Short-Term Stimulation of Growth Induced by the Apical Application of IAA to Intact Maize Coleoptiles

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Indole-3-acetic acid (IAA), mixed with lanolin, was applied to the surface of defined parts of the intact coleoptile of maize (Zea mays L.) seedlings, and the elongation growth of the coleoptile zone was monitored for a period of 8 h. When IAA was applied to a subapical region, the growth of zones located below was stimulated only temporarily, although stimulation was continuous when IAA was applied directly to the zone that was monitored for growth. This short-term stimulation was observed at all concentrations tested (0.03-30 mg of IAA per g of lanolin). The duration of stimulated growth, which varied from 1 to 5 h, was longer as the distance from the site of IAA application was reduced and the concentration of IAA was increased. Growth during the post-stimulation phase after application of a high dose of IAA could not be enhanced again by applying IAA near the site of the first application, but it was enhanced when IAA was applied directly to the zone used to measure growth. These results suggest that the supply of IAA from the site of application to lower zones is controlled internally such that the elevated concentration of IAA in these zones returns to the concentration before IAA application. Either the feedback regulation of auxin transport or the feedforward regulation of auxin metabolism may account for the suggested control of IAA supply.

Key words: Auxin — Coleoptile — Growth — Indole-3-acetic acid — Zea mays.

Results

Growth of coleoptile zones—The coleoptile was...
Fig. 1 Elongation growth of successive zones of intact maize coleoptiles. The coleoptiles, 22 to 24 mm in length, were marked with India ink at 5-mm intervals from the top, and the lengths of individual zones were measured at 30-min intervals. Zones are numbered from top to bottom. The mean increments (±SE) of length determined from 10 plants are plotted. Linear regression lines obtained between 1 and 9 h are shown for zones 2, 3 and 4 (r > 0.999 in all cases).

Marked with India ink at 5-mm intervals from the top, and the length of each zone was measured at 30-min intervals for 15.5 h to determine the time course of zonal elongation growth. The first measurement (the length at time 0) was obtained within 15 min after marking. As shown in Figure 1, the elongation rate (the rate of increase in length) in each zone was nearly constant from 1 to 9 h and decreased thereafter. Within the first hour, the elongation rates in zones 2–4 were lower than the ensuing steady rates. This result probably reflects the effects of handling. The elongation rate was greater for zones closer to the base of the coleoptile (see also lino and Briggs 1984).

For the time-course experiments described below, plants were allowed to stand for 1 to 1.5 h after marking before the first measurements were obtained. All subsequent measurements were made during the next 8 h, during which elongation rates were nearly constant.

Growth of zone 3 after apical and after direct application of IAA—In the first set of experiments, an IAA-lanolin paste was applied as a horizontal ring around the basal region of zone 1, i.e., the region just above the line 5 mm below the top of the coleoptile. The width of the ring in contact with the coleoptile surface was about 1 mm. After the application of IAA, increases in length of zone 3 were measured at 1-h intervals. It was confirmed in separate experiments that application of a ring of lanolin alone, 1–2 mm in width, around the base of zone 1 had no significant effect on the growth of zone 3. In the second set of experiments, an IAA-lanolin paste was applied to zone 3 and growth of this zone was measured similarly. In this case, the lanolin-IAA paste was applied as vertical stripes on the two narrow sides (i.e., the sides nearest to the vascular bundles) of the coleoptile. The IAA-lanolin stripe on each side extended over the entire length of the zone, and its

Fig. 2 The growth of zone 3 after application of IAA to the bottom of zone 1 (apical application). A mixture of IAA and lanolin was applied as a ring (about 1 mm wide). The numbers indicate the concentrations of IAA in mg per g of lanolin. The number 0 refers to lanolin without IAA. The means (±SE) obtained from 8 plants are shown.

Fig. 3 The growth of zone 3 after application of IAA to this zone (direct application). A mixture of IAA and lanolin was applied to the two narrow sides of the zone as vertical stripes (about 1 mm wide). The numbers indicate the concentrations of IAA in mg per g of lanolin. The number 0 refers to lanolin without IAA. The means (±SE) obtained from 8 plants are shown.
width in contact with the surface of the coleoptile was about 1 mm. This method of application was chosen because more extensive application of lanolin alone to zone 3 resulted in a significant inhibition of the growth of this zone. The application of a ring of IAA-lanolin to the base of zone 1 is referred to hereafter as apical application and that of stripes of IAA-lanolin to zone 3 (the zone subjected to growth measurements), as direct application.

