THE SIGNIFICANCE OF
THE ROOT CAP FOR GEOTROPISM

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SUMMARY

The geotropic curvature of pea roots decapitated at 0.3 or 0.4 mm was retarded and no positive geotropic response occurred when the roots were decapitated at 0.5 mm or more. The elongation of the roots was not affected by these decapitations. Caffeic acid and 2,4-dichloro-phenol, when applied to the root cap, inhibited the geotropic curvature initially, probably because these compounds decreased the lateral distribution of the auxin in the root cap. 2,3,5-Triiodobenzoic acid, when applied to the root cap prevented the geotropic curvature almost completely. None of these treatments altered the elongation of the roots. It is concluded from the results presented in this and a previous paper (KONINGS 1967) that the part of the root cap between 0.2 and 0.5 mm from the apex, where the starch-containing columella cells are localized, controls both geotropism and the lateral distribution of auxin.

1. INTRODUCTION

It has been reported recently (KONINGS 1967) that IAA-^{14}C, when applied to the tips of horizontal pea roots became distributed laterally in these tips. When a part of 0.3 mm length of the root tip was cut off, the transverse distribution of the applied auxin was less and no unequal distribution occurred when 0.5 mm (the whole root cap) or more was removed. It was concluded that the root cap controlled the transverse distribution of the auxin. It is well known that unequally distributed auxin will cause, in the extension zone, unequal elongation of the upper and lower side (CHOLODNY 1924; NAVEZ 1933). If the transverse distribution of IAA, as found in the root cap, is connected with root geotropism, then the cap will also control the growth response of the roots to gravity. The effect of removal of the root cap or part of it on the geotropic curvature, there-fore, has been investigated now. It will be shown that, in fact, the root cap controls the positive geotropic curvature of the roots. In the aforementioned paper was also stated that the transverse distribution of applied auxin was less in roots treated with caffeic acid (CA) or 2,4-dichlorophenol (DCP). It was suggested that the two compounds affected the mechanism which was respon-sible for the transverse distribution of the auxin in the root cap. The influence of these two compounds on the geotropic response, therefore, has been studied also. Their influence on the geotropic curvature parallelled that on the lateral distribution of auxin, which suggests that the two phenomena are related. Finally, a study of the effect on geotropism of 2,3,5-triiodobenzoic acid (TIBA), a potent inhibitor of polar auxin transport, has been included.

2. MATERIAL AND METHODS

Two day old pea roots var. Vlijmse Gele Krombek of about 4 cm length were used. The seeds were soaked for 16 hours in aerated tap water, then put in moist sand over vertical holes and allowed to germinate for 45 hours at a temperature of 24 °C. The relative humidity was 95 per cent in the room and saturated in the chambers in which the roots were kept during the experiments. All manipulations were carried out under red light and the shadowgraphs were made with orange light. The apparatus shown in *fig. 1* was used for cutting off small parts of the root tips. The seedlings were placed in a holder (1) with a macro- (2) and a micro-adjustment (3). Lower on the same frame (4) a one mm thick revolving disc (5) was mounted. Through this disc, near the edge, a number of tapered holes of slightly different diameter had been drilled (see drawing, *fig. 1*). The holder with the root was moved downwards till the tip of the root slipped in the adequate hole in the disc. The position where the tip of the root was on a level with the brim of the bottom opening of the hole was taken as zero
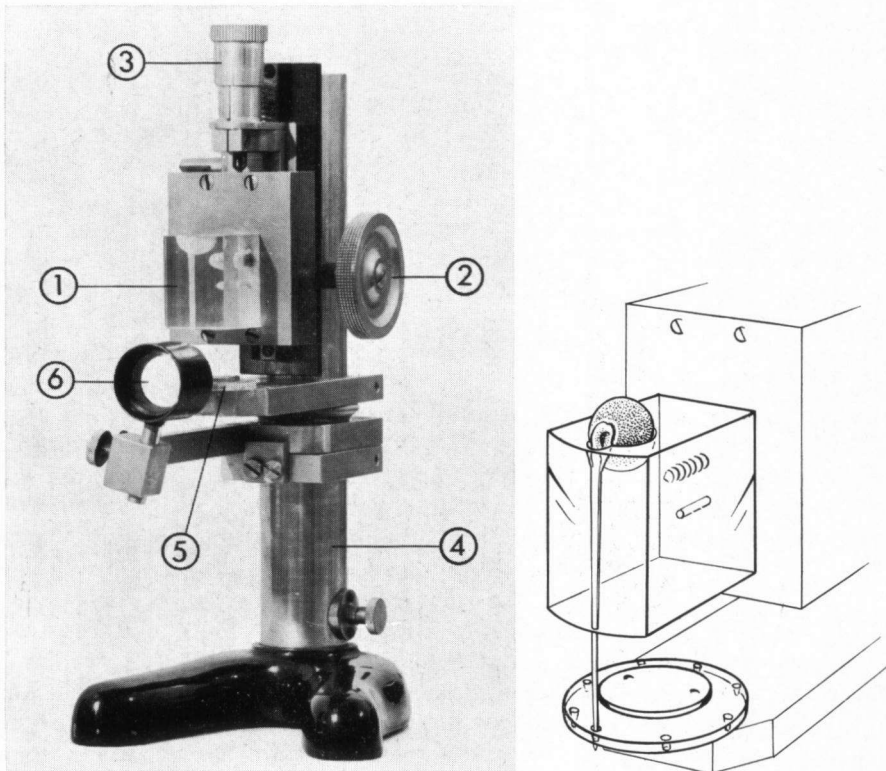
Fig. 1. Apparatus used for cutting off small parts of the root tips. Left: photograph of apparatus with 1, holder; 2, macro-adjustment; 3, microadjustment; 4, frame; 5, revolving disc with tapered holes, and 6, magnifying-glass. Right: drawing to scale of 1 and 5 which shows the tapered holes and the position of the root just before cutting.

