ERRATA

Page 16, line 21 : 'light flux' should read 'radiant energy flux'.

Page 32, line 20 : '[8-methylene-^{14}C]gibberellic acid' should read '[17-^{14}C]gibberellic acid'.

Page 48, paragraph 1 : delete last sentence.

Page 62, line 7 : '2nd internode' should read '3rd internode'.

Figure 17, abscissa : 'mm' should read 'cm'.

Page 74, line 26 : delete 'and, apparently, of the primary root'.

Page 158, line 6 : '7th internode' should read 'A 7th, B 6th internode'.

Page 166, line 3 : 'colour print' should read 'colour slide'.

ADDENDUM

Page 31, line 25 : after 'column' insert '9 feet long'.

APICAL DOMINANCE IN LEGUMINOUS PLANTS

A thesis submitted to the University of Glasgow

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by

JONATHAN CHRISTOPHER WHITE

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CONTENTS

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Abbreviations

Unless quoted from other sources the units used in this thesis are based on S.I. the international system of units (1970), abbreviated as quoted by Ellis (1971). Standard mathematical and chemical symbols have been employed as well as other commonly used abbreviations:-

s = second
min = minute
h = hour
d = day
m = metre
in = inch
g = gram
l = litre
ct = count (radioactive)
d = disintegration (radioactive)
Ci = Curie
mol = mole = gram molecule
ppm = parts per million
pH = log hydrogen ion concentration
% = percent
$\underline{d}$ = relative density = specific gravity
W = Watt
eV = electron Volt
Pa = Pascal
lbf = pounds force
^{o}C = degree Celsius = degree centigrade
rev = revolution
m/e = mass/charge ratio (mass spectrometry)

$$Rf = \frac{\text{distance from origin}}{\text{distance of solvent front from origin}}$$ (chromatograms)

p = pico = 10^{-12}
n = nano = 10^{-9}
μ = micro = 10^{-6}
m = milli = 10^{-3}
c = centi = 10^{-2}
k = kilo = 10^{3}

c. = circa = approximately
e.g. = example given
i.e. = id est = that is

Abbreviations (continued)

G.M.T.	=	Greenwich Mean Time
S.E.	=	Standard Error
u.v.	=	ultraviolet
t.l.c.	=	thin layer chromatography
g.l.c.	=	gas-liquid chromatography
ABA	=	abscisic acid
IAA	=	indole acetic acid
GA	=	gibberellic acid
TIBA	=	2,3,5-triiodobenzoic acid
CFM	=	2-chloro-9-hydroxyfluorene-9-carboxylic acid-methyl ester
BSA	=	bis-trimethylsilyl acetamide
PPO	=	2,5-diphenyloxazole
OFN	=	oxygen-free nitrogen
TTC	=	2,3,5-triphenyltetrazolium chloride
TMS	=	trimethylsilyl

GENERAL INTRODUCTION

The development of the plant body is ultimately determined by the genotype and is modified by the environment. The regulation of cell differentiation and extension is achieved not only by a direct effect of these determining factors upon each cell but also through effects of one cell upon another, and one group of cells upon another group. Thus the various parts of a plant are interrelated in their development and the sum of these 'correlations' determines the form of that plant.

An important aspect of this integrated development in both shoots and roots is the ability of the leading organ to modify the development of lateral organs. This 'apical dominance', which is of considerable physiological and agricultural interest, has received relatively little attention in monocotyledons, roots and woody shoots. Conversely, much consideration has been given to the shoots of herbaceous dicotyledons, especially legumes, in which apical dominance is chiefly manifest as a complete or partial 'correlative inhibition' of lateral bud growth by the main shoot. This inhibition is produced by the apical bud or, more precisely, by the young, growing leaves of the upper shoot in Phaseolus (Weiskopf, 1927) and Pisum (Snow, 1929b). Mature leaves may inhibit the growth of buds in their own and other leaf axils of non-leguminous plants (Goebel, 1880) and were reported by Weiskopf (1927) to have a slight effect in Phaseolus and Pisum, although this was not confirmed by Snow (1929b) for Pisum. Stem internodes (Weiskopf, 1927), cotyledons (Dostál, 1909), roots (Goebel, 1908) and other buds (Loeb, 1915) have all been reported to inhibit bud growth in various species.

Early investigators interpreted most correlative phenomena in terms of nutrient availability. Thus it was believed that the main shoot as a result of its growth consumed all available nutrients or 'growth factors', thereby restricting the supply of these to the lateral buds

which therefore did not grow (see Goebel, 1900). Loeb (1915 ; 1923) demonstrated that nutrient availability was a limiting factor in the outgrowth of both axillary and foliar buds of Bryophyllum and concluded that his data supported the 'nutritive' theory. Both Snow (1925) and Thimann (1939) have pointed out the naivety of this conclusion. Nevertheless, compensatory growth effects do occur in plants other than Bryophyllum (see e.g. Jacobs and Bullwinkel, 1953) and the promotive effect of various nutritive factors upon bud growth has been demonstrated in a number of species, including Phaseolus (McIntyre, 1973) and Pisum (Nakamura, 1965). The nutritive status of the plant, therefore, may well influence the degree of apical dominance and must be considered as possible factor in the mechanism of apical dominance (Gregory and Veale, 1957 ; Audus, 1959).

McCallum (1905 a; b) demonstrated, however, that the cotyledon axillary buds of Phaseolus could grow out even under unfavourable nutritive conditions and that this outgrowth was not a response to wounding: he concluded that inhibition was not the result of restriction of nutrient supply, especially in view of the close proximity of these buds to the cotyledons, which are the major nutrient source in young Phaseolus seedlings. Other experiments with Vicia (Mogk, 1913) and Phaseolus (Child and Bellamy, 1920 ; Harvey, 1920) were taken as evidence against the 'nutritive' theory and led Snow (1925) to experimentally investigate the possibility, already suggested by Errera (1904) and considered by Dostál (1909) and Loeb (1917), that inhibitory substances might be responsible for correlative inhibition. Snow's approach was to trace the pathway through which the inhibition of lateral bud growth could travel. He discovered that inhibition could pass through a zone of stem internode from which all tissues outside the xylem had been removed. He further showed the passage of inhibition through pith alone and xylem alone, and also confirmed Harvey's (1920) demonstration that

living tissue is required for the transmission of inhibition. Snow finally claimed to have shown the movement of inhibition across a moist protoplasmic discontinuity and interpreted his results as unequivocally indicating the involvement in apical dominance of the movement of an inhibiting substance.

Although Snow's (1925) experimental data were inadequate his conclusion seemed to have been justified upon the discovery of the effect of indole acetic acid, then known as growth substance or hetero-auxin, on apical dominance. Thimann and Skoog (1933 ; 1934) found that isolated terminal buds of Vicia released growth substance (detected by the Avena curvature bioassay) into agar and that similar amounts of growth substance isolated from Rhizopus and reapplied to the cut surface of plants with their terminal buds removed could partially replace the terminal bud with respect to inhibition of lateral bud outgrowth. Growth substance was also produced by developing leaves and rapidly growing buds but not by inhibited buds or older organs. Pure hetero-auxin could also inhibit bud growth on decapitated plants (Skoog and Thimann, 1934) and it has since been generally accepted that indole acetic acid (IAA) does play a major role in apical dominance. Jacobs (1959), however, has outlined six requirements that need to be met before it can be concluded that a particular substance normally controls a given biological process.

Firstly, the presence of the substance in the intact organism must be demonstrated. The presence of auxin in young, green tissues of leguminous plants has been amply confirmed by both extraction (e.g. Forman, 1938 ; Shoji et al., 1951 ; Kefford, 1955 ; Scott and Briggs, 1960) and collection in agar (e.g. Le Fanu, 1936 ; Scott and Briggs, 1960 ; Libbert, 1964a) but its identification as IAA has been based on non-specific tests such as co-chromatography with authentic IAA, bioassay and chromogenic reactions.

Secondly, removal of the substance should stop the process. It is mainly the young growing tissues of the shoot which contain much auxin (e.g. Berman, 1938 ; Shoji et al., 1951 ; Kefford, 1955) and it is the removal of these which breaks apical dominance (Weiskopf, 1927 ; Snow, 1929b).

Thirdly, exact quantitative substitution of the endogenous substance with artifical substance after the removal of the former should lead to reimposition of the process. Complete inhibition of lateral bud growth has been maintained for at least a week by exogenous IAA applied to decapitated plants in aqueous solution (Skoog and Thimann, 1934 ; Scott and Pritchard, 1968) or in lanolin paste (Šebánek, 1966 ; Phillips, 1968). The concentrations of IAA used in these studies, however, were high compared with endogenous auxin levels (e.g. Kefford, 1955) and even these have often been found to be only partially effective (Went, 1939 ; Phillips, 1971a ; Shein and Jackson, 1972). Concentrations of applied IAA thought to be more akin to those found endogenously either had little effect on (Scott and Pritchard, 1968 ; Hillman, 1970) or actually promoted lateral bud growth (Meinl and von Guttenberg, 1954 ; Šebánek, 1966). Few attempts have been made at quantitatively replacing apically produced auxin with IAA. Thimann and Skoog (1934) found that 10 times the auxin 'diffusing' from shoot tips of Vicia had to be applied to completely prevent bud growth and Jacobs et al. (1959) obtained no effect on bud growth of Coleus with a concentration of IAA which exactly replaced the upper shoot with respect to both 'diffusible' auxin and the promotion of xylem differentiation in the stem. Conversely, Libbert (1964a), using a similar technique, claimed that lateral bud growth of Pisum was inhibited by a concentration of IAA which exactly replaced the upper shoot. These undertainties surrounding the effect of applied IAA have led to the expression of serious doubts about the role of IAA in apical dominance (Meinl and von Guttenberg, 1954 ; Jacobs et al., 1959 ;

Shein and Jackson, 1972).

Fourthly, the substance should influence the process in isolation from the rest of the organism: IAA does inhibit the growth of buds on isolated nodal segments of Phaseolus (Meinl and von Guttenberg, 1954) and Pisum (Wickson and Thimann, 1958). Fifthly, the generality of the effect should be shown: the review of Thimann (1939) indicates the wide range of plant species in which auxin affects lateral bud growth. Lastly, the effect should be specific to the substance under study: this is not strictly true for IAA in apical dominance but the significance of inhibition of bud growth by other chemicals (e.g. Meinl and von Guttenberg, 1954) is doubtful.

Before the discovery that IAA inhibited lateral bud growth it had been shown that the movement of IAA in isolated segments of plant tissue was primarily in the basipetal direction (van der Weij, 1932). Thimann and Skoog (1934) envisaged the action of IAA in apical dominance involving the basipetal translocation of IAA from the terminal bud towards the lateral buds and this view is implicitly accepted in most of the theories on IAA action in correlative inhibition. That the translocation of applied IAA in segments of plant tissue is predominantly basipetal has been repeatedly demonstrated for many plant tissues (see Goldsmith, 1969), including the young hypocotyl (Jacobs, 1950 ; Hay, 1956), epicotyl (Hay, 1956 ; Whitehouse and Zalik, 1967) and petiole (McCready and Jacobs, 1963) of Phaseolus. There is far less evidence that basipetal transport of endogenous auxin occurs. The work of Scott and Briggs (1960) suggested that there was a basipetal flow of endogenous auxin in stems of Pisum: the movement of auxin from isolated shoot tips into agar is also, perhaps, indicative of this.

The application of radioactively labelled IAA to intact plants has usually involved its introduction either directly or indirectly into the vascular system (Fang and Butts, 1957 ; Little and Blackman, 1963 ;

Whitehouse and Zalik, 1967 ; Eschrich, 1968 ; Long and Basler, 1973). In these cases radioactivity was distributed both acropetally and basipetally, but the results may not be relevant to the normal movement of endogenous IAA. The much more useful approach of radioactive IAA application to the apical region has recently provided evidence of a system for the basipetal translocation of IAA in intact plants of Pisum (Morris et al., 1969 ; Morris and Kadir, 1972 ; Morris et al., 1973) and Vicia (Bonnemain, 1971 ; 1972 ; Bourbouloux et al., 1973). None of these studies, however, was directly related to apical dominance.

Some information on the transport of both applied and endogenous auxin in apical dominance has been obtained from the use of 2,3,5-triiodobenzoic acid (TIBA), which inhibits IAA transport (Niedergang-Kamien and Skoog, 1956). Kuse (1953) found that TIBA applied to the petiole of a young leaf of Ipomoea blocked the inhibition of bud growth by both the leaf blade and IAA applied to the debladed petiole. Similarly, TIBA prevented the inhibition of lateral bud growth by IAA applied to the top of a decapitated plant of Pisum (Libbert, 1959) and Vicia (Panigrahi and Audus, 1966). Recently, a similar breakage of IAA-induced inhibition of bud growth was shown to be caused by one of the morphactins (Krelle and Libbert, 1968) which also inhibits IAA transport (Bridges and Wilkins, 1973). Only Panigrahi and Audus (1966), however, checked that IAA transport was inhibited, the other authors using bud growth as an indication of the effect of the chemicals on IAA transport rather than the other way round.

Thimann and Skoog (1934) proposed a mechanism of apical dominance in which apically produced auxin moved down the stem and thence into the lateral buds, there preventing the buds' own production of auxin. Thimann and Skoog maintained that this concept was supported by the fact that lateral buds released no auxin into agar unless they were growing, and Sachs and Thimann (1967) have developed the idea, suggesting that the

balance between external and internal auxin supply to the buds might, be mediated by cytokinin levels. Thimann (1937) modified the 'direct' theory of auxin action, proposing that the amount of auxin which reaches the lateral buds from the main shoot is supraoptimal and thus inhibitory to bud growth. The 'direct' theory in both its forms, although supported by Skoog (1939), has received much criticism. Its most active antagonist was Snow, who in a series of experiments demonstrated that inhibition could travel where auxin apparently could not (Snow, 1929a ; 1931b ; 1936 ; 1937 ; 1938 ; 1939 ; 1940). Snow's arguments, however, as pointed out by Audus (1959), were based on the belief in almost totally basipetal transport of IAA, which may not in fact be the case in intact plants. Furthermore, Wickson and Thimann (1960) showed that in Pisum the amount of [^{14}C]IAA moved into lateral buds was directly proportional to the amount of inhibition produced by IAA, although the significance of this observation is clouded by the fact that the experiments were carried out with isolated nodal segments.

The most telling evidence against the 'direct' theory has been obtained from investigations involving the extraction of growth substances from inhibited lateral buds, which on the basis of the 'direct' theory should contain high levels of auxin. Small amounts of auxin activity only were extracted from inhibited lateral buds of Lupinus (Ferman, 1938) and Pisum (van Overbeek, 1938) and none, using chromatographic procedures, from lateral buds of Vicia (Kefford, 1955) or Pisum (Denizci, 1966). That low concentrations of applied IAA can actually promote bud growth on decapitated plants (Ferman, 1938 ; Went, 1939 ; Šebánek, 1966) also militates against the 'direct' theory. Further evidence difficult to explain by the 'direct' theory is the demonstration in Pisum that inhibition of bud growth by either young leaves (Snow, 1931a) or applied IAA (Went, 1939) increased with distance of the bud from the inhibiting region, although Thimann (1937), also with Pisum, could detect

no such increase of IAA-induced inhibition with distance. Both Audus (1959) and Phillips (1969a) have concluded that the weight of evidence against the 'direct' theory suggests it is no longer tenable.

With a series of experiments, the results of many of which he found difficult to explain in terms of the 'nutritive' or 'direct' theories, Snow (1925 ; 1929a ; 1931a;b ; 1932 ; 1936 ; 1937 ; 1938 ; 1939 ; 1940) developed what has become known as the 'correlative inhibitor' theory: it was proposed that, as a result of auxin action, by a process perhaps related to growth, there occurs the formation of an inhibitor, which unlike auxin can move into lateral buds; although the inhibitor is present in both main stem and lateral buds its effect is only evident in the latter because basipetally moving auxin in the main shoot protects it from inhibition. Unlike the theories already considered the 'correlative inhibitor' theory as envisaged by Snow, or with only slight modifications, can explain most of the available evidence on correlative inhibition. Almost as a corollary of this statement, there is very little positive evidence to support the theory: Snow's experiments, rather than proving his own theory, were largely designed to disprove others.

Thimann (1939) concluded that the 'correlative inhibitor' theory remained open and little has occurred since to change that view. In a series of experiments with Pisum Libbert (1955a, b, c ; 1964b) claimed to have isolated a substance which exhibited the characteristics required of a correlative inhibitor but this work has been criticized by Audus (1959) on the grounds that these characteristics were determined on crude extracts. Furthermore, no attempt was made to estimate changes in the level of this inhibitor in lateral buds before and after decapitation of the main shoot (all Libbert's extracts were from the main shoot).

Kefford (1955) extracted seedlings of Vicia and detected IAA-like activity in the main shoot but inhibitory activity like inhibitor B

(Bennet-Clark et al., 1952) in the lower lateral bud. Dörffling (1965 ; 1966) has isolated at least 2 inhibitors, one like inhibitor β, from stems of Pisum: neither of these inhibitors resembled Libbert's, although both could inhibit lateral bud growth. Unfortunately, although Dörffling (1963, 1964) has correlated levels of inhibitor β in buds with correlative inhibition in Acer he has not done this with Pisum.

