

THE INFLUENCE OF ACROPETALLY TRANSPORTED INDOLEACETIC ACID ON THE GEOTROPISM OF INTACT PEA ROOTS AND ITS MODIFICATION BY 2, 3, 5-TRIIODOBENZOIC ACID

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SUMMARY

The geotropic response of horizontal intact pea seedling roots was rapidly influenced by indole-3-acetic acid (IAA), which was applied in narrow rings of lanolin paste a few cm from the apex. The applied auxin thus likely was transported in the acropetal direction in these roots. The influence of IAA was strongly reduced when 2, 3, 5-triiodobenzoic acid (TIBA) was applied to the roots at the apical side of the IAA-ring. When TIBA was applied alone at various distances from the apex of the horizontal roots it also inhibited the geotropic curvature. The transport of auxin in the apical region of horizontal roots probably occurred both in the acropetal and in the basipetal direction.

1. INTRODUCTION

A few decades ago CHOLODNY (1929), BOYSEN-JENSEN (1933), THIMANN (1934), and others demonstrated that root tips contained a growth substance similar to that found in grass coleoptiles. BOYSEN-JENSEN (1933), CHOLODNY (1934) and VAN RAALTE (1937) presented some evidence that in isolated root tips auxin was produced if the tips were supplied with glucose. CHOLODNY (1931, 1934) further found that the growth substance from the coleoptile tips inhibited the root elongation strongest when it was applied just at the apical side of the elongation zone and that segments of maize roots curved geotropically only when coleoptile tips of maize were placed at the apical cut surface, but not when placed at the basal end. These results led Cholodny and others to the conception that the auxin was produced in the root tip and was transported in the basipetal direction only.

There are, however, serious objections to this idea. The conditions under which some auxin synthesis may have occurred in isolated root tips are not necessarily relevant to the tips of intact roots. Besides, THIMANN (1934) already obtained evidence that the auxin in the tips of *Avena* roots was not synthesized in these tips but arrived there from other parts of the roots. It was further found that in pea roots in addition to a basipetal transport of applied indoleacetic acid (IAA), also an acropetal transport could occur (CZAJA 1935; THIMANN 1936). Finally, FABER (1936) found that in sections cut from the elongation zone of *e.g.* maize roots, the applied auxin moved acropetally as well as basipetally.

Recently, several investigators found that IAA-¹⁴C, when applied to root segments was either transported predominantly in the acropetal direction (PILET 1964; BONNETT & TORREY 1965; KIRK & JACOBS 1968; WILKINS & SCOTT 1968 and SCOTT & WILKINS 1968), or equally well in both directions (HERTEL & LEOPOLD 1963; YEOMANS & AUDUS 1964).

In this paper evidence for a rapid acropetal transport of indole-3-acetic acid (IAA) in horizontal pea roots was obtained from experiments where the effect of IAA on the geotropic curvature was studied when it was applied at various places along the axis of intact seedling roots. 2,3,5-Triiodobenzoic acid (TIBA) reduced the IAA effects and depressed the geotropic curvature of otherwise untreated roots. Besides, the probability of a basipetal auxin transport in the apical region of the horizontal roots will be discussed.

2. MATERIAL AND METHOD

Two-day-old pea of roots cultivar. "Vlijmse Gele Krombek" of about 4 cm length were used. The seeds were soaked for about 20 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 in the room was about 90 per cent and saturated in the chambers in which the roots were kept during the experiments. All manipulations were carried out under red light. The shadowgraphs were made with orange light at hourly intervals on Gevaert copyline rapid paper. Indoleacetic acid lanolin paste was prepared by mixing equal weights of the IAA solution and lanolin. The concentration of IAA in the lanolin paste was either 10⁻⁵ g/g or 10⁻⁴ g/g. TIBA was dissolved in one ml of ethanol and then mixed with 25 g of lanolin. The concentration of TIBA in the lanolin paste was 10⁻³ g/g. A mixture of equal weights of water and lanolin was taken as control for the IAA treatments and a mixture of one ml of ethanol and 25 g of lanolin as the control for the TIBA treatments. The experiments were repeated at least three times and the data are averages of 60 to 80 roots. The vertical bars in the figures represent the standard errors of the mean.