adjustment. The root was subsequently moved further down till the part of the root which protruded the hole had the desired length. This part then was cut off with a razor blade. The displacement of the tip of the root during adjustment was observed through a ten-fold magnifying glass (6).

The water-soluble compounds were applied in lanolin paste which was prepared by mixing equal weights of an aqueous solution and lanolin. TIBA was dissolved in one ml of ethylalcohol and then mixed with 25 g of lanolin.

3. RESULTS

3.1. The geotropic curvatures of pea roots which were decapitated at different distances from the tips

The roots were decapitated as described in section 2 at 0,2, 0.3, 0.4, 0.5, 0.7 and 1.0 mm from the tip respectively and then placed horizontally. The effects of such decapitations on the geotropic responses are shown in *fig. 2*. Decapitation at 0.2 mm did not influence the geotropic curvature of the roots. When 0.3 mm was cut off, then the geotropic curvature was clearly retarded during the first hour, but the curvature reached the level of that of the intact roots after 3 hours. Removal of 0.4 mm delayed the geotropic response for one hour and the curvature of these roots did not become equal to that of the intact roots. The rate of curvature of the roots decapitated at 0.3 and 0.4 mm was almost equal to that of the intact roots, during the second hour. Although a part of the root cap was removed these roots were still capable of developing a growth difference between the upper and lower side of the root tip, which was equal to that of the roots with an intact cap. It took, however, longer to build up such a difference. Cutting off 0.5 mm, which includes the whole root cap, deprived the roots of their
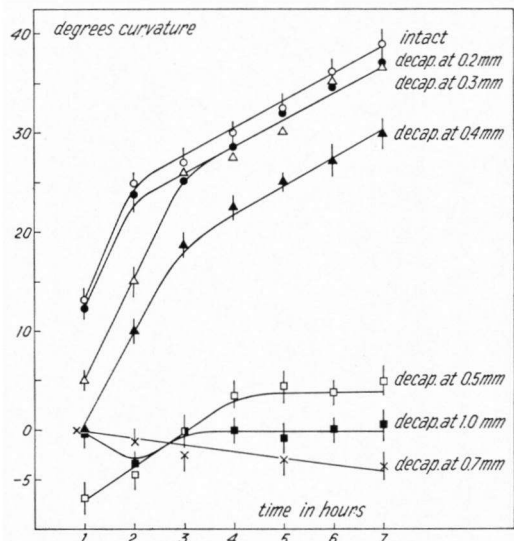
Fig. 2.
The effects of decapitation at different distances from the tip on the geotropic curvature of pea roots.

ability to respond to gravity with a positive geotropic curvature. The roots showed a negative curvature initially, followed by a weak positive one. The roots decapitated at 0.7 and 1.0 mm kept almost straight. The roots, therefore, apparently require the major part of the root cap to achieve a normal positive geotropic curvature.

The elongation of the decapitated roots, measured over a 6-hour period, was equal to that of the intact roots, although the growth rates measured at hourly intervals sometimes differed (*table 1*).

