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Abstract

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Formula on page 30 corrected.

Full paper (scanned from the original) follows.

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ABSTRACT

Phaseolus vulgaris L. decapitated at the third internode showed accelerated growth of the uppermost axillary bud remaining on the stem (the first trifoliate axillary bud) after a lag period of 3–5 h. Much of the initial growth increment could be attributed to cell expansion.

Key words: *Phaseolus vulgaris* L., dwarf bean, correlative inhibition, cell expansion.

INTRODUCTION

Correlative inhibition of lateral bud development is most effectively released by removal of the apical shoot. The time lag between shoot decapitation and growth response in the bud could give some indication of the mechanisms governing the maintenance and release of correlative inhibition but considerable variation has been reported. Such differences are perhaps to be expected since they reflect not only the variation between species, but also the experimental approach used in the different investigations. Studies on the rapid growth response in lateral buds have relied either on direct measurement of the bud or on the detection of an increase in mitotic division in the bud. Using the former approach, the outgrowth of the lateral bud in *Pisum* was observed 6–10 h (Wardlaw and Mortimer, 1970) or 8–12 h (Nagao and Rubinstein, 1976) after decapitation of the shoot. By extrapolation of the data of Wardlaw and Mortimer (1970), it has been shown that onset of bud outgrowth could have taken place as early as 4 h following decapitation. Work by Hall and Hillman (1975) on *Phaseolus* showed an even shorter time lag from decapitation; using a photographic method, bud outgrowth was detected within 30 min of decapitation. Since there is obviously a lower limit to the measurable increment in growth, the time of the earliest detectable growth is likely to be influenced by the size and extent of development of the inhibited bud. McIntyre (1977) proposed that the very rapid increase in bud length following decapitation of the apex was due primarily to cell extension resulting from an increase in water potential in the buds.

Mitotic division in the zone of quiescent cells at the tip of the lateral bud in *Tradescantia* did not occur until 2 days after decapitation (Naylor, 1958). In *Glycine*, an increase in cell division was detected from about the 25th hour following decapitation of the shoot (Ali and Fletcher, 1970), or excision of the bud from the plant (Peterson and Fletcher, 1974). Cell division in the lateral bud is stimulated even earlier in *Pisum*, and increased mitoses were noted within 12 h of decapitation, shortly after an increase in lateral bud length was discerned (Nagao and Rubinstein, 1976).

We report here the early growth response in the first trifoliate axillary bud of *Phaseolus vulgaris* L. following decapitation of the shoot. The time lag between the shoot decapitation stimulus and the onset of bud internode extension was first determined.

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Estimates were then made of the extent by which cell extension, both during the initial growth phase and subsequently in the developed shoot, could account for the elongation of the bud internode.

MATERIALS AND METHODS

Plants of the dwarf French bean, *Phaseolus vulgaris* L. cv. Canadian Wonder, were grown in 75 mm pots in the greenhouse and transferred into a growth room 1 day before the start of each experiment. The growth room was continuously illuminated by fluorescent tubes (*c.* 12 W m^{-2}) and the temperature was maintained at $298 \pm 1{\cdot}5$ K ($25 \pm 1{\cdot}5$ °C). Humidity was not precisely controlled, but relative humidity generally fell within the range of 70–80 per cent. The plants were placed in a growth cabinet within the growth room. This had an open front and transparent top and side walls and reduced the cyclic fluctations in temperature (by *c.* 75 per cent) and relative humidity (by *c.* 40 per cent) that were due to thermostat switching.

Rapid axillary bud growth in decapitated plants

A photographic method, basically similar to that described by Hall and Hillman (1975), was used to determine the time lag between shoot decapitation and onset of an increase in growth rate of the first trifoliate leaf axillary bud. Two plants were used in each experiment: one which was decapitated through the third internode and the other serving as a control. The plants were secured to supporting stakes to ensure that no movement of the stem, and hence of the axillary buds, occurred during the course of the experiment. Two single-lens reflex cameras were used, each assigned to the control or decapitated plant. The cameras were fitted with extension bellows or extension rings and were focused on the internode of the first trifoliate leaf axillary bud.