3. RESULTS

3.1. Modifications of the geotropic curvature of pea roots following the application of IAA at various distances from the tip

Indoleacetic acid was applied in about 1.5 mm wide rings of lanolin paste to horizontal roots at 10, 20 or 30 mm from the tip. The geotropic responses were followed and the results are illustrated in the *figs. 1* and *2* where the amounts of IAA applied to each root were 0.05 µg and 0.5 µg respectively. Application of 0.05 µg of IAA at 20 or 30 mm from the apex caused after an hour already, a strong promotion of the geotropic curvature (*fig. 1*). It was judged that in case of a modification of the geotropic response the applied IAA had reached the responsive cells in the elongation zone. It then follows that the uptake of the IAA, its acropetal transport from the place of application to the site of action

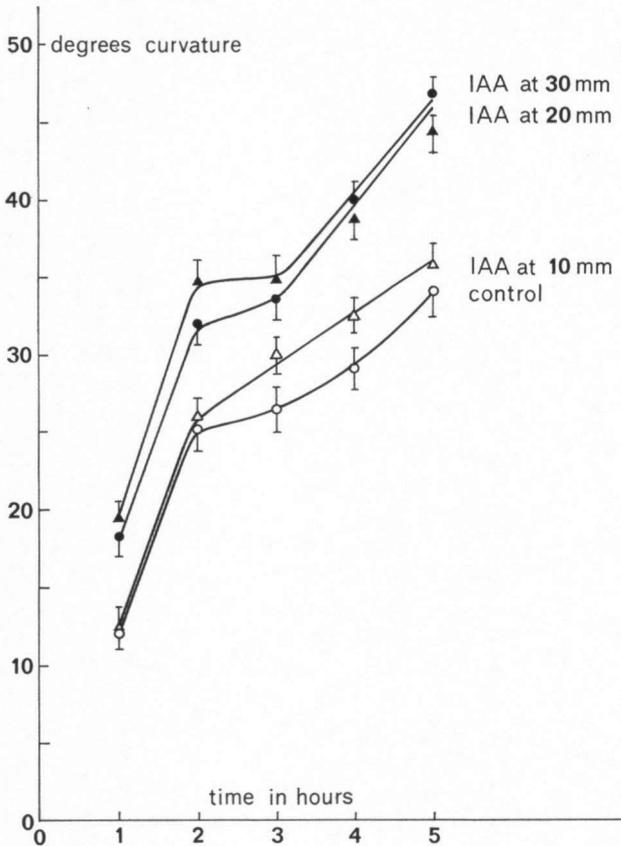


Fig. 1. The influence of 0.05 μg of IAA on the geotropic curvature of intact pea roots, when applied at various distances from the tip.

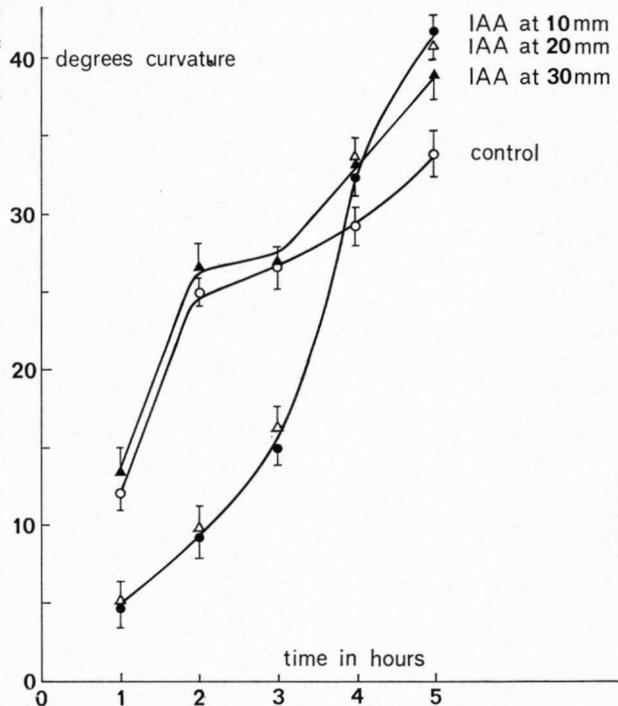
(in the region of 2–5 mm behind the apex), and the appearance of its stimulating effect altogether took less than an hour. The transport velocity thus was at least a few centimeters per hour in the acropetal direction. This velocity is about ten times greater than the values found by others in experiments with root sections, which were only a few millimeters per hour.

