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Development of Chrysanthemum Cuttings: The Influence of Age and Position of the Axillary Buds

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In three experiments (two *in-vivo*, one *in-vitro*), an attempt was made to separate the possible effects of age and position of axillary buds of chrysanthemum on bud outgrowth and the subsequent quality of cuttings.

In the *in-vivo* experiments, bud age and bud position were not significant factors in bud outgrowth and subsequent quality of cuttings. Nevertheless, most outgrowth parameters showed slightly higher values for the lower positioned buds and the time needed to produce a cutting tended to decrease with the age of the axillary bud.

In the *in-vitro* experiment, the relationship between age and the various parameters showed an optimum.

Key words: Age, axillary bud, *Chrysanthemum morifolium*, cutting, *Dendranthema grandiflora*, chrysanthemum, position.

INTRODUCTION

Chrysanthemum cuttings are usually harvested from stock-plants. The cuttings originate from an axillary bud situated in the axil of a leaf. Axillary buds are able to develop into shoots after release from inhibition by some factor emanating from the growing shoot tip. When these developing shoots have reached a certain length, the top (the 'cutting') is taken. The axillary buds on the remaining part of these shoots are able to sprout to give the next generation of cuttings.

In the commercial production of chrysanthemum cuttings, homogeneity of cuttings is a requisite since uniform, well grown cuttings offer uniformity and predictability in harvesting and flowering. This requirement of homogeneity is not always satisfied, probably because axillary buds differ in age and in position along a shoot. These factors are linked, and their relative importance for bud outgrowth and subsequent quality of cuttings is not easy to determine. In an experiment where chrysanthemum shoots were pruned above the basal four or eight nodes, the apical axillary bud produced the highest number of cuttings (total of all generations) and the basal produced the lowest (Heins and Wilkins, 1979). Similarly, in *Nicotiana tabacum* it was shown that the number of nodes produced by an axillary bud was a function of its position on the stem (McDaniel and Hsu, 1976). In an *in-vitro* culture study, explants of *Vitis rotundifolia*, originating from the ten basal nodes of a shoot with at least 25 nodes, gave better shoot proliferation than those originating from the ten distal nodes (Sudarsono and Goldy, 1991). Cuttings from *Schefflera arboricola* from subapical positions rooted more slowly and produced fewer roots with a lower rooting percentage than cuttings from the more basal regions (Hansen, 1986). Comparison of the structure of axillary buds along a rose shoot showed several anatomical and morphological differences (Zamski, Oshri and Zieslin, 1985). Cockshull and Horridge (1977) suggested that the bud-inhibition gradient along a shoot originates from differences in the anatomical structure laid down during the early stages of bud development.

In the present study of two *in-vivo* and in one *in-vitro* experiments, an attempt was made to separate the factors of age and position and to study their possible influences upon the outgrowth of an axillary bud and the subsequent quality of cuttings. The following quality parameters of the cuttings were considered: diameter, number of leaves, total leaf area and fresh- and dry-weight.

MATERIALS AND METHODS

Experiment 1

This experiment was designed to assess the influences of position and age on bud outgrowth. Axillary buds on an intact shoot (Fig. 1C) were, therefore, compared with isolated buds (Fig. 1B).

At the end of Sep. 1993, cuttings (5 cm in length) of *Chrysanthemum morifolium* Ramat (*Dendranthema grandiflora* Tzvelev.) cv. Cassa (Fides, De Lier) were rooted in a mixture of sand and peat (1:1 by volume). Each cutting was pinched either at leaf five (6 Oct., expt 1.1) or at leaf eight (11 Oct., expt 1.2) counting from the soil surface, when there was 5 cm of shoot above buds 5 or 8, respectively (Fig. 1A) and excised at the soil surface.