Table 1. The effects of decapitation at different distances from the tip on the elongation of horizontal pea roots.

mm removed	elongation (mm) in subsequent hours						total elongation
	0–1	1–2	2–3	3–4	4–5	5–6	(mm) in 6 hours
0.0	0.4	1.0	1.2	1.2	0.8	0.8	5.4
0.2	0.4	0.8	1.0	0.7	1.2	1.2	5.3
0.3	0.4	0.8	0.9	0.9	1.2	0.7	4.9
0.4	0.4	0.7	0.8	1.1	1.1	0.8	4.9
0.5	0.7	0.8	1.0	0.8	1.1	1.0	5.4
0.7	0.4	0.8	1.1	0.8	1.0	0.9	5.0
1.0	0.3	1.0	1.0	0.9	0.9	0.9	5.0

The data are averages of 40 to 50 roots from 3 to 5 experiments.

3.2. The effect of cutting off the root cap at different times during geotropic bending

The root cap, or part of it, is indispensable for a geotropic curvature to begin (section 3.1.). During the increase of the curvature, however, the position of the root cap altered with regard to gravity and the rate of increase of the curvature slowed down after about two hours (*fig. 2*). It could be expected, therefore, that the root cap controlled the curvature continually. To clear this point the roots were decapitated at 0.5 mm at different times after the roots were placed horizontally. As *fig. 3* shows, all roots straightened to some extent after this decapitation and did not show any further increase in curvature afterwards. The roots elongated at the same rate as before under their newly obtained angle. The straightening of the root tips occurred immediately after the removal of the root cap when the decapitation was made after 1.5 hours or more, but when the decapitation was made at an earlier stage of the geotropic curvature the bending continued for some time, though at a lower rate, before the straightening began. The actual reversible part of the growth difference between the upper and lower side of the tip was almost equal in all cases, but it became relatively less as the curvature increased and a greater part of it was fixed. Evidently the presence of the root cap is required during the whole course of the geotropic curvature.
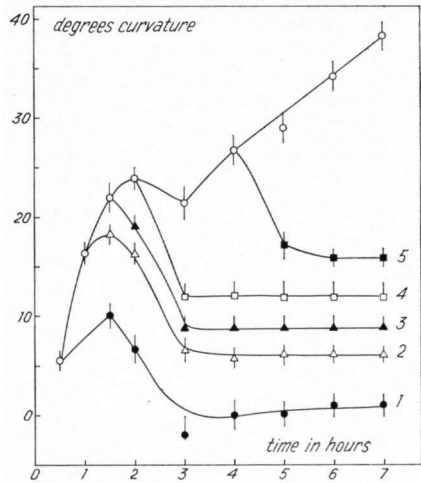
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Fig. 3.
The effects of cutting off the root cap of pea roots at different times during their geotropic curvature. 1: cap cut off 30 min after the roots were placed horizontally. 2: cut after 1 hour. 3: cut after 1.5 hours. 4: cut after 2 hours. 5: cut after 4 hours. The open circles show the course of the curvature of the intact roots. The apparatus of *fig. 1.* was not used in these cases, but 0.5 mm was cut off as judged at tenfold magnification without altering the position of the roots.

3.3. The influence of caffeic acid (CA), 2, 4-dichlorophenol (DCP) and 2,3,5-triiodobenzoic acid (TIBA) on the geotropic curvature, when applied to the root cap

Caffeic acid and 2,4-dichlorophenol both decreased the lateral distribution of applied auxin in the root cap (KONINGS 1967). The influence of the two compounds on the geotropic curvature was tested now. They were, therefore, applied to the root cap in lanolin. The concentrations in the lanolin were 10^{-6}, 10^{-5}, and 10^{-3} g/g respectively. The amounts applied to each root then were 0.5 ng, 5 ng and 0.5 µg. The results are presented in *fig. 4*. Both CA and DCP retarded the geotropic curvature initially. The parallellism between the decrease of the transverse distribution of the auxin, found earlier, and the retardation of the geotropic curvature suggests that the latter could well be the result of interference of the two compounds with the lateral distribution of the endogenous growth regulator in the root cap. TIBA is a well known inhibitor of polar auxin transport. It inhibited the geotropic curvature of wheat roots stronger then their elongation, when the roots were growing on the surface of agar, which contained this compound (KEITT 1961). This suggests an influence of TIBA on the lateral distribution of the endogenous growth regulator. TIBA, therefore, was applied to the root cap of intact roots in the same way as CA and DCP were. The concentration of TIBA in the lanolin was 10^{-3} g/g, the amount applied per root was 0.5 µg. The result is shown in *fig. 4* also. No geotropic curvature appeared during two or three hours and a very weak curvature only afterwards. TIBA clearly prevented the occurrence of different growth rates at the upper and lower side of the root tip and thus likely prevented the lateral distribution of the endogenous growth substance. Because TIBA was dissolved in alcohol and then mixed with lanolin, the alcohol/lanolin mixture was taken as the control for the TIBA treatment. The curvatures of the roots with alcohol/
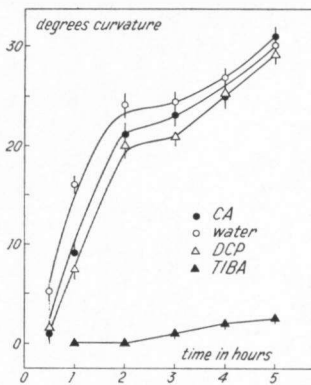
Fig. 4. The effects of caffeic acid (CA), 2,4-dichlorophe-
nol (DCP) and 2,3,5-triiodobenzoic acid (TIBA)
on the geotropic curvature of pea roots. The
compounds were applied to the root cap in lano-
lin.