Dörffling (1967) further claimed to have demonstrated the existence of abscisic acid (ABA) in Pisum and Arney and Mitchell (1969), having shown ABA-induced inhibition of lateral bud growth in Pisum, suggested the possibility that ABA might be the correlative inhibitor proposed by Snow. Hillman (1970), however, detected no such effect of ABA in Phaseolus, although ABA could increase the inhibitory effect of other hormones. In non-legumes the levels of inhibitory substances like ABA (Tucker and Mansfield, 1972 ; 1973) or unlike ABA (Goodwin and Cansfield, 1967 ; Blake and Carrodus, 1970) have been correlated with apical dominance.

Went (1936) suggested a modification of the 'nutritive' theory that has become known as the 'nutrient diversion' theory: endogenous or applied auxin creates a flow of bud growth factors towards its point of production or application, thus creating the same type of starvation of lateral buds suggested in the 'nutritive' theory. Went (1938 ; 1939) further obtained evidence that root-produced factors were required for bud growth and that factors promotory to bud growth accumulated in IAA-treated decapitated internodes. Although many of Snow's (1925 ; 1929a ; 1932 ; 1937 ; 1940) experiments are difficult to explain on the 'nutrient diversion' theory and Thimann (1939) discounted the idea, it has been revived in recent years. There is now a considerable body of evidence that applied IAA can rapidly induce accumulation of ^{14}C or ^{32}P labelled compounds at the region of IAA application (e.g. Booth et al., 1962 ; Davies and Wareing, 1965 ; Šebánek, 1965b) and that bud growth and

nutrient availability are correlated (e.g. Gregory and Veale, 1957 ; Nakamura, 1965 ; Husain and Linck, 1966 ; McIntyre, 1973). Thimann (1937) has demonstrated, however, that IAA application leads to a lowering of the overall increase in dry weight of decapitated shoots rather than merely a diversion of material from the lateral buds to the decapitated internode. Phillips (1968) obtained similar results on examination of the levels of nitrogen, phosphorus and potassium in IAA and non-IAA treated plants. A more important objection to the involvement of IAA-induced nutrient diversion in apical dominance was the discovery that two concentrations of IAA, one that promoted and the other that inhibited lateral bud growth, both induced accumulation of ^{32}P at their point of application (Šebánek, 1966). Most of the objections to the 'nutritive' theory also apply to the 'nutrient diversion' theory and Thimann (1939) has pointed out that the fact that buds on isolated nodal sections grow out is difficult to explain if nutrients or factors from other parts of the plant are required for bud growth.

The theory originally devised by Went (1936 ; 1938 ; 1939) involved the diversion of specific root-produced bud growth factors rather than nutrients away from the buds and Phillips (1969a) has suggested that perhaps root-produced cytokinins are required for bud growth and are diverted towards the upper shoot. Direct application of a cytokinin to an inhibited lateral bud does partially release the bud from correlative inhibition (Sachs and Thimann, 1964 ; Panigrahi and Audus, 1966 ; Ali and Fletcher, 1970b), cytokinins appear to be produced in the roots (Loeffler and van Overbeek, 1964 ; Sitton et al., 1967) and IAA can influence the movement of applied kinetin in plants (Seth et al., 1966 ; Morris and Winfield, 1972) but there is little positive evidence that endogenous cytokinins might be involved in apical dominance.

Van Overbeek (1938) suggested that growth factors, rather than being diverted towards the upper shoot, were prevented from entering

the lateral buds by a direct effect of auxin on the vascular system. Gregory and Veale (1957) modified this idea with the proposal that auxin might prevent differentiation of vascular strands into the lateral buds. IAA does influence vascular differentiation (Jacobs and Morrow, 1957) and a correlation between vascular development and bud outgrowth has been noted in Pisum (Sorokin and Thimann, 1964), Phaseolus (Moreland, 1934) and Vicia (Panigrahi and Audus, 1966). This theory, however, could only account for the inhibition of weakly developed buds, not of well grown shoots.

Recently, scepticism about the evidence for all the classical theories of apical dominance has led to the proposal of a 'hormone balance' theory: lateral bud growth is controlled by the general background hormone balance of the plant (Shein and Jackson, 1971, 1972 ; Jackson and Field, 1972). The experimental evidence for this idea, however, is limited. The evidence for auxin involvement has been described but is doubted by Shein and Jackson (1972). The limited evidence for ABA and cytokinin involvement in apical dominance has also been outlined.

Gibberellins can influence the expression of apical dominance (e.g. Marth et al., 1956 ; Brian et al., 1959 ; Ruddat and Pharis, 1966) but these effects are all explainable in terms of a gibberellin-induced promotion of the growth of already-elongating shoots (Phillips 1969a), except perhaps the reports that gibberellin can enhance IAA-induced inhibition of lateral bud growth (Jacobs and Case, 1965 ; Scott et al., 1967). This latter effect, however, could not be confirmed by numerous workers (e.g. Kato, 1958 ; Nakamura, 1965 ; Hillman, 1970 ; Ali and Fletcher, 1971) and, after a detailed examination of the problem, Phillips (1969b ; 1971a, b) concluded that gibberellin could sometimes enhance inhibition by IAA but only by promoting the growth of the decapitated internode.

Applied plant hormones often show interactions in their effect on bud growth (Davies et al., 1966 ; Hillman, 1970 ; Ali and Fletcher, 1970b, 1971 ; Shain and Jackson, 1971, 1972 ; Jackson and Field, 1972) and interactions between known plant hormones and other chemicals have been implicated in apical dominance (Libbert, 1954b ; Giertych, 1964 ; Tomaszewski, 1964 ; Garg, 1966). The possibility that a balance of hormonal factors controls apical dominance cannot be discounted, but most available evidence can be interpreted on the basis of other theories and the postulation of a 'hormone balance' theory without details on the mode of action of each hormone does not aid the interpretation of apical dominance phenomena.

INTRODUCTION

Since the discovery of the possible involvement of auxin in correlative inhibition, investigators into this problem have tended to concentrate on elucidating the mechanism of auxin action. Most of the theories on this mechanism accept the involvement of the basipetal transport of indole acetic acid (IAA) from its point of production in the upper shoot towards the lateral buds that it inhibits. The application of Jacobs' (1959) rules to the problem, however, reveals major flaws in the evidence for the regulation of apical dominance by IAA. Firstly, the auxin isolated from green shoots has never been conclusively identified as IAA. Secondly, much greater amounts of IAA than those isolated from shoot tips are usually required to replace the shoot tip with respect to inhibition of lateral bud growth. Thirdly, the basipetal translocation of endogenous auxin has rarely been demonstrated; in addition the well known basipetal polarity of transport of applied IAA in segments of plant tissue has not been adequately confirmed in intact plants.

Of the various mechanisms proposed for the maintenance of correlative inhibition the 'correlative inhibitor' and 'nutrient diversion' theories seem to be the most generally acceptable. Much of the evidence for the former theory and against the latter has emanated from the work of Snow, which although conceptually and technically brilliant, was often unsound in experimental design.

The aims of the current investigation were twofold; to examine the involvement of indole acetic acid in apical dominance and to reassess the importance of Snow's investigations in relation to the 'correlative inhibitor' and 'nutrient diversion' theories. Seedlings of Phaseolus vulgaris were chosen as the experimental material in view of the predominance of apical dominance work already performed with this species

and other legumes. The work was split into four sections. In Section 1 the characteristics of apical dominance in Phaseolus were carefully analysed. In Section 2 the involvement of indole acetic acid in correlative inhibition was investigated by the application of exogenous IAA to decapitated plants, the qualitative analysis of the IAA-like auxin extracted or collected in agar from the major inhibiting region of the upper shoot and the analysis of endogenous auxin transport by the application of IAA transport inhibitors to intact plants. In Section 3 the distribution of radioactivity after application of [^{14}C]IAA to carefully selected regions of intact plants has been quantitatively and qualitatively scrutinized and compared with that after similar application of ^{14}C labelled sucrose, abscisic acid and gibberellic acid. In Section 4 some of Snow's more critical experiments have been repeated and analysed.

EXPERIMENTAL PROCEDURES

Unless it is stated otherwise the following experimental procedures were adopted:-

1. PLANT MATERIAL

Seeds of dwarf french bean, *Phaseolus vulgaris* variety 'Canadian Wonder', were obtained from Hurst Gunson Cooper Taber Ltd., Witham, Essex, U.K. and stored at 10°C in darkness. Bean plants were grown singly in plastic pots (8.0 cm diameter, 8.5 cm depth) containing a standard compost mixture of topsoil:'Humax' *Sphagnum* moss peat: coarse sand::1:1:1.

Seeds of garden pea, *Pisum sativum* variety 'Thomas Laxton', were supplied by Thomas Cullen and Son Ltd., Witham, Essex, U.K. and stored at 10°C in darkness. They were soaked for 6 h in running tap water and grown for 5 d in damp vermiculite (Alexander Products Ltd., Burnham-on-Sea, Somerset, U.K.) at 25°C under continuous light from Atlas 'Super Five White' 65/80 W fluorescent tubes (4 W m^{-2} intensity at plant level) before transfer into the standard compost in 8 cm diameter plastic pots.

Plants were grown in a heated glasshouse with maximum and minimum temperatures of 35°C and 10°C respectively. Between the months of October and May inclusively, 16 h supplementary lighting was supplied daily from 0300 h to 1900 h G.M.T. by 400 W 'GES MBFR Kolorlux' high pressure mercury vapour reflector lamps in Atlas 'HBR/G400' fittings (Thorn Lighting Ltd., Glasgow, U.K.). During the rest of the year similar supplementary lighting was supplied daily between 0300 h and 0700 h G.M.T. The lamps were spaced 0.8 m apart and hung 1.0 m above the plants.

Batches of 3000 bean plants were grown in the standard compost mixture in wooden fish-boxes, 40 x 25 x 9 cm, (50 plants per box) in a glasshouse, either in natural daylight or with the supplementary lighting. Batches of 10000 bean plants were grown in soil in plots, 3.5 x 1.0 m (5000 plants per plot) under 16 h supplementary light daily from 1000 W 'GES MBFR/U Kolorlux' high pressure mercury vapour reflector lamps (Thorn Lighting Ltd.), 2 per plot, 1.3 m apart and 1.8 m above the plants.

The qualities of the light sources employed are shown in Figure 1 A,B.

The terminology used for the various structures of bean and pea plants is shown in Figures 2 and 3, which can be compared with photographs of these plants (Plates 1 and 2).

2. GROWTH EXPERIMENTS

A. Experimental conditions

Experiments involving the measurement of growth were carried out either in the glasshouse under 400 W lamps (see above, 'PLANT MATERIAL') or in a controlled environment cabinet at 25 $\pm$ 2°C under continuous or 16 h daily illumination from a bank of 65/80 W fluorescent tubes (Atlas 'Warm White':Mazda 'Universal Daylight'::1:1) 1 m above plant level. The light flux at plant level in the controlled environment cabinet was 5 W m^{-2}. The quality of light emitted by the fluorescent tubes is shown in Figure 1 C,D.

Plants were moved into the cabinet at least 24 h before the beginning of an experiment and were watered twice daily.

B. Mensuration

When possible, growth measurements were taken daily, at the same

Figure 1 : Spectral distribution of illumination from experimental light sources; A 'MBFR Kolorlux' mercury vapour lamp, B 'White', C 'Warm White', D 'Daylight' fluorescent tubes.

Wavelength (nm)	Colour
380 - 420	Far violet
420 - 440	Violet
440 - 460	Blue
460 - 510	Blue Green
510 - 560	Green
560 - 610	Yellow
610 - 660	Light Red
660 - 760	Dark Red

Data from catalogue (Thorn Lighting Ltd.)

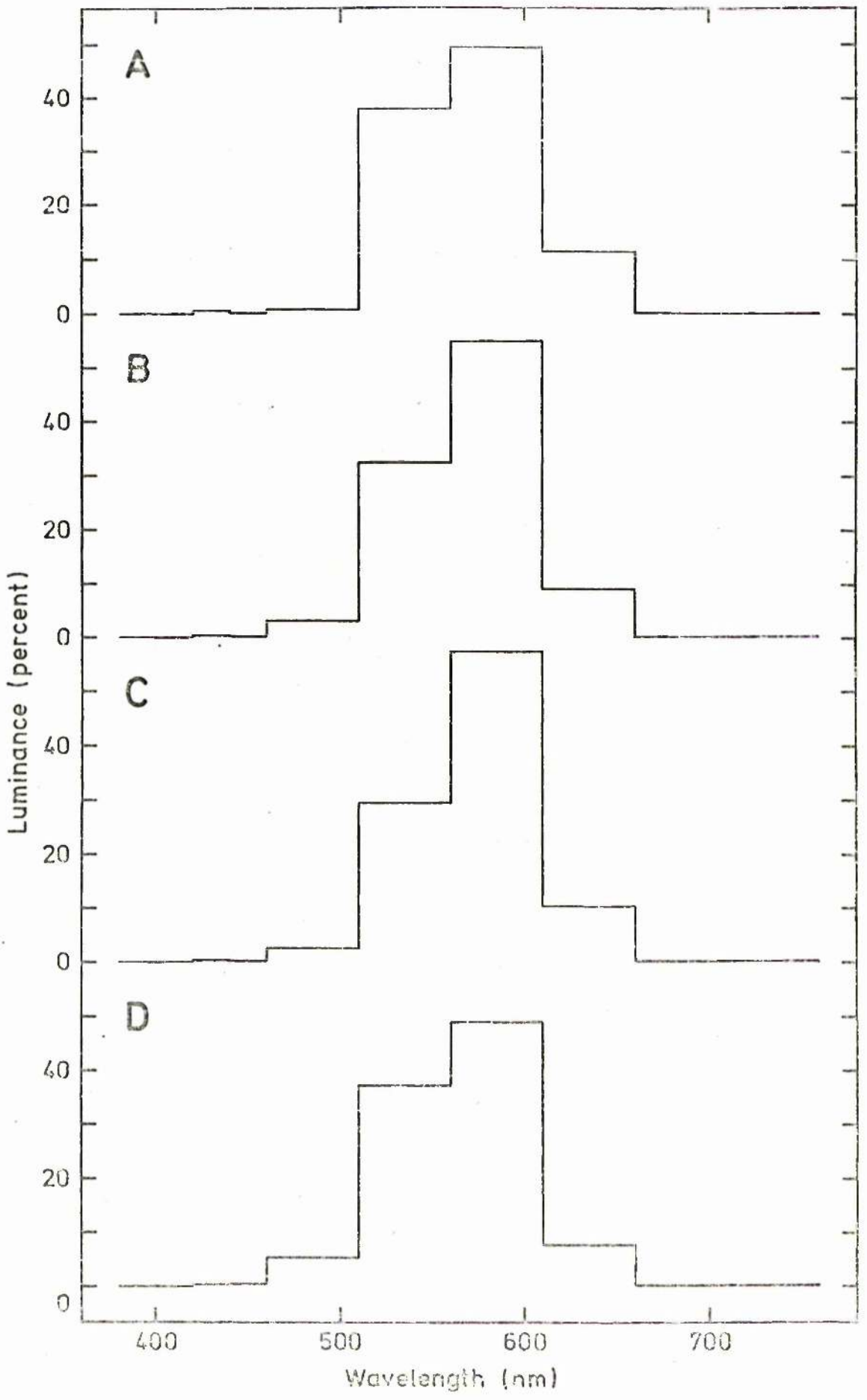

A
B
C
D
40
20
0
Luminance (percent)
400
500
600
700
Wavelength (nm)

Figure 2 : Diagrammatic representation of an approximately 3 week old plant of *Phaseolus vulgaris*.

Numbers = node numbers.