Figures 2 and 3 show representative time courses of zonal elongation that followed the apical and the direct application of IAA, respectively. Results of all measurements are presented in Figures 4 and 5 in the form of a dose-response curve for each 1-h interval. These results demonstrate that growth was enhanced by applied IAA, and that the extent of the enhancement was greater with higher concentrations of IAA. The time courses did, however, differ between the two methods of IAA application as described below.

The stimulation of growth induced by the apical application of IAA was temporary (Fig. 2, 4). Growth was maximally stimulated during the second hour after IAA application and, following a phase of growth stimulation, the growth rate fell to the rate in control plants. The length of the stimulatory phase was longer as the concentration of IAA increased. By contrast, the stimulation of growth induced by the direct application of IAA continued over the entire 8-h period of the experiment (Fig. 3, 5).

With either method of IAA application, the highest rate of growth recorded was in the neighbourhood of 0.55 mm h\(^{-1}\), being about twice the rate in control plants (Fig. 4, 5). The results indicated that the growth of plants that have not been treated with IAA is maintained at a rate that is about half of the maximal rate achieved by application of IAA.

Fig. 4 Dose-response curves for the effects of IAA applied to the base of zone 1 on the growth of zone 3. Growth rates during each 1-h interval after the application of IAA were determined from time-course measurements (see Fig. 2). The interval (h) from the time of IAA application is indicated in each panel. The means (±SE) obtained from 8 plants are shown. Dashed lines represent the means of control values obtained after application of lanolin without IAA.

Fig. 5 Dose-response curves for the effects of IAA applied to zone 3 on the growth of this zone. Growth rates during each 1-h interval after the application of IAA were determined from time-course measurements (see Fig. 3). The interval (h) from the time of IAA application is indicated in each panel. The means (±SE) obtained from 8 plants are shown. Dashed lines represent the means of control values obtained after application of lanolin without IAA.
The dose-response curve obtained for the second hour after apical application of IAA (Fig. 4) indicated that the maximal rate of growth was achieved at a concentration as low as 0.3 mg g\(^{-1}\). With direct application, the corresponding rate was established at higher concentrations, 3-10 mg g\(^{-1}\) (Fig. 5). The results show that, paradoxically, apical application was more effective than direct application.

**Growth of zone 3 after application of IAA to the region just above this zone**—In the next experiment, a ring of lanolin-IAA (about 1 mm wide) was applied to the base of zone 2, which was just above the zone used to measure growth. As in the case of apical application, the growth rate decreased after an initial enhancement, approaching the control rate (Fig. 6). The phase of stimulated growth was, however, longer than that observed after the apical application at corresponding concentrations. This result indicates that the time at which the growth rate decreases after the stimulatory phase is related to the distance between the site of IAA application and the zone in which growth is measured, the decrease occurring later as the distance is reduced.

The maximal enhancement of the growth rate was achieved at 0.1 mg g\(^{-1}\) (see rates between 1 and 3 h after the application of IAA). This result indicates that, although the site of IAA application in this case was closer to the site of direct application than to that of apical application, the effective range of concentrations was similar to that found with apical application (Fig. 2). Therefore, the high degree of effectiveness observed with apical application (see above) is attributable to the application of IAA-lanolin as a ring.

**Effects of a second application of IAA**—An IAA-lanolin ring (10 mg g\(^{-1}\)) was applied to the top of zone 2. After 3 h and 20 min, another IAA-lanolin ring (10 mg g\(^{-1}\)) was applied just above the first one (i.e., at the base of zone 1). As shown in Figure 7, the time course after one application of IAA at time 0, which was essentially identical to the time course shown in Figure 2 (the data for 10 mg g\(^{-1}\)), was unaffected by the second application of IAA. It appears, therefore, that the decrease in the growth rate observed after the apical application was not caused by a reduction in the amount of IAA available for uptake.

The effect of a second application of IAA made directly on zone 3 was investigated next. The concentration of IAA for apical application was 10 mg g\(^{-1}\), and that for direct application, made 3 h and 20 min after the apical application, was either 1 or 10 mg g\(^{-1}\). As shown in Figure 8, the decrease in the growth rate, which became apparent about 5 h after the apical application, was prevented by the second application, and the resulting time courses with two applications of IAA were approximately linear up to 8 h.