When the same amount of IAA (0.05 μg) was applied at 10 mm from the apex then only a slight promotion of the curvature was observed after 3 hours of geotropic exposure.

Application of a greater amount of IAA (0.5 μg) at 10 or 20 mm from the apex resulted in the inhibition of the geotropic curvature for about two hours, but subsequently the curvature sharply increased and the value obtained after 5 hours of geotropic exposure was greater than that of the control roots (*fig. 2*). The amount of auxin reaching the elongation zone was apparently too high initially and caused inhibition, but after a few hours this amount was probably lowered by conjugation, binding or destruction to a promotive level. Application of 0.5 μg of IAA at 30 mm from the tip did not affect the curvature initially and slightly promoted the curvature after 3 hours.

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Fig. 2. The influence of 0.5 μg of IAA on the geotropic curvature of intact pea roots, when applied at various distances from the tip.



In summary, the effect of 0.05 μg of IAA shifted from a weak promotion when applied at 10 mm, to a strong promotion when it was applied at 20 or 30 mm from the tip and the effect of 0.5 μg of IAA shifted from an initial inhibition of the geotropic curvature when applied at 10 or 20 mm, to a slight promotion when it was applied at 30 mm from the tip. These results could be explained by assuming that the quantities of IAA reaching the responsive cells decreased when the IAA was applied at increasing distance from the tip.

3.2. The depression of the IAA effects on the geotropic curvature by TIBA

The inhibition of the polar transport of IAA by TIBA has been demonstrated many times (e.g. NIEDERGANG-KAMIEN & SKOOG 1956; CHRISTIE & LEOPOLD 1965; HERTEL & FLORY 1968). The assumption that in the pea roots an acropetal transport of the applied IAA in fact occurred would be supported if the IAA effects described in the preceding section could be depressed by TIBA. This compound was applied, 5 μg in lanolin paste to each root, at the apical side of the IAA-ring and 2 or 5 mm apart from it. Fig. 3 shows the influence of TIBA on the IAA effects. When TIBA was applied at 15 mm from the apex and 0.05 μg of IAA at 20 mm, then the promotive effect of the IAA was still present during the first hour of the geotropic exposure, but it disappeared during the second hour and subsequently the curvature decreased to an average value far below

