

## THE AUXIN CONCENTRATION RULE FOR THE GEOTROPISM OF AVENA COLEOPTILES

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### INTRODUCTION

In a previous investigation (ANKER, 1954) it was found that the geotropic response of decapitated *Avena* coleoptiles is confined to a limited range of growth-substance concentrations, and that the optimal concentrations differ considerably for different auxins.

The experiments discussed in this paper were undertaken in order to find out what relation there is between the optimal auxin concentration required for the geotropic reaction and that required for straight growth when as auxins either indole acetic acid (IAA), indole aceto nitrile (IAN) or naphthyl acetic acid (NAA) were used.

The account of these experiments is followed by a study of the effect exercised by the extent of decapitation (the length of the part removed) on the geotropic reaction.

The results of both sets of experiments are discussed in connection with the lateral distribution hypothesis proposed by CHOLODNY (1927) for the explanation of the geotropic reaction.

Finally an attempt is made to estimate the auxin production of the tip of a coleoptile.

### METHODS

The method of investigation, fully described at an earlier occasion (ANKER, 1954), consists of measuring the curvature of decapitated coleoptiles after a horizontal stay of 75 minutes in an aerated auxin solution; the length of the removed part was 1 mm. The coleoptiles, from which the primary leaves had been pulled out were cut to a uniform length of 19 mm, and then placed on metal pins; two apparatuses were now available, each with ten pins. The figure given for the curvature is the average calculated for a set of ten coleoptiles. The curvatures were shadowgraphed with phototropically inactive light.

The *growth* of the coleoptiles was studied by the same method, the coleoptiles now being placed vertically in the same solutions and for the same period. The photographic paper was kept in a plate holder by means of a glass negative marked in square millimeters, so that with the aid of a lens the increase in length could easily be estimated with an accuracy of a tenth of a millimeter.

The experiments were carried out in phototropically inactive orange light in a room kept at a relative humidity of 90–96 % and at a temperature of 23° C.

THE RELATION BETWEEN THE OPTIMAL AUXIN CONCENTRATION FOR GROWTH AND THAT FOR GEOTROPISM

The relation between the optimal concentrations found for growth and for geotropism is shown in figures 1, 2 and 3. In the highly active IAN the curvature reached its highest value at a concentration of 0.01 mg/l. In the case of IAA and NAA the optimal concentrations were 0.075 and 0.4 mg/l respectively. At higher concentrations the geotropic response decreased until at saturation values for straight growth the coleoptiles remained uncurved in the horizontal position.

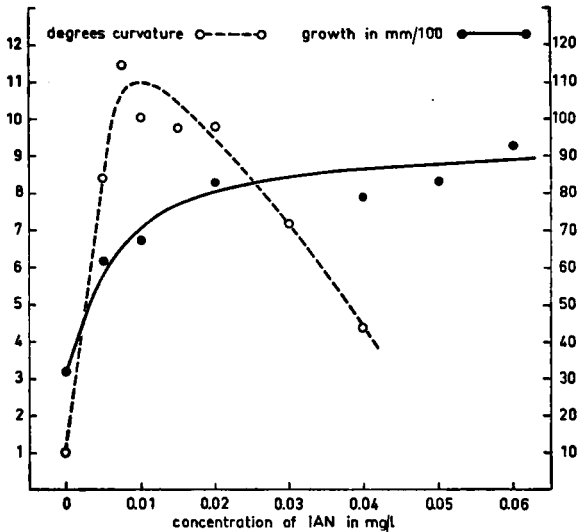


Fig. 1. Growth and geotropic curvature of decapitated *Avena* coleoptiles after 75 minutes in indole aceto nitrile solutions of various concentration.

This is shown for IAA in fig. 2. The same happened with IAN and NAA, as described in the previous publication. Since the molecular weights of IAN, IAA and NAA do not differ very much—being 156, 175 and 188—, comparison of figures 1, 2 and 3 provides us with a rough idea of their relative activity.

