THE PERMEATION OF INDOLEACETIC ACID THROUGH THE CUTICLE OF THE AVENA COLEOPTILE AND ITS EFFECTS ON THE GROWTH AND THE GEOTROPISM

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SUMMARY

The auxin production by the tip of the *Avena* coleoptile seems too high for a complete geotropic response (a curvature of 90°) under the present experimental conditions (weak red light).

It is sufficient for a maximum *rate* of geotropic curvature, but it is too low for a maximum rate of elongation.

The entrance of indoleacetic acid through the cuticle is 2500–3000 times as difficult as that via the cut surface of a decapitated *Avena* coleoptile.

1. INTRODUCTION

In previous investigations of the geotropic reaction of decapitated *Avena* coleoptiles it was found that the rate of the curvature is very sensitive to the concentration of the auxin administered (ANKER 1954, 1956). Because of this high sensitivity the occurrence of the geotropic response is restricted to that region of concentrations where auxin is limiting the rate of straight growth.

In the above experiments the geotropic reaction was almost completely dependent on the so-called exogenous (supplied) auxin since the amount of residual endogenous auxin is very small in a decapitated coleoptile.

During the last few months similar experiments were carried out with non-decapitated coleoptiles. In this case the coleoptiles had two auxin sources at their disposal, an endogenous and an exogenous one. The effect of the extra auxin on both the growth and the geotropic reaction was studied. A few experiments with decapitated coleoptiles were included in order to compare the entrance of auxin through the cuticle with that through the cut surface.

2. METHODS

Avena seedlings were grown after the method described by WIEGAND & SCHRANK (1959), modified by BLAAUW & BLAAUW-JANSEN (1964). In order to inhibit mesocotyl growth, incandescent light from a 40 W bulb, filtered through red selenium glass was given for the first three days of germination and growth. On the fourth day the seedlings were put in absolute darkness, a measure which prevents the untimely breaking through of the primary leaves. At the beginning of the fifth day the plants were returned into the red light.

From the coleoptiles that had reached a length of 4–5 cm the primary leaves

were removed and the apical parts (18 mm) were slipped each over one of 12 pins of the instrument described earlier (ANKER 1954). Finally they were submerged, in vertical or horizontal position, in an aerated auxin solution, the temperature of which was adjusted to 23 °C beforehand.

A second group of 12 coleoptile segments, serving as the control, was put vertically or horizontally in aerated water. An accommodation time of half an hour was observed. The preparation of the experimental material took place in a climate room at 23 °C and a relative humidity of about 80%.

3. RESULTS AND DISCUSSION

The geotropic reaction. The speed of the geotropic reaction expressed as degrees curvature per hour cannot be increased by the addition of extra auxin (*fig. 1*). Apparently the natural tip produces enough auxin for an optimal response.

The only positive effect of external auxin, being observed at the 0.1 mg/l concentration, is an earlier start of the geotropic reaction. This effect was confirmed by triplicating the experiments, but strangely enough it was not present at the neighbouring auxin concentrations.

The second peculiarity, also illustrated by *fig. 1*, is the incompleteness of the geotropic reaction. The segments do not curve 90°, but the highest degree of curvature attained is 70°, and is performed by the control plants in water. As to the cause of this incompleteness, it could be precluded that the limit of 70° is determined by the shortness of the segment. For, transferring the coleoptiles after 4 hours in the position deviating 135° from the normal vertical position causes a continuation of the curvature (*fig. 2*).

Apparently a deviation of the tip of only 20° from the vertical is no longer sufficient to cause an auxin gradient steep enough for differential growth. This result suggests that even in water the internal auxin concentration is too high for a full geotropic response, a hypothesis which is supported by the well-known fact that *Avena* seedlings cultivated in darkness show a very poor geotropic reaction. The reaction can be improved by illumination with red light, which, according to BLAAUW-JANSEN (1959), causes, among other things, a reduction of the IAA content. The weak red light used in the present experiments will have reduced the auxin concentration to a level that was, as far as the geotropic reaction is concerned, not low enough.

The growth. The experiments of *fig. 3* confirm earlier reports that the growth rate of the *Avena* coleoptile is controlled by the limited production of auxin. Externally supplied auxin can more than double the rate of elongation.