For re-stimulation of growth by the second application of IAA, 1 mg g\(^{-1}\) was only slightly less effective than 10 mg g\(^{-1}\) (Fig. 8). This dose-response relationship was similar to that found with direct application alone (Fig. 3), suggesting that the sensitivity to IAA was not modified in the post-stimulation phase.

**Growth of successive zones after apical application of IAA**—To obtain more information about the short-term stimulation of growth observed in zone 3 after apical application...
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Fig. 8 The growth of zone 3 after application of IAA to the base of zone 1 with a second application of IAA to zone 3. The first application was made at time 0, and the second application, at 3 h and 20 ± 5 min (dashed vertical line). The concentration of IAA for the first application was 10 mg g⁻¹ and that for the second application was either 1 mg g⁻¹ (△) or 10 mg g⁻¹ (■). Control measurements were made with lanolin alone for both applications (○), and with 10 mg g⁻¹ IAA for the first application and lanolin alone for the second application (●). The means (±SE) obtained from 8 plants are shown.

As shown in Figure 9, growth was stimulated by IAA in all zones. Stimulation was initiated after a lag period, the length of which increased towards the base of the coleoptile. The short-lived nature of stimulation was evident in the zones located between 7.5 and 20 mm from the top. In these zones, the transition from the enhanced to the reduced growth rate occurred within the period 2 to 3 h after IAA application. The transition was sharper as the distance from the site of IAA application increased. In the top zone (5.0-7.5 mm), growth remained stimulated to the end of the 4.5-h experiment. However, a gradual reduction in the growth rate was also evident. It appeared that the stimulation of growth was temporary even in this zone, the reduction of the growth rate occurring more gradually than that in the lower zones.

The gradual delay of the onset of growth stimulation, which was observed as the distance from the site of IAA application increased, probably reflects the time required for the basipetal transport of IAA. To evaluate the velocity of IAA transport from the data of Figure 6, an estimate was made of the time at which growth stimulation was initiated in each zone, and this time was plotted against the initial distance of the center of each zone from the top of the coleoptile. The apparent velocity, as calculated from this relationship, was 17 mm h⁻¹. This value is somewhat greater, as reported earlier (Baskin et al. 1985), than the mean velocity estimated by measuring the transport of radio-labelled IAA (e.g., Hertel and Leopold 1963) or from measurements of endogenous IAA (lino 1991).

The time of the transition from the enhanced to the reduced growth rate did not evidence any basipetal migration that reflects the basipetal transport of IAA. Simple kinetic analysis was difficult, however, because of the gradual nature of the growth-rate change observed in the upper zones. The data in Figure 6 tend to indicate that the phase of stimulated growth becomes shorter in more basal zones.

Discussion

The mechanism underlying the temporary stimulation of growth—The elongation growth in a zone below the site of IAA application was characterized by an initial enhance-
ment in rate that was followed by a decrease to a steady rate comparable to that in control plants (Fig. 2, 4, 6, 9). These results indicate that the stimulation of growth by apically applied IAA is overcome by a regulatory mechanism that returns the stimulated growth rate to the pre-stimulation rate. Since no such change in growth rate was observed when IAA was applied directly to the zone used to monitor growth (Fig. 3, 5), it is suggested that the supply of IAA from the site of application to a lower zone is regulated such that the initially enhanced level of IAA in the zone is reduced to the original level prior to IAA application. The change in growth rate from the enhanced to the control rate was most evident when the distance from the site of IAA application to the top of the monitored zone was 5 mm or more. When the site of IAA application was immediately above the monitored zone, the change was delayed and more gradual (Fig. 6, 9). These results suggest that the reduction in the supply of IAA is achieved when it is transported basipetally.

Two possibilities may be considered to account for the suggested control of the supply of IAA: (1) the feedback regulation of the basipetal transport of IAA; and (2) the feedforward regulation of the metabolism of IAA. In the former possibility, it is thought that the amount of IAA transported to lower parts is reduced in response to an elevated level of IAA in the transport system. In the latter possibility, it is thought that the metabolism of IAA, which can be either catabolism or conjugation, is enhanced in response to an elevated level of IAA in tissues.

Control of the sensitivity of tissues to IAA may also be considered (see, e.g., Vesper and Evans 1978). The following two observations, however, argue against a role of a sensitivity control as a basis for the observed decrease in growth rate. First, growth stimulation was continuous when IAA was applied directly to a monitored zone (Fig. 3, 5), as was also the case when isolated coleoptile segments were incubated in solutions of IAA (Cleland 1972). Second, the doses of IAA needed to re-stimulate growth by direct application after apical application (Fig. 8) were not higher than the doses needed for comparable stimulation of growth by direct application alone (Fig. 3).

