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Auxin-Gibberellin Interaction in Apical Dominance[1, 2]

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Summary. Indoleacetic acid and gibberellic acid were added to decapitated light-grown 'Alaska' pea seedlings as substitutes for the intact apex in the control of apical dominance. Of various concentrations and combinations tried, a combination of 1 % indoleacetic acid + 1 % gibberellic acid was the most inhibiting to side bud growth. The greatest degree of hormonally induced side bud inhibition was achieved when seedlings were deprived of soil nutrients.

In order to demonstrate the degree to which lateral bud growth is inhibited by the main shoot apex, it is normally necessary to remove the apex surgically. Efforts to compensate for the metabolic imbalance which necessarily ensues have traditionally involved the application of synthetic plant growth regulators near the apex. Unfortunately, direct treatment of inhibited lateral buds has yielded equivocal results. Indoleacetic acid (IAA) may induce growth of lateral buds of *Pisum* (11) or not (26). Direct application of kinetin has only recently proved to be effective in releasing buds on seedlings which have not been decapitated (19). Direct application of gibberellic acid (GA), on the other hand, has not been shown to be stimulatory (9, 19). In spite of the obvious shortcomings, it continues to appear that pursuing a classical approach to the problem through the use of material in which only the apex has been removed is the best compromise between intact plants and isolated stem segments for gaining an understanding of the control mechanism(s) involved.

The basis for the present study was formed from the suggestion that auxin does not control, or is not the exclusive agent in the control, of apical dominance. Meinl and von Guttenberg (16) have pointed out that only relatively high concentrations of added auxin inhibit the lateral buds of *Phaseolus*, and they therefore questioned whether endogenous levels of auxin are that high. Jacobs et al. (7) later demonstrated that IAA added in the amount sufficient to replace the level of native auxin was not sufficient to maintain the correlative inhibition of lateral buds of *Coleus*. In order to determine if the same is the case for the frequently studied *Pisum* seedling, we added auxin to decapitated (but otherwise intact) plants using the classical lanolin carrier technique. In addition, studies were conducted to show the extent to which gibberellin, alone or together with auxin, is able to substitute for the apex and to what extent relative starvation is a limiting factor in the regulation of dominance. Experiments which consisted of checking the growth of all side shoots over an extended period of time indicate that all the factors mentioned above are related to apical dominance in *Pisum*, a seedling in which dominance is normally complete.

Materials and Methods

Twelve-day-old Alaska pea seedlings (*Pisum sativum* L.) were used throughout this study. Seeds were soaked for 3 hours and were planted in shallow, approximately one foot square clay pans containing either unsterilized vermiculite or a vermiculite and sterilized compost-soil mixture. Intact and treated seedlings were grown and maintained in a growth chamber at 24 ± 1° on a short-day cycle (8 hrs) at a light intensity of 2000 ft-c. Plants with 6 internodes ranging 13 to 15 cm in height were used.

Treatment mixtures consisted of IAA and 75 % potassium salt of GA incorporated in anhydrous lanolin. Crystals of IAA and GA were mixed thoroughly (together or separately) with warmed, melted lanolin. Pastes were applied to the cut-surface at the top of the sixth internode immediately following the excision of the apical bud. Reapplications were made at 3 day intervals on the freshly cut surface

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resulting from the removal of approximately the apical 2 mm of tissue.

Daily growth measurements, to the nearest mm, were made using calipers and a ruler with mm subdivisions. The length of each lateral bud was measured as the distance between the point of attachment of the base and the tip of the stipules enclosing the apical bud. All measurements were pooled for each treatment and the data are expressed as the average of the combined side shoot length(s) per plant. The internode lengths of the main seedling axis were taken between India ink reference spots made at the nodes and the apical cut-surface beneath the paste application. Controls consisted of the application of plain lanolin and treatments were compared to intact seedlings of the same experimental lots unless specified otherwise. All experiments were repeated at least once and all values represent the average of at least ten plants unless specified otherwise. Statistical methods and terminology follow Snedecor (24).

Results

In the normal Alaska pea seedling, the presence of the apical bud is correlated with a lack of outgrowth of lateral buds for at least the first 3 weeks; dominance is normally complete for this period. If the apical bud is excised 12 days following planting, small buds (1 mm or more) may be detected at 1 or more nodes between 1 and 2 days. Normally, elongation of 1 lateral bud gains in acceleration over that of the others, although at least some growth may take place at more than 1 node.