Nine experiments were carried out: five experiments using plants with the second trifoliate leaf entering its grand phase of growth and four experiments involving plants with the third trifoliate beginning its grand phase of growth. Short lengths (*c.* 4 mm) of finely drawn glass were inserted into the proximal and distal ends of the internode of the axillary bud 1 day before the experiment, to act as markers. Photographs of the axillary bud were taken at 20 min intervals for 2·7 h prior to decapitation of the shoot and at 30 min intervals for 10 h thereafter. Exposures of 1 s were made in available light. Measurements were made from the images of the buds magnified (× 16) using a photographic enlarger. All measurements were made at least twice, and generally, measurements consistent to about 0·3 mm in the enlarged image were achieved.

Growth rates at selected time intervals were compared in control plants and in decapitated plants using the Wilcoxon match-pairs signed-ranks test (Wilcoxon, 1945).

Cell extension in the bud internode

Plants with the second trifoliate leaves expanding were selected in batches of three. The first trifoliate leaf axillary bud in the three plants were screened for similarity in the length of the bud internode, and in the general size and shape of the bud. Two plants were decapitated and the third left intact as a control. After 23–26 h, longitudinal hand sections were made of the internode of the first trifoliate leaf axillary bud from the control plant and from one of the two decapitated plants. The sections were mounted in water and the length of the internode was measured under the microscope using a graduated eye-piece. The average length of the pith cells in the bud internode from the sub-apical portion of the internode to its mid-point was estimated by measuring files of cells under the microscope. Cells from this region were selected as they were generally uniform in

shape and size. After 6 days, the internode of the then developed axillary shoot in the second decapitated plant was measured and the length of pith cells at the midpoint of the internode or at the proximal end of the internode, 3–6 mm from the axil, were measured under the microscope as before. Growth extension of the bud internode and pith cells was represented by the differences in these measurements between control and decapitated plants. The proportionate increase in cell length and the proportionate increase in internode length after decapitation are given by $\Delta C/C_i$ and $\Delta L/L_i$ respectively, where:

ΔC = Difference in cell length in the bud internode of the intact plant and of the decapitated plant
C_i = Cell length in the bud internode of the intact plant
ΔL = Difference in the bud internode length of the intact plant and of the decapitated plant
L_i = Bud internode length of the intact plant

RESULTS

Timing of the rapid growth

While active growth of the first trifoliate axillary bud was inhibited by the apical shoot in intact *Phaseolus*, the buds in most plants were found to maintain very slow but measurable growth. The basal growth rate (estimated from the gradient of the linear regression of bud internode lengths over time) was found to be variable between

TABLE 1. *Estimated growth rates ($\mu m\ h^{-1}$) of bud internodes at various time intervals in control and decapitated plants*

	Control				Decapitated		
Experiment	−2·7 to 10 h	−2·7 to 0 h	1 to 3 h	5 to 7 h	−2·7 to 0 h	1 to 3 h	5 to 7 h
1	7	11	8	−2	−3	−20	10
2	0	−7	−21	30	0	0	11
3	2	−1	0	8	8	0	0
4	−2	−22	20	0	−2	0	4
5	1	4	−6	−2	3	−2	24
6	1	−9	−4	6	3	4	32
7	5	2	16	−2	−10	2	−4
8	32	31	26	3	20	16	64
9	1	0	8	6	9	16	24

Growth rates were approximated from the gradient of the linear regression of a mean of 27, 8, 5 and 5 internode measurements over time for the time intervals −2·7 to 10 h, −2·7 to 0 h, 1–3 and 5–7 h respectively. Decapitation was carried out at 0 h.

individual plants. Pre-decapitation recording was therefore necessary to differentiate the inherent basal growth rate from the increased growth activity resulting from shoot decapitation.

The basal growth rate of the bud internode in intact control plants estimated over 12·7 h ranged from −2 to 32 $\mu m\ h^{-1}$ (Table 1). In six out of nine plants, growth rate did not exceed 2 μm^{-1} and in eight out of nine plants, growth rate did not exceed 7 $\mu m\ h^{-1}$. In the ninth plant (Expt 8, Table 1), growth was exceptionally vigorous, attaining 32 $\mu m\ h^{-1}$. In one plant, an apparent negative growth rate was obtained, but this was

due mainly to a transient but marked decreasing rate in the first 3 h of measurement (Expt 4, Table 1). Generally, therefore, the basal growth rate of the internode of the first trifoliate leaf axillary bud was of the order of 1–5 μm h^{-1}. While the bud internodes were found to increase in length when observed over a period of 12·7 h, both positive and negative growth rates were encountered when estimated over shorter intervals of 2·7 h or 3 h (Table 1). These fluctuations might be due to nutational movement of the bud, although measurement errors could have been partly responsible.