The first batch of excised cuttings was kept intact and put into the rooting medium in a rooting tray covered by a glass lid (Fig. 1C) such that buds 1–5 in the five-bud plants and buds 4–8 in the eight bud plants were of the same age, but were not in the same position, whereas in each treatment

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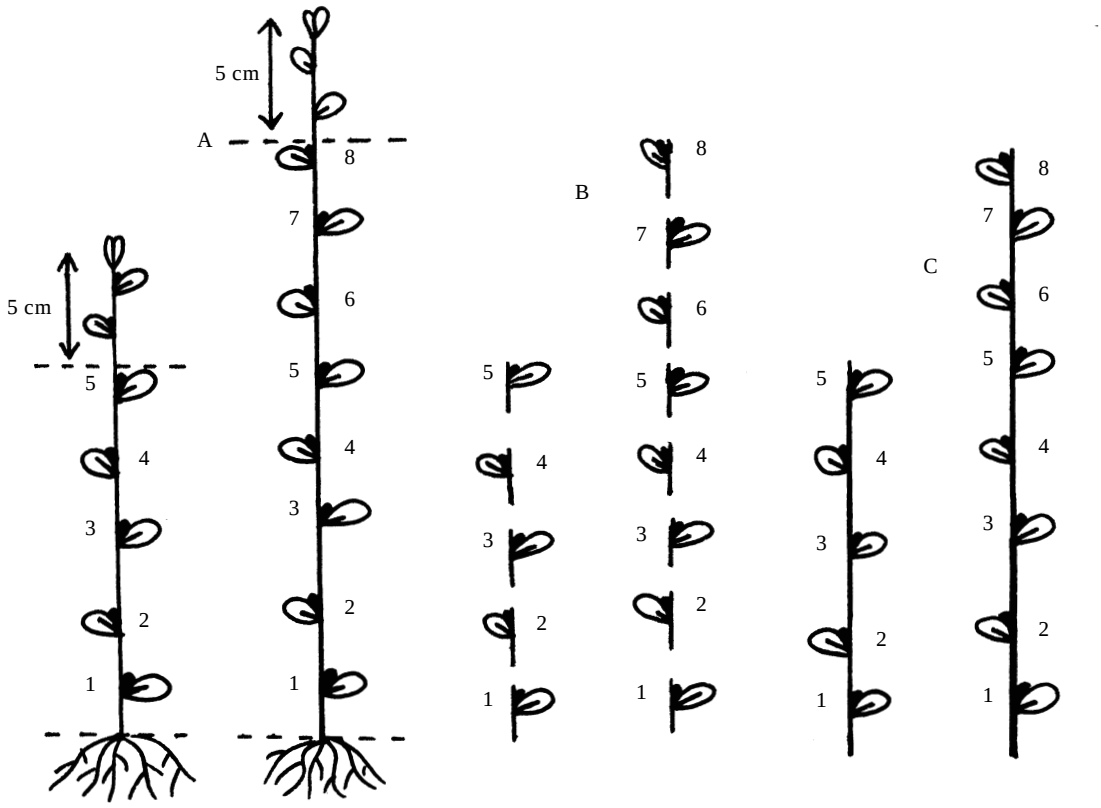

FIG. 1. Schematic representation of the plant material used in expt 1 (see text for details).

buds 1–5 were in the same position but differed in age (Fig. 1). The second batch of pinched cuttings (Fig. 1 B), with five or eight internodes (including one bud and attached leaf), were severed at a few millimetres above the axillary buds and each separate segment was put into the rooting medium in a rooting tray covered by a glass lid (Fig. 1 B).

The rooting trays were kept closed for 2 weeks. Four weeks after commencing the experiment, the following parameters of the axillary sprouting buds or shoots were recorded: length, diameter, number of leaves (over 0·5 cm in length), total leaf area and fresh- and dry-weight.

The experiments were carried out under natural day lengths in the greenhouse. In the first 2 weeks, average day/night temperatures were approx. 24/22 °C respectively, relative humidity approx. 100 % and mean irradiance 160 J cm^{-2} d^{-1}. In the last 2 weeks, average day/night temperatures were approx. 23/21 °C respectively, relative humidity approx. 70 % and mean irradiance 780 J cm^{-2} d^{-1}. If the total global irradiance outside the rooting tray fell below 30 W m^{-2} (between 0600 and 1200 h), additional irradiance of 70 W m^{-2} (PAR, SON-T 400W, Philips) at plant level was switched on, but above 50 W m^{-2} the lamps were switched off. In both experiments there were four replicated groups of four plants in each treatment, positioned at random.

Experiment 2

In this experiment bud position was the same as in expt 1, but bud age varied.