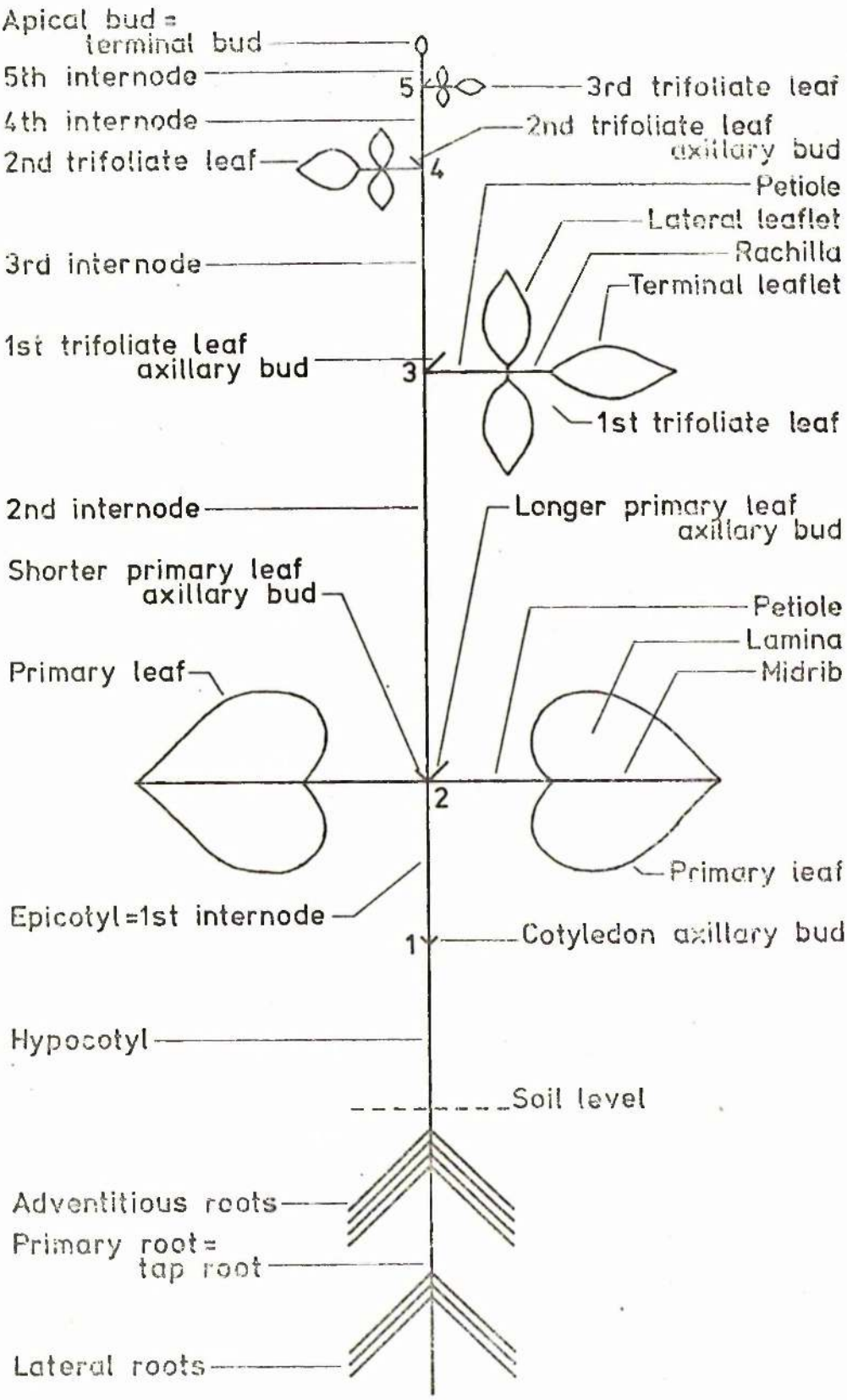

Apical bud = terminal bud
5th internode
4th internode
2nd trifoliate leaf
3rd internode
1st trifoliate leaf axillary bud
2nd internode
Shorter primary leaf axillary bud
Primary leaf
Epicotyl = 1st internode
Hypocotyl
Adventitious roots
Primary root = tap root
Lateral roots
5
4
3
2
1
3rd trifoliate leaf
2nd trifoliate leaf axillary bud
Petiole
Lateral leaflet
Rachilla
Terminal leaflet
1st trifoliate leaf
Longer primary leaf axillary bud
Petiole
Lamina
Midrib
Primary leaf
Cotyledon axillary bud
Soil level

Figure 3 : Diagrammatic representation of an approximately 2 week old plant of *Pisum sativum*.

Numbers = node numbers.

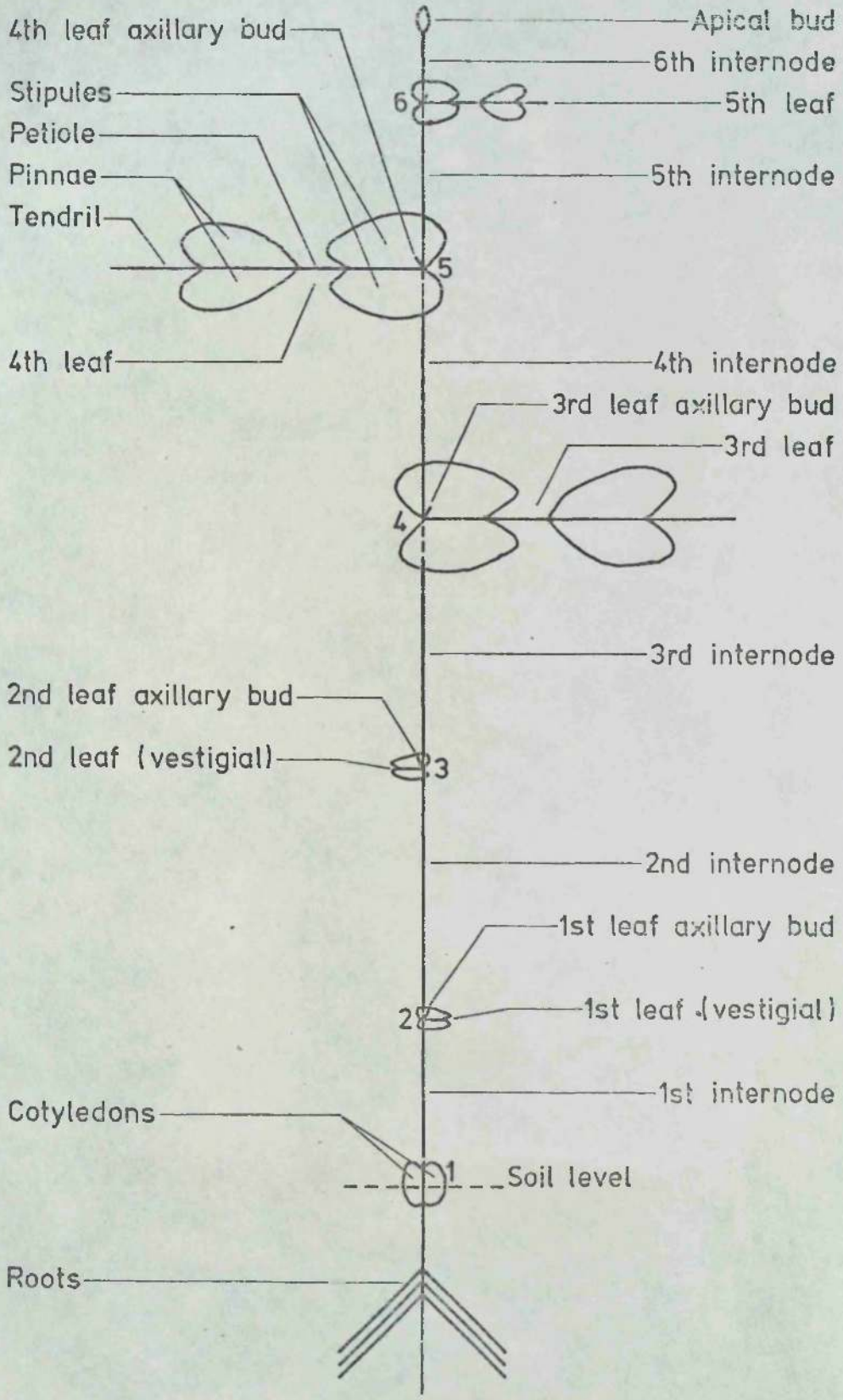
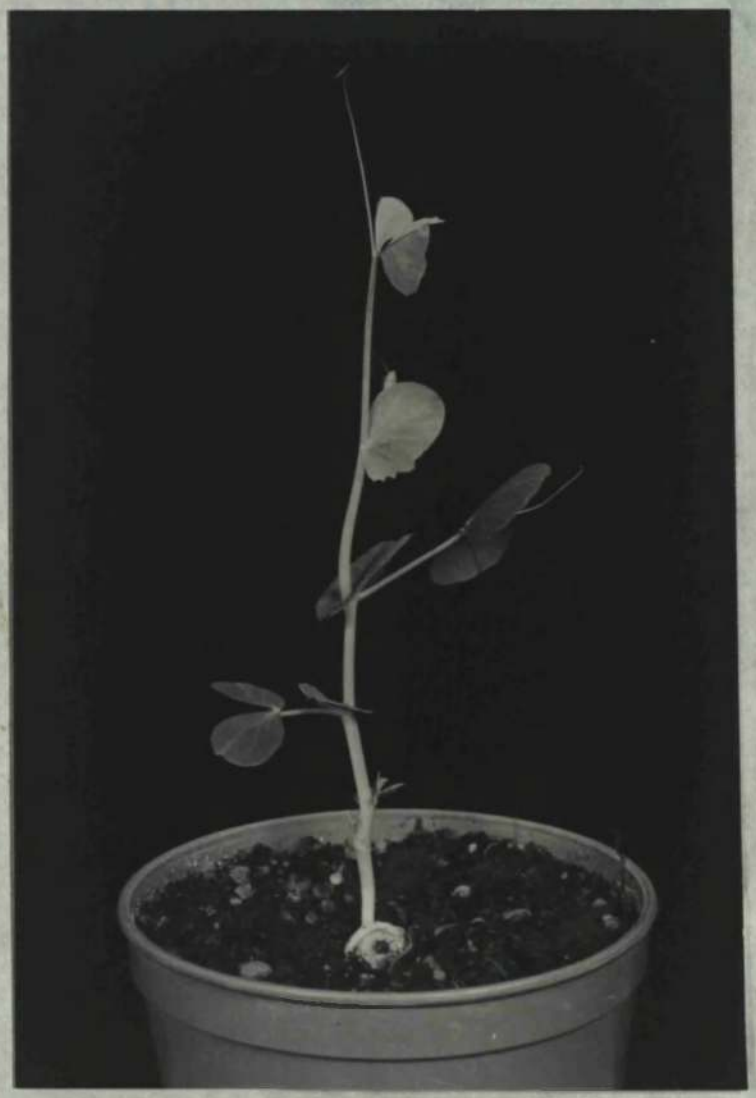

4th leaf axillary bud
Apical bud
6th internode
Stipules
6
5th leaf
Petiole
Pinnae
5th internode
Tendril
5
4th leaf
4th internode
3rd leaf axillary bud
3rd leaf
4
3rd internode
2nd leaf axillary bud
2nd leaf (vestigial)
3
2nd internode
1st leaf axillary bud
2
1st leaf (vestigial)
1st internode
Cotyledons
1
Soil level
Roots

Plate 1 : A 21 d old plant of *Phaseolus vulgaris*.

Plate 2 : A 17 d old plant of Pisum sativum.



time of day. Lengths of < 100 mm were measured, to the nearest millimetre, with a pair of dividers and a ruler, those of > 100 mm with a ruler only. The length of a bud was measured from the point of attachment to the main stem to the base of the stipules at the first node of the bud (i.e. the first internode of the bud). At the end of each experiment the length of the bud from its base to the centre of its apical bud was also measured (i.e. the total length of the bud).

Primary leaf lengths were taken from the junction between lamina and petiole to the tip of the lamina. Trifoliate leaf measurements were taken from the tip of the terminal leaflet to the base of its rachilla. Internodes were measured between the bases of the stipules at the lower and upper nodes, and petioles between the junction of lamina and petiole and the junction between petiole and main stem.

Dry weights were assessed after drying the plant material in an oven at 150°C for 48 h.

C. Surgical treatments

(i) General

Surgery was performed using a sharp scalpel washed with methanol. Leaves and petioles were cut off at the base of the petiole. Laminae were removed at the junction of the lamina with the petiole. Internodes were excised through the lower node. When disbudded nodes showed redevelopment of secondary axillary buds or the formation of adventitious buds these were removed as soon as possible.

Specific information on surgical treatments is provided with the experimental results.

(ii) Derooting

Each plant was removed from its pot and, after excess compost was washed off, was placed with the roots and the lower part of the hypo-

cotyl submerged in tap water in a 250 ml conical flask wrapped in silver foil. The roots were excised by cutting underwater through the hypocotyl 1 cm above the uppermost adventitious root. The roots were removed from the flask and the shoot was supported, with the cut edge of the hypocotyl 2 cm below the water surface, by a wad of non-absorbent cotton wool around the hypocotyl in the neck of the flask. Control plants, with their roots left intact, were supported similarly in conical flasks.

(iii) Ringing

Plants were ringed by careful removal of the epidermis, cortex, phloem and interfascicular cambium around a 2 cm length of internode as described by Snow (1925). The plants were subsequently supported by tying loosely to a cane with string above the ring (Plate 3).

(iv) Steam girdling

A jet of pressurized steam from a steam can was played for 10 s around a 2 cm length of internode. The rest of the plant was protected by layers of tissue paper. The plants were subsequently supported in the same way as ringed plants (Plate 4).

D. Chemical treatments

(i) Chemicals

Indole-3-acetic acid (IAA) and 2,3,5-triiodobenzoic acid (TIBA) were supplied as 'pure' reagents by Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K. RS(±) abscisic acid (ABA) was donated by F. Hoffmann-La Roche AG, Basle, Switzerland, and the morphactin, 2-chloro-9-hydroxyfluorene-9-carboxylic acid-methylester (CFM) by E. Merck AG, Darmstadt, West Germany (code number IT 3456). The latter compound contained c. 20% impurity, consisting mainly of 9-hydroxyfluorene-9-carboxylic acid-methylester and a small amount of 2,7-dichloro-9-

Plate 3 : A 21 d old plant of Ph. vulgaris surgically ringed at the 2nd internode.

Arrow indicates ringed region.

Plate 4 : A 21 d old plant of Ph. vulgaris steam girdled at the 2nd internode.

Arrow indicates girdled region.

hydroxyfluorene-9-carboxylic acid-methylester.

The chemicals were stored at -8°C in darkness.

(ii) Preparation

IAA was dissolved in a minimal volume of redistilled methanol which was dispersed into either distilled water or hydrous lanolin. Before dispersal into lanolin, ABA and CFM were dissolved in a minimal volume of redistilled methanol, and TIBA in a minimal volume of 2-methoxyethanol. Hydrous lanolin was prepared from anhydrous lanolin (BDH Chemicals Ltd., Poole, Dorset, U.K.) by stirring in distilled water vigorously until in excess. After the extra water was decanted and the lanolin weighed, the appropriate weight of chemical was dissolved in solvent and stirred vigorously into the lanolin for at least 10 min. The lanolin was subsequently kept at 40°C for 24 h with occasional stirring to evaporate the solvent. Lanolin pastes were stored at 2°C in darkness.

(iii) Application

Aqueous solutions were applied to decapitated plants using the method of Hillman (1970). A 3 cm length of rubber tubing (2 mm^2 internal area) was pushed 5 mm onto the cut stump and sealed at the base with hydrous lanolin. The reservoir so formed was filled with solution at the start of the experiment and subsequently refilled daily. Every 2 d a 2 mm section was removed from the cut stump and the reservoir replaced.

Lanolin pastes were dispensed with a 1 ml disposable syringe, either directly onto an intact plant as a ring around an internode, or into half a gelatin capsule (number 2, Parke, Davis and Co., Hounslow, London, U.K.) used to cap a decapitated internode.

E. Histological techniques

(i) Internode sections

Transverse sections, cut as thin as possible by hand with a sharp razor blade, were stained with dilute thionine and mounted on a glass slide in distilled water under a coverslip. The preparations were ringed around the edge of the coverslip with DPX mountant (BDH Chemicals Ltd.). Within 3 d of preparation these temporary mounts were photographed using transmitted light, either directly or through a microscope.

(ii) Node sections

Primary leaf nodes, including the primary leaf axillary buds, primary leaf pulvini and 5 mm of the 1st and 2nd internodes, were detached from the plant. Approximately 0.25 mm thick longitudinal sections were hand cut from the node in the plane of insertion of the primary leaves. The sections were placed in lactic acid, which was brought to the boil and then allowed to cool. After 12 h the sections were placed on a glass slide in lactic acid, covered with a glass coverslip and photographed directly using dark field illumination.

(iii) Vital staining

Segments of stem tissue were infiltrated under vacuum for 15 min with 10 g l^{-1} 2,3,5-triphenyltetrazolium chloride (BDH Chemicals Ltd.) in distilled water, and subsequently incubated in this solution in darkness at room temperature for a further 24 h. The segments were blotted dry and photographed in direct light.

3. AUXIN ISOLATION EXPERIMENTS

A. Extraction and partitioning

Terminal portions of shoot, consisting of the apical bud, a young trifoliate leaf (5 to 30 mm long) and 5 mm of the internode below the leaf, were excised from 10 to 20 d old bean plants. They were extracted

in darkness at room temperature twice with 500 ml redistilled 'Analar' methanol (enough to cover the plant tissue). Each extraction was for 12 h. The bulked extract was evaporated under vacuum at 40°C on a rotary evaporator (Rotavapor-R, Buchi, Switzerland) until no more methanol distilled over. The aqueous residue (25 to 30 ml) was acidified to pH 3.0 with 2 mol l^{-1} HCl and extracted 3 times with 50 ml redistilled diethyl ether. The ether was then extracted 3 times with 50 g l^{-1} $NaHCO_3$ solution (100 + 75 + 50 ml). After acidification to pH 3.0 with concentrated HCl, the aqueous phase was extracted 3 times with 175 ml ether. The ether extract was stored overnight at -15°C, decanted while <0°C and evaporated almost to dryness under vacuum at 30°C on a rotary evaporator. The residue was dissolved in redistilled 'Analar' methanol (1.0 + 0.5 + 0.5 ml), which was subsequently evaporated under a stream of oxygen-free nitrogen (OFN) to a volume of c. 0.25 ml.

All glassware was rinsed 3 times with redistilled methanol as well as with any other solvent with which it would be used.

