

SYMPOSIA
OF THE SOCIETY FOR
EXPERIMENTAL BIOLOGY

NUMBER XI

THE
BIOLOGICAL ACTION
OF
GROWTH SUBSTANCES

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SYMPOSIA

- I NUCLEIC ACID
- II GROWTH, DIFFERENTIATION AND MORPHOGENESIS
- III SELECTIVE TOXICITY AND ANTIBIOTICS
- IV PHYSIOLOGICAL MECHANISMS IN ANIMAL BEHAVIOUR
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- X MITOCHONDRIA AND OTHER CYTOPLASMIC INCLUSIONS

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PREFACE

The eleventh Symposium of the Society for Experimental Biology was held at Aberystwyth in September 1956, at the invitation of the University College of Wales. In addition to the formal sessions evening discussions were held on 'Some problems of phototropism', opened by Dr C. L. Mer, and 'Control of growth from embryo to adult', opened by Dr M. Abercrombie.

The Society is indebted to the University College of Wales for hospitality and for the provision of facilities, and as well to many members of the staff and technical staff for invaluable help both in the organization and during the course of the meeting.

The British Council, Messrs Fisons Ltd, Messrs Glaxo Ltd and Messrs May and Baker Ltd contributed towards the expenses of the Symposium and the publication of this volume. The Rockefeller Foundation provided funds for expenses of the speakers from the United States of America. The Society expresses its gratitude for this financial help.

The Editor wishes to record her appreciation of the help afforded by the Cambridge University Press in the preparation of this volume.

H. K. PORTER

*Editor of the eleventh Symposium of the
Society for Experimental Biology*

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Additional Communication to the meeting not included in this volume:

Chelating Agents as Plant Growth Substances *by* O. V. S. HEATH *and*
J. E. CLARK. (See *Nature* (1956), 177, 1118, and 178, 600.)

A REASSESSMENT OF THE PROBLEM OF APICAL DOMINANCE

BY F. G. GREGORY AND J. A. VEALE*

Research Institute of Plant Physiology, Imperial College of
Science and Technology, London, S.W. 7

The phenomenon of apical dominance is by no means universal and varies in different species from extreme cases in which no branching normally occurs to freely divaricating forms. The phenomenological aspects of the subject have been recently reviewed by Söding (1952). In seeking a causal explanation of such 'correlative inhibition' two diverse views have held the field: first, that the inhibition is occasioned by lack of nutritive or plastic materials which are present in limited supply and for which preformed meristems compete, and of which the flow is directed to the most active region of growth. This concept is of a purely quantitative kind. The second explanation postulates the presence of specific inhibiting substances produced in growing regions and translocated about the plant.

NUTRITIVE HYPOTHESIS

The notion of competition for nutrients as the main factor in correlative inhibition was developed by the older botanists, Goebel, Jost and Pfeffer, and was investigated in a systematic way by Loeb (1924).

His work with *Bryophyllum calycinum* has become classic. His conclusions were based on quantitative data of the most convincing kind. Loeb established the following points: Equal masses of tissue of either isolated leaves or portions of stem always produce equal masses of regenerated roots and shoots. A piece of stem left attached to the isolated leaf restricts the growth of buds on the leaf, and the gain in weight of the stem balances the reduction in weight of the shoots and roots on the leaf. Starting with a hormone hypothesis Loeb eventually abandoned it, and held that the immediate cause of the sustained inactivity of buds on the attached leaf and in the leaf axils is due to the drain of nutrients to the stem, and, of course, in the intact plant to the apical region. Loeb emphasized the fact that the buds are not inherently dormant, as on the isolated leaf or portions of stem all the buds begin to develop, and only later those less favourably situated are arrested by mutual competition. Physiological isolation of the

* Now at Lincoln College, Christchurch, New Zealand.

leaf by low temperature is as effective as complete separation (Child & Bellamy, 1920). Loeb was not unaware of the limitations of his hypothesis, particularly in regard to the effect of gravity on the determination of the sites of the active buds, a matter which he fully discusses.