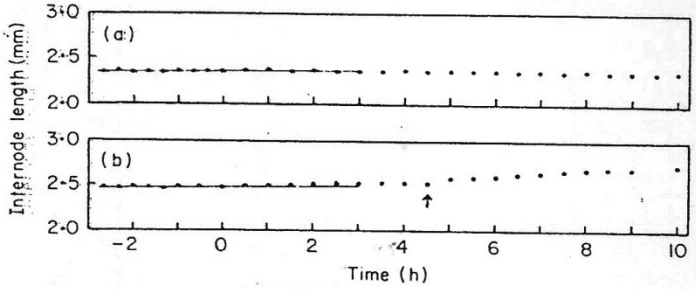

FIG. 1. Growth of the internode of the first trifoliate axillary bud in (a) the control intact plant and (b) the decapitated plant. Decapitation was carried out at 0 h. The straight line superimposed on each curve is the linear regression of the pre-decapitation measurements over time extrapolated to 3 h after decapitation. The arrow indicates the time at which a measurable growth response to decapitation is discernible. Results presented are from Expt 5 (see Table 1).

TABLE 2. *Extension of pith cells in the internode of the first trifoliate axillary bud and its contribution to the increase in the internode length*

	Day 1		Day 6	
	Intact	Decapitated	Decapitated	
Internode length (mm)	2·33 ± 0·07	2·95 ± 0·10	35·02 ± 2·24	
			base*	middle*
Cell length (μm)	15·4 ± 0·5	18·7 ± 0·6	81·8 ± 3·5	118·0 ± 6·9
	(126)	(128)	(108)	(87)
Increase due to cell extension as % of the total increase in internode length	—	105·0	37·4	51·2

* Measurements of cells from the base of the internode (3–6 mm from the axil) or from the middle of the internode.

The measurements are the means ± standard errors calculated from 32 buds per treatment. Figures in brackets denote the average number of cells measured for each bud. The percentage increase in internode length due to cell extension is the mean of values calculated individually for each pair of control and decapitated plants.

A first estimate of the time lag between decapitation of the shoot and response in the bud was made by inspection of the growth curves (Fig. 1). A sustained increase over basal growth rate was taken as an actual growth response to decapitation.

Of nine decapitated plants, a growth response to decapitation was distinct in six plants and ambiguous in a seventh, the time lag between decapitation and response varying between 3 and 5 h. In two plants, no response to decapitation was evident in the 10 h following removal of the apical shoot. In the nine control plants which were left intact, none showed any change in growth rate of internode length. There was no evidence of

any consistent difference in the time lag from decapitation to axillary bud growth response between plants of the two physiological age groups (plants with an expanding second trifoliate leaf and with an expanding third trifoliate leaf).

Having determined tentatively by inspection of the growth curves that visible growth response to shoot decapitation occurred after 3–5 h, confirmation was derived by statistical analysis. Estimates of growth rates in bud internodes of control and decapitated

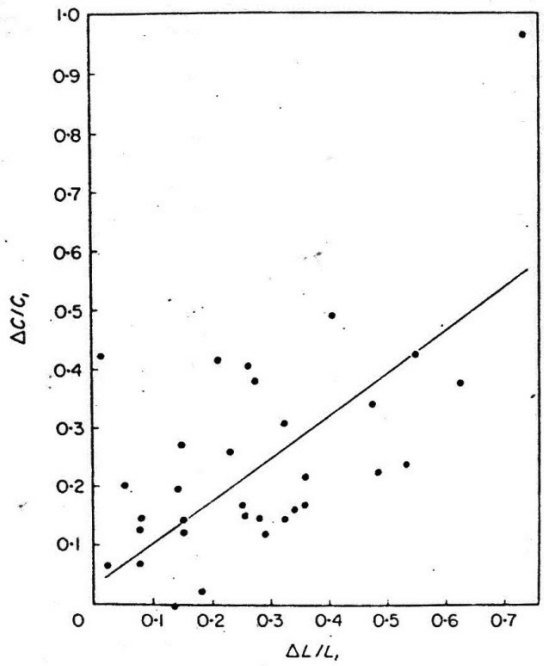

FIG. 2. Relationship between $\Delta L/L_i$ and $\Delta C/C_i$ in the internode of the first trifoliate axillary bud one day after decapitation. ΔL, L_i, ΔC and C_i are defined in the text. $y = 0{\cdot}73x + 0{\cdot}03$; $r = 0{\cdot}704$; $n = 32$.

