second node	1.7 ± 0.3 (6)	1.2 ± 0.2 (6)	2.3 ± 0.6 (6)
second internode	5.6 ± 1.0 (10)	2.8 ± 0.9 (6)	42.8 ± 5.2 (10)
third note	3.1 ± 0.3 (10)	2.3 ± 0.3 (8)	1.8 ± 0.3 (8)
third internode	2.2 ± 0.8 (5)	1.7 ± 0.7 (3)	3.2 ± 1.3 (6)
fourth node	4 (1)	2.5 ± 0.7 (4)	2.0 ± 1.0 (4)
fourth internode	0	1	0
whole cutting	39.0 ± 0.7	39.0 ± 3.2	55.0 ± 5.8

TABLE XV

The number of roots in exp. 2 after half an hour's treatment of 1 cm of the basal part of the cuttings with mercuric chloride, counted after 14 days (alternating light and dark periods of 12 hours). In brackets the number of cuttings that did root (out of a total of 10 cuttings); mean values of these cuttings only, and the standard error of the mean. Experiment in summer

concentration of HgCl₂ / localization	control	0.01%	0.1%
lowest node	6.5 ± 0.7 (10)	6.3 ± 0.4 (10)	0
lowest internode	19.5 ± 3.0 (10)	19.0 ± 2.8 (10)	30.3 ± 7.5 (8)
second node	1.6 ± 0.3 (5)	2.0 ± 0.4 (7)	2.0 ± 0.4 (7)
second internode	6.2 ± 0.8 (10)	6.0 ± 1.4 (8)	17.3 ± 5.6 (9)
third node	2.2 ± 0.5 (6)	1.8 ± 0.4 (6)	1.9 ± 0.4 (8)
third internode	6.1 ± 1.1 (9)	4.0 ± 0.9 (8)	6.3 ± 1.1 (10)
fourth node	1.5 ± 0.5 (2)	1.3 ± 0.3 (3)	1.2 ± 0.2 (5)
fourth internode	2 (1)	5.0 ± 1.9 (4)	4.5 ± 0.3 (4)
fifth node	0	0	0
fifth internode	0	5.8 ± 0.9 (6)	12.0 ± 3.6 (6)
sixth node	0	0	0
sixth internode	0	11 (1)	4.0 ± 1.7 (3)
whole cutting	40.3 ± 3.4	43.2 ± 3.4	59.8 ± 8.2

DISCUSSION

Part of the stimulation of root formation by potassium hydroxide and sulphuric acid may be related to the removal of the "basal dominance", as the lowest node and part of the lowest internode are damaged in some of the experiments.

However, in some cases the promotion is evident, even up till and including the fourth internode (Table IX) and the total number of adventitious roots attains values twice that of the control batch (Table VIII). These two phenomena are difficult to explain by removal of the "basal dominance" alone.

Mercuric chloride too, promotes the formation of adventitious roots. In Table XIV the greatest promotion is seen in the second internode only, in Table XV in the second as well as in the lowest internode.

In both experiments, however, the increase of the number of roots as compared to the control values is evident. The solution of 0.01% of mercuric chloride has a promoting effect in one of these experiments. In both experiments the solution of 1% is lethal, the 0.1% solution is lethal for the lowest part of the cutting, and therefore "just sub-lethal" for the region above the discoloured part.

So, only the concentration of this poison that is "just sub-lethal" to the tissue promotes the formation of adventitious roots.

The observation in the experiment of Table XV of the formation of adventitious roots on the fifth and sixth internode, might be seen as a result of a higher sensitivity of the younger tissue. In this case the "just sub-lethal" concentration for the fifth and sixth internode must be supposed to be lower than that for the fourth internode.

In conclusion it may be said, that the type of injury that causes a higher number of adventitious roots to be formed is not specific.

Low pH-values as well as high pH-values and mercuric chloride show a promotion of the formation of adventitious roots by those concentrations that have a visible injurious effect on the lowest part of the cutting.

CHAPTER VII

GENERAL DISCUSSION

THE EPINASTIC CURVATURE OF THE PETIOLES

In the concentration range of 10^{-7} to 10^{-5} indoleacetic acid we see a concentration dependent increase of the epinastic curvature of isolated petioles of *Coleus*. In this range, therefore, the epinastic curvature could be used as a bio-assay for indoleacetic acid, with a series of known concentrations as a standard.

The petioles of the fourth leaf pair, counted from the apical bud downward, are most suitable for the bio-assay. The leaves are usually just fully expanded and the petioles are comparatively long. The curvature of older petioles in a solution of indoleacetic acid is smaller than that of petioles of the fourth leaf pair.

For one experiment, only plants of the same age, grown under the same conditions, should be used. Supplementary illumination by fluorescent light tubes was not sufficient to secure the absence of seasonal fluctuations in the response to indoleacetic acid.