B. Collection in agar

Ionagar No. 2 (Oxoid Ltd., London, U.K.) was dissolved in boiling distilled water to provide a 15 g l^{-1} solution of agar which was moulded into 2 mm thick blocks. Terminal portions of bean shoots (as described above, 'Extraction and partitioning') were excised and placed with the cut end of the internode just inserted into the agar. The agar blocks were on glass plates on wet tissue paper in sandwich boxes enclosed in transparent polythene bags. After 6 to 8 h at 25°C in continuous light the plant material was removed and the agar extracted 3 times at 2°C with enough redistilled 'Analar' methanol to cover it. The pooled extract was evaporated almost to dryness under vacuum, and the residue

dissolved in redistilled 'Analar' methanol, which was subsequently evaporated under OFN to a volume of c. 0.25 ml.

All glassware was rinsed 3 times with redistilled methanol.

C. Chromatographic purification

(i) Paper chromatography

Methanolic extracts were loaded under OFN onto 5 cm wide strips of methanol-washed Whatman 3 MM chromatography paper. The chromatograms were developed, without prior equilibration, for 30 cm beyond the origin in the machine direction, with a descending solvent, in darkness at room temperature. The solvent was either butan-1-ol: glacial acetic acid:distilled water::25:5:11 (acidic solvent) or propan-2-ol:ammonia (d=0.88):distilled water::8:1:1 (basic solvent). All reagents were 'Analar' grade.

Marker chromatograms had 10 µg IAA loaded from 0.1 ml methanol. The position of the IAA on the developed chromatograms was ascertained by scanning the pattern of fluorescence under ultraviolet light or by testing chromogenically (see below, 'Assay procedures'). Often, 0.5 µl 0.1 mmol l^{-1}[1-^{14}C] IAA (c. 9 ng IAA) was added to the origin of extract chromatograms as an internal marker. These chromatograms were run through a chromatogram scanner (see below, 'Radioassay').

The region of each chromatogram which should include IAA was eluted with a descending flow of c. 1 ml redistilled 'Analar' methanol. The eluants were evaporated under OFN to c. 0.25 ml in preparation for further paper chromatography or thin layer chromatography (t.l.c.).

Chromatograms for bioassay were 2 cm wide strips of methanol-washed Whatman 3 MM paper developed for 15 cm in the basic solvent.

(ii) Thin layer chromatography

5 x 20 cm sheets of 0.25 mm thick silica gel on plastic (Polygram Sil G, Macherey-Nagel & Co., Düren, West Germany) were

washed with redistilled methanol. Extracts and marker IAA were loaded under a stream of OFN onto a line 3 cm from the base of a sheet. The chromatograms were developed in ascending solvent to 10 cm from the origin. The solvent was either chloroform:glacial acetic acid::19:1 (acidic solvent) or methyl acetate:propan-2-ol:ammonia (d=0.88):: 9:7:4 (basic solvent). All reagents were of 'Analar' quality.

D. Assay procedures

(i) Ultraviolet scanning

After developing and drying all chromatograms were viewed under 254 nm and 350 nm wavelength ultraviolet light from a Universal UV Lamp Type TL-900 (Camag, Muttenz, Switzerland) and the positions of fluorescent or absorbent areas noted.

(ii) Chromogenic assay

T.l.c. sheets and marker paper chromatograms were sprayed with Ehrlich's reagent (10 g l^{-1} p-dimethylaminobenzaldehyde in 2 mol l^{-1} HCl plus a few drops of ethanol) which was evaporated under a hot air stream from a hair dryer.

(iii) Bioassay

a. Preparation. Developed paper chromatograms were cut into segments, each of which was put into a 2 dram vial (diameter 23 mm, height 35 mm) to which was added 0.75 ml phosphate-phosphate buffer (9.9 mmol l^{-1} KH_2PO_4, 0.1 mmol l^{-1} Na_2HPO_4) or phosphate-citrate buffer (10 mmol l^{-1} K_2HPO_4, 5 mmol l^{-1} citric acid) at pH 5.0. Standard quantities of IAA were introduced to the vials in 1 ml methanol which was evaporated under OFN before buffer was added. Sections of developed chromatography paper were also added.

The vials were left overnight at 2°C in darkness.

b. Incubation. Fruits of Avena sativa variety 'Victory 1', supplied by the General Swedish Seed Co. Ltd., Svalöf, Sweden, were

soaked in running tap water for 4 h, sown in damp vermiculite in the early evening and placed in darkness at 25°C. On the morning of the 4th day after sowing, 5 mm segments were excised from 1 mm behind the tips of 1 to 2 cm long coleoptiles and floated in distilled water for 1 to 3 h. This process was carried out under dim green light. Ten segments were placed in the buffer in each vial and incubated for 24 h at 25°C in darkness.

c. Shadowgraphing. Segments were blotted dry and placed on a glass plate. They were magnified x 5 with a photographic enlarger (Gnome Universal Alpha II) and the shadows recorded on sheets of Ilfobrom IB4 1P photographic paper (Ilford Ltd., Ilford, Essex, U.K.). The shadowgraphs were developed with Ilford Contrast FF developer:: water::1:4, fixed with Kodafix fixer (Kodak Ltd., London, U.K.): water:: 1:4, washed in running water and dried in a Kodak glazing machine (Model 15 TC).

The shadows were measured with a similarly shadowgraphed ruler scale.

(iv) Gas-liquid chromatography

Purified methanolic samples were evaporated under O_2^N, redissolved in 0.25 ml twice redistilled 'Analar' diethyl ether and stored at -8°C. After evaporating the ether, 50 µl bis-trimethylsilyl acetamide (BSA) was added and kept at 40 to 50°C for 1 h. 5 µl samples were introduced by on-column injection into a Pye Series 104 gas chromatograph (Pye Unicam Ltd., Cambridge, U.K.). A 30 g l^{-1} SE-30 column was used at 195°C with a flow of 30 ml min^{-1} N_2 carrier gas.

(v) Mass spectrometry

Purified methanolic samples were introduced by a direct probe into an AEI MS12 mass spectrometer (AEI Scientific Apparatus Ltd., Manchester, U.K.). The samples were scanned with an ionization

voltage of 70 eV, the ion source being at 150°C.

Purified samples in ether were treated with BSA (as for 'Gas-liquid chromatography') and 2 to 5 µl samples introduced onto a 30 g l^{-1} SE-30 column at 195°C in a Pye 104 gas chromatograph with an outlet to an AEI MS 30 single beam mass spectrometer equipped with a membrane separator. The samples were carried in a flow of 30 ml min^{-1} He gas. Chosen peaks of activity from the gas-liquid chromatography (g.l.c.) were introduced to the mass spectrometer and scanned for 10 s $decade^{-1}$. The ionization voltage was 24 eV, the ion source was at 200°C, and the membrane separator at 200°C.

4. RADIOACTIVE EXPERIMENTS

A. Experimental conditions

Plants of dwarf french bean grown in pots were used. One day before the application of radioactivity, 14 to 21 d old plants were transferred to a controlled environment cabinet at 25 ± 2°C in continuous or 16 h d^{-1} light.

B. Radiochemical application

(i) Radiochemicals

[1-^{14}C]IAA and [2-^{14}C]IAA were supplied in benzene:acetone:: 9:1 by the Radiochemical Centre, Amersham, Bucks., U.K. [8-methylene-^{14}C] gibberellic acid ([^{14}C]GA), in solid form, and [U-^{14}C] sucrose, in aqueous solution, were also obtained from the Radiochemical Centre. Solid [2-^{14}C]ABA was donated by F. Hoffmann-La Roche AG.

(ii) Preparation

a. Solutions. The solvent was evaporated from [^{14}C]IAA under O_2-N_2 and the residue was dissolved in 1 drop of absolute ethanol before dilution with distilled water to give stock solutions of 0.1 mmol l^{-1} IAA. Aliquots of these were further diluted to give working solutions

of 2 or 3 μmol l^{-1} IAA.

[^{14}C]GA was dissolved directly in distilled water to give a stock solution of 1 mmol l^{-1} GA.

[^{14}C]ABA was dissolved in a minimal volume of methanol before dilution with distilled water to give a stock solution of 0.1 mmol l^{-1} ABA. An aliquot of this was further diluted to provide a working solution of 20 μmol l^{-1} ABA.

[^{14}C]sucrose was diluted with distilled water to give a stock solution at 10 μmol l^{-1}, further diluted for a working solution at 0.25 or 0.50 μmol l^{-1} sucrose.

All the solutions were stored in darkness, stock solutions (except [^{14}C]GA) at -15°C and working solutions at -8°C. [^{14}C]GA was stored at 2°C.

The chromatographic properties of the working solutions after storage for 2 to 8 months are shown in Figure 4.

b. Agar blocks. Ionagar No. 2 was incorporated into distilled water to give a 30 g l^{-1} agar solution which was autoclaved at a pressure of 20 lbf in^{-2} (138 kPa) at 125°C for 10 min before storage at 2°C. An aliquot of the appropriate working solution was incorporated into an equal volume of molten 30 g l^{-1} agar and moulded into blocks 26 x 20 x 1 mm. These were divided into 8 smaller units each measuring 10.0 x 6.5 x 1.0 mm. Specific activities, concentrations and estimated amounts of the radiochemicals in these blocks are displayed in Table 1 and their chromatographic properties with 2 developing solvent systems in Figure 5.

(iii) Application

The agar blocks were applied, using the method of Hocking et al. (1972), either to the basal end of an expanded primary leaf just to one side of the midrib (Plate 5A), or to the basal end of the terminal

Figure 4 : Chromatography of working solutions of A [1-^{14}C]IAA, B [2-^{14}C]IAA, C[^{14}C]GA, D [^{14}C]ABA.

Solutions stored for A 2, B 2, C 6, D 8 months.

Acidic solvent = butanol:acetic acid:water::25:5:11.

Basic solvent = isopropanol:ammonia:water::8:1:1.

Chromatograms assayed by liquid scintillation spectrometry.

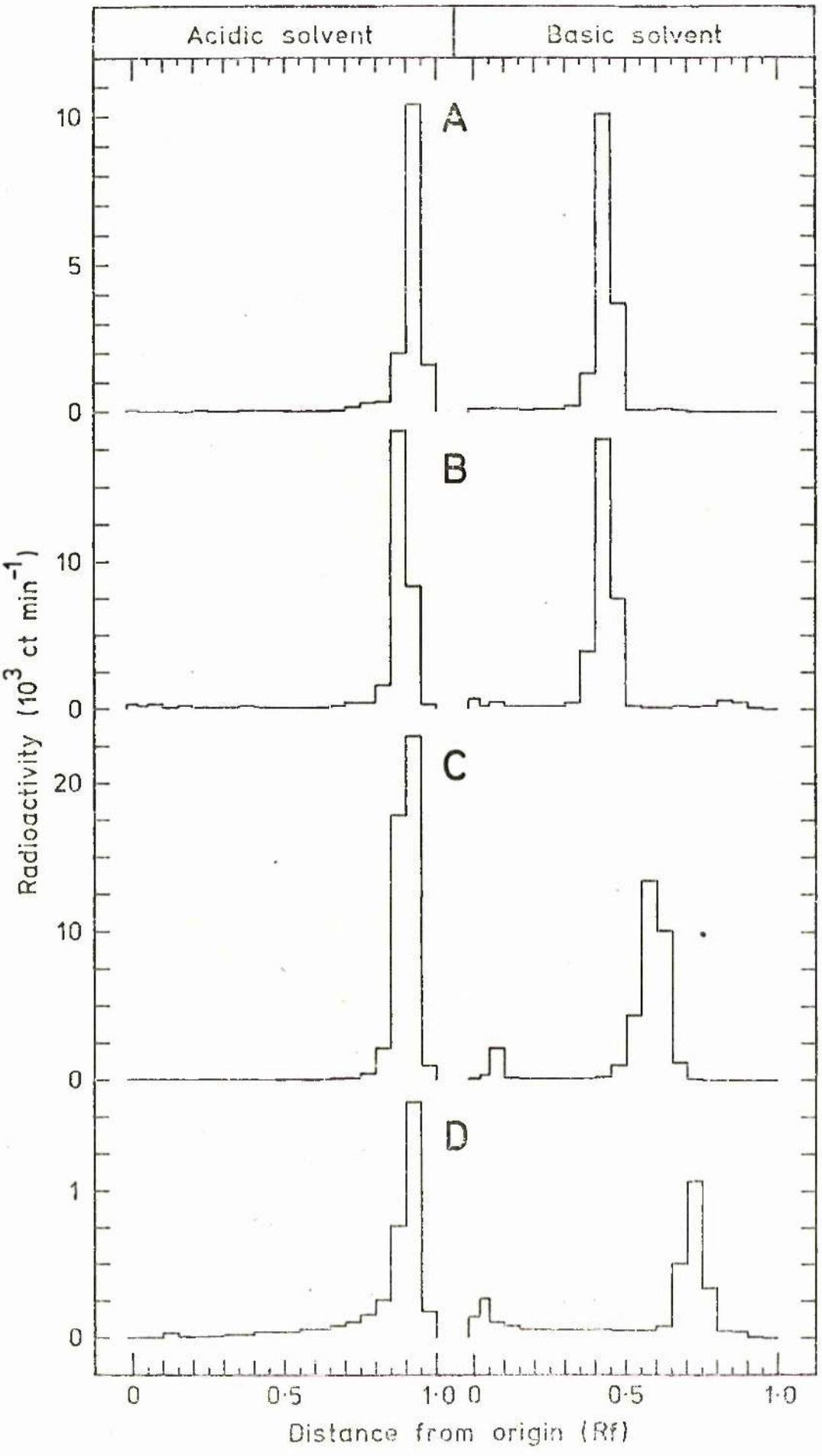

Acidic solvent
Basic solvent
A
B
C
D
10
5
0
10
0
20
10
0
1
0
Radioactivity (10^3 ct min^{-1})
0
0·5
1·0
0
0·5
1·0
Distance from origin (Rf)

Table 1 : Specific activity, concentration and estimated quantity of radiochemicals in agar blocks.

Tracer	Ci mol^{-1}	µmol l^{-1}	d min^{-1} $block^{-1}$	pmol $block^{-1}$
[^{14}C]IAA	57	1.0	8225	65.0
		1.5	12338	97.5
		10.0	82251	650.0
[^{14}C]GA	1.54	50	11111	3250
		500	111111	32500
[^{14}C]ABA	11.94	5	8614	325
		10	17229	650
[^{14}C]sucrose	600	0.125	10823	8.125
		0.250	21645	16.250

Figure 5 : Chromatography of extracts from donor blocks of A [1-^{14}C]IAA, B [2-^{14}C]IAA, C [^{14}C]GA.

Acidic and basic solvents as in Figure 4.

Chromatograms assayed by liquid scintillation spectrometry.

Donor blocks of [^{14}C]ABA have not been assayed, but Hocking (1973) has demonstrated no apparent difference between the radioactivity in stock solutions and donor blocks of [^{14}C]ABA.

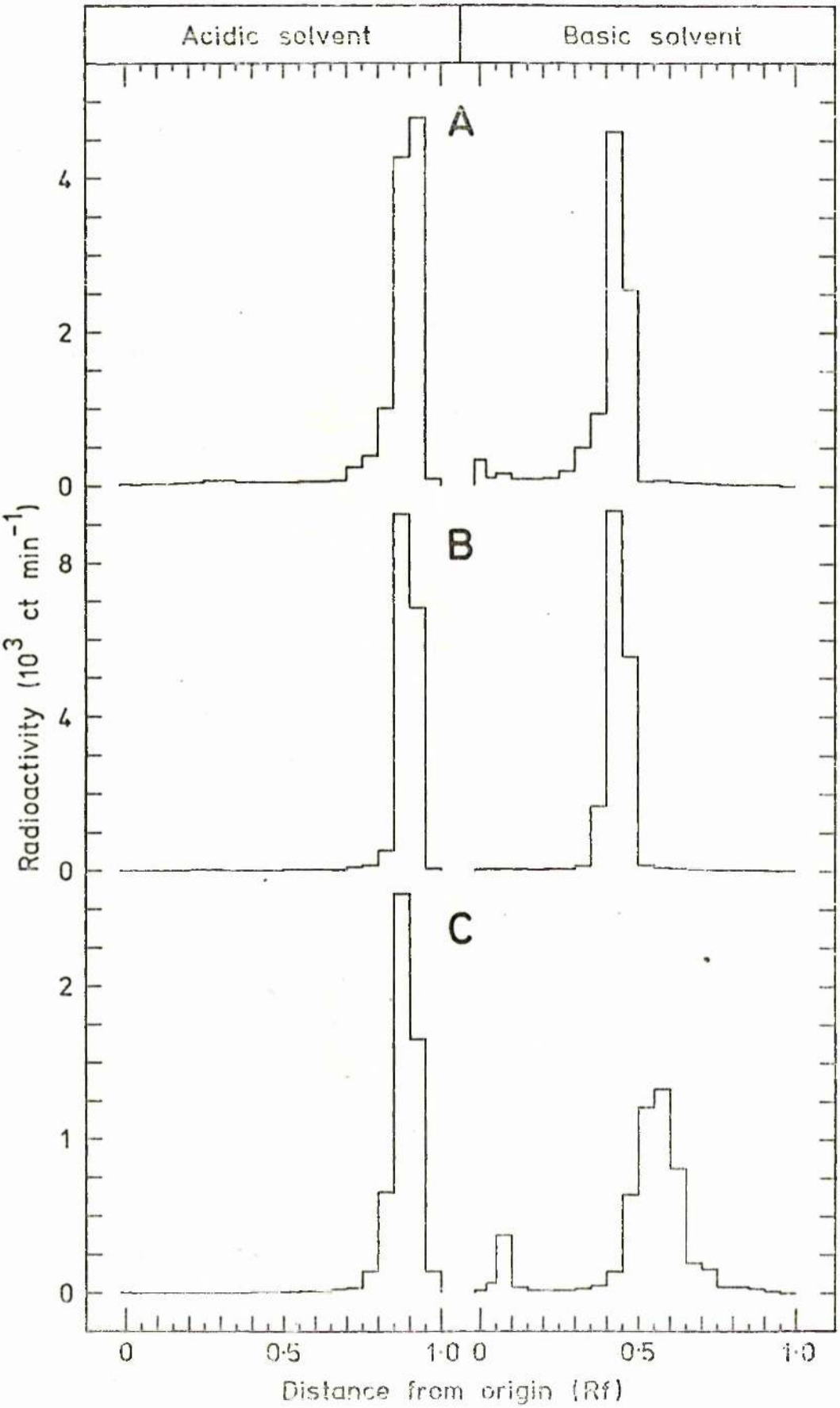

Acidic solvent
Basic solvent
A
B
C
Radioactivity (10^3 ct min^{-1})
0
2
4
0
4
8
0
1
2
0
0·5
1·0
0
0·5
1·0
Distance from origin (Rf)

Plate 5 : Application of an agar block to the lamina of A a mature primary leaf, B a young trifoliate leaf of Ph. vulgaris.