The magnitude of the geotropic curvature depends upon the difference in growth rate of the upper and lower side of the coleoptile. According to CHOLODNY's hypothesis this difference is caused by a lateral displacement of the auxin towards the lower side. Such a mechanism will only be effective so long as the absolute auxin concentration inside the coleoptile remains below the saturation value for the promotion of growth. This was actually found in the experiments we have just described, the largest curvatures being obtained

in the range of solutions where a small difference in the concentration causes the largest possible difference in the growth rate of the coleoptile. At saturation concentrations for growth the geotropic curvatures were small or absent.

The results, however, can not be regarded as "proof" of this theory. An alternative explanation of the results is possible, viz. that under the influence of the gravitational force the lower side of the coleoptile becomes more sensitive to *equally* distributed auxin in some way or another. A higher or lower sensitivity of a tissue to auxin means that it reacts as if the concentrations really were higher or lower. This mechanism, too, will operate at the lower concentrations only.

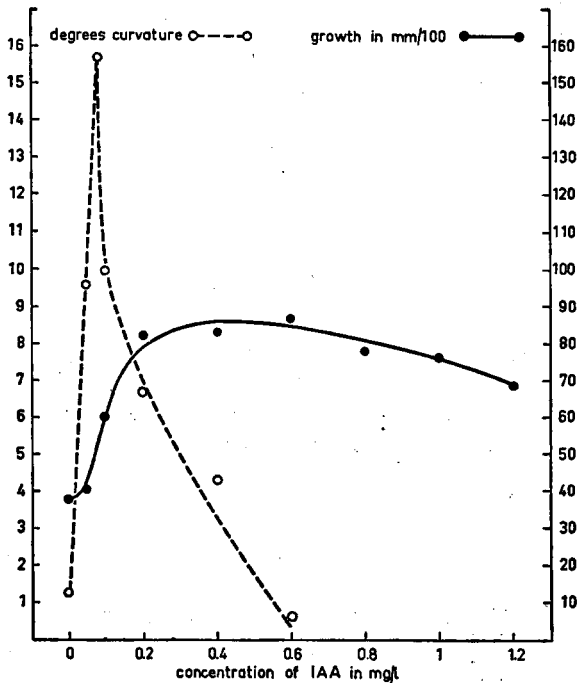


Fig. 2. Growth and geotropic curvature of decapitated *Avena* coleoptiles after 75 minutes in indole acetic acid solutions of various concentration.

The lateral displacement theory is mainly supported by the observations of DOLK (1930) with *Avena* coleoptiles and of DIJKMAN (1934) with *Lupinus* hypocotyls. They found that more auxin diffused into agar blocks from the lower side than from the upper one.

The value of this support, however, is limited by the possibility that the amount of free, diffusible auxin is larger in the lower side, whereas the total amount of auxin (free plus bound) in the upper and lower side may be the same. Growth would then be proportional to the amount of free auxin and not to the total amount of auxin.

It is difficult to design experiments which would prove the lateral

displacement theory of geotropism unless one could make use of the direct method of applying a radioactive auxin. It is doubtful, however, whether the differences would be large enough to be measured in this way (see BÜNNING *et al.* 1956). Perhaps the results of the next section will provide additional support to the lateral distribution hypothesis.

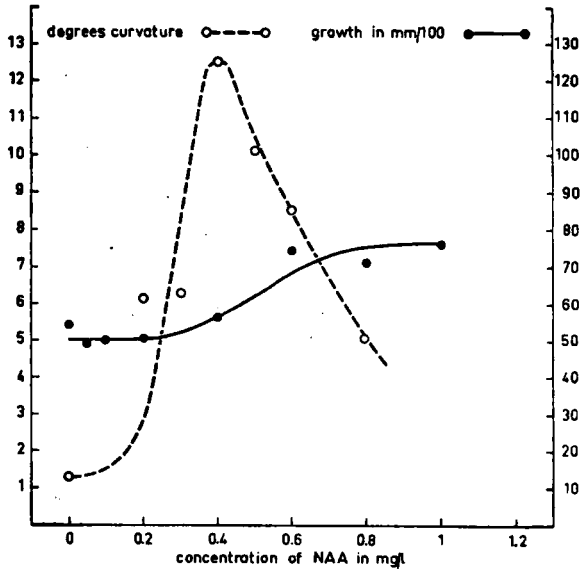


Fig. 3. Growth and geotropic curvature of decapitated *Avena* coleoptiles after 75 minutes in naphthyl acetic acid solutions of various concentration.