Although the occurrence of a small maximum followed by a small minimum in the curvature vs. concentration curve may be of interest for further experiments upon the mechanism of the epinastic curvature and the endogenous substances playing a part in it, this response to low concentrations of indoleacetic acid interferes with the use of the epinastic curvature as a bio-assay in this concentration range.

BLAAUW–JANSEN (1959) described the occurence of a similar response pattern in oat coleoptiles. Irradiation with red light causes an increase of the lower maximum, which occurs at low indoleacetic acid concentrations. Extraction of coleoptiles irradiated with red light produced a substance which caused an increase of this maximum in non-irradiated coleoptiles. This substance was termed the "red light factor". It was shown that this substance did not evoke a response similar to that of gibberellic acid (GA_3) in oat coleoptiles, but no further indication of the identity of the "red light factor" was given.

THE NATURE OF REACTIONS ON SUPRA-OPTIMAL GROWTH SUBSTANCE CONCENTRATIONS

VON GUTTENBERG & MEINL (1952) and VON GUTTENBERG & BEYTHIEN (1952) have shown a destruction of the cytoplasm of epidermis cells of leaves of *Rhoeo discolor* by a concentration of 10^{-3} indoleacetic acid within twenty minutes. Furthermore, in the latter paper, the independence of this destruction of the pH-values was established.

As this destructive effect of high concentrations of indoleacetic acid is obvious when a concentration of 10^{-3} indoleacetic acid is used, a less marked injurious effect can be expected in somewhat lower concentrations, even if these effects might not be directly discernible.

The discolouration of one or both ends of the petioles may be regarded as an indication for such an injurious effect.

The seemingly contradictory views of HOUSLEY, BENTLEY & BICKLE (1954) and HANCOCK (1959), can be explained by the difference in exposure time to solutions of high concentrations of indoleacetic acid. During a short period of contact of the tissue with such a solution, only a small amount of growth substance enters the tissue, not damaging the cell mechanism.

Distinctly damaging concentrations of indoleacetic acid and injurious substances such as potassium hydroxide, sulphuric acid and mercuric chloride do promote the formation of adventitious roots, as

has been reported in this paper. Therefore, the fact that high concentrations of indoleacetic acid promote the formation of roots, cannot be regarded as an indication that these high concentrations are not injurious to the tissue, as supposed by BOTTELIER (1959).

THE FORMATION OF ADVENTITIOUS ROOTS

The enhancement of the formation of adventitious roots, caused by high concentrations of indoleacetic acid, is correlated with the injurious effect of the solutions. After a 24 hours' treatment with 10^{-3} or $2 \cdot 10^{-3}$ indoleacetic acid, the whole shoot is covered with roots, the lowest two or three internodes excepted, which are necrotic.

After a treatment with potassium hydroxide, sulfuric acid and mercuric chloride, there is an increase of adventious roots formed. This increase is, however, less than that caused by high concentrations of indoleacetic acid.

So, in the action of indoleacetic acid we have to distinguish two different effects: the direct injurious effect of high concentrations on the lowest part of the cuttings, *and* the effect of concentrations that became lower during the transport, in higher parts of the cuttings.

An indication that the actual concentration in the tissue in the higher parts of the cuttings is lower than in the lower parts, which are in direct contact with the medium, is of course the fact that the higher parts do not grow necrotic.

The effect of concentrations of indoleacetic acid that do not cause necrosis must again be distinguished in: the effect of concentrations that *just* do not cause necrosis, and the effect of still lower concentrations. The "just sub-lethal" concentrations are the concentrations that cause the exuberant formation of adventitious roots, just above the necrotic parts.

When we apply sulphuric acid, potassium hydroxide or mercuric chloride, the effect of "just sub-lethal" concentrations is similar to that of "just sub-lethal" concentrations of indoleacetic acid: just above the necrotic part there is a promotion of the formation of adventitious roots.

However, the additional effect of indoleacetic acid is absent, the amount of growth substance available, i.e. the endogenous growth substance, is limited. The promotion as compared to the control values is effected by the same, limited, amount of endogenous substances.

Therefore, the cells just above the necrotic parts must be more susceptible to the morphogenetic influence of endogenous growth substance than the cells that had not been treated with the poisons mentioned.

In other words, these treated cells must be strongly influenced by these substances, which results in a loss of the present organisation or differentiation. This loss of the differentiation makes the cells once again "meristematic", susceptible for new differentiation.

Working with tissue cultures of carrot root phloem, STEWART, MAPES & MEARS (1958) did not find the organized formation of roots in primary explants. After frequent sub-culturing of the callus growth from these explants, however, organized root formation was observed. This fact is comparable to the situation with *Coleus* cuttings that had been treated with injurious solutions. In both cases the cells must "forget" their differentiation before they become susceptible to new differentiation. Citing Stewart *et al.*: "it may be said that the differentiated phloem tissue must first "de-differentiate" — whatever that may mean".