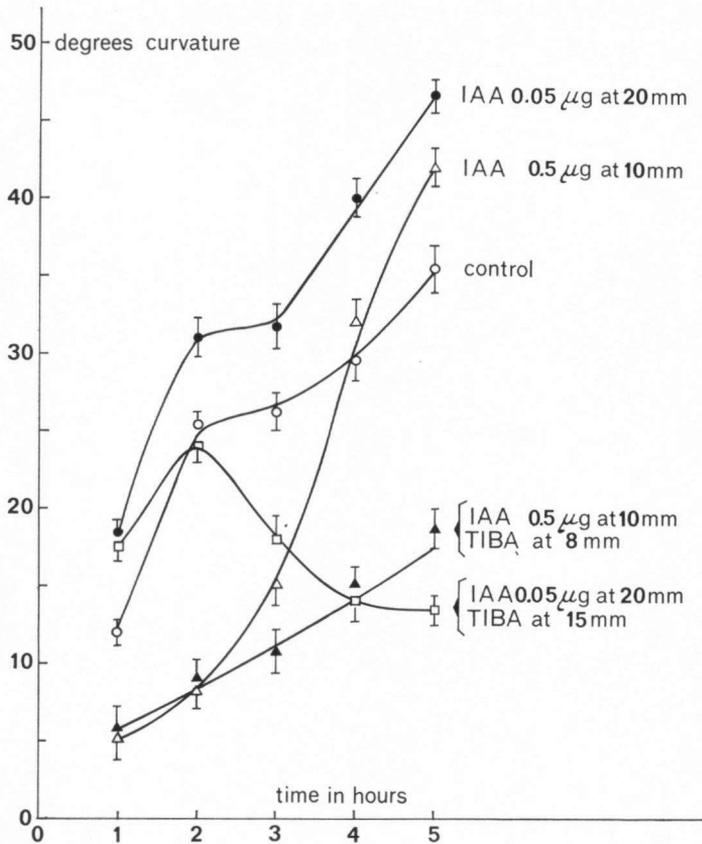


Fig. 3. The depression by TIBA of the influence of IAA on the geotropic curvature of intact pea roots.

that of the control and the auxin treated roots. A decrease of the positive geotropic curvature has been found to be the result of an increase of the growth rate at the lower side of the elongation zone (AUDUS & BROWNBRIDGE 1957; KONINGS 1964) which probably occurred because the transverse auxin gradient in the root tip leveled out *e.g.* during the rotation on the horizontal clinostat (in the experiments of Audus and Brownbridge) or after removal of the root cap (KONINGS 1968). When the curvature finally settled at a lower value, then the growth rates of the upper and lower side were equal and no unequal distribution of auxin probably existed anymore. TIBA thus likely decreased the transverse auxin gradient below the critical value for geotropism.

When TIBA was applied at 8 mm from the apex and 0.5 µg of IAA at 10 mm, then the sharp increase of the geotropic curvature after 3 hours as observed at roots treated with this amount of auxin alone did not occur, but the curvature

increased only slowly. TIBA thus clearly decreased the effect of 0.5 μg of IAA but could not entirely suppress it.

The depression of the IAA effects by TIBA applied at the apical side of the auxin, suggests that the acropetal transport of IAA was somehow inhibited by TIBA (WINTER 1967; HERTEL & FLORY 1968), although alternative explanations e.g. the inhibition of the lateral distribution of IAA in the tip or the decrease of the response of the cells to IAA cannot be excluded.

The elongation of the roots was not affected by the TIBA treatments.

3.3. The effect of TIBA on the geotropism of intact pea roots, when applied at various distances from the apex

TIBA was applied at 4, 10, 20 or 30 mm from the apex (5 μg to each root). *Fig. 4* shows the results. No curvature at all appeared during the experimental period of 6 hours when TIBA was applied at 4 mm from the apex (the curvature of the roots thus treated began after 8 to 10 hours). The delay of the curvature coincided with a strong growth retardation. The roots to which TIBA was applied at 10, 20, or 30 mm curved as much as the control roots during the first hour of geotropic exposure, but the presence of TIBA became apparent during the second hour and the curvatures decreased and settled at a lower