A

B

leaflet of an expanding trifoliate leaf just to one side of the midrib (Plate 5B). The area of application was abraded gently for about 15 s using one fingertip and a paste of water and 'Aloxite' aluminium oxide medium size optical smoothing powder (Carborundum Co. Ltd., Manchester, U.K.). The leaf was rinsed with distilled water and blotted dry before the agar block was pressed onto the abraded area and covered with a 3 cm^2 piece of transparent polythene.

The radioactivity was applied to the plants between 0900 h and 1300 h G.M.T.

C. Harvest

(i) Translocation experiments

After 24 h incubation, plants were cut into small segments which were placed in 2 ml 95% ethanol in scintillation vials. These were stored at 2°C for between 2 and 21 d and the ethanol was evaporated under vacuum prior to radioassay.

(ii) Metabolism experiments

After 24 h incubation, plants were divided into leaf of ^{14}C application (donor leaf), shoot above donor leaf (upper shoot), stem below donor leaf (lower shoot) and roots. The bulked sections were extracted twice with methanol at 2°C. The pooled extract was evaporated under vacuum almost to dryness and the residue dissolved in 2 ml methanol which was evaporated under OFN to c. 0.5 ml. A small aliquot of donor leaf extract and as much of the other extracts as possible were each loaded onto two 5 mm wide strips of Whatman 3 MM chromatography paper. It was discovered that the paper would accept more of the extracts when the origin was streaked a few times with distilled water beforehand. Chromatograms of stock radioactive compounds were prepared both with and without being loaded with a plant extract treated in the same way as the radioactive extracts.

The chromatograms were developed in the machine direction for 30 cm from the origin at room temperature in darkness. One chromatogram of each extract was developed with butan-1-ol:glacial acetic acid:distilled water::25:5:11 (acidic solvent), the other with propan-2-ol:ammonia (d=0.88):distilled water::8:1:1 (basic solvent).

The chromatograms were either cut into sections, each of which was placed in a scintillation vial, or passed through a chromatogram scanner (see below, 'Radioassay'). Peaks of radioactivity from the scanned chromatograms were eluted with c. 1 ml methanol and reapplied to chromatograms for development in another solvent system. These chromatograms were then prepared for scintillation counting.

D. Radioassay

(i) Scintillation counting

Scintillation vials containing plant segments from translocation experiments or chromatogram sections from metabolism experiments were filled with 10 ml toluene (Asschem, Falkirk, Stirlings., U.K.) containing 4 g l^{-1} 2,5-diphenyloxazole, PPO, (Fisons Scientific Apparatus, Loughborough, Leics., U.K.). The vials were stored at 2°C in darkness for 0 to 3 d before assay.

Samples containing plant tissue were assayed in a Tri-Carb liquid scintillation spectrometer (Model 3380) with an absolute activity analyser (Model 544) manufactured by the Packard Instrument Co. Inc., Illinois, U.S.A. Each sample was counted once for either 5 or 10 min, or 10^4 counts. Background radioactivity was determined with vials containing various amounts of material from untreated plants and the background subtraction on the spectrometer was set at 5 ct min^{-1} above the mean ct min^{-1} of the background vials. Other controls were preset by the manufacturers. The spectrometer uses an external standard to calculate quenching and corrects for background radioactivity. The absolute

activity analyser automatically corrects for quenching and radioactivity is calculated as disintegrations min^{-1} (d min^{-1}).

Samples containing chromatogram sections were assayed once for 10 min or 10^4 counts in either a Packard Model 3380 (without an absolute activity analyser) or a Corumatic 200 (Tracerlab G.B. Ltd., Weybridge, Surrey, U.K.) liquid scintillation spectrometer. These spectrometers calculate quenching but only correct for background, estimating radioactivity as counts min^{-1} (ct min^{-1}). The background subtraction was set at 5 ct min^{-1} above the mean ct min^{-1} of vials containing sections of chromatogram from behind the origin. Other controls were preset by the manufacturers.

The spectrometers were calibrated for efficiency at different quench levels by counting variably acetone - and ethanol-quenched samples containing known amounts of [1-^{14}C]n-hexadecane (1.1 µCi g^{-1}). The calibration curves are shown in Figure 6.

Scintillation vials were 20 ml low potassium glass disposable vials with double-screw caps. Between experiments they were boiled twice in water with 'Pyroneg' detergent (Diversey Ltd., Barnet, Herts., U.K.) for at least 1 h each time. They were then rinsed together 3 times with hot water, left soaking overnight and rinsed individually 6 times in hot, running, water. Caps were washed 3 times in warm water and 'Pyroneg', and rinsed 3 times in warm water.

(ii) Chromatogram scanning

Intact chromatograms were assayed using a chromatogram scanner with a strip chromatogram scanning attachment RCMS-3 (Panax Equipment Ltd., Redhill, Surrey, U.K.). A flow of propane:argon:: 1:49 (British Oxygen Co. Ltd., London, U.K.) was passed through the detection chamber at a pressure of 34.5 kPa (5 lbf in^{-2}). The chromatograms were passed through at a speed of 60 cm h^{-1}. The aperture was set at 3 mm width, dead geiger time at 200 µs, time constant at

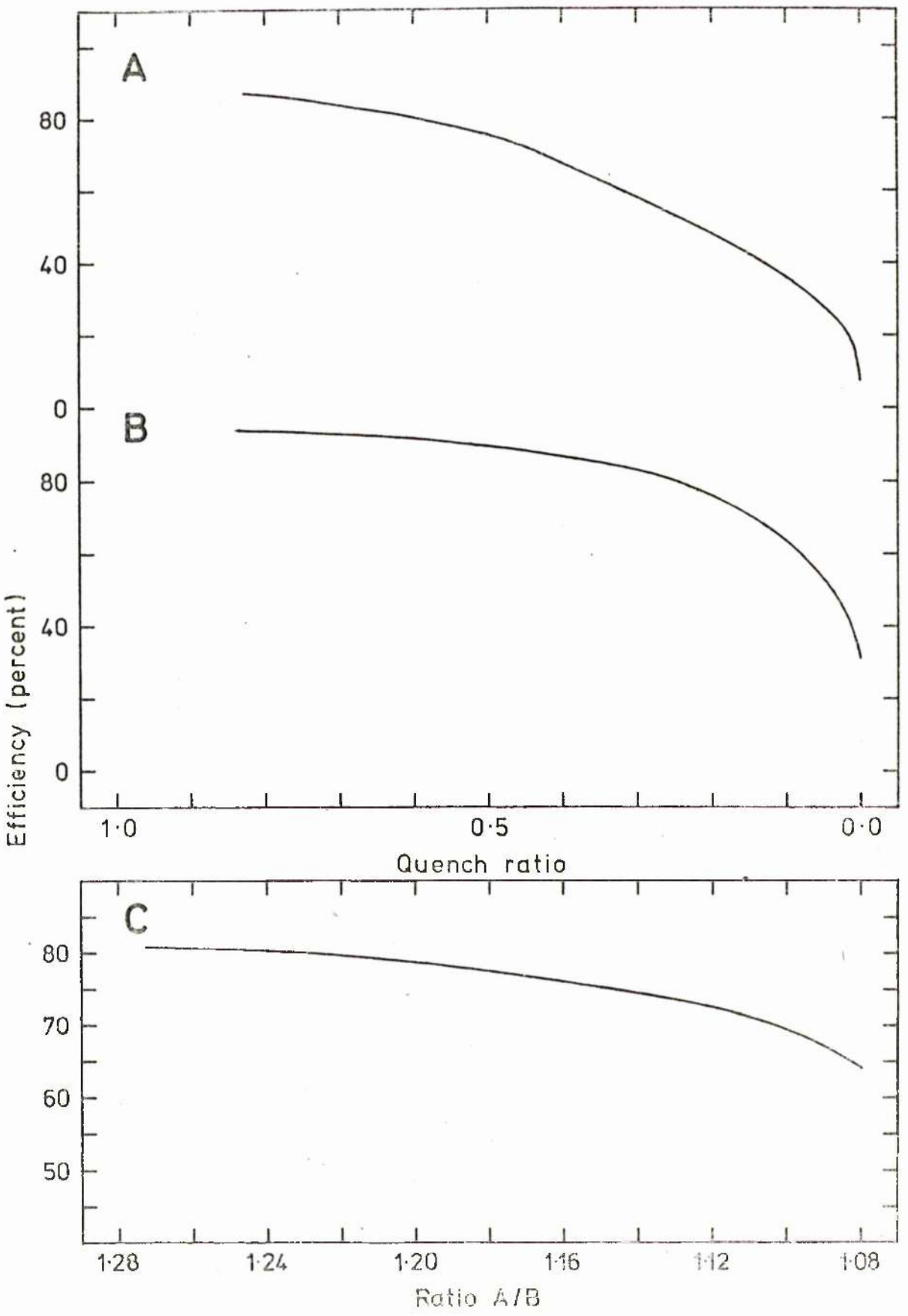
Figure 6 : Calibration curves for efficiency of A Packard Tri-Carb (with absolute activity analyser), B Packard Tri-Carb (without absolute activity analyser), C Tracerlab Corumatic 200 liquid scintillation spectrometers at different levels of quenching in samples.

$$\text{Efficiency} = \frac{\text{Ct min}^{-1} \text{ detected in sample}}{\text{D min}^{-1} \text{ supplied in sample}} \times 100$$

$$\text{Quench ratio} = \frac{\text{Ct min}^{-1} \text{ detected in external standard}}{\text{D min}^{-1} \text{ supplied in external standard}}$$

$$\text{Ratio A/B} = \frac{\text{Ct min}^{-1} \text{ detected in external standard in Channel A}}{\text{Ct min}^{-1} \text{ detected in external standard in Channel B}}$$

Channel A counts through sample bottle, Channel B by-passes sample bottle.


A
B
C
80
40
0
80
40
0
Efficiency (percent)
1·0
0·5
0·0
Quench ratio
80
70
60
50
1·28
1·24
1·20
1·16
1·12
1·08
Ratio A/B

3 s and range at 3 s^{-1}. Other controls were preset by the manufacturers.

No compensation was made for quenching or background and a trace of percentage radioactivity was produced on a 'Servoscribe' RE 511 .20 potentiometric chart recorder.

5. BIOMETRY

A. Experimental design

Experiments involving the measurement of growth were performed using 10 plants in each treatment. Within the controlled environment cabinet plants were arranged at random. Because the design of heating and lighting in the glasshouse (Figure 7A) probably caused gradients of temperature and light intensity across the bench, in most experiments in the glasshouse the plants were arranged in 'organized' rows such that each treatment was evenly distributed in relation to these factors (Figure 7B).

In experiments involving the translocation and metabolism of radioactive chemicals a variable number of plants in each treatment were situated at random within a controlled environment cabinet.

All but a few experiments were performed on at least 3 occasions.

B. Statistical analysis

Standard error

The standard error of the mean value for a series of observations was calculated using an Olivetti programma 101 desk top computer from the formula

$$\text{Standard error} = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n\,(n-1)}}$$

where X = value of each individual observation and n = number of observations (Snedecor and Cochran, 1967). Standard errors were calculated

Figure 7 : Diagrammatic representation of a bench in the glasshouse showing A position of high pressure mercury vapour lamps and heating pipes, B layout of treatments in 'organized' rows.

A : H = heating pipes, L = lamp.

B : 2 = 2 treatments A,B

3 = 3 treatments A,B,C

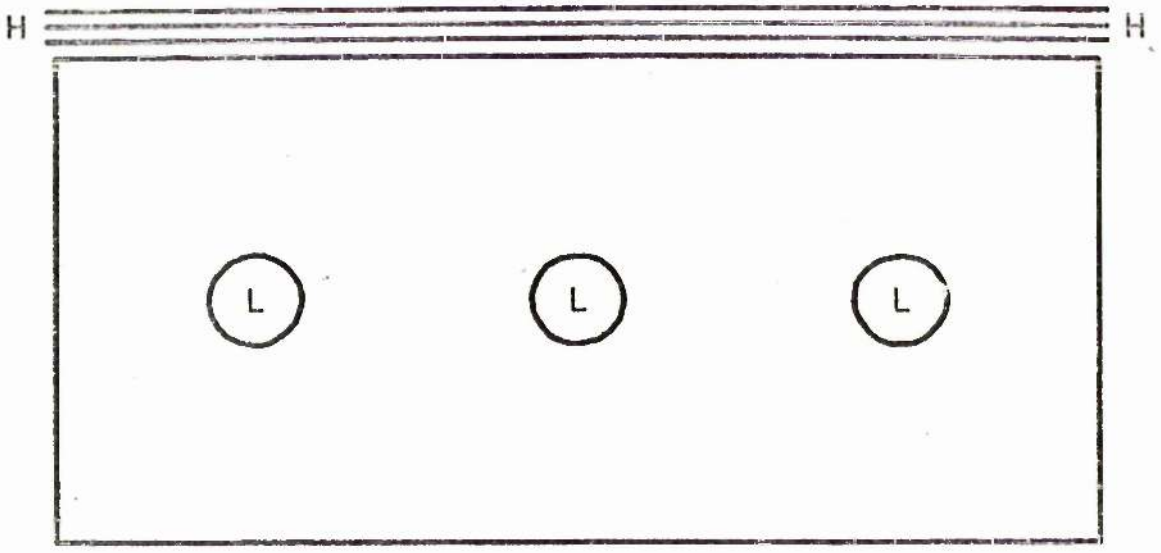
4 = 4 treatments A,B,C,D

5 = 5 treatments A,B,C,D,E

etc.

Each letter represents a single plant of that treatment. Position of experiment on bench in the longitudinal direction selected at random.

A



B

2	3	4	5	etc.
A B	A B C	A B C D	A B C D E	
B A	C A B	D A B C	E A B C D	
A B	B C A	C D A B	D E A B C	
B A	A B C	B C D A	C D E A B	
A B	C A B	A B C D	B C D E A	
B A	B C A	D A B C	A B C D E	
A B	A B C	C D A B	E A B C D	
B A	C A B	B C D A	D E A B C	
A B	B C A	A B C D	C D E A B	
B A	A B C	D A B C	B C D E A	

for growth data, bioassay data and quantitative data on the distribution of radioactivity within plants. In tables the standard error (S.E.) was presented as $\pm$ S.E. after the mean; in graphs and histograms as a vertical bar above and/or below the point of the mean.

C. Presentation of results

Growth data are presented, either in graphic or tabular form, as mean values, at a particular time, of absolute length measurements for each treatment of a single experiment. When essentially the same results were obtained on separate occasions, the results of only one experiment are presented. Otherwise the results of each replicate experiment are given separately.

Bioassay results are graphed as a histogram of the mean of 10 coleoptile segments for each section of the chromatogram. G.l.c. traces were copied onto a graph. Peak heights of mass spectra were measured and presented as a percentage of the highest peak, which was assigned 100%; peaks appearing in background spectra were omitted.

Quantitative estimates of radioactivity in each part of the plant are presented in tabular form as mean values of either d min^{-1} or percentage of the total radioactivity in the plant. Results from individual experiments are given separately. Radioactivity on chromatograms is plotted as ct min^{-1} in histogram form or copied from a chromatogram scanner trace onto a graph.

Histological examinations were recorded photographically.

EXPERIMENTAL RESULTS

1. CHARACTERIZATION OF CORRELATIVE PHENOMENA

As a basis for further investigation the characteristics of apical dominance in french bean plants have been examined. In particular, the influence of various parts of the plant body upon the growth of the lateral buds has been analysed, using surgical techniques.

A. Growth patterns

The initial experiments were designed to elucidate the patterns of growth found in young bean plants. The growth of intact seedlings has been followed and the characteristics of growth of a number of lateral buds have been investigated on decapitated and defoliated plants.