There is another difference between the growth in water and in auxin solutions. While the growth of the segments in water is fairly constant, that in auxin solutions shows a sudden increase after the second hour (except at the very high concentration of 10 mg/l IAA). This change is already visible at as low an external IAA concentration as 0.05 mg/l, and it is still present at the relatively high concentration of 5 mg/l. At the concentration of 1 mg/l the increase

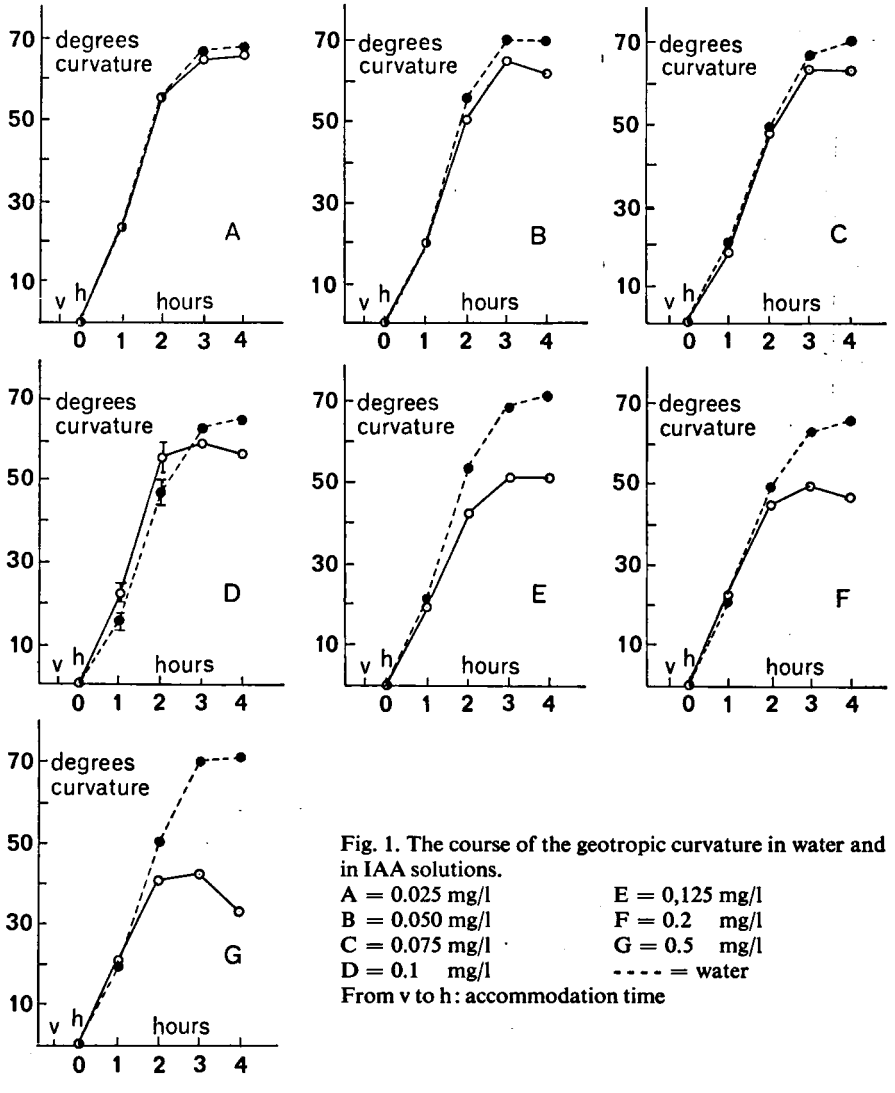

Fig. 1. The course of the geotropic curvature in water and in IAA solutions.

A = 0.025 mg/l	E = 0,125 mg/l
B = 0.050 mg/l	F = 0.2 mg/l
C = 0.075 mg/l	G = 0.5 mg/l
D = 0.1 mg/l	---- = water

From v to h: accommodation time

amounts to 35%. It seems that after about two hours some property of the segment changes under the influence of added auxin. It does not do so in water.

The nature of the change was not elucidated here by experiments. It is not impossible that the decrease in the speed of the geotropic curvature observed after two hours is due to the same cause (see *fig. 1*).