#### THE INFLUENCE OF THE EXTENT OF DECAPITATION ON THE MAGNITUDE OF THE GEOTROPIC CURVATURE

In a study on the effect which decapitation exercises on the magnitude of the geotropic curvature obtained in an auxin solution it appeared that the latter depends upon the length of the part that is removed. This is illustrated by fig. 4, which shows that the larger the removed part the smaller is the geotropic curvature. In other words: the *difference* in growth rate of upper and lower side of a coleoptile decreases when the length of the removed part increases.

The extent of decapitation did not influence the *growth rate* of the stump in an auxin solution. When the elongation of such coleoptile stumps of which a part either 1 or 2 mm long was removed and which were placed vertically in the same solution of 0.1 mg/l IAA and for the same period of 75 minutes was measured an exactly equal increase in length was observed. In two experiments the growth of the first set of decapitated coleoptiles (length of removed part 1 mm) amounted to  $0.66 \pm 0.05$  mm and  $0.61 \pm 0.04$  mm, whereas the growth of the second set of decapitated coleoptiles (length of removed part 2 mm) was  $0.68 \pm 0.05$  and  $0.59 \pm 0.05$  mm respectively. This indicates that the slower curving in the second set of decapitated stumps is not

due to a decreased growth capacity but only to a decrease in the difference in growth rate at the upper and the lower side.

Concerning the shape of the curved coleoptiles, it was noted that in the second set of decapitated stumps (length of removed part 2 mm) the curvature was situated nearer to the base of the stump than in

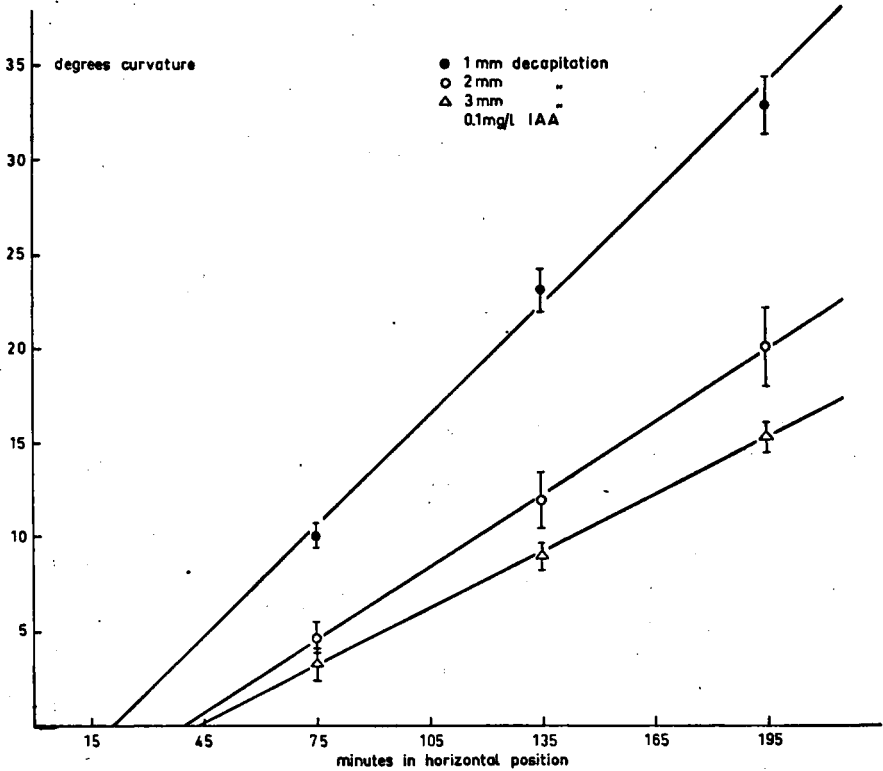


Fig. 4. The influence of the extent of decapitation on the geotropic reaction in a 0.1 mg/l IAA solution.