In the case of treatment with an injurious substance, this substance may be regarded as effecting only the "de-differentiation" comparable with the effect of indoleacetic acid in "just sub-lethal" concentrations. The new differentiation is then a result of the action of the endogenous growth substance.

When we apply high concentrations of indoleacetic acid, this substance has both the primary "de-differentiating" property as well as the ability to promote the formation of roots.

In the case in which substances are applied that may have an influence on the amount of endogenous growth substance, the promotion of adventitious root formation may have a more complicated character.

If we assume that the endogenous growth substance is indoleacetic acid, as has been established for *Coleus blumei* by SCOTT & JACOBS (1964), those substances that cause an increase in the internal concentration by inhibiting the destruction, may eventually promote root formation, if the internal concentration reaches the level required for root formation.

This may be the explanation for the promotion of the formation of adventitious roots by carbon monoxide gas, for instance, as has been reported by ZIMMERMAN, CROCKER & HITCHCOCK (1933). As in these experiments the plants were exposed to daylight, and the inhibition of iron-enzymes by carbon monoxide is reversible by light, whereas the inhibition of copper-enzymes is not, the abundant formation of roots in these experiments might be regarded as an indication that the important enzyme system responsible for the inactivation of the endogenous growth substance (assuming that the growth substance is indoleacetic acid) is of the copper containing phenoloxidase type.

The promotion of the formation of roots after a treatment with potassium permanganate, CURTIS (1918), might, however, be interpreted in favour of a peroxidase type of enzyme. This is, however, less tndicative, as the treatment with potassium permanganate effects the iissue directly, and may exert an effect comparable to that of potassium hydroxide, sulfuric acid and mercuric chloride, as described and discussed in the present paper.

ACKNOWLEDGEMENTS

REFERENCES

BLAAUW-JANSEN, G. 1959. Acta Bot. Neerl. **8**: 1.
BOTTELIER, H. P. 1954. Ann. Bogor. **1**: 185.
————. 1956a. Ann. Bogor. **2**: 175.
————. 1956b. Ann. Bogor. **2**: 183.
————. 1958. Pers. comm.
————. 1959. Proc. Kon. Akad. Wet., Ser. C, **62**: 493.
CURTIS, O. F. 1918. Cornell Univ. Agric. Exp. Stat. Mem. **14**: 75.
DORE, J. 1965. *In*: Encyclopedia of Plant Physiology 15 (2).
FISCHNICH, O. 1935. Planta **24**: 552.
GUTTENBERG, H. VON & A. BEYTHIEN. 1952. Planta **40**: 36.
———— & G. MEINL. 1952. Planta **40**: 431.
HANCOCK, C. R. 1959. Ann. Bot. **23**: 107.
HOUSLEY, S., J. A. BENTLEY & A. S. BICKLE. 1954. Jour. Exp. Bot. **5**: 373.
KNIEP, H. 1910. Jahrb. f. wiss. Bot. **48**: 1.
LAIBACH, F. & O. FISCHNICH. 1936. Zentralbl. **56**: 62.
LUNDEGÅRDH, H. 1915. Flora **107**: 433.
————. 1918. Lunds Univ. Årsskr. N.F., Avd. 2, **14**: 1.
MAI, G. 1934. Jahrb. f. wiss. Bot. **79**: 681.
MOUREAU, J. 1940. Bull. Soc. Roy, Sci. Liège **9**: 41.
RAALTE, M. H. VAN. 1950. Ann. Bogor. **1**: 13.
SCOTT, T. K. & W. P. JACOBS. 1964. *In:* Régulateurs naturels de la croissance végétale. Paris.
SKOOG, F., CH. L. SCHNEIDER & P. MALAN. 1942. Amer. Jour. Bot. **29**: 568.
SMITH, E. P. 1925. Trans. Bot. Soc. Edinburgh **29**: 145.
SOEKARJO, R. 1961. Proc. Kon. Akad. Wet., Ser. C, **64**: 655.
————. 1965. Acta Bot. Neerl. **14**: 266.
STEWART, F. C., M. O. MAPES & K. MEARS. 1958. Amer. Jour. Bot. **45**: 705.
TERPSTRA, W. 1956. Acta Bot. Neerl. **5**: 157.
THIMANN, K. V. 1956. Amer. Nat. **90**: 145.
VENDRIG, J. C. 1960. Wentia **3**: 1.
————. 1961. Acta. Bot. Neerl. **10**: 190.
VRIES, H. DE. 1872. Arb. Bot. Inst. Würzburg **1**: 223.
ZIMMERMAN, P. W., W. CROCKER & A. E. HITCHCOCK. 1933. Contrib. Boyce Thompson Inst. **5**: 1.