(i) Intact plants

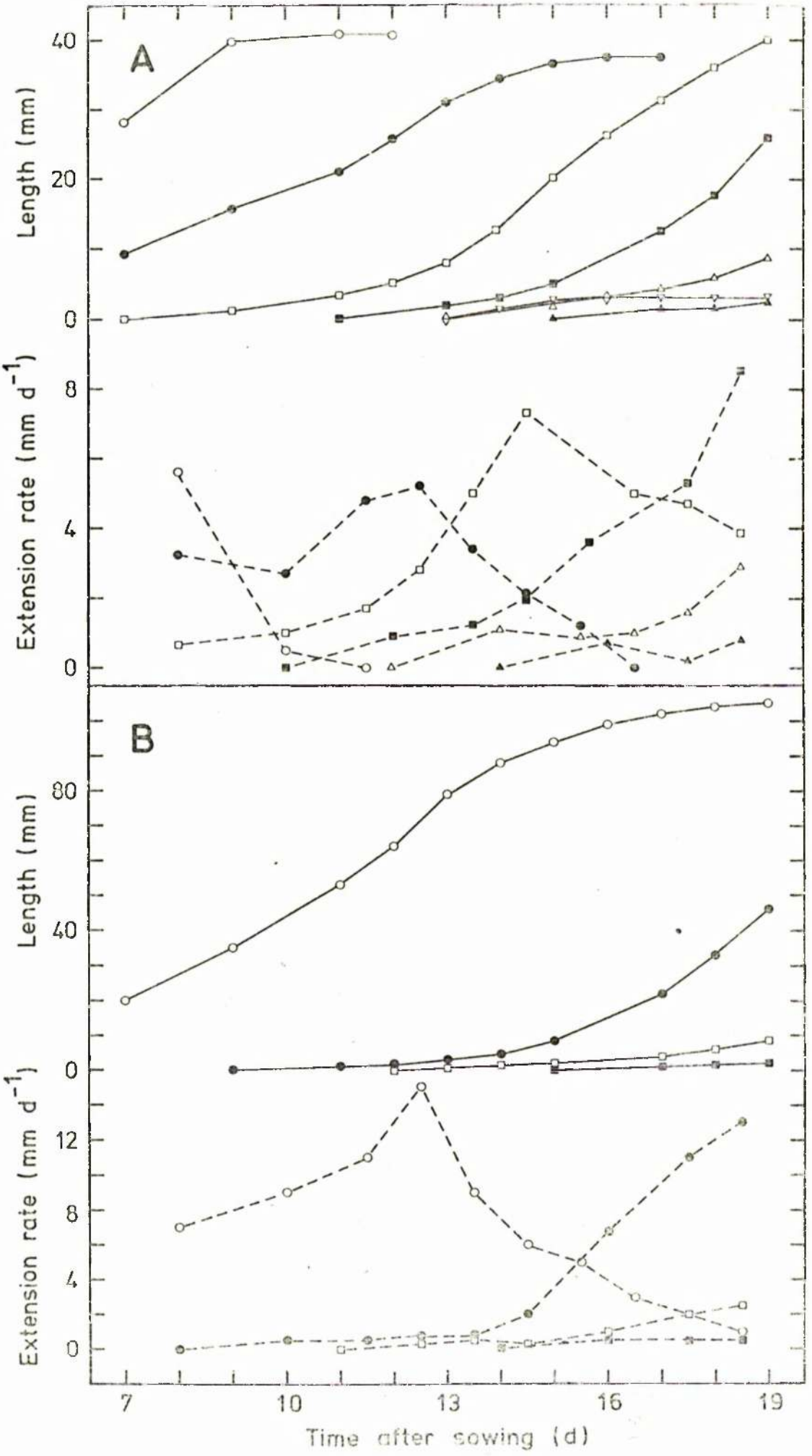
The pattern of growth of intact bean seedlings has been studied by taking measurements of the length and the dry weight of all their parts over a period of 19 d from sowing (Figure 8).

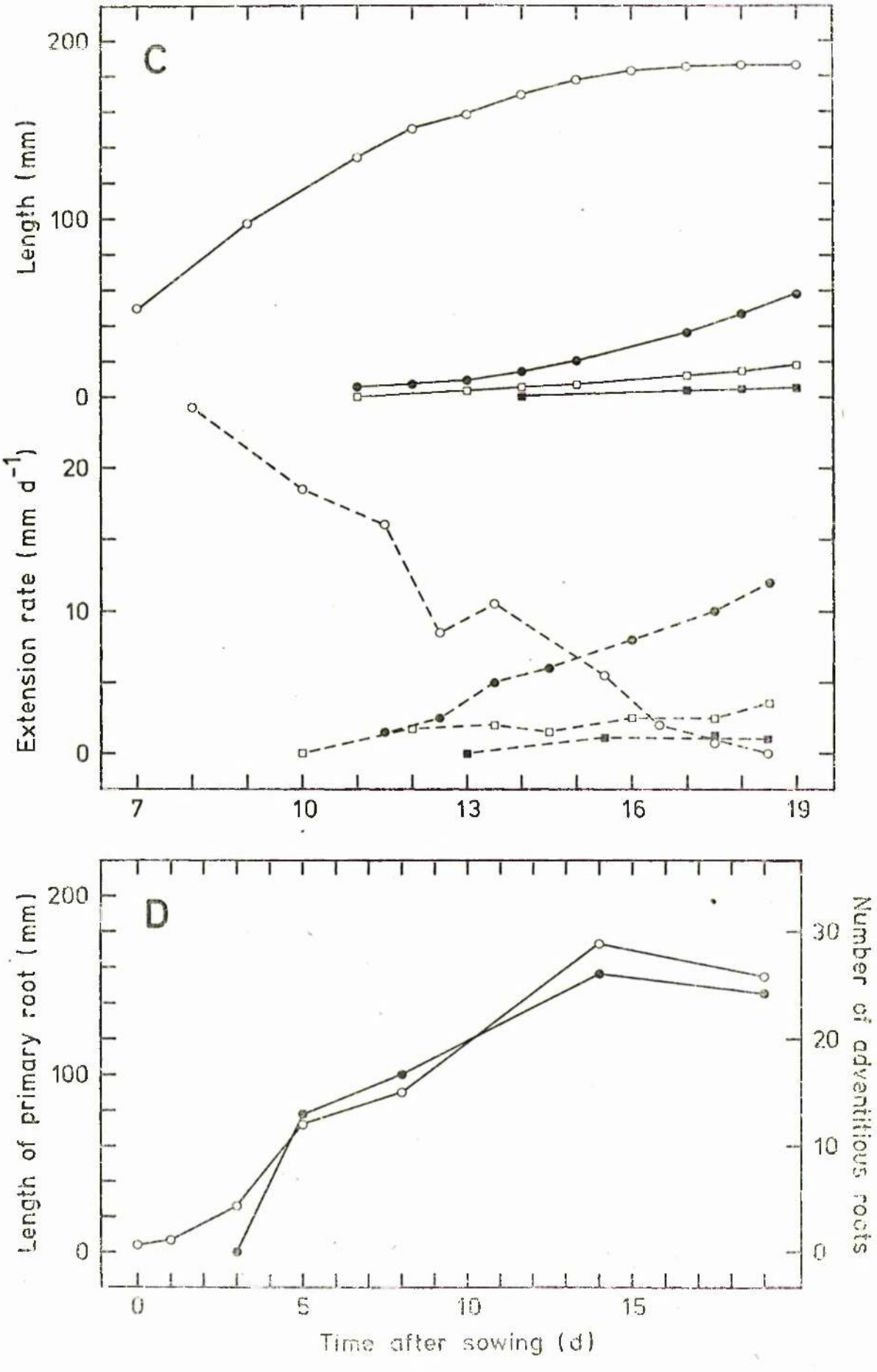
Although up to 4 internodes were growing at one time, the greatest extension rate of the stem passed sequentially from the lowest internode upwards (Figure 8A). The growth rate of any internode was declining before the internode above reached its period of greatest extension. Similarly, the extension rate of both the petiole and the lamina of the first trifoliate leaf increased when that of the primary leaf petiole and lamina decreased, although the second and third trifoliate leaves were also growing slightly (Figure 8B,C). Generally, the growth of each leaf closely followed that of the internode immediately below it. From 12 to 16 d after sowing the primary leaf axillary buds increased slightly in length but they ceased growth over the next 3 d (Figure 8A): they were, therefore, developing while the internode

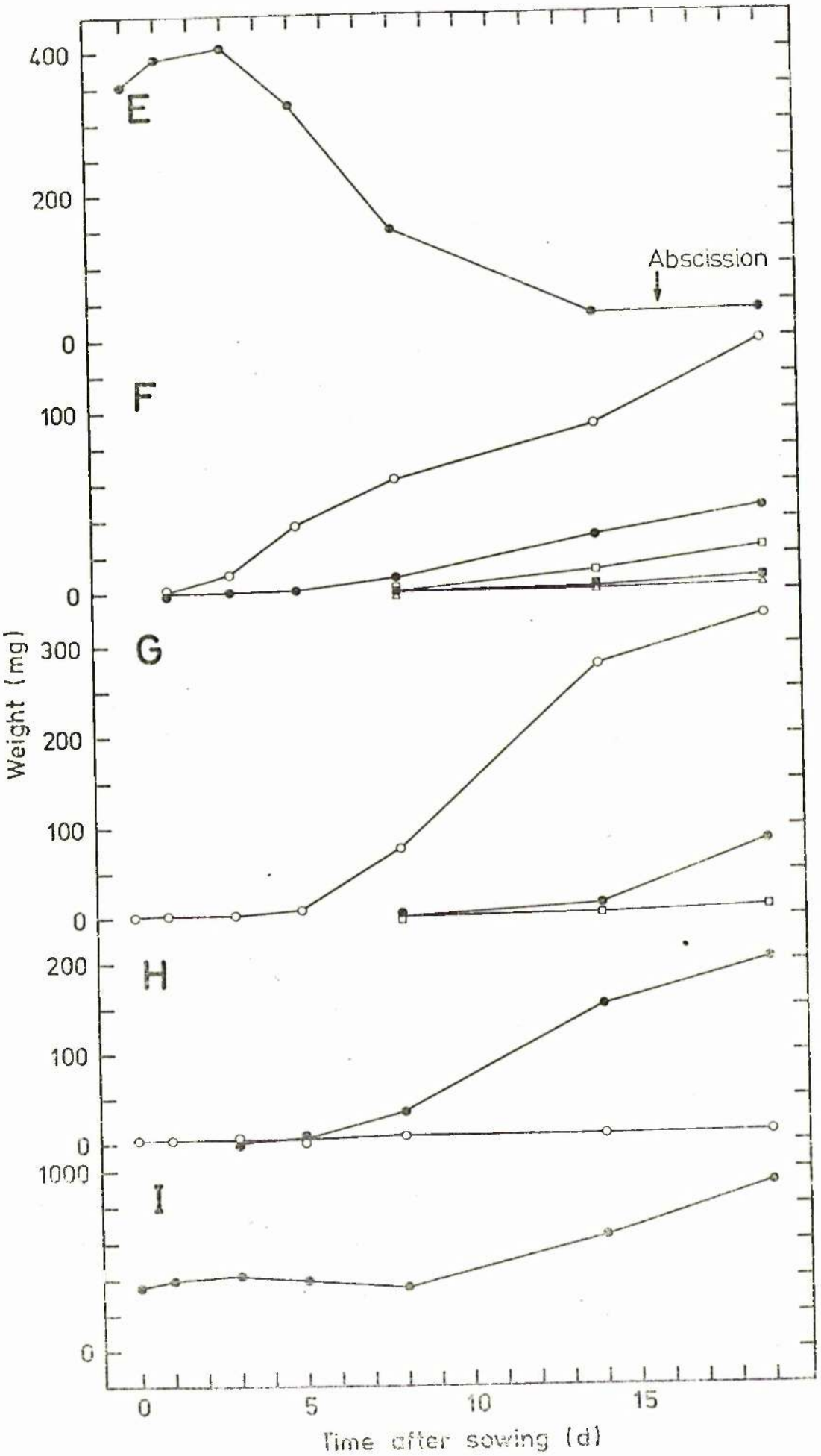
Figure 8 : Growth in length of A stem internodes, B petioles, C laminae expressed as length (———) and extension rate (---); increase in length and number of D roots; change in weight of E cotyledons, F stem internodes, G leaves, H roots, I whole plant.

18.3.71 : Ph. vulgaris : glasshouse : randomized : A,B,C = means from successive measurements of 10 plants; D,E,F,G,H,I = means from destructive sampling of 10 plants at each time point : length of hypocotyl measured from level of compost surface to 1st node; length of lamina of terminal leaflet measured for trifoliate leaves : key:-

Symbol	Stem (A,F)	Leaves (B,C,G)	Roots (D,H)
○	Hypocotyl	Primary	Primary
●	1st internode	1st trifoliate	Others
□	2nd internode	2nd trifoliate	
■	3rd internode	3rd trifoliate	
△	4th internode		
▲	5th internode		
▽	Primary leaf axillary buds		


A
B
Length (mm)
Extension rate (mm d^{-1})
Time after sowing (d)
0
20
40
4
8
80
12
7
10
13
16
19


C
Length (mm)
Extension rate (mm d^{-1})
200
100
0
20
10
0
7
10
13
16
19
D
Length of primary root (mm)
Number of adventitious roots
200
100
0
30
20
10
0
0
5
10
15
Time after sowing (d)


E
F
G
H
I
400
200
0
100
0
300
200
100
0
200
100
0
1000
0
Abscission
Weight (mg)
0
5
10
15
Time after sowing (d)

and leaf immediately above them were also extending. Both the length of the primary root and the number of adventitious roots increased from the 3rd day after sowing and appeared to have reached a maximum by the 15th day (Figure 8D). The lateral roots were too numerous to count but appeared to increase steadily in number and length after the 5th day.

The dry weight of the cotyledons decreased rapidly from the 3rd to the 14th day after sowing (Figure 8E), during which time the hypocotyl, epicotyl and primary leaves were increasing in weight (Figure 8F,G). The cotyledons were almost depleted by the 14th day, by which time the primary leaves were almost fully extended (Figure 8A). The dry weight accumulation of internodes and leaves resembled their sequential pattern of extension growth, although the hypocotyl and epicotyl continued to increase in weight after their elongation had ceased. The latter increase in weight was also manifest as an increase in thickness of the stem. The weight of the roots increased from the 5th day onwards (Figure 8H); the greater weight increment was shown by the lateral and adventitious roots rather than the tap root.

The total weight of the plant remained fairly steady until the 8th day after sowing, and increased thereafter (Figure 8I). The early growth of the seedling, therefore, appears to involve a transfer of material from the cotyledons to the hypocotyl, epicotyl, primary leaves and roots. By the 14th day the cotyledons were exhausted and the plants had increased in dry weight. At this time the primary leaves were almost fully grown and probably photosynthetically functional; the root system was also well developed.

(ii) Decapitated plants

Removal of the upper shoot by excision through the 3rd node had little effect on the total dry weight of the plant 8 d later

(Table 2). This decapitation caused a redistribution of dry matter, partially into the primary leaf axillary buds but also, apparently, into the stem and primary leaves. The outgrowth on decapitated plants of the lateral buds in the axils of the primary leaves was also evident as an increase in their length (see also Plate 6; Figure 9). Decapitation, however, did not greatly affect the elongation of stem or leaf other than preventing further extension of the 2nd internode. The growth of the tap root appeared to be curtailed by the removal of the upper shoot, although this effect was only partially confirmed in one of two repeat experiments.

The outgrowth of the primary leaf axillary buds on decapitated plants was evident as an increase in both the length of their 1st internodes and their total length (Figure 9A,B). The increases in 1st internode length and in total length were similar over the first 3 or 4 d after decapitation, indicating that the initial outgrowth of the stem of these buds occurred in the 1st internode. For this reason in subsequent experiments the 1st internode of each bud, which can be measured with greater accuracy than the total length, was followed over 7 d. Their total lengths were measured at the end of each experiment but never gave a pattern of results differing from that obtained with 1st internode measurements and have not, therefore, been presented.