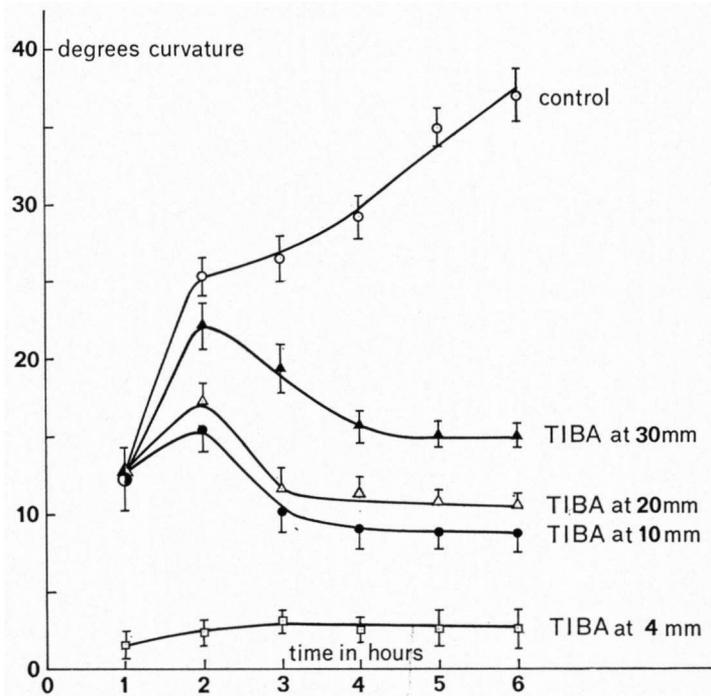


Fig. 4. The influence of TIBA on the geotropic curvature of intact pea roots, when applied at various distances from the tip.

value during the subsequent hours. The elongation of these roots was not affected. Finally, when TIBA was applied at 10 mm from the tip 2 hours before the roots were placed horizontally, no curvature followed on exposure. The presence of TIBA thus sooner or later made a geotropic curvature i.e. the unequal elongation of the upper and lower side impossible, whereas in most cases the straight growth was not affected. The decrease of the rate of curvature during the second hour as shown in *fig. 4* could be explained in two ways. One possible explanation is that TIBA reduced the acropetal transport of the endogenous auxin. The curvature would then probably not stop abruptly, but continue for some time with the auxin available in the part of the root at the apical side of the TIBA-ring. This amount would probably be less in a 10 mm part than in a 30 mm part, so that the curvature would stop earlier in the first case. A second possibility is that TIBA moved to the tip and exerted its effect there. It would take less time to cover 10 mm than 30 mm, so that here too, the inhibition would appear earlier in the first case.

3.4. The importance of the root cap for the lateral distribution of the acropetally transported auxin

It has been demonstrated that IAA when applied to the apex of horizontal intact pea roots became unequally distributed in the root cap (KONINGS 1967) and also that the root cap controlled the geotropic curvature (KONINGS 1968). These results led to the assumption that the auxin probably moved from the root cap basipetally in unequal amounts to the upper and lower side of the elongation zone. In geotropism, therefore, a basipetal transport in the apical region of the root seems likely. The following results may support this view.

First, when 0.05 μg of IAA was applied at 20 mm from the apex of roots from which the root cap had been removed no curvature appeared, whereas the same amount of IAA promoted the curvature of intact roots (*fig. 1*). The decapitation did not affect the elongation of the roots. The auxin which reached the elongation zone did apparently not become transversely distributed.

Second, a 1 mm wide ring of lanolin with TIBA (1 μg) applied at 2 mm from the apex i.e. at the apical side of the expanding cells, inhibited the geotropic curvature for several hours. After 3 to 4 hours the cells just basally of the TIBA ring began to swell and produced abundant root hairs. Such phenomena suggest ethylene liberation following auxin accumulation. Possibly TIBA blocked the acropetal transport of the endogenous auxin, so that it could not reach the root cap and did not become laterally distributed, but accumulated in the young cells.

Third, when TIBA was applied at the root cap (KONINGS 1968), it prevented the appearance of a geotropic curvature entirely (up to 30 hours measured) without clearly affecting the elongation of the roots. In contrast, application of TIBA at 2 or 4 mm delayed the curvature for 8 to 10 hours only, although the elongation was strongly reduced. The effect of TIBA applied at 10, 20 or 30 mm from the tip did not become apparent before the second hour of the horizontal exposure.

All these facts suggest that the critical place for the transverse distribution of auxin is the root cap. If this is true, then the auxin has to move basipetally to the responsive cells in the elongation zone in order to induce a geotropic curvature. Earlier, the possibility has been considered that in the root cap some phenolic compound became transversely distributed, which was supposed to move basipetally to the elongating cells and to affect the action of the auxin there. This possibility, however, seemed unlikely (KONINGS 1967).