HORMONE HYPOTHESIS

Dostal (1909, 1926) first suggested that inhibitory factors were operative in correlative inhibition, but this aspect of the problem has been systematically studied by Snow (1925, 1929), who showed that inhibitory effects originating in the apical bud could pass between two shoots bound together at a cut surface, and across a ringed zone of the stem. References to Snow's work will be made in the course of later discussion, in particular to the 'distance effect' (Snow, 1931 *a, b*) which established the paradox that the more distant the site of the bud from the apex the greater the inhibition. This observation was the basis of Snow's later experimental work. In the meantime Thimann & Skoog (1934) had shown that dominance of the apical bud could be replaced by applying indole-3-acetic acid (IAA) to the decapitated stem, and the inhibitory influence was therefore identified with auxin.

The mechanism of inhibition has since been the subject of controversy; critical reviews of possible alternatives were published by Snow (1937), Thimann (1939, 1948) and Söding (1952). Three possible mechanisms have been propounded, all based upon the fundamental role of auxin.

(1) The theory of direct auxin inhibition proposed by Thimann (1937) attributed inhibition of lateral buds to their high sensitivity to auxin so that the normal content of the shoot is supra-optimal. Either too high or too low a level of auxin arrests growth. Skoog (1939), working with isolated apical buds of *Pisum*, showed that low concentrations of IAA (below 0.1 mg./l.) had little or no effect, whereas high concentrations reduced, but did not prevent, growth. Allsopp (1956), working with *Marsilea drummondii*, has concluded that 'direct inhibition of auxin plays little or no part in correlative inhibition of lateral buds'.

(2) Theories of indirect action of auxin as inhibitor are of two kinds. Went (1936, 1939) favoured the view that under the influence of auxin, produced in actively growing regions, nutrient factors (including specific 'caulines') are preferentially transported to these regions. That nutrients of all kinds accumulate in regions of applied auxin is well established. A modification of this hypothesis was suggested by Ferman (1938), who supposes that an auxin precursor behaves in this way.

Main support for indirect inhibition comes from the work of Snow conspicuous for the ingenuity of his experimental approach. He suggests that auxin, in passage through stem tissues, by a secondary reaction is either converted into or stimulates the production of an inhibitor. Auxin and inhibitor act antagonistically, excess of auxin overcoming the effect of the inhibitor so that growth of the main axis can continue. The polar transport of auxin prevents its entry into the lateral buds, whereas the inhibitor is free to move into the buds and inhibits them. Snow (1940) has provided evidence of transmission of inhibition across a tissue discontinuity, and Uhrova (1934) showed that the inhibiting effect of the leaf of *Bryophyllum crenatum* on its axillary bud can be transmitted through a layer of agar.

There is now ample evidence of the presence of inhibitors in tissues (Bennet-Clark & Kefford, 1953; Hemberg, 1949*a*) and particularly in inactive buds (Hemberg, 1949*b*).

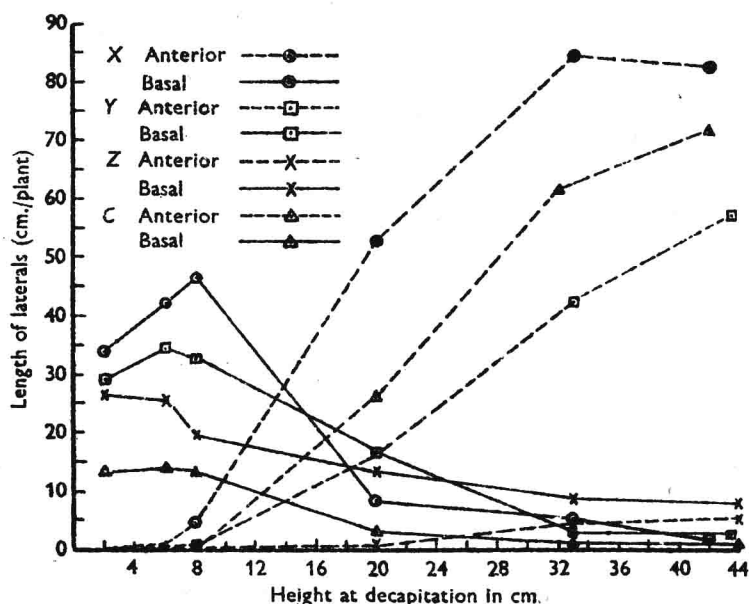
(3) The 'blockage' hypothesis of van Overbeek (1938) attributes the inactivity of lateral buds to the impedance of transport of nutrients through the vascular system, and focuses attention on the anatomical relations of the bud to the shoot. It is not intended in this paper to examine critically the evidence for and against these various hypotheses, but rather to present a body of experimental data, and to endeavour to interpret the results in the light of current theory. In some respects this work is a return to the methods of Loeb. It appeared desirable to study the extent to which apical dominance is related to the nutritional status of the plant as this has not been systematically undertaken hitherto. The main work has been conducted with two varieties of flax, though other plants have also been examined to extend the findings to plants which have been used by other investigators.