The temporary stimulation of growth described above is reminiscent of an observation made by Hatfield and LaMotte (1984). These authors found that when an agar block that contained a relatively high concentration of IAA was placed on the cut apical surface of a decapitated maize coleoptile, growth of the coleoptile stump was stimulated for only a short period of time. The highest growth rate was observed about 2 h after application of IAA. Although these authors interpreted their result in terms of the sensitivity of the tissue to IAA, the result does not contradict the present conclusion, which is based on a change in the level of IAA.

Studies with intact seedlings of dicotyledons indicated that exogenously applied IAA stimulates growth for only a short period of time (Hall et al. 1985, Carrington and Esnard 1988). Unlike the temporary response in the present study, the response was recorded in pea internodes (Hall et al. 1985) and in watermelon hypocotyls (Carrington and Esnard 1988) to which IAA had been directly applied. More recently, Yang et al. (1993) used intact pea seedlings to investigate growth of the internodes to which solutions of IAA was applied continuously. Although most of the measurements indicated that the elongation of internodes was stimulated for a relatively long period of time, the internodes of dwarf pea showed a temporary stimulation of growth when the concentration of IAA was low, the highest growth rate occurring 2 h after the onset of IAA application. This short-term stimulation was again observed during application of IAA to the entire length of elongating internodes. These temporary responses reported for dicotyledonous, therefore, appear to differ from the present case in which separation from the site of IAA application was required.

Some implications of the dose-response relationship—The maximal growth rates observed at saturating concentrations were similar, irrespective of whether IAA was applied apically or to the monitored zone (Fig. 4, 5). The application of IAA to the region just above the monitored zone also resulted in a similar maximal rate (Fig. 6). These results suggest that the maximal growth rate represents the saturation of the auxin action at the target tissue. Since the growth rate of intact coleoptiles that were not treated with IAA was about half of the maximally enhanced rate, it is further suggested that the concentration of IAA in the coleoptile (at least in zone 3) is regulated to maintain its action at about half the saturation.

The dose-response curve for the period 1-2 h after the apical application of IAA was nearly flat from 0.1 to 30 mg g⁻¹ (Fig. 4). If this saturation of growth response were caused by the action of IAA at the target tissue and not by the transport of IAA, as discussed above, then it would become likely that the actual level of IAA in the target tissue increases with increasing doses of applied IAA even in the growth-saturating range. The longer stimulatory phase found with higher concentrations of IAA might represent the time needed to reduce the level of IAA in the target tissue below the saturating concentration. A similar explanation can be applied to describe the longer stimulatory phase found in zones that were closer to the site of IAA application.

The application of IAA as a horizontal ring was far more effective for growth stimulation than was application as vertical stripes. The uniform distribution of IAA in the target tissue (e.g., the epidermis) may be necessary for effective stimulation of growth, and it may be better achieved by the application as a ring. It is also possible that a certain radial or vertical gradient of IAA within the coleoptile is
necessary for effective stimulation of growth, and that this
gradient is better achieved by a ring than stripes. Further
careful studies of the difference in effectiveness might
reveal important aspects of the action of IAA.

Concluding remarks—The present study demonstrates
that growth of a coleoptile zone is stimulated for only a
short term when IAA is applied above the monitored zone,
and that the initially enhanced rate of growth is reduced to
the pre-stimulation rate, or the rate in control plants. It
also appears that the growth rate in control plants is main-
tained at about half of the maximal rate achieved by ap-
plication of IAA. These results indicate that the maize cole-
optile is equipped with a mechanism that somehow adjusts
the growth rate to a more or less specific rate, which is
about half of the rate at saturation. When the level of aux-
in is enhanced, by exogenous application of IAA in the
present case, the regulatory mechanism becomes operative,
and the enhanced rate is reduced to the pre-stimulation
rate. The duration of stimulated growth depends on the
concentration of IAA, ranging from about 1 to 5 h. The
shorter duration, observed at low concentrations of IAA,
is likely to correspond to the duration of growth stimula-
tion that occurs in the intact plant in response to an in-
crease of IAA within the physiological range of concentra-
tions. It is suggested that the primary role of auxin in plant
growth is to mediate growth responses that occur within
a time span of a few hours.

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