lanolin on their root cap was equal to that of roots with water/lanolin on the
caps. These controls, therefore, are not shown in *fig. 4*.

The elongation of the roots treated with CA, DCP or TIBA, measured over a
5-hour period, was equal to that of the control roots (*table 2*). The poor growth
of the roots treated with CA and DCP in the period from three to four hours
probably represents a situation in which the concentration of these compounds
in the elongating cells was high enough to cause inhibition.

Table 2. The elongation of horizontal pea roots when caffeic acid (CA), 2,4-dichlorophenol
(DCP) and 2,3,5-triiodobenzoic acid (TIBA) were applied to the cap.

treatment	elongation (mm) in subsequent hours					total elongation
	0–1	1–2	2–3	3–4	4–5	(mm) in 5 hours
water	0.6	1.1	1.4	0.9	1.1	5.1
CA 10^{-3} and 10^{-5} g/g	0.8	1.2	1.3	0.4	1.4	5.1
DCP 10^{-3} and 10^{-5} g/g	0.7	1.6	0.6	0.4	1.6	4.9
alcohol 3.10^{-2} g/g	0.7	1.5	1.0	0.9	1.4	5.5
TIBA 10^{-3} g/g	0.6	1.3	1.0	1.0	0.9	4.8

The data are averages of 50 to 60 roots from 4 or 5 experiments.

4. DISCUSSION

The results show clearly that the positive geotropic curvature is guided from
the part of the root cap situated between 0.2 and 0.5 mm from the apex.
Removal of part of this region lengthened the reaction time of the positive geo-
tropic curvature and no positive response occurred when the whole region was
cut off. This region coincides with the part of the root cap where the columella
(statolith) cells are found, which suggests a close relation between the number of
columella cells present and the length of the reaction time of the geotropic
curvature. In a previous paper (KONINGS 1967) results were presented which
demonstrated that removal of 0.3 mm of the root cap reduced the transverse
distribution of applied IAA–^{14}C and that no unequal distribution of the auxin

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was found in tips decapitated at 0.5 mm. It is very likely, therefore, that the columella cells of the root cap are the sites where the lateral distribution of auxin takes place. The increase of the length of the reaction time after removal of a part of the columella cells then could well be the result of the reduction of the quantity of laterally distributed (endogenous) auxin per unit length of time.

The effect of removal of parts of different length of the root tips on the geotropic curvature has been studied also by JOST (1912) and by SYRE (1938). Jost decapitated roots of *Lupinus albus*, whose columella was about 1 mm long, at 0.3 to 0.7 mm and found that the geotropic curvature was postponed for 4 to 5 hours. Cutting off 0.7 to 0.9 mm prevented the curvature for at least 24 hours. Syre, however, reported that after removal of 0.7 mm of *Lupinus albus* roots and 0.8 mm of *L. luteus* roots still 65 and 33 per cent respectively of these roots were curved after 12 or 14 hours. Decapitation at 0.3 or 0.4 mm did not influence the geotropic curvature of maize roots measured after 24 hours, but the roots which were decapitated at 0.5 or 0.6 mm (all columella cells were cut off in these cases) remained straight during this time. GORTER (1932) and VON GUTTENBERG (1933) briefly reported that roots of *Pisum sativum* and *Zea mais* still bend downwards after decapitation at 1 mm. Unfortunately none of the above authors presented data on the time-course of the geotropic curvature so that their results and ours are in fact incomparable. YOUNIS (1954) did not observe any influence of decapitation at 0.5 mm on the geotropic curvature of roots of *Vicia faba* although the reaction was followed from the beginning. Based on our experience with pea roots one would expect a prolongation of the reaction time in this case. The reason for this discrepancy is obscure.