In Jul. 1991, chrysanthemum cuttings were rooted as in expt 1 and after 14 d planted into plastic pots (14 cm diameter) containing a mixture of peat, river clay, Swedish peat moss and peat dust (40:15:20:24 by volume) (Lentse potgrond, No. 4, Coöp Tuinbouwcentrum Lent). The cuttings were divided according to length into three groups of approx. 12, 10 and 8 cm, respectively. When the distance between the growing point and the sixth axillary bud (counting from the soil surface) was 1, 3, 5, 7 and 9 cm, four groups of four plants each were chosen randomly from each length group and topped just above the sixth bud (Fig. 2). In this way the sixth axillary bud differed in age at the moment it was allowed to sprout. Throughout the experiment, the temperature was 21 °C, relative humidity approx. 70 % and irradiance (fluorescent tubes, Philips TLD 84HF, 50W) approximately 30 W m^{-2} (PAR); day-length was 16 h. The plants were supplied once every 2 weeks with a nutrient solution containing either N, P and K (18:18:18) or N, P, K and Mg (15:3:15:5).

Measurements were taken when there was 5 cm stem (the cutting) above four leaves. Parameters measured were: diameter, number of leaves (over 0·5 cm in length), total leaf area and fresh- and dry-weight.

Experiment 3

In this experiment bud age was also varied but, unlike expt 2, bud outgrowth occurred *in-vitro*, isolated from the rest of the plant.

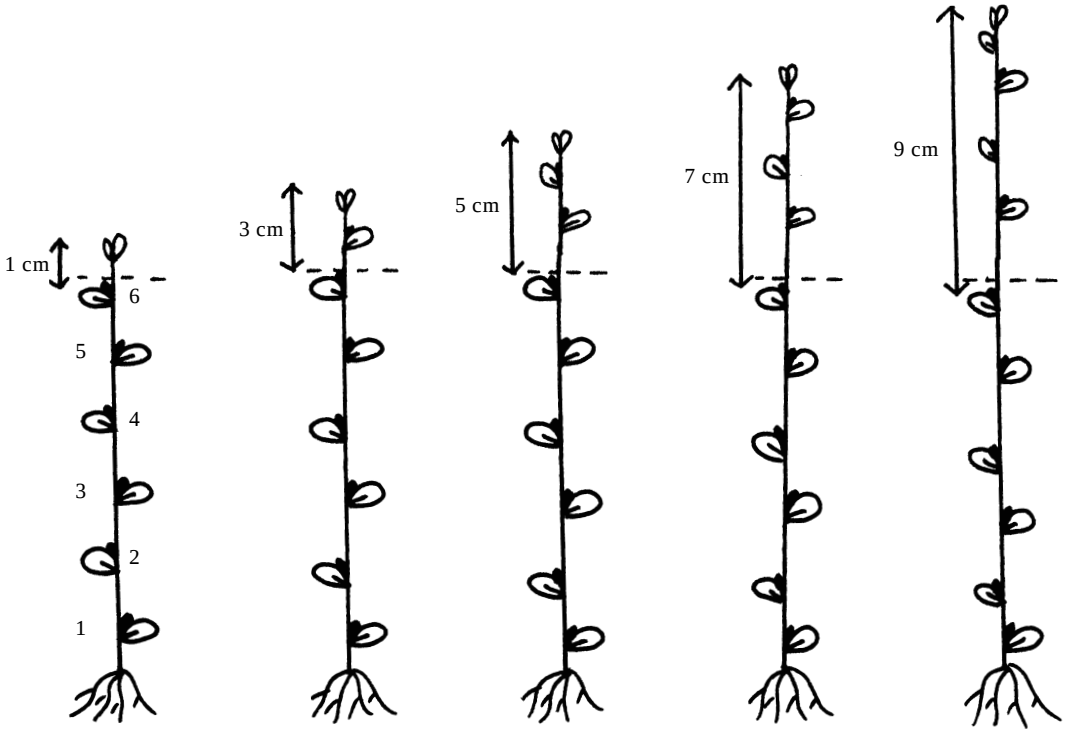

FIG. 2. Schematic representation of the plant material used in expt 2 (see text for details).