The buds on the primary leaf node are usually of unequal length (Plate 1; Figure 2) and upon their outgrowth after decapitation of the plant a difference in length remains. Thus, the 1st internode length and the total length of the initially longer bud on intact and decapitated plants were greater throughout the experiment than the equivalent lengths of the shorter bud (Figure 9A,B). These two buds, however, always exhibited similar qualitative responses and their

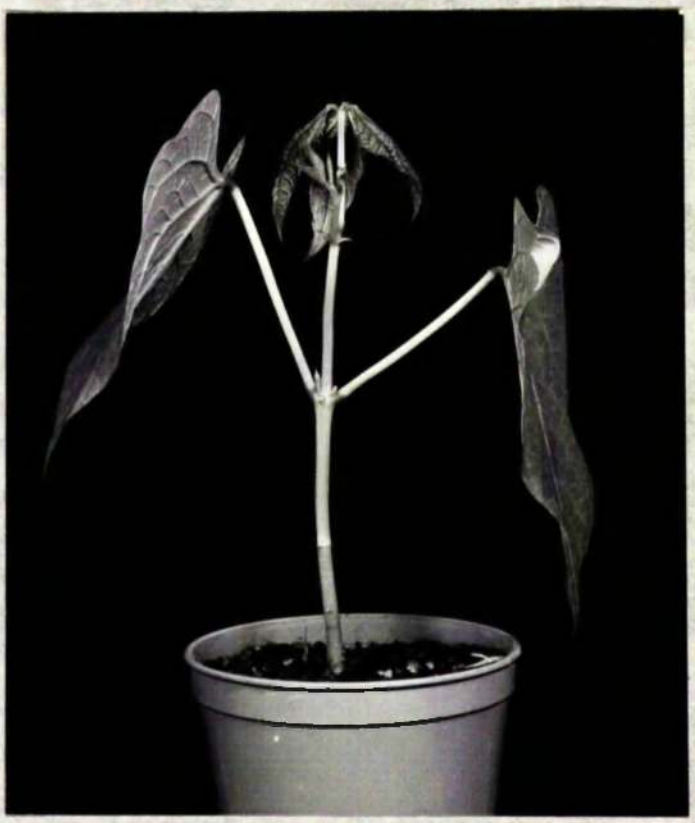
Table 2 : Dry weights and lengths of segments of intact and decapitated plants.

Segment	Dry weight (mg)		Length (mm)	
	Intact	Decapitated	Intact	Decapitated
Upper shoot	58.5 ± 5.3			
2nd internode	9.0 ± 1.0	0.1	39.5 ± 1.4	2.1 ± 0.4
Primary leaf axillary buds	0.5	18.6 ± 3.3	0.3	17.5 ± 1.7
Primary leaf laminae	157.7 ± 11.0	178.9 ± 7.7	155.9 ± 3.7	159.2 ± 4.7
Primary leaf petioles	14.2 ± 0.9	18.7 ± 0.7	72.1 ± 2.1	75.8 ± 2.0
Epicotyl	18.3 ± 1.2	21.4 ± 0.9	40.3 ± 2.0	40.7 ± 1.9
Cotyledons	50.5 ± 1.8	50.4 ± 4.7		
Hypocotyl	75.4 ± 3.7	88.7 ± 2.1	84.4 ± 2.3	80.7 ± 2.3
Roots	168.6 ± 16.1	161.6 ± 10.9		
Total	552.7 ± 31.3	538.4 ± 22.8		

23.2.71 : Ph. vulgaris : glasshouse : decapitation at 3rd node on 7th day after sowing; harvest on 14th day after sowing : 10 plants/ treatment; randomized : results = mean ± S.E.; confirmed once.

Plate 6 : 21 d old plants of Ph. vulgaris A intact, B decapitated through 2nd internode 3 d previously.

A



B

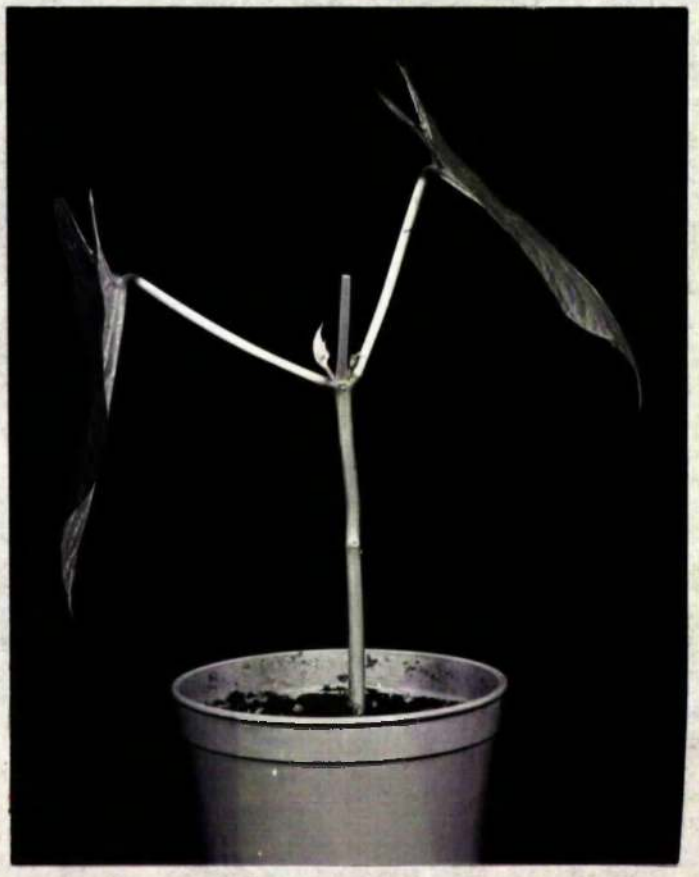
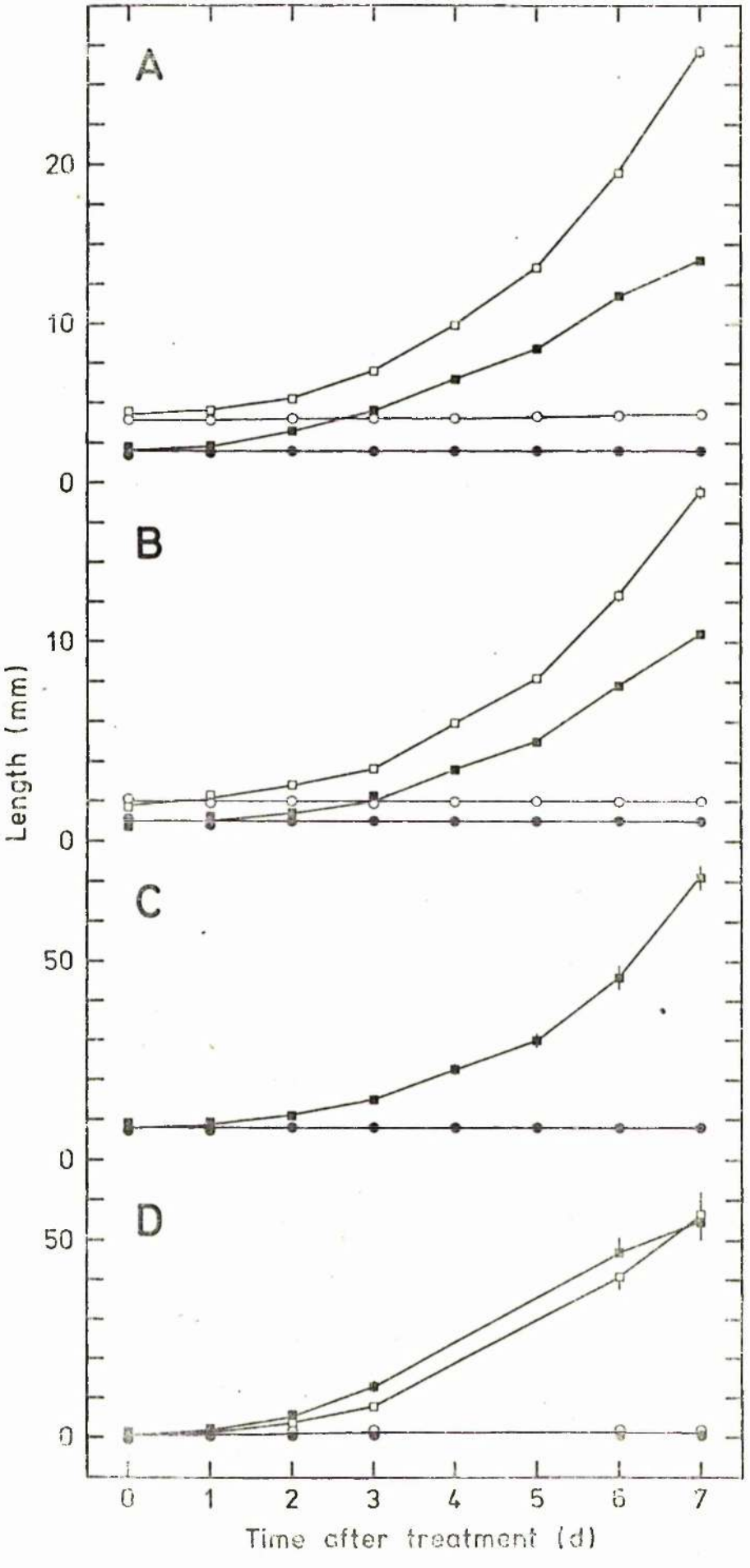


Figure 9 : Growth of primary leaf axillary buds on intact (● ○) and decapitated (■ □) plants of Ph. vulgaris.

A,B : 25.1.71 : 20 d old plants : glasshouse : decapitation through 2nd internode : 10 plants/treatment; randomized : points = mean ± S.E. of length of 1st internode (closed symbols) or total length (open symbols) of A longer, B shorter primary leaf axillary buds.

C : as above but points = mean ± S.E. of combined lengths of terminal leaflets of 1st trifoliate leaves on both primary leaf axillary buds.

D : 23.2.71 : 14 d old plants : decapitation through 2nd internode : plants in glasshouse (open symbols) or controlled environment cabinet (closed symbols) : 10 plants/treatment; randomized : points = mean ± S.E. of combined lengths of 1st internodes of both primary leaf axillary buds.


A
B
C
D
20
10
0
10
0
50
0
50
0
Length (mm)
0
1
2
3
4
5
6
7
Time after treatment (d)

lengths were, therefore, added together for presentation of the results of most subsequent experiments. Bud dry weights never gave a pattern of results differing essentially from that shown by length measurements, but have been presented for those experiments in which they were assessed.

The outgrowth of the buds on decapitated plants also involved extension of their 1st trifoliate leaves (Figure 9C). The growth of the primary leaf axillary buds was similar under both glasshouse and controlled environment cabinet conditions (Figure 9D).

Plants decapitated through successive nodes from 1 to 6 showed different patterns of bud outgrowth (Figure 10). The buds in the cotyledon axils extended slowly on plants decapitated just below the 2nd node but much more quickly on plants decapitated just above the 2nd node with their primary leaf axillary buds removed (Figure 10A). In the presence of the primary leaf axillary buds or part of the upper shoot above the primary leaf node no growth of the cotyledon axillary buds was recorded. Presumably the primary leaves improved the growth of the cotyledon axillary buds by providing nutrients. Both the longer and the shorter primary leaf axillary buds showed strong outgrowth on plants decapitated at the 3rd node, less on plants decapitated at the 4th node and none on plants decapitated higher up (Figure 10B,C). The 1st trifoliate leaf axillary bud grew out strongly on plants decapitated at the 4th node, less on those decapitated at the 5th node and very slowly on those decapitated at the 6th node or left intact (Figure 10D). The 2nd trifoliate leaf axillary bud extended slowly on plants decapitated at the 5th node and even more slowly on those decapitated at the 6th node or left intact (Figure 10E).

The same results presented in a different fashion (Figure 10F to J) indicate that on intact plants and on those decapitated at the 6th node the buds in the axils of the 1st, 2nd and 3rd trifoliate leaves

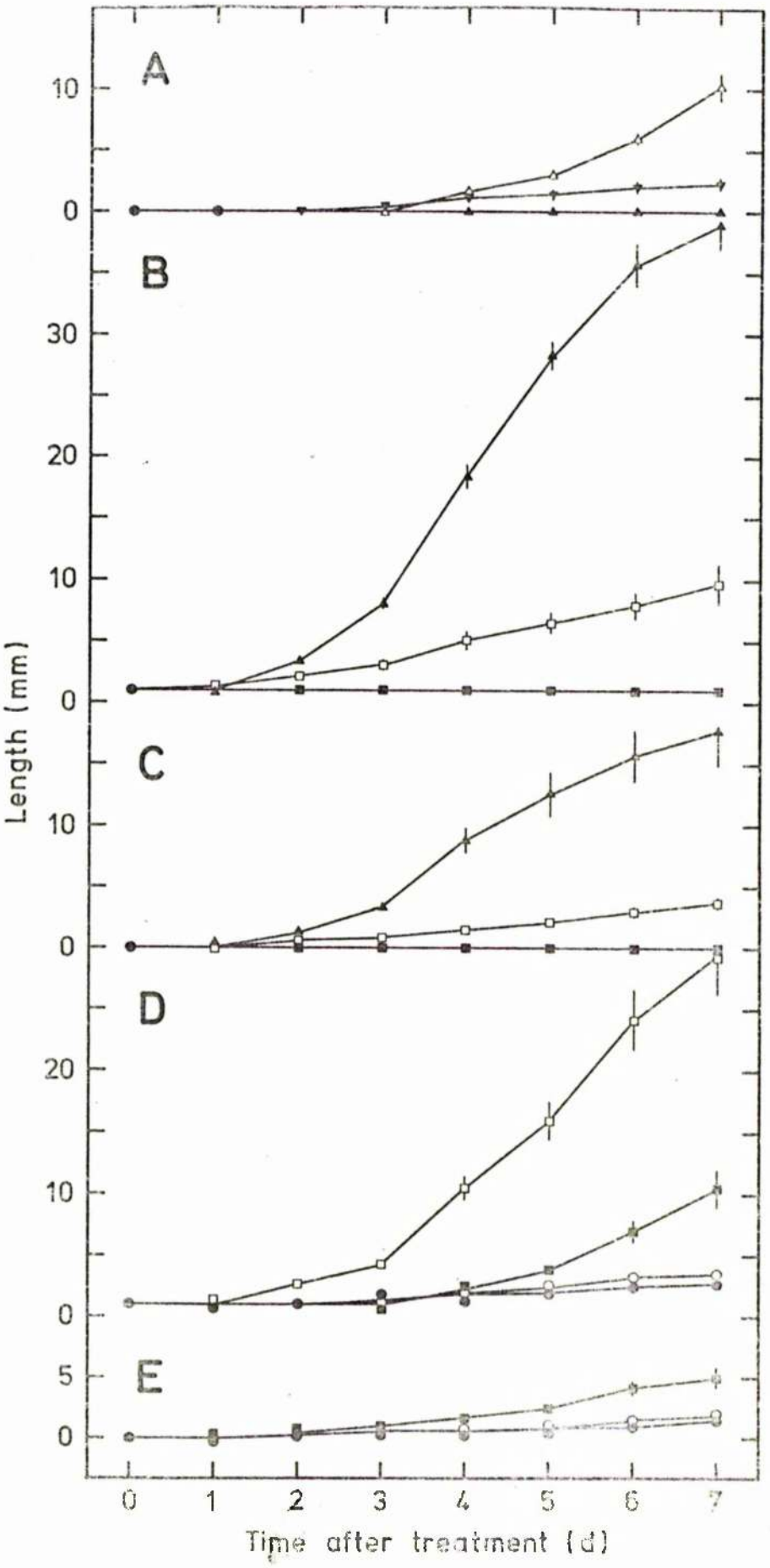
Figure 10 : Growth of lateral buds on plants decapitated at different nodes; A both cotyledon axillary buds, B longer primary leaf axillary bud, C shorter primary leaf axillary bud, D 1st trifoliate leaf axillary bud, E 2nd trifoliate leaf axillary bud; plants F intact, G decapitated at 6th node, H decapitated at 5th node, I decapitated at 4th node, J decapitated at 3rd node (_____) or just above 2nd node with primary leaf axillary buds present (---) or excised (····).