SYRE (1938) was able to remove the root cap of maize roots in 7 cases without doing damage to the meristem. Of the 7 roots thus treated 2 were curved after 7 hours, the others after 24 to 36 hours. The latter results were also obtained by JUNIPER *c.s.* (1966), who beautifully demonstrated that removal of the root cap of maize roots, which could be done accurately because these roots possess a well marked boundary between the root cap and the root proper, completely prevented the perception of the gravitational stimulus. The roots remained straight till the major part of the root cap was regenerated. Then the ability to perceive gravity was restored. Syre reported similar observations at roots of *Zea mais* and *Lupinus*. In our experiments the pea roots which were decapitated at 0.5 mm were geotropically curved after 1.5 or 2 days. These roots were also found to have produced a number of columella-type cells.

The weak negative curvatures of the roots decapitated at 0.5 mm or more were the result of growth accelerations at the lower side of the root tip. The origin of this growth action is not known.

The overall elongation of the pea roots was not affected by the decapitations. The elongation of the decapped roots in the experiments of JUNIPER *c.s.* (1966) was not altered either. Similar results were obtained by YOUNIS (1954) with roots of *Vicia faba* decapitated at 0.5 or 1.0 mm, by SYRE (1938) with roots of *Lupinus luteus* decapitated at 0.5 or 0.7 mm, and by a number of other workers several decades ago (see *e.g.* GORTER 1932, for the older literature). Growth

retardations of seedling roots of *e.g. Vicia faba, Lupinus* sp., *Pisum sativum, Phaseolus* sp. and *Zea mais* were generally found when 1.5 mm or more of the root tip was cut off (see *e.g.* NAVEZ 1933; YOUNIS 1954 and many others). In these cases the decapitated part often included the major part of the meristem or even part of the young expanding cells. Reports on growth accelerations following decapitation are scanty. CHOLODNY (1926) stated a 12 per cent increase in elongation over a 4–5 hour period for roots of *Lupinus* without giving any further data. BüNNING (1928) reported growth accelerations of some parts of the elongation zone of *e.g.* roots of *Vicia faba*, which were decapitated at 0.5 or 1.0 mm, but the elongation of pea roots was not altered after such decapitations. SYRE (1938) found that the elongation of maize roots and sometimes that of *Lupinus* roots also, was promoted after decapitations varying from 0.4 to 0.6 mm, but it is not clear whether the differences are statistically significant. Taking all available recent and older data together it seems justified to conclude that removal of the root cap does not affect the elongation of the roots.

The results obtained with decapitation of horizontal roots at different times during their geotropic curvature also demonstrate convincingly the significance of the root cap for geotropism. When the root tips were partly straightened after the decapitation, the two sides of the extension zone elongated at a rate which was equal to the rate of elongation before the decapitation. Because endogenous growth substances become transversely distributed in horizontal roots (HAWKER 1932; BOYSEN-JENSEN 1933) and decapitated and intact roots respond to unequally applied auxin (CHOLODNY 1924; NAVEZ 1933; AMLONG 1936, and own not published results with pea roots), it is evident that the auxin in these pea roots is no longer transversely distributed after removal of the root cap. The phenomenon of straightening itself cannot be explained as yet, but it will be studied in the near future.

Caffeic acid and 2,4-dichlorophenol both retarded the initial phase of the geotropic curvature, when they were applied to the root cap. The lateral distribution of applied IAA–^{14}C was decreased when the roots were pretreated with solutions of these compounds. The close parallelism between these two effects suggests an interference of CA and DCP with the transverse distribution, in the root cap, of the natural growth regulator. TIBA in the concentration used completely prevented the geotropic curvature of the roots, although their elongation was not affected. TIBA has been found to be a potent inhibitor of polar auxin transport. The results obtained in the other experiments reported in this paper strongly suggest that TIBA, when applied to the root cap, prevented the lateral distribution of the endogenous auxin in the cap. It will be noted that the absence of a geotropic curvature of the roots treated with TIBA is not related with the movement of starch granules in the columella cells, for these showed a normal displacement.

The general conclusion from the results presented in this and the previous paper is, that factors which influence the geotropic response of the roots (removing the root cap partly or wholly, application of CA, DCP or TIBA to the root cap), also affect the transverse distribution of the auxin in the root cap.

The root cap obviously controls both the transverse distribution of auxin and geotropism.

ACKNOWLEDGEMENT

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