4. DISCUSSION

The most striking result is that small amounts of IAA, which were applied a few cm from the root apex, rapidly modified the geotropic curvature. This suggests a transport velocity of IAA in the acropetal direction of several cm per hour, whereas the velocities calculated from auxin passage through root sections were only a few mm per hour (PILET 1964; BONNETT & TORREY 1965; KIRK & JACOBS 1968; SCOTT & WILKINS 1968). This difference between the transport velocity of IAA in intact and in excised tissue could be explained by the presence of active phloem tissue in the intact roots. LITTLE & BLACKMAN (1963) for instance found a transport velocity of 20–24 cm per hour for IAA in the phloem of intact plants of *Phaseolus vulgaris*. In root segments however, the phloem is no longer functional shortly after excision (WEATHERLEY *c.s.* 1959), so that auxin transport in sections probably occurred in the parenchyma cells only. In pea root sections the transport of IAA has been found to be particularly weak (WILKINS & SCOTT 1968). A poor transport was also found to occur in root sections of *Vicia faba*, which led YEOMANS & AUDUS (1964) to suppose that IAA has probably nothing to do with root growth at all. The great discrepancy between the results obtained with intact roots and with root sections makes it clear that in the latter the conditions for auxin transport are far from natural.

The stimulation of the geotropic curvature by IAA applied at 30 or 20 mm from the apex indicated that the supply of auxin to the root tip in untreated roots was not optimal for geotropism.

The depression by TIBA of the effect of auxin applied to plant organs, as was demonstrated for roots in this paper, has been found to occur also in shoots (LIBBERT 1959; VARDAR 1959), in branches (LYON 1963a) and in petioles (KUSE 1953, LYON 1963b). The reactions of plants following the application of TIBA *e.g.* the release of buds from apical dominance (WHITING & MURRAY 1948), the prevention of lateral root formation at the apical side of the place of TIBA application (BONNETT & TORREY 1965) and the inhibition of the geotropic curvature (this paper) could be interpreted by assuming that the translocation of the endogenous auxin was inhibited by TIBA in the same way as it inhibited the transport in sections of plants (NIEDERGANG-KAMIEN & SKOOG 1956; CHRISTIE & LEOPOLD 1963; HERTEL & FLORY 1968).

The root cap has been found to be indispensable for the lateral distribution of auxin in horizontal roots (section 3.4 and KONINGS 1967) and also for geotropism (KONINGS 1968). These facts forced to believe that, at least during geo-

tropic exposure, auxin moved basipetally from the root cap to the elongation zone. Direct evidence for a basipetal transport of auxin in the cells of the elongation zone was obtained by FABER (1936), by means of bioassay, with maize root sections and, with the aid of IAA-¹⁴C, by HERTEL & LEOPOLD (1963) with maize, by YEOMANS & AUDUS (1964) with bean (*Vicia faba*) root sections and by WILKINS & SCOTT (1968) with sections of pea roots. In all these cases the auxin was equally well transported basipetally as acropetally. Basipetal translocation of IAA-¹⁴C applied to the tips of *intact* roots has also been found to occur (KONINGS 1967). In most cases the transport velocity was estimated at about 4 mm per hour. Further, indirect evidence was obtained by CHOLODNY (1931) and by NAGAO & OHWAKI (1968) who put coleoptile tips and IAA in lanolin paste respectively at different places of the apical region of roots (*Zea mays* and *Vicia faba* respectively) and found that the inhibition of the elongation was much stronger when the source of auxin was applied at the apical side of the elongation zone than when applied at the basal side. Their conclusion, therefore, was that the transport of the applied auxin was predominantly in the basipetal direction to the expanding cells.

From the above it is clear that the transport of auxin in the elongation zone, of some roots at least, can occur in both the acropetal and the basipetal direction.

On basis of the present knowledge I would suggest that in seedling roots the auxin moves from the cotyledons to the root cap, then becomes laterally distributed and subsequently moves basipetally in unequal amounts to the upper and lower side of the expansion zone, which finally results in a geotropic curvature.

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