the first set (length of removed part 1 mm). As there is no reason to assume a migration from the zone of optimal sensitivity to gravity in the basal direction, the difference in shape of the curved zone could best be explained in terms of the lateral distribution hypothesis of CHOLODNY: the shift in the position of the curved zone would be due to a decreased capacity for lateral displacement in the second set of decapitated coleoptiles (length of removed part 2 mm) so that a sufficient difference in growth-substance concentration and hence in growth is reached at a greater distance from the top of the stump. The smaller curvature of the more apical parts, therefore, is not caused by a decreased sensitivity to gravity or by a decreased growth, but is due to the fact that the *difference* in auxin content is in this part less than in the more basal regions of the stump.

A diffusion of auxin through the cuticle may be left out of consideration in the latter experiments, since in a 0.1 mg/l solution of IAA the value found for the growth nor that found for the geotropic curvature of *non-decapitated* coleoptiles were different from those obtained in water. There is, however, a considerable permeation through the cuticle in a solution which contains ten times as much auxin (1 mg/l). Ten *non-decapitated* coleoptiles, placed vertically in the latter solution showed after 75 minutes an increase in length of  $0.84 \pm 0.11$  mm. In a solution containing 5 mg/l the growth was  $0.87 \pm 0.05$  mm and in the controls (in water)  $0.48 \pm 0.03$  mm. This further shows that the amount of auxin produced in the tip of a coleoptile remains below the saturation value for growth.

#### ESTIMATION OF THE AUXIN PRODUCTION BY THE TIP

Although it is evident that the auxin content of the growing zone is below the saturation value, it is difficult to determine the true auxin production in the tip. An estimation, however, is possible from the following facts. 1. A non-decapitated coleoptile shows an elongation of approximately 0.50 mm per 75 minutes in water. A 1 mm decapitated coleoptile shows the same increase in length in a solution of 0.075 mg/l IAA. 2. A non-decapitated coleoptile shows an average geotropic curvature of  $15.5^\circ$  in 75 minutes in water. This value was calculated from seven experiments with ten coleoptiles each:  $20.7^\circ \pm 0.8$ ,  $18.5^\circ \pm 1.5$ ,  $15.9^\circ \pm 1.1$ ,  $15.0^\circ \pm 1.4$ ,  $12.9^\circ \pm 1.3$ ,  $12.7^\circ \pm 1.1$ ,  $12.6^\circ \pm 1.4$ . The same curvature was again found at the optimal concentration of 0.075 mg/l IAA with decapitated coleoptiles. If we may assume with WILDMAN and BONNER (1948), REINERT (1950) and TERPSTRA (1954) that IAA constitutes the bulk of the natural auxin in *Avena*, and, moreover, that the uptake and transport are not much disturbed by decapitation, the natural auxin stream—not to be confused with the natural auxin content—from the tip to the growing zone will have the same concentration as when the tip is replaced by a solution containing 0.075–0.1 mg/l. This shows that the tip produces auxin in amounts which provide for a maximal rate of geotropic curvature rather than for maximal growth.

#### SUMMARY

Experiments with three different auxins viz. indole acetic acid, indole aceto nitrile and naphthyl acetic acid revealed that the magnitude of geotropic curvature of *Avena* coleoptiles is regulated according to the following rule:

A geotropic curvature only appears in auxin concentrations which are sub-optimal for coleoptile growth. The largest curvatures occur in the range of solutions where small differences in the concentration cause large differences in the growth of the coleoptile.

The natural auxin production in an *Avena*-coleoptile tip provides for a maximal geotropic reaction and not for maximal growth.

The larger the part removed by decapitation the smaller the geotropic curvature of the stump and the nearer the zone of maximal curvature approaches the base.

Supplementary observations support the interpretation of the results according to the lateral distribution hypothesis proposed by CHOLODNY.

The stimulating discussions held with Prof. Dr. V. J. Koningsberger and with Dr. H. P. Bottelier on this topic are recognized with pleasure.

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