THE PATTERN OF LATERAL BRANCHING IN THE INTACT AND DECAPITATED PLANT

Flax plants (*Linum usitatissimum* var. Liral Prince) were sown at weekly intervals in soil, and at the time the last set had reached 2-2.5 cm. all the series were treated according to the following scheme: C, intact controls; X, terminal bud only removed; Y, upper third of stem removed; Z, upper two-thirds removed. Each variant comprised twenty plants, and the lateral branches were measured 4 weeks after treatment. Two types of laterals were separately recorded: basal branches and anterior branches arising behind the apex.

In Text-fig. 1 total length of laterals of the two types is plotted against

the height of the plants at the time of decapitation. The following results appear: In plants decapitated before reaching a height of 6–8 cm. lateral growth is entirely basal, and total growth made is least in the intact controls, and greatest in treatment *X* with apical bud alone removed. The more stem that is removed the less is the basal growth, but always more than in intact plants. As the source of auxin is removed in all treatments, variation in lateral growth must be due to other factors. Anterior branching begins in



Text-fig. 1. Effect of removing various amounts of stem tissue on the branching response of flax plants decapitated at various stages. *X*, terminal bud removed. *Y*, upper one-third of stem removed. *Z*, upper two-thirds of stem removed. *C*, control. Anterior branches: --- 4 weeks after treatment. Basal branches: — 4 weeks after treatment.

control plants 8 cm. high, somewhat earlier in type *X* (6 cm.), and much later in type *Z* (20 cm.).

Maximal basal growth occurs in the intact plant at 8 cm. and later declines owing first to arrest and later death of the basal branches. In the decapitated plants maximal basal growth also occurs when treated before reaching 8 cm., and later decapitation leads to progressive decline in basal growth. In all cases delay in decapitation leads to increasing anterior growth, though in type *Z* little anterior growth is made. Tendency to produce anterior branches is well marked in the intact plants, and the removal of the apical bud alone intensifies this behaviour to a small extent. On the other hand, removal of a portion of the stem greatly reduces this

tendency. This indicates that either (a) the stem is important as a source of nutrient, (b) the removal of the leaves carried by the amputated stem or (c) the buds removed are important factors. So far as the bud number is concerned this is not the decisive factor, as even in type Z only a small proportion of the remaining nodes produce branches. The situation of the buds on the stem, as will be seen later, is the important factor.

Removal of portions of the stem entails removal of leaves, and to separate these effects the following experiment was performed. Serial sowing provided plants of varying height at the time of decapitation. The following treatments were given:

- X apical bud alone removed.
 X (YD) apical bud removed and one-third of stem defoliated.
 X (ZD) apical bud removed and two-thirds of stem defoliated.
 Y apical bud removed and one-third of stem removed.
 Y (ZD) apical bud removed, one-third of stem removed and a further one-third defoliated.
 Z apical bud removed and two-thirds of stem removed.

The resulting lateral growth for means of twenty-one plants after 3½ weeks is shown in Table 1.

Table 1. *Comparison of effect on branching in flax of total removal of portions of stem and of defoliation leaving stem tissue in situ*

Height at decapitation (cm.) ...	Length of basal branches cm.				Length of anterior branches cm.			
	6-8	12-16	20-27	30-40	6-8	12-16	20-27	30-40
Treatment								
X	9	36	3	20	2	16	65	105
X(YD)	9	34	28	49	0.4	2	9	30
Y	10	37	27	51	0.3	0.6	18	34
X	9	36	3	20	2	16	65	105
X(ZD)	9	34	31	41	0.5	0.4	1	7
Z	7	28	29	50	0.8	0.4	0.5	2
Y	10	37	27	51	0.3	0.6	18	34
Y(ZD)	7	31	32	46	1	0.4	0.6	5
Z	7	28	29	50	0.8	0.4	0.5	2

The effect of defoliation on basal branching is the same as that of removing the whole portion of the stem, indicating that assimilates from the leaves may have an effect on basal growth, but as all regeneration growth on the defoliated part of the stem was removed, the effect may have been due to elimination of competition of anterior branches. The latter conclusion is supported by the general relation between basal and anterior growth, for basal growth in the taller plants is *increased* by stem removal and defoliation, whereas anterior growth is greatly reduced. Competition

would appear to be the chief factor, though assimilation by the leaves is concerned as defoliation reduces the total lateral growth; nutrients stored in the stem clearly have no added effect.