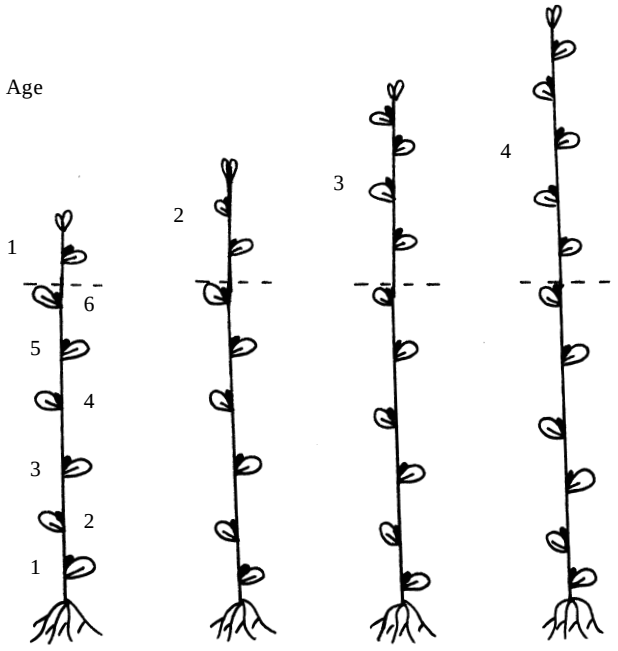

FIG. 3. Schematic representation of the plant material used in expt 3 (see text for details).

At the end of Jul. and at the beginning of Sep., cuttings were rooted as in expt 1 and 14 d later planted into plastic pots as in expt 2. Thereafter the plants were grown in a controlled-environment room at a constant temperature of 18 °C, a relative humidity of approx. 70% and an irradiance of 30 W m^{-2} (PAR) provided by fluorescent tubes (Philips TLD50W/84HF). Daylength was 16 h. The plants were supplied with mineral nutrients as in expt 2. Starting when the distance between the growing point and the sixth axillary bud (counting from the soil surface) was about 4 cm, in four successive weeks (Age 1 to 4, Fig. 3), the sixth axillary bud of 16 plants in four randomly chosen groups of four plants each was taken. In this way bud age increased with time of sampling. The buds were sterilized in 70% alcohol for a few seconds, followed by 15 min in 1% NaOCl to which a few drops of Tween 20 had been added. Explants were then washed three times with sterile water. The axillary buds were inoculated individually in Pyrex glass tubes (20 mm diameter) containing 10 ml of culture medium, and after inoculation covered with a cotton plug and Vitafilm. The axillary buds were grown on a basic culture medium of macrosalts and microsalts at full strength according to Murashige and Skoog (1962) to which NaFeEDTA 37·5 mg l^{-1}, 4% saccharose and 0·7% Daichin agar in distilled water was added. The pH of the medium was adjusted to 6·0 (before addition of agar). The tubes with the axillary buds were incubated in a growth chamber at 23 °C at an irradiance of 6 W m^{-2} (PAR) given by fluorescent tubes (Philips TLD36W/84). The daylength was 14 h. Four weeks after the start of bud culture, the following measurements were made: shoot length, diameter, number of leaves (visible to the naked eye) and fresh- and dry-weight.

In all experiments results were statistically analysed by analysis of variance followed by mean separation according to Tukey's HSD-test.

RESULTS

Experiment 1

Although some difference in outgrowth could be observed when axillary buds were separated, these were mostly slight compared to those occurring when axillary buds grew out on the shoot.

The length and diameter of the shoots growing out from five or eight isolated axillary buds showed no clear pattern.

TABLE 1. *Effect of position and age of axillary buds* (*isolated or* in situ) *on bud outgrowth after 4 weeks* (*expt 1; 5-bud plants*)

Position Age	Shoot		Leaf		Weight	
	Length (cm)	Diameter (mm)	Number	Area (cm^2)	Fresh (g)	Dry (g)
Isolated						
5	11·3	2·49	7·71	55·02	2·73	0·25
4	11·8	2·51	8·29	60·21	3·05	0·25
3	11·3	2·50	8·57	56·58	2·86	0·26
2	12·0	2·63	9·19	61·04	3·16	0·29
1	12·2	2·37	9·06	61·08	3·12	0·26
l.s.d. ($P = 0·05$)	0·6	0·32	0·68	2·43	0·58	0·02
In situ						
5	16·4	2·63	8·44	62·85	3·44	0·34
4	13·4	2·58	7·80	56·66	2·74	0·26
3	6·1	2·27	5·94	27·01	1·24	0·11
l.s.d. ($P = 0·05$)	1·1	0·40	0·93	3·20	0·62	0·03