plants were made for the time intervals −2·7 to 0 h, 1–3 h and 5–7 h where 0 h was the time of decapitation (Table 1). The growth rates between each time interval were compared in control plants and in decapitated plants. No significant difference in decapitated plants was found when the growth rates between −2·7 to 0 h and 1–3 h were compared. This confirms the finding from visual assessment of the growth curves that detectable growth response did not occur within 1 h from decapitation. The growth rate at 5–7 h, however, was significantly higher than that at −2·7 to 0 h or at 1–3 h ($P < 0{\cdot}01$). This is in agreement with the finding from inspection that an increase in axillary bud internode growth occurred between 3 and 5 h following decapitation. No significant difference was found when growth rates at the three time intervals were compared in control plants, again confirming that growth rate in the bud internodes of control plants did not change significantly.

Cell extension during the initial growth

The internode length of the axillary bud and the length of the pith cells in the internode measured 1 day after decapitation in the intact plant and in one of the two decapitated

plants are given in Table 2. Similar measurements taken in the axillary shoot (i.e. the developed axillary bud) of the second decapitated plant after 6 days are also presented in the table. When $\Delta C/C_i$ was plotted against $\Delta L/L_i$ for values of ΔC and ΔL obtained one day after decapitation, a significant positive correlation was evident ($r = 0{\cdot}704$***, Fig. 2). Similar linear regressions were carried between $\Delta C/C_i$ and $\Delta L/L_i$ for values of ΔC and ΔL obtained 6 days after decapitation, by which time, growth of the axillary shoot was complete or near completion. When the values of ΔC were based on measurements of cells at the base of the internode no significant correlation between $\Delta C/C_i$ and $\Delta L/L_i$ was found ($r = -0{\cdot}145$ NS, Fig. 3a). These two variables were found

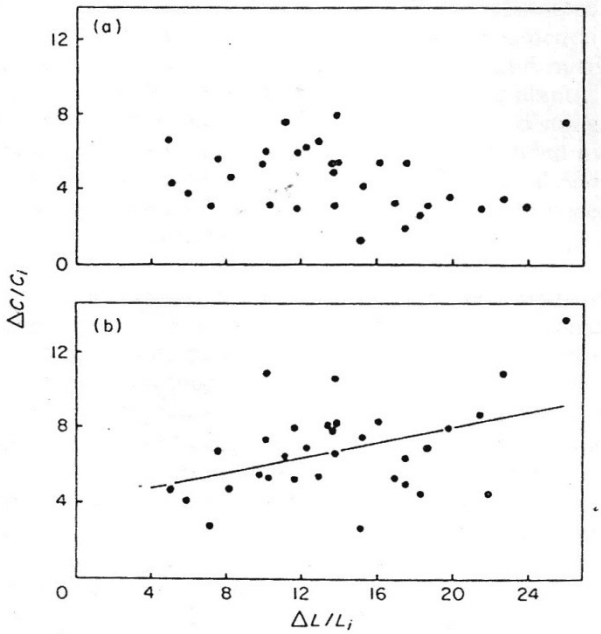

FIG. 3. Relationship between $\Delta L/L_i$ and $\Delta C/C_i$ in the internode of the first trifoliate axillary shoot 6 days after decapitation. Values of ΔC were calculated from lengths of cells located at (a) the proximal end and (b) the middle of the shoot internode. ΔL, L_i, ΔC and C_i are defined in the text. (*a*) $r = -0{\cdot}145$ NS; $n = 32$; (*b*) $y = 0{\cdot}20x + 3{\cdot}94$; $r = 0{\cdot}423$; $n = 32$.

to be related when ΔC was calculated from the lengths of cells in the middle of the internode [$r = 0{\cdot}432$*, Fig. 3(b)]. There was, however, a substantial loss in the extent of correlation when the values of ΔC and ΔL were taken after 6 days. The variation in $\Delta C/C_i$ could account for 50 per cent of the variation in $\Delta L/L_i$ 1 day after decapitation, but could explain only 19 per cent of the variation 6 days later.