THE GRADIENT OF 'GROWTH POTENTIAL' IN THE STEM

As already mentioned, lateral growth varies as between different regions of the stem and this is reflected in the pattern of development. To investigate the gradient in 'growth potential' of various nodes, these were isolated and

Table 2. *Bud development on isolated nodes of the stem of flax*

Serial no. of node from base	No. of cuttings	No. of buds just visible	Number of buds			Total no. of buds	Percentage with buds
			0.3- 0.5 mm.	0.5- 1 mm.	1-2 mm.		
3 and 4	110	30	24	16	10	80	36
5 and 6	108	0	0	0	0	0	0
8	106	2	0	0	4	6	0.6
12	106	2	2	2	0	6	0.6
16	104	4	2	6	6	18	17.3
20	98	2	4	8	10	24	24.5
24	98	16	12	16	28	72	73.5
28	100	0	14	24	38	76	76

planted as cuttings. In the first experiment plants measuring 12 cm. were used. Of the cuttings comprising the cotyledonary node and nodes of leaves 1 and 2, 96 % grew out into shoots; nodes 3 and 4 gave 50 % with developing buds, while higher nodes gave very few. The experiment was repeated using plants 15 cm. high. As the cotyledons at this time were dead the lowest nodes were not used, but the results with higher nodes are shown in Table 2. The behaviour of the various isolated nodes closely resembles that of the nodes *in situ* on the intact plant. A marked gradient in growth potential is apparent, with a minimum at leaf 6 followed by a steady rise in the higher nodes. As seen in Text-fig. 1 the change-over from basal to anterior lateral growth occurs when the plant is about 8 cm. high, at which time some twenty mature leaves are present on the stem, and the nodes with higher growth potential are already formed. These observations elucidate the effects on apical dominance noted above, consequent upon removal of varying lengths of stem at different stages of development of the plant; removal of the apical portion after 10 cm. height has been reached removes buds of increasing growth potential, and the more of the stem that is removed the lower the growth potential at the remaining nodes.

The results obtained with flax were found to apply to *Pisum sativum*;

here the change-over from basal to anterior branching occurs at a plant height of 40–50 cm. at a time when 9–10 leaves have been produced. This may account for the 'distance effect' noted by Snow (1925) which led him to the paradoxical conclusion that apical dominance increases with distance from the apical bud, and that the closer the lateral buds are to the apex the less the inhibition. This was the basis of his hypothesis that passage of auxin through the stem tissues leads to a secondary reaction with production of an inhibitor which by virtue of the fact that it is not subject to polar transport can enter the lateral buds, whereas auxin cannot do so.

THE EFFECT OF NUTRIENT SUPPLY ON APICAL DOMINANCE

In the literature there are frequent references to the effect of 'vigour' of the plant on the degree of apical dominance, and this was confirmed with flax.

Table 3. *Interaction of nitrogen level and day length on maximum basal and anterior growth in decapitated flax plants*

	Basal growth (cm.)			Anterior growth (cm.)		
	Long days	Short days	Mean	Long days	Short days	Mean
High nitrogen level	23.0	12.4	17.7	12.5	7.6	10.1
Low nitrogen level	12.4	10.0	11.2	13.6	12.5	13.1
	17.7	11.2	—	13.1	10.1	—

The nutritive factors likely to be concerned are (1) nitrogen supply, (2) carbohydrate supply. The former was studied by growing plants in sand culture and irrigating with nutrient solution. Three levels were used providing a supply of nitrogen per forty plants of 0.09, 0.8 and 2.4 g. respectively spread over 6 weeks. Carbohydrate supply was varied by growing half the plants in a day length of 17 hr., the rest in 10 hr. days. In addition, half the plants in all treatments were decapitated at different heights, removing the stem apex and all leaves which had not at the time unfolded to a horizontal posture. The experiment was arranged as nearly as possible factorially, but the numbers of available plants in the height ranges varied from fifteen to thirty-nine with a mean number of twenty-two replicates.