TABLE 2. *Effect of position and age of axillary buds* (*isolated or* in situ) *on bud outgrowth after 4 weeks* (*expt 1; 8-bud plants*)

Position Age	Shoot		Leaf		Weight	
	Length (cm)	Diameter (mm)	Number	Area (cm^2)	Fresh (g)	Dry (g)
Isolated						
8	11·2	2·61	7·06	49·88	2·49	0·21
7	11·5	2·40	7·25	51·39	2·49	0·22
6	11·1	2·35	7·25	51·39	2·48	0·21
5	10·5	2·38	7·38	46·72	2·26	0·20
4	10·7	2·33	7·75	51·41	2·44	0·21
3	10·6	2·59	8·06	56·03	2·69	0·23
2	11·6	2·36	8·86	63·27	3·07	0·24
1	11·0	2·34	8·94	58·84	2·85	0·24
l.s.d. ($P = 0·05$)	0·9	0·34	0·52	2·62	0·44	0·02
In situ						
8	14·3	2·55	7·93	55·40	3·07	0·31
7	11·9	2·58	6·73	48·79	2·51	0·22
6	7·4	2·35	5·40	30·47	1·45	0·10
l.s.d. ($P = 0·05$)	1·2	0·32	0·56	2·70	0·48	0·04

TABLE 3. *Effect of length of cut-off shoot above the sixth axillary bud* (*age of the sixth axillary bud*) *on the outgrowing 5 cm cutting* (*expt 2*)

	Length (cm) of cut-off shoot					l.s.d. ($P = 0·05$)
	1	3	5	7	9	
Length at start: 12 cm						
Diameter (mm)	3·3	3·1	3·5	3·3	3·4	0·3
Number of leaves	5·4	5·4	5·7	5·4	5·4	0·4
Leaf area (cm^2)	45·4	41·0	50·8	43·3	44·5	7·6
Fresh weight (g)	1·7	1·5	1·9	1·7	1·8	0·3
Dry weight (g)	0·20	0·19	0·22	0·18	0·21	0·03
Days	24	25	22	21	21	2
Length at start: 10 cm						
Diameter (mm)	3·2	3·2	3·1	3·3	3·0	0·2
Number of leaves	5·6	5·6	5·4	5·4	5·1	0·5
Leaf area (cm^2)	44·7	45·9	40·9	44·0	35·8	8·6
Fresh weight (g)	1·7	1·7	1·6	1·7	1·4	0·3
Dry weight (g)	0·20	0·19	0·18	0·18	0·16	0·03
Days	24	23	23	20	20	2
Length at start: 8 cm						
Diameter (mm)	3·3	3·3	3·2	3·1	3·2	0·2
Number of leaves	5·8	5·3	5·4	4·9	4·8	0·5
Leaf area (cm^2)	48·4	41·7	40·0	37·0	36·2	7·0
Fresh weight (g)	1·7	1·6	1·5	1·5	1·5	0·2
Dry weight (g)	0·20	0·19	0·17	0·17	0·18	0·03
Days	26	27	24	23	22	2

The number of leaves over 0·5 cm, however, increased from position 5 or 8 to position 1, and most of the other parameters also tended to be slightly higher for the lower-positioned buds (Tables 1 and 2).

Outgrowth parameters (not statistically tested) of buds of the same age (buds 1–5) in the two experiments showed little difference, if any. Similarly, there was no effect of position, as shown by the comparison of bud outgrowth of buds 1–5 in expt 1.1 with 4–8 in expt 1.2, although it should be kept in mind that the experimental conditions were not identical for the two experiments.

When the axillary buds grew out from the shoot, the values for all parameters, except diameter, decreased with increasing distance from the top. Measurements were taken only from the topmost three axillary buds, because lower buds showed little growth (Tables 1 and 2).

Experiment 2

Most outgrowth parameters tended to decrease with bud age, especially at the cutting length of 10 and 8 cm at the start of the experiment, but, in general, the differences were of no statistical significance (Table 3). It is noteworthy that in all three length classes the time needed to produce a 5-cm-cutting declined with increasing bud age.