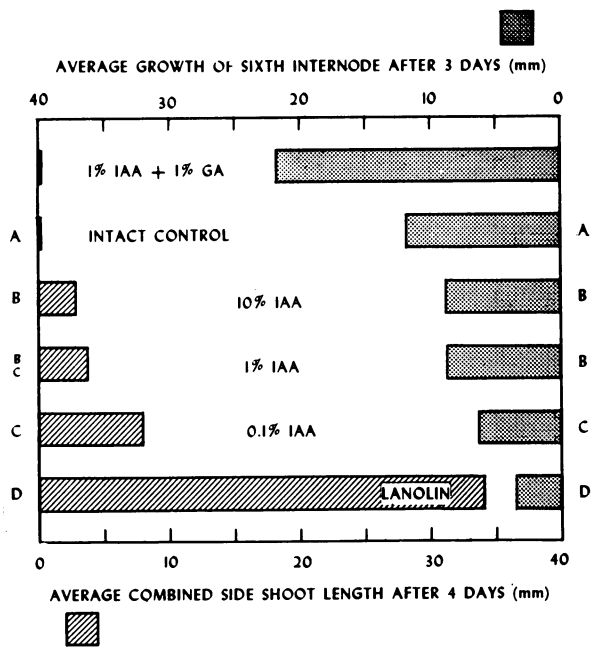

FIG. 1. The effect of lanolin, IAA, and IAA + GA on side shoot growth of decapitated pea seedlings 4 days following treatment (left) and on the growth of the sixth internode of the same seedlings 3 days following treatment (right). Treatments are compared with intact controls (n=9). Values significantly different at the 5 % level, as determined by the 't' test, are designated by letters which differ preceding the histograms. Letters which are the same signify no difference.

Excision and Substitution Experiments; Seedlings Planted in Vermiculite. Measurements of averages of combined side shoot lengths 4 days following decapitation are compared to the intact control in figure 1. High concentrations of IAA in lanolin (10 % and 1 %) inhibit growth of side shoots by a factor of 10 over that of lanolin alone but growth is nevertheless significantly more than the intact control. A 0.1 % concentration of IAA is less inhibitory. A mixture of 1 % IAA + 1 % GA is the most effective of the chemical treatments tried in preventing lateral bud growth; there being essentially none as in the case of the intact control. Note: data for the 1 % IAA + 1 % GA treatment are pooled from 2 experiments different from the others shown in figure 1. They are included here for comparative purposes since the seedlings were grown and handled in an identical fashion and average values for intact and lanolin controls are comparable for the 2 experimental lots.

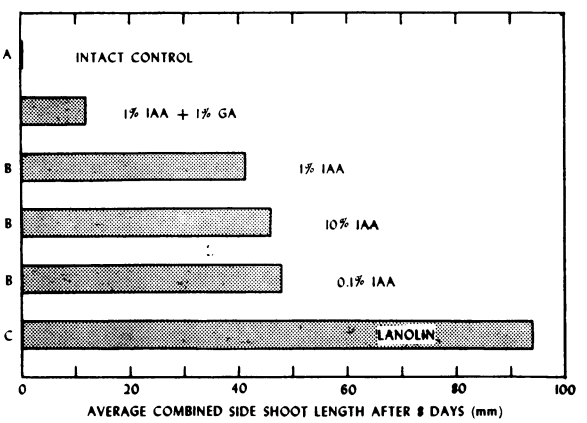

FIG. 2. The effect of lanolin, IAA, and IAA + GA on side shoot growth of decapitated pea seedlings 8 days following treatment, as compared to intact controls. Significance designations as in figure 1.

Also shown in figure 1 is the amount of elongation of the sixth (apical) internode 3 days following the initiation of the experiment. This designation has been made since it represents the time of maximum growth of this internode in treated as well as intact seedlings; growth of this internode has ceased 15 days following germination. The greatest amount of growth of the sixth internode takes place when 1 % IAA + 1 % GA are substituted for the apex (see Note above). Conversely, the least growth occurs with only lanolin applied. The 3 IAA treatments fall within the extremes with the amount of growth differing significantly from the lanolin treatment. In addition, it should be noted that a striking inverse relationship exists between the amount of internodal

growth and summated side shoot growth when one compares all of the treatments.

The average combined side shoot lengths of the same experimental seedlings, 8 days following treatment, are shown in figure 2. Essentially similar relationships exist between the IAA treatments as previously, with all 3 being equally inhibitory. The differences in the growth patterns after the additional 4 days are: 1) an acceleration of side shoot growth in IAA treatments such that they represent one-half of that of lanolin alone; 2) a substantial amount of growth with the 1 % IAA + 1 % GA treatment is now evident although it continues to be the least of all the treatments and therefore the most equivalent to the intact controls.