To show the treatment effects on decapitated plants the maximum values of total growth of basal and anterior branches are entered in Table 3 for the extreme nitrogen levels. Both factors increase maximum basal growth to the same degree; there is no interaction and the factors thus act independently. Anterior growth is decreased by nitrogen level, but is increased

by day length. There is marked compensatory growth between basal and anterior branches with varying nitrogen supply, but day length affects both types of branches in the same sense, with a greater effect at high nitrogen level. The effects of the nitrogen level and day length on total lateral growth (basal and anterior) in relation to height at decapitation are shown in Table 4, and the ratio of total anterior/basal growth in Table 5. There is no

Table 4. *Total growth (cm. per plant) of laterals of flax (basal and anterior) and interactions of nitrogen level and day length*

Height at decapitation (cm.) ...	0-1	1-5	5-7.5	7.5-12	12-20	20-26
High nitrogen level:						
Long days	12.0	16.8	18.2	24.7	24.8	18.5
Short days	8.3	10.9	13.8	15.7	21.6	19.5
Low nitrogen level:						
Long days	9.8	11.2	14.9	24.9	7.5	9.7
Short days	6.5	8.7	11.6	14.7	6.2	13.5
Means	9.2	11.9	14.6	20.0	15.0	15.3
Interaction of nitrogen and day-length	18.5 18.1	25.5 22.1	29.8 28.7	39.4 40.6	31.0 29.1	32.0 28.4

Table 5. *Effects of factors (nitrogen level and day length) on ratio of anterior to basal lateral growth in flax*

Height at decapitation (cm.) ...	0-1	1-5	5-7.5	7.5-12	12-20	20-26
HN/LD	0	0	0.03	0.07	0.24	0.40
HN/SD	0	0.07	0.12	0.62	0.43	0.63
LN/LD	0	0.02	0.22	1.2	4.0	48
LN/SD	0	0.06	0.16	2.7	8.0	13

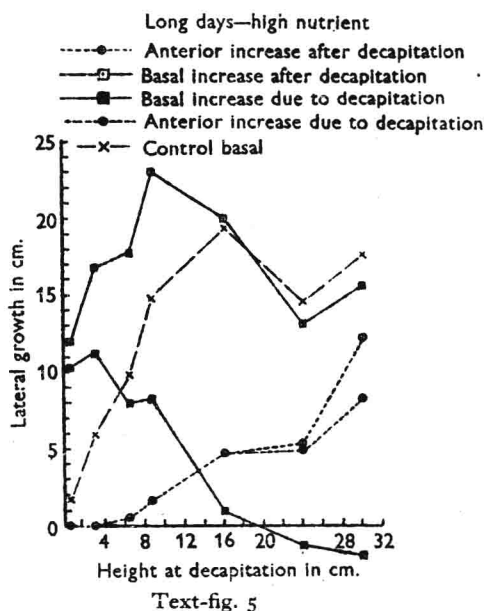
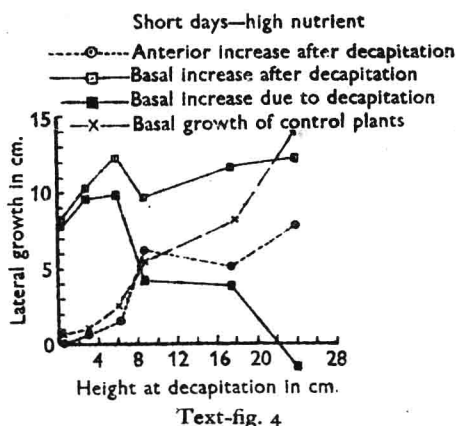
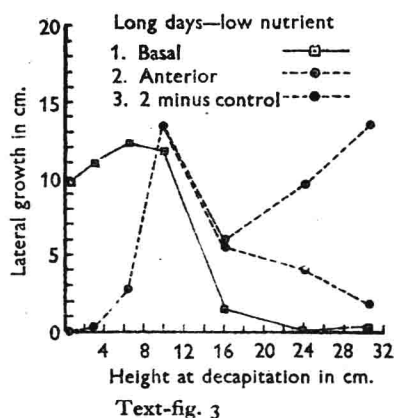
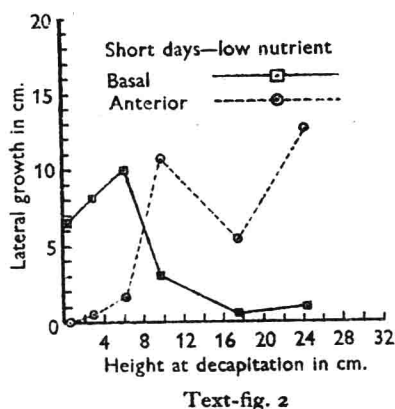
HN=high nitrogen level.
LN=low nitrogen level.