Experiment 3

In this experiment bud outgrowth occurred *in-vitro*, isolated from the rest of the plant. As Table 4 shows the

TABLE 4. *Effect of age of the sixth axillary bud on bud outgrowth* in vitro *after 4 weeks* (*results of two identical experiments*) (*expt 4*)

Age at	Length (cm)	Diameter (mm)	Number of leaves	Weight	
				Fresh (g)	Dry (g)
Week 1	4·1	1·48	10·0	0·53	0·05
Week 2	6·1	1·54	11·7	0·73	0·07
Week 3	4·4	1·41	10·5	0·55	0·05
Week 4	4·3	1·51	10·3	0·67	0·06
l.s.d. ($P = 0·05$)	0·8	0·18	0·8	0·16	0·01

values for the various growth parameters for 'age 2' exceeded those for 'age 1, 3 and 4'.

DISCUSSION

The data from expt 1 (Tables 1 and 2) do not provide any evidence for the view that bud age and bud position are important factors for bud outgrowth. Nevertheless, most outgrowth parameters showed slightly higher values for the lower-positioned buds. This is in line with findings of Keppeler (1968), also for chrysanthemum. In contrast, Zieslin, Haaze and Halevy (1976) reported for rose that sprouting ability is highest in the apical axillary buds. The slightly better performance of basal buds in expt 1 may be due to more rapid rooting of lower-positioned shoot sections. Basal leaf-bud cuttings of *Schefflera arboricola* rooted more rapidly and produced more roots than cuttings from the more apical positions (Hansen, 1986). Hansen and Kristensen (1990) found a relationship between the number of roots, the bud position and the height of the plant. Basal axillary buds rooted more quickly and produced longer shoots. Light conditions in the basal regions are usually less favourable and, as found for a majority of plant species, more roots are produced with decreasing irradiance (Biran and Halevy, 1973; Hansen and Eriksen, 1974; Poulsen and Andersen, 1980). Another factor explaining the slight gradient in outgrowth of buds along the shoot could be the quality of the sustaining leaves. The basal leaves developed on the mother plant whereas the more apical leaves developed during rooting i.e. they were formed under different environmental conditions. In some way this could have affected leaf structure, rooting ability and bud outgrowth.

In expt 2, removing different lengths of shoot above the same axillary bud did not markedly influence bud outgrowth (Table 3) again indicating that, in young shoots, bud age is not relevant for bud outgrowth. However, the data suggest strongly that the time needed to produce a cutting of a certain length decreased with bud age.

In the *in-vitro* experiment (Table 4), where similar axillary buds were forced to grow out at four successive weeks, 'age-2' buds performed better than the buds of age 1, 3 or 4. This unexpected deviation is not easy to explain. It should be realized that in this experiment the bud only is placed *in-vitro* and its outgrowth must have been determined almost completely by its own potential. In expt 2 and, although to a lesser degree, in expt 1, the outgrowing bud forms part of an intact plant, which will certainly affect bud behaviour to a high degree, and equalize the individual potential of the bud. The importance of individual bud potential was shown for rose by Zieslin and Halevy (1978); they found that upper buds were inhibited when budded on the basal part of the stem but that basal buds retained part of their inhibition when inserted in the upper stem. Furthermore, the age range in expt 3 was markedly larger than in expt 2. Growing conditions being the same, the distance between the sixth axillary bud of age 3 and the apical meristem was about 21 cm in expt 3, compared with only 9 cm in expt 2.

The pattern found for 'intact' shoots in expt 1: a strong decrease in outgrowth of axillary buds from position 5 or 8 to position 1 (Tables 1 and 2), is in accordance with the 'hormonal' theory of apical dominance, assuming that the most apical growing point is the source of some correlative signal of hormonal nature, probably auxin, which restricts development of lower meristems (Martin, 1987; Cline, 1994). In addition, a role is also attributed to cytokinins owing to their ability to stimulate outgrowth of axillary buds. According to this theory it is not surprising that when isolated from each other, every bud along the shoot sprouts readily. The second important concept to explain the mechanism of apical dominance is the 'nutritive' theory which assigns a prominent role to the internal competition for nutrients and carbohydrates among the growing points along the shoot. However, it is unlikely that lack of carbohydrates restricted growth of the axillary buds in the 'intact' shoot. Otherwise, total dry weight of the shoots produced by the five or eight isolated buds would not have greatly exceeded that of the intact shoot bearing the same number of buds (Tables 1 and 2). Intact cuttings and cutting segments were rooted again. The shoots had roots!

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