Having established that cell extension contributed significantly to the early stages of elongation of the axillary bud internode, estimates were then made of the increase in internode length that was due to cell extension. This is given by $\Delta C/C_i \times L_i$, and is expressed in Table 2 as percentage of the total increase in internode length: ~~$\Delta C/C_i \times \Delta L/L_i \times 100$~~. A mean close to 100 per cent was obtained for measurements made 1 day after decapitation. As the axillary bud developed further, the internode elongation due to cell expansion dropped to about 50 per cent after 6 days.

$$\frac{\Delta C}{C_i} \times \frac{L_i}{\Delta L} \times 100$$

DISCUSSION

In the first trifoliate axillary bud, the unexpanded leaf portion of the bud is held at an angle to the internode, making measurement of the length of the entire bud difficult. Nutation movement in the unexpanded leaf in the bud was suspected and this would have further complicated precise measurement. It was decided, therefore, to monitor the extension growth of the bud internode alone.

The detection of a slow growth in the first trifoliate axillary bud is comparable to a similar observation in the primary leaf axillary bud of *Phaseolus* (Hall and Hillman, 1975). Although the basal growth rate of the axillary bud internode was estimated as being in the order of 1–5 μm h^{-1}, these values might vary according to environmental conditions. McIntyre (1973) has shown that with favourable light, humidity and nutrient supply, spontaneous growth of axillary buds can occur in intact *Phaseolus* plants.

A small amount of internode extension above the insertion of the distal glass marker took place in some decapitated plants when observations were extended over a period of 3–4 days. This suggests that the active growth at the extreme distal end of the bud internode might, in some cases, be excluded by measuring the distance between markers. Some of the growth rates of bud internodes calculated in this study are therefore likely to be underestimated.

Although basal growth rates vary considerably between axillary buds of individual plants, the time lag between shoot decapitation and bud growth response was fairly consistent, being about 3–5 h. As this period represents the interval between stimulus and measurable response, the actual time lag is probably even shorter. Working with *Phaseolus vulgaris* and using a basically similar method of measurement, Hall and Hillman (1975) observed axillary bud outgrowth within 30 min of decapitation. The discrepancy between their results and those in the present study might be due partly to the fact that Hall and Hillman studied a different axillary bud (that of the primary leaf) and that the entire bud length was measured, whereas growth in the internode was investigated in the present study. Growth increment in the entire bud in *Phaseolus vulgaris* would effectively include both the growth of the bud internode as well as the unexpanded trifoliate leaf in the bud. In *Pisum sativum*, Nagao and Rubinstein (1976) found the onset of active growth of the leaf in the axillary bud to precede that of the bud internode by 4 h following decapitation, the time lag for the two events being about 8 and 12 h respectively. Decapitation of *Vicia faba* (*Faba vulgaris*) led to leaf growth in the axillary bud after about 4 h. In the bud internode, however, only limited growth occurred during the first 8 h; thereafter, active growth was observed (Couot-Gastelier, 1978). The detection of growth in the entire bud of *Phaseolus* (Hall and Hillman, 1975) approximately 4 h before growth in the bud internode alone (as determined in the present study) is therefore compatible with findings using the two other leguminous plants, *Pisum* and *Vicia*.

In view of the very rapid growth response of the axillary bud to decapitation, the possibility of the initial growth being due mainly to cell extension, as suggested by McIntyre (1977), was investigated. The measurements of cell extension are consistent with this proposition and the increase in cell length could account, to a large extent, for bud internode elongation 1 day after decapitation of the shoot. As the bud developed further, the increase in bud internode length could no longer be adequately explained by the increase in cell length alone, and other factors (e.g. cell division) were apparently involved.

Much of the deviation of values from the linear regressions between $\Delta C/C_i$ and $\Delta L/L_i$ (Figs 2, 3) arose from two basic assumptions made in the statistical analyses of the data. In the first instance, the buds of the intact plant and decapitated plant were assumed

to be identical (with respect to the growth behaviour of the bud internodes) in determining ΔC and ΔL. Secondly, cells from a selected region of the bud internode only were measured. In attempting the correlations between $\Delta C/C_i$ and $\Delta L/L_i$, it was assumed that the remaining cells of the internode underwent extension growth by a proportion similar to that for the cells measured. Estimates of the increase in bud internode length that was due to cell extension are also liable to errors arising from the latter assumption.

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