When searching the literature for other reports on changes occurring in the *Avena* coleoptile two hours after the addition of IAA, we encountered the paper of PRESTON & HEPTON (1960). These authors studied the effect of a constant

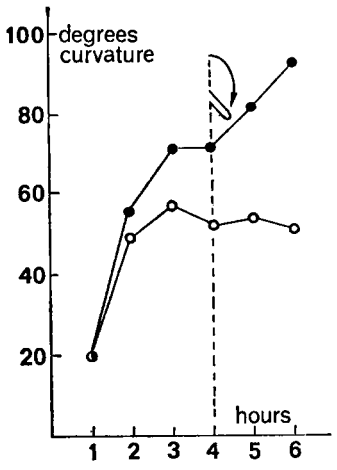

Fig. 2. The development of the geotropic curvature of horizontal coleoptile segments in water (—●—), and in an IAA solution of 0.2 mg/l. After 4 hours the segments are put in the position deviating 135° from the vertical.

load on the extension of *Avena* coleoptile sections immersed in an IAA solution of 1 mg/l. It appeared that the extension of the cell walls rose to a new level, also at the beginning of the third hour, and the rise, being roughly 35%, is equal to the rise in the growth rate found in the present experiments at the same auxin concentration. If these processes would appear to be causally related, then the decrease in the speed of geotropic curvature after two hours could have been caused by a yielding of the cell walls of both the upper and the lower side of the coleoptile segment.

As to the maximum rate of growth attainable there is a considerable difference between non-decapitated segments and those from which a tip of 1 mm has been removed (compare *figs. 3* and *4*). Those with intact tips grew 35% faster (1.70 mm/hour versus 1.25 mm/hour).

The limitation of the growth rate of decapitated coleoptiles will probably be the effect of more than one cause, 1. the removal of part of the zone which normally contributes to the elongation, and 2. the removal of unknown growth factors, other than auxin, produced by the tip, and 3. the injury done to the coleoptile by decapitation.

The observation that the maximum speed of elongation of the decapitated 18 mm segments is already attained at an IAA concentration slightly above 0.075 mg/l exactly agrees with the results of WEINTRAUB (1938) who studied the growth of de-tipped *Avena* coleoptiles not separated from the seedling. The maximum speed of elongation of intact coleoptiles is 35% higher, but this requires a multiple of the just-mentioned concentration of auxin. The high resistance of the cuticle to IAA permeation will certainly be one of the reasons for the different auxin requirement.

The permeation of IAA through the cuticle. In this section the entrance of auxin through the cuticle is compared with that via the cut surface of decapitated co-