The effects of 1 % GA alone and in combination with the 3 concentrations of IAA were also tested. After 8 days of treatment, seedlings with 1 % GA exhibited side shoot growth in considerable excess of those treated with lanolin alone (fig 3). However, when GA was applied together with IAA, growth was equal to or less than the plain lanolin treatment. Here, 1 % IAA is significantly more effective than the 10 % concentration in inhibiting side shoot growth when applied with GA, while 0.1 % IAA is far less so than either. By comparing figures 2 and 3, it may be concluded that a low concentration of IAA does not alter the GA influence nor, conversely, does GA influence the response to a high concentration of IAA. Only when both are added in 1 % concentrations does one find a significant change from either one alone.

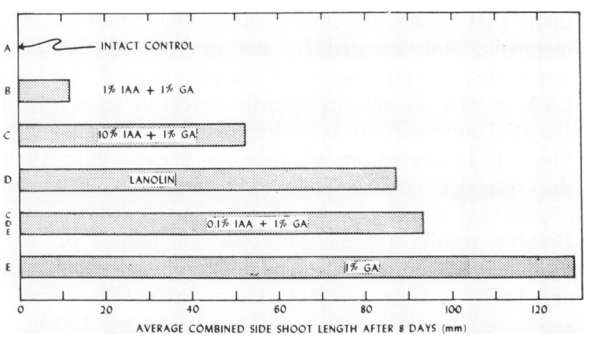

FIG. 3. The effect of lanolin, GA, and GA + IAA on side shoot growth of decapitated pea seedlings 8 days following treatment, as compared to intact controls. Significance designations as in figure 1.

Excision and Substitution Experiments; Seedlings Planted in Vermiculite and Soil. The addition of a sterilized compost-soil mixture to the vermiculite is correlated with a more vigorous growth of the seedlings in all treatments, as measured by the growth of the side shoots (fig 4). (Relative degrees of differences may be inferred by comparing points for days 4 and 8 of the vermiculite curves with significance designations of figs 1 and 2). It is also apparent from the data that once lateral buds begin to elongate, IAA does little to retard their growth. The

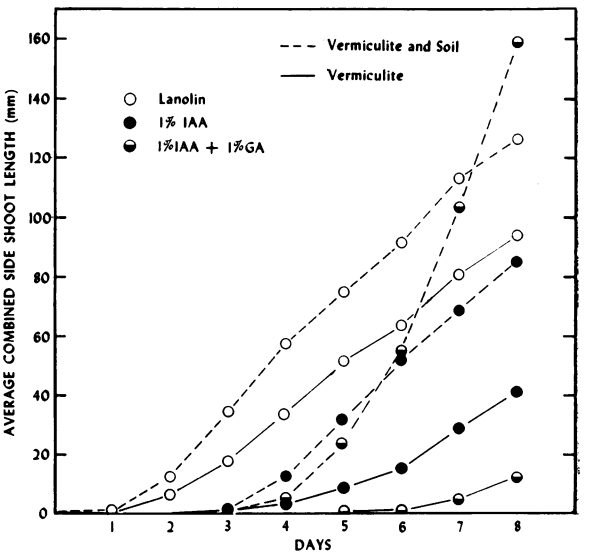

FIG. 4. The effect of a vermiculite and soil potting mixture, compared to vermiculite alone, on side shoot growth of treated decapitated pea seedlings over a period of 8 days.

one curve which is not in general alignment with the others is that for 1 % IAA + 1 % GA for plants in vermiculite and soil. The overall increase in side shoot growth shown is not a result of an earlier time at which growth commenced. Rather, as is the case for 1 % GA alone, measurable buds first appeared at 2 to 3 days but then grew at a faster rate. The addition of soil to vermiculite did not stimulate any of the intact plants to produce lateral bud growth.

Discussion

Since the review of Champagnat (2), further reports have appeared which continue to relate auxin to the phenomenon of apical dominance, either directly or indirectly (1, 3, 8, 12, 13, 14, 19, 27). Gibberellin, too, has been shown to influence dominance in a variety of plants, as pointed out in the review by Paleg (17). The interesting possibility is suggested, therefore, that an interaction between these 2 growth regulators may, at least in part, be responsible for this feature of development in higher plants (1, 8, 22).