LD=long days.
SD=short days.

evidence of interaction on total lateral growth of nitrogen level and day length in any of the series. The balance of basal and anterior growth requires comment. Under conditions of high nitrogen anterior growth never overtakes basal growth (within the duration of the experiment), while under low nitrogen this occurs in plants decapitated at a height of about 10 cm. Flax is a long-day plant and onset of flowering is associated with anterior branching. With excessive nitrogen, however, as in the high-nitrogen series, flowering fails even in long days. In the low-nitrogen series only those in long days flowered, and this is associated with a preponderance of anterior growth.

The interaction of the factors is shown graphically in Text-figs. 2-5. In the low-nitrogen/short-day set (Text-fig. 2) the intact controls showed

no lateral growth, and the data entered therefore represent the direct result of decapitation. Under conditions of nitrogen starvation, apical dominance in intact plants is thus complete, which is not the case in



Text-figs. 2-5. Effects of day length and nutrient supply on the lateral growth responses of flax plants decapitated at various stages.

plants grown in soil at higher nitrogen level (cf. Text-fig. 1). In the high-nitrogen series (Text-figs. 4, 5) the intact controls showed varying amounts of lateral growth, which for the basals is indicated in the diagrams. Basal growth in the high-nitrogen series increased with delay in decapitation.

In both cases this is associated with a slow rise in anterior growth; quite evidently competition between the lateral buds is the main factor.

The net effect of decapitation on basal growth in the high-nitrogen series is shown by the solid squares in Text-figs. 4 and 5 and hollow squares in Text-figs. 2 and 3. The striking result appears that in spite of large differences in total basal growth the *net effect of decapitation* is always the same, reaching a similar maximum in plants which were decapitated at a height around 6 cm. and falling to zero in plants decapitated around 20 cm. The basal branches once established are not affected by decapitation, but in turn actively compete with the anterior branches. At low nitrogen level, plants which at the time of decapitation have not branched after early decapitation produce mainly basal branches, but when decapitated after reaching a height of approximately 10 cm. produce anterior branches which compete with and finally prevent the growth of the basal buds. The uniform effect of decapitation on basal growth is regarded as a competitive effect for nutrients between the terminal bud and the lateral buds.

EXPERIMENTS WITH APPLIED AUXIN

Since it was demonstrated by Laibach (1933) with extracts of orchid pollinia and by Thimann & Skoog (1933, 1934) with extracts of *Rhizopus* and with pure IAA, the fact that auxins can replace the stem apex in maintaining apical dominance has been well established. In previous work, however, the role played by nutritive factors has not been systematically investigated. Experiments were therefore carried out to determine the effects of nitrogen level and carbohydrate supply on the inhibition of lateral growth in decapitated plants by the application of IAA. The auxin was applied as lanolin paste. This was found to be stable at summer temperatures, and applications were renewed every 3 days after removing a thin slice of tissue below the old paste. Nitrogen supply was varied by irrigating sand cultures with culture solutions to give three levels as stated above. Carbohydrate level in the plants was varied by differential shading, using gauze of different gauge and, when necessary, butter muslin in addition.

(i) *Effect of removing cotyledonary tissue from seedling plants of flax on the degree of inhibition of basal growth by IAA*

Seedlings of flax (var. Liral Prince) were decapitated to six leaves, treated with 10^{-1} M auxin paste, and varying amounts of cotyledonary tissues removed: viz. both cotyledons, one cotyledon, half of each cotyledon, and intact plants as controls. The mean values of basal growth for approximately 100 plants in each treatment after 3 weeks' growth are shown in Table 6.