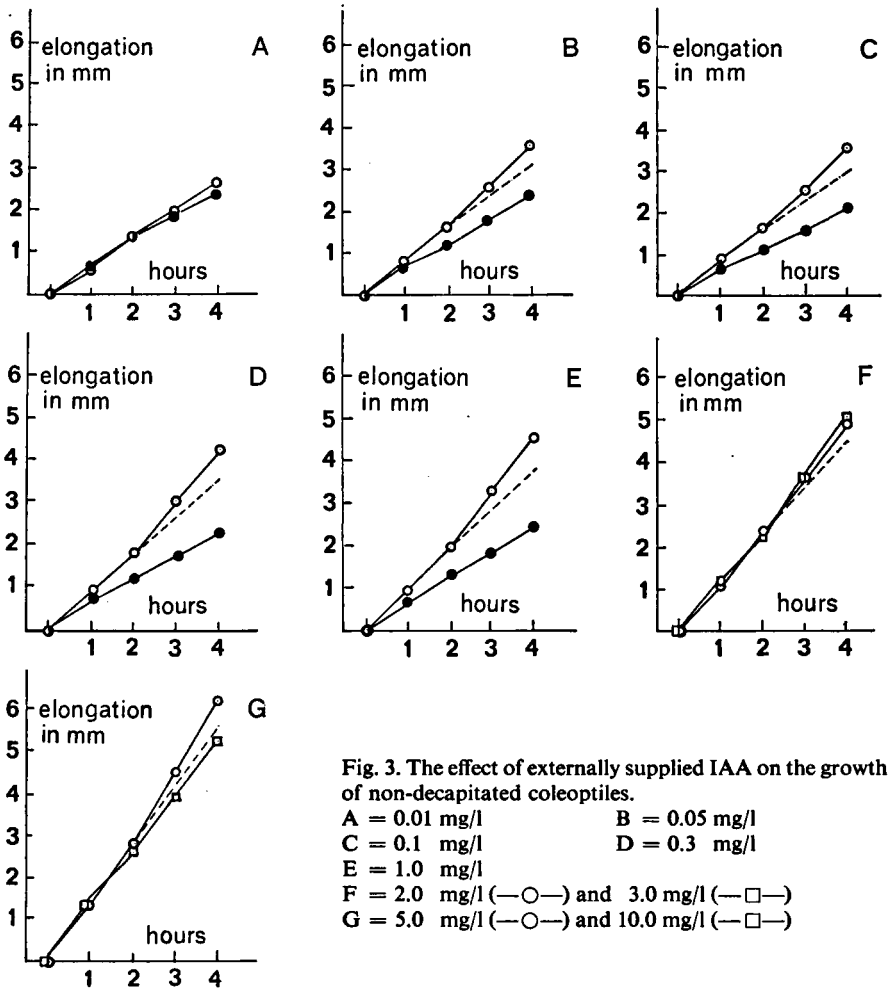

Fig. 3. The effect of externally supplied IAA on the growth of non-decapitated coleoptiles.
A = 0.01 mg/l B = 0.05 mg/l
C = 0.1 mg/l D = 0.3 mg/l
E = 1.0 mg/l
F = 2.0 mg/l (—○—) and 3.0 mg/l (—□—)
G = 5.0 mg/l (—○—) and 10.0 mg/l (—□—)

leoptiles. The comparison and the following calculation are based on the assumption that at low concentrations the elongation of the coleoptile is proportional to the internal auxin concentration. This assumption is sufficiently supported by the literature (THIMANN & BONNER 1933; WEINTRAUB 1938). Considering the high sensitivity of the de-tipped segments to auxin (*fig. 4*) it is further assumed that the sensitivity to auxin is not much reduced by decapitation. If it be assumed that the equality of the growth rate of the decapitated coleoptiles in the 0.05 mg/l solution with that of the non-decapitated ones in the 0.3 mg/l solution (1.25 mm/hour) points to an equal internal auxin concentration, the following calculation can be made.

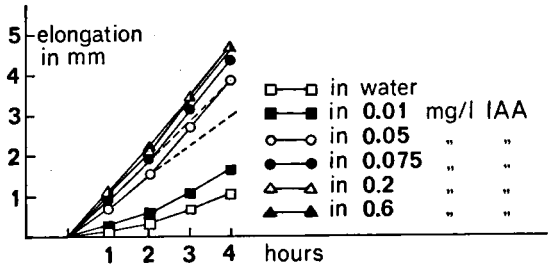

Fig. 4. The growth of decapitated coleoptiles (1 mm removed) in water and in IAA solutions.

Let the amount of auxin produced by the tip of the non-decapitated coleoptiles be called a, then it follows from the fact that the growth rate of the segments is about doubled by the external solution, that the same amount of auxin (a) has passed the cuticle. From the similarity of growth rate it follows that the same amount of auxin ($=2\ a$) has entered the decapitated segments via the cut surface and the cuticle. If it be assumed that the amount of auxin molecules passing the cuticle is proportional to the external concentration, then $^1/_6\ a$ has passed the cuticle of the decapitated coleoptiles (0.3/0.05), and $2\ a - {}^1/_6\ a = 1^5/_6\ a$ has gone through the cut surface.

On the basis of the anatomical data on the *Avena* coleoptile published by AVERY & BURKHOLDER (1936) it can further be calculated that the wound surface is 0.4 mm^2 and the surface of the cuticle of the 18 mm segments is 100 mm^2. Since the amount of auxin taken up through the cut surface was 11 times ($1^5/_6$:$^1/_6$) as much as that gone through the cuticle, the resistance of the cuticle to the entrance of auxin, calculated per mm^2, is 11 times 250 (100/0.4) = 2750 times as great as that of the wound.

As a matter of course the result of this calculation is no more than an approximation because the possible influence of several factors has not been taken into account. Among them are the influence of the wound and the possibility that the auxin transport in the radial direction, from the epidermis into the parenchyma cells, is slower than that in the basipetal direction etc. The question whether the calculation is invalidated by the regeneration of a physiological tip can be answered in the negative. According to SÖDING (1925) and THIMANN & BONNER (1933) the apical cells of the decapitated coleoptiles will not produce auxin themselves when the natural tip is replaced by an artificial auxin source.

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