Hypotheses advanced to account for correlative inhibition on a hormonal basis have commonly involved other factors, in addition to auxin and gibberellin, as related causative agents. For example, there are reports that kinetin acts as an antagonist of auxin-induced inhibition when both compounds are applied together (28) or separately (19) while, on the other hand, it may act to enhance auxin-induced inhibition when applied simultaneously with auxin (3). In addition, it has been suggested that other balanced interactions occur between auxin and inhibitors (11, 25), mono- and poly-phenols (27) and auxin precursor(s) (4, 16). Contrasted with the above, is the theory which accounts for the same phenomenon on

a nutritional basis, while maintaining hormonal (or auxin) action to be indirect (5, 15).

That auxin is a participant in and not of itself the controlling factor in the maintenance of apical dominance (7, 16) seems clearly indicated. In the present study, a concentration of 0.1 % IAA did not prevent the expansion of side shoots. Scott and Briggs (21) have shown that this amount of auxin applied in lanolin produces diffusible and extractable auxin yields (as determined by the *Avena* curvature test) from the light-grown pea seedling which are equal to or are in excess of endogenous levels. Presumably, all IAA concentrations used in the present study adequately replaced the native auxin. Libbert's findings indicate that exact auxin replacement closely correlates with lateral bud inhibition in the light-grown pea (12). However, his experimental period never exceeded 2 days and therefore the results are not comparable though not necessarily in conflict with ours. The minimum of 2 days before the onset of bud expansion of IAA-treated seedlings in the present study indicates a longer time course is required before release (or lack of it) is manifested and reliably recorded.

A further delay in the onset of bud elongation may be achieved by adding GA in combination with IAA (fig 4). The effect was especially pronounced if the treatment consisted of 1 % IAA and 1 % GA and if the seedlings were grown in plain vermiculite (compare figs 2 and 3). Little growth resulted after 8 days under these conditions. The evidence of Jacobs and Case (8) suggests that this combination may result in the most effective restoration of apical dominance because more auxin is present in the basal region of the epicotyl as a consequence of GA being present in the application mixture. The explanation that more IAA is detectable at a greater distance by virtue of a GA induced increase or enhancement of auxin transport has recently gained experimental support (18).

The actual role auxin plays in bud inhibition remains puzzling. Circumstantial evidence provided by this study indicates auxin action is indirect and that whatever the action, it may be amplified by GA and most dramatically when nutrition is limiting. Three points may be advanced which argue that growth in 1 part of the seedling effectively prevents growth in another. First, as long as the apical bud is intact and internodal development and elongation take place normally, lateral buds do not elongate. Second, if the apical bud is removed, 1 bud at 1 of the 5 nodes usually does all of the elongating (occasionally 2 buds will elongate, seldom more). Finally, as figure 1 shows, during the time which the sixth internode retains the ability to elongate, the elongation induced by IAA or IAA + GA is qualitatively the reciprocal of the lateral bud growth.

If, then, compensatory growth explains the phenomenon (7), what is the mechanism of the GA interaction in this system? A reasonable explanation would seem to involve auxin transport. Regions where maximum transport of auxin takes place regularly correspond to regions of maximum elongation of growing tissues and it is, accordingly, felt by several authors that transported auxin may control elongation growth (6, 10, 20). It may follow, then, that if GA increases the transport of applied auxin (18) the consequence is increased growth (fig 1). The increased distribution of IAA at the bottom of the stem, following its application together with GA, found by Jacobs and Case (8), might be an unrelated event which just happens to be correlated with bud inhibition. However, this view is too simple, since the same authors showed that GA caused increased amounts of IAA at the level of the inhibited buds even in cases where there was no significant increase in the elongation of the main axis (their table 1). It is also important to note that the early work of Skoog (23) and that of MacQuarrie (14) warn that it is impossible to generalize about compensatory growth in dark-grown peas. In each study the inverse relationship, though conspicuous, did not always obtain and stem swelling occurred under conditions of maximum bud growth, both with applications of high (23) and low (14) auxin concentrations.

The nutritional status of the pea seedling is, also, clearly an important parameter of artificially imposed dominance. As Gregory and Veale reported for Linum (5), inhibition by auxin application is more negligible if conditions of nutrition are more favorable (fig 4). The presence of soil in the potting mixture advanced the times of bud release as well as, perhaps, accelerating the rate of bud expansion (compare 2 IAA + GA curves). It would appear, then, that under the more favorable conditions, the metabolic sink created by the growth of the sixth internode, in response to the application of IAA or IAA + GA, is not sufficiently effective in diverting essential nutrients so as to impose as severe an inhibition. It is interesting to note the great similarity in the response curves of similar treatments made on decapitated potato shoots (fig 8; 1). Like our own, Booth's results reflect the complex nature of correlative bud inhibition. Undoubtedly, the interaction and balance of many factors are involved. Clearly, auxin, gibberellin, and nutrition are among them.

Acknowledgments

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