11.3.71 : Ph. vulgaris; 16 d old : controlled environment cabinet : decapitation through a particular node removed the leaf and bud at that node as well as the shoot above : 10 plants/treatment; randomized : points = mean ± S.E. of length of 1st internode of bud : results confirmed in 2 further experiments : key:-

Symbol	Node of decapitation (A,B,C,D,E)	Measured axillary bud (F,G,H,I,J)
●	None	Cotyledon (both) ..
○	6th	Longer primary leaf ..
■	5th	Shorter primary leaf ..
□	4th	1st trifoliate leaf ..
▲	3rd	2nd trifoliate leaf ..
△	Above 2nd (primary leaf axillary buds removed)	3rd trifoliate leaf ..
▼	2nd	

Mean dry weight (mg) of axillary buds on the 7th day after treatment:-

Node of decapitation	None	6th	5th	4th	3rd	2nd
Cotyledon (both) ..	0.0	0.0	0.1	0.2	0.2	0.4
Longer primary leaf ..	0.3	0.4	0.7	7.7	33.2	
Shorter primary leaf ..	0.1	0.2	0.3	1.7	9.9	
1st trifoliate leaf ..	0.9	1.7	7.9	31.8		
2nd trifoliate leaf ..	0.6	1.1	3.7			
3rd trifoliate leaf ..	1.0	1.4				
Total	2.9	4.8	12.7	41.1	43.3	0.4


A
B
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Length (mm)
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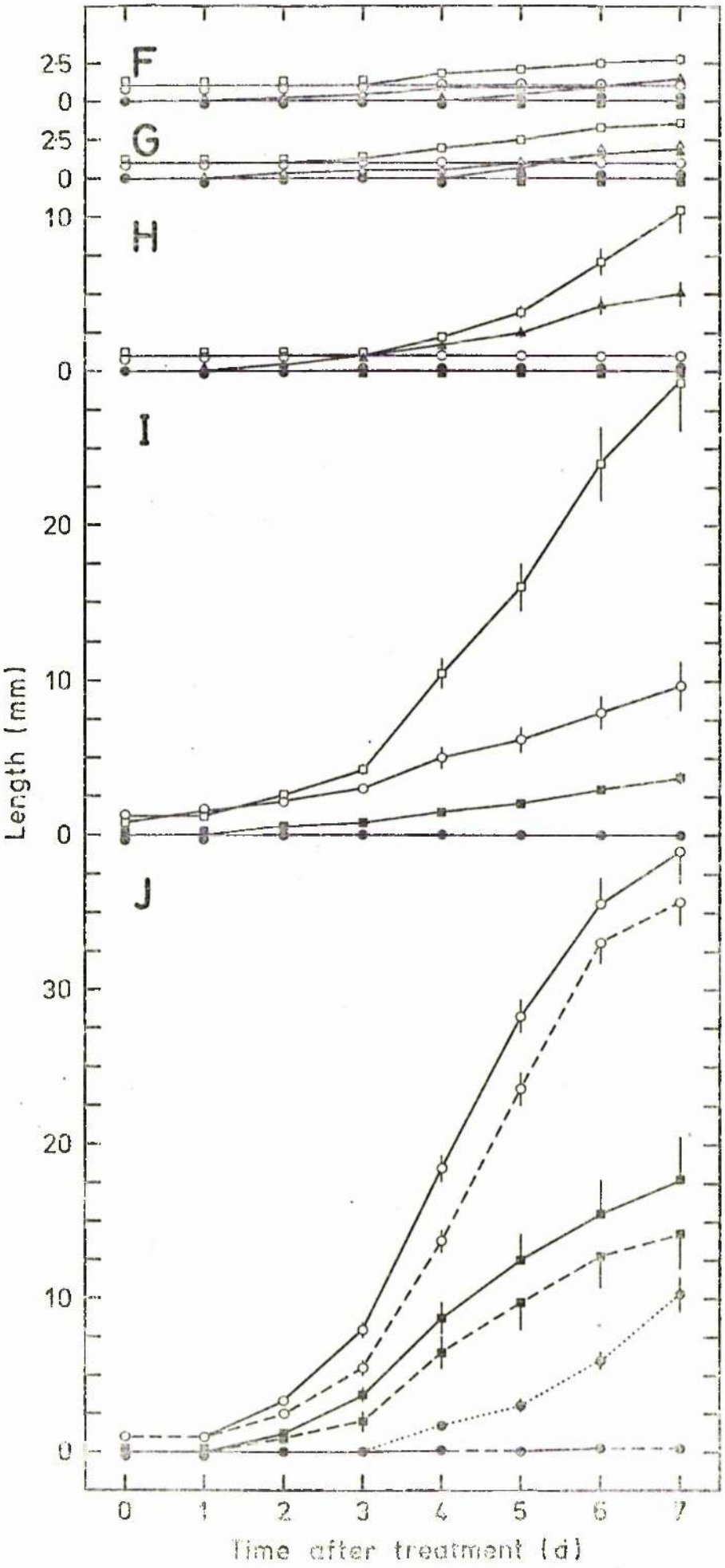
7
Time after treatment (d)


F
G
H
I
J
25
0
25
0
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0
20
10
0
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20
10
0
Length (mm)
0
1
2
3
4
5
6
7
Time after treatment (d)

were growing slowly while those in the primary leaf and cotyledon axils were not detectably extending (Figure 10F,G). On plants with the shoot removed at the 5th node considerable growth was shown by the 1st trifoliate leaf axillary bud and some by the 2nd trifoliate leaf axillary bud (Figure 10H). Decapitation at the 4th node led to vigorous outgrowth of the 1st trifoliate leaf axillary bud and marked extension of the two buds in the primary leaf axils (Figure 10I). The primary leaf axillary buds grew vigorously on plants decapitated at the 3rd node or just above the 2nd node, and subordinated the cotyledon axillary buds which only grew when the primary leaf axillary buds were removed (Figure 10J). The differences in bud length on all plants were reflected by the bud dry weights.

The 1st trifoliate leaf axillary bud, when present, grew out to a greater extent than the other buds in all treatments. The 2nd trifoliate leaf axillary bud, although extending slowly in intact plants, responded only slightly to removal of the shoot above it. The primary leaf axillary buds, conversely, exhibited no detectable growth on intact plants but extended rapidly when the shoot was removed one or two nodes above them. The cotyledon axillary buds scarcely grew out unless all growing tissues above them were removed. Apart from the 2nd and 3rd trifoliate leaf axillary buds, which may have been immature, any bud on these plants showed greater growth than the buds below. Bud growth was clearly influenced by the presence of the upper shoot.

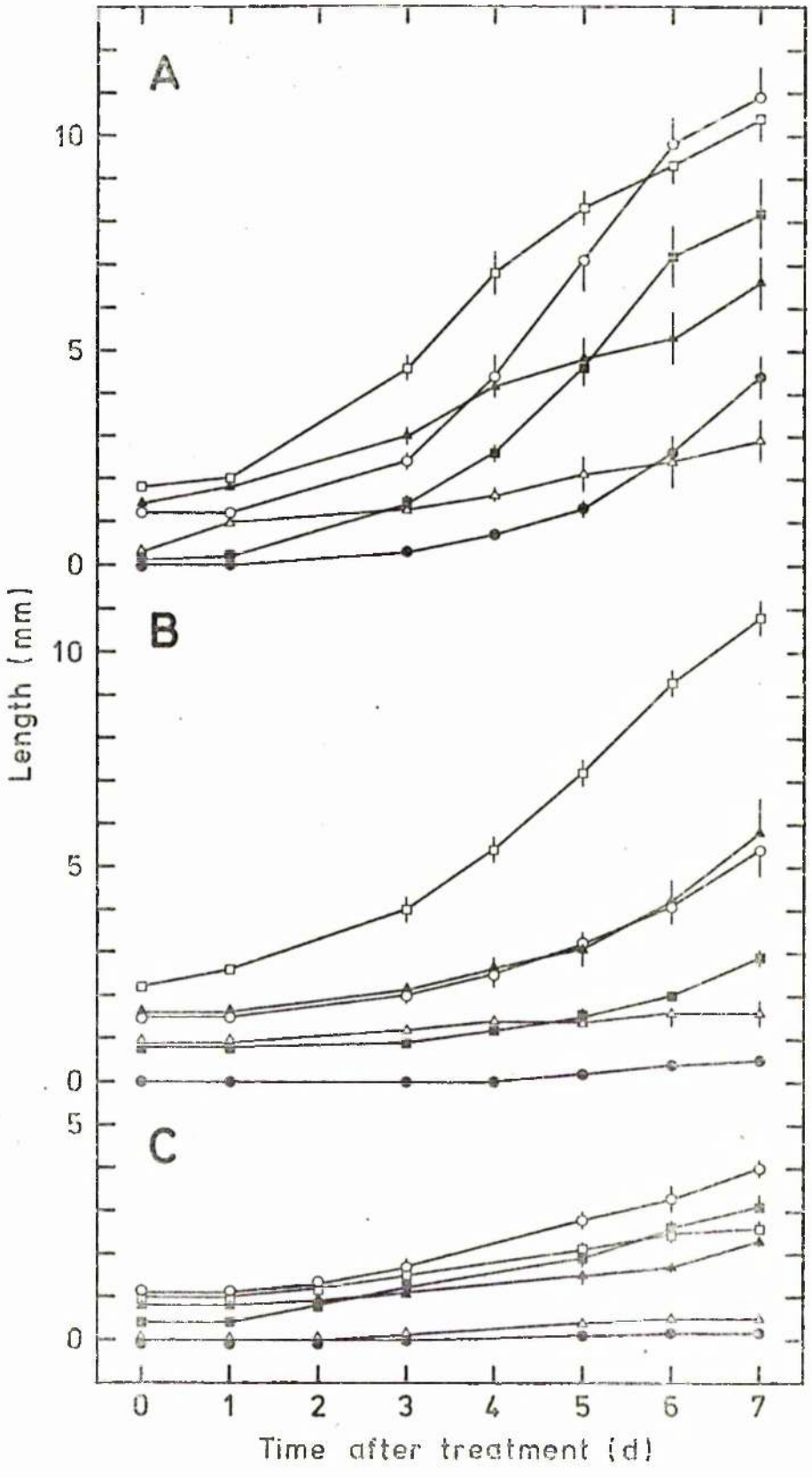
(iii) Defoliated plants

The growth of individual buds alone on plants decapitated just above the 5th node, and completely defoliated, was followed in 3 replicate experiments (Figure 11). The growth pattern varied between the experiments and in one (Figure 11C) the buds grew very poorly. These experiments were carried out in the glasshouse in different months

Figure 11 : Growth of lateral buds on decapitated and defoliated plants of Ph. vulgaris; A,B,C = 3 experiments.

A : 1.10.71 : 21 d old plants, B : 26.11.71 : 25 d old plants, C : 15.12.71 : 26 d old plants : glasshouse : each bud alone on a plant decapitated through a 5th internode and completely defoliated : A 9, B,C, 10 plants/treatment; organized : points = mean $\pm$ S.E. of length of 1st internode of each bud : key:-

Symbol	Axillary bud
●	Cotyledon ..
○	Longer primary leaf ..
■	Shorter primary leaf ..
□	1st trifoliate leaf ..
▲	2nd trifoliate leaf ..
△	3rd trifoliate leaf ..


A
B
C
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10
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5
0
Length (mm)
0
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6
7
Time after treatment (d)

and much of the variation between them may be a result of environmental factors. The plants in each experiment were also at slightly different ages, both temporally and physiologically. Despite the variation several features were evident. The cotyledon and 3rd trifoliate leaf axillary buds consistently grew poorly. In general, the 1st trifoliate leaf and the longer primary leaf axillary buds grew well while the 2nd trifoliate leaf and the shorter primary leaf axillary buds showed less vigorous extension. The outgrowth of these buds relative to each other was, however, rather variable.

The results suggest that there may be inherent factors, possibly relating to age, influencing the growth of individual buds, although their position in relation to stem internodes and roots or to gravity may be important.

It has already been shown (Figure 10) that the removal of the terminal bud itself by excision through the 6th node did not release any of the measured lateral buds from correlative inhibition. Removal of more of the upper shoot, which allowed some of the lateral buds to grow, involved the excision of leaves, buds and stem from the plant.

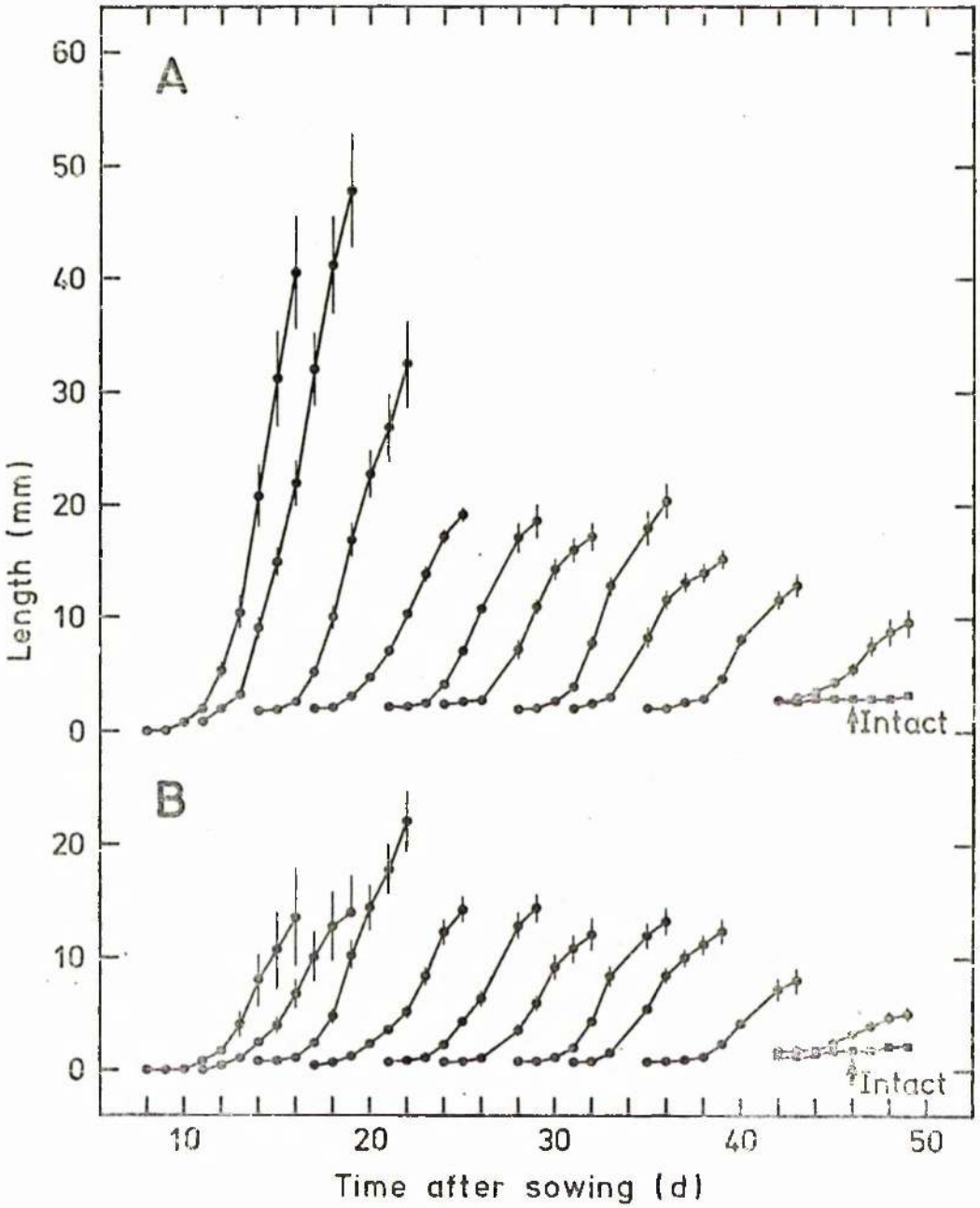
In the following 4 subsections the effects of age and of leaves, buds and other organs are considered.

B. Effect of age

The influence of age upon the growth of the primary leaf axillary buds has been tested in 3 experiments. In the first the growth of these buds was followed on plants from one batch decapitated just above the 2nd node at intervals over a period of 6 weeks. The greatest growth shown by the longer buds over 8 d was on plants decapitated when 11 d old (Figure 12A) while that of the shorter bud was on plants decapitated when 14 d old (Figure 12B). The growth of buds on older plants than these decreased with increasing age. This experiment, however, was performed in the glasshouse during summer and it is possible that the deterioration of bud growth

Figure 12 : Growth of A longer, B shorter primary leaf axillary buds on plants decapitated through the 2nd internode at different times after sowing.

1.6.71 : Ph. vulgaris : glasshouse : one batch of plants with decapitation at 8, 11, 14, 17, 21, 24, 28, 31, 35 and 42 d after sowing : 10 plants/treatment; randomized : points = mean $\pm$ S.E. of length of 1st internode of bud over 8 d after decapitation.


A
B
Intact
Intact
Length (mm)
Time after sowing (d)
60
50
40
30
20
10
0
20
10
0
10
20
30
40
50

with age was a reflection of environmental conditions, perhaps in relation to a transfer of the plant from a vegetative to a reproductive state. Two other experiments, however, in which 2 to 5 week old plants were decapitated at the same time, suggested that, on February- and April-grown plants at least, the growth of the primary leaf axillary buds improved with age up to 5 weeks (Figure 13). It may be that the difference between these experiments and the first reflects the slower development of plants grown in winter or spring compared with summer. Anyway, it seems that these buds have the potential to grow out vigorously for 2 to 3 weeks after their development and it is not possible from these data to implicate bud age as a major factor affecting the pattern of bud outgrowth on intact and decapitated or defoliated plants.

C. Effects of leaves

Snow (1929b) showed that in Pisum sativum the leaf was the major organ contributing to the correlative inhibition of lateral bud growth. Using surgical techniques similar to those of Snow the involvement of leaves in apical dominance in french bean has been analysed.

(i) Primary leaves

Removal of mature primary leaves from otherwise intact plants had no effect on the growth of their axillary buds (Figure 14). The upper shoot, which consisted of only young leaves and stem in these plants, could, therefore, completely inhibit lateral bud growth in the absence of mature leaves. The presence of the primary leaves on plants decapitated just above the 2nd node greatly enhanced the outgrowth of their axillary buds (Figure 14). The petioles of these leaves had no effect on their own. The outgrowth of the buds on decapitated and defoliated plants, and their additional growth in the presence of the primary leaves, was also evidenced by their dry weight measurements. The primary leaves

Figure 13 : Growth of primary leaf axillary buds on plants of Ph. vulgaris of different ages intact (closed symbols) or decapitated through the 2nd internode (open symbols); A,B = 2 experiments.

A : 9.2.71 : ●○14, ■□21, ▲△28 d old plants : controlled environment cabinet : 10 plants/treatment; randomized.

B : 10.4.73 : ■□22, ▲△29, ▼▽36 d old plants : glasshouse : 10 plants/treatment; organized.

Points = mean ± S.E. of combined length of 1st internodes of primary leaf axillary buds.

Mean dry weight (mg) of both buds on the 7th day after treatment (Experiment A):-

Age	Intact	Decapitated
14 d	0.7	27.6
21 d	0.5	67.4
28 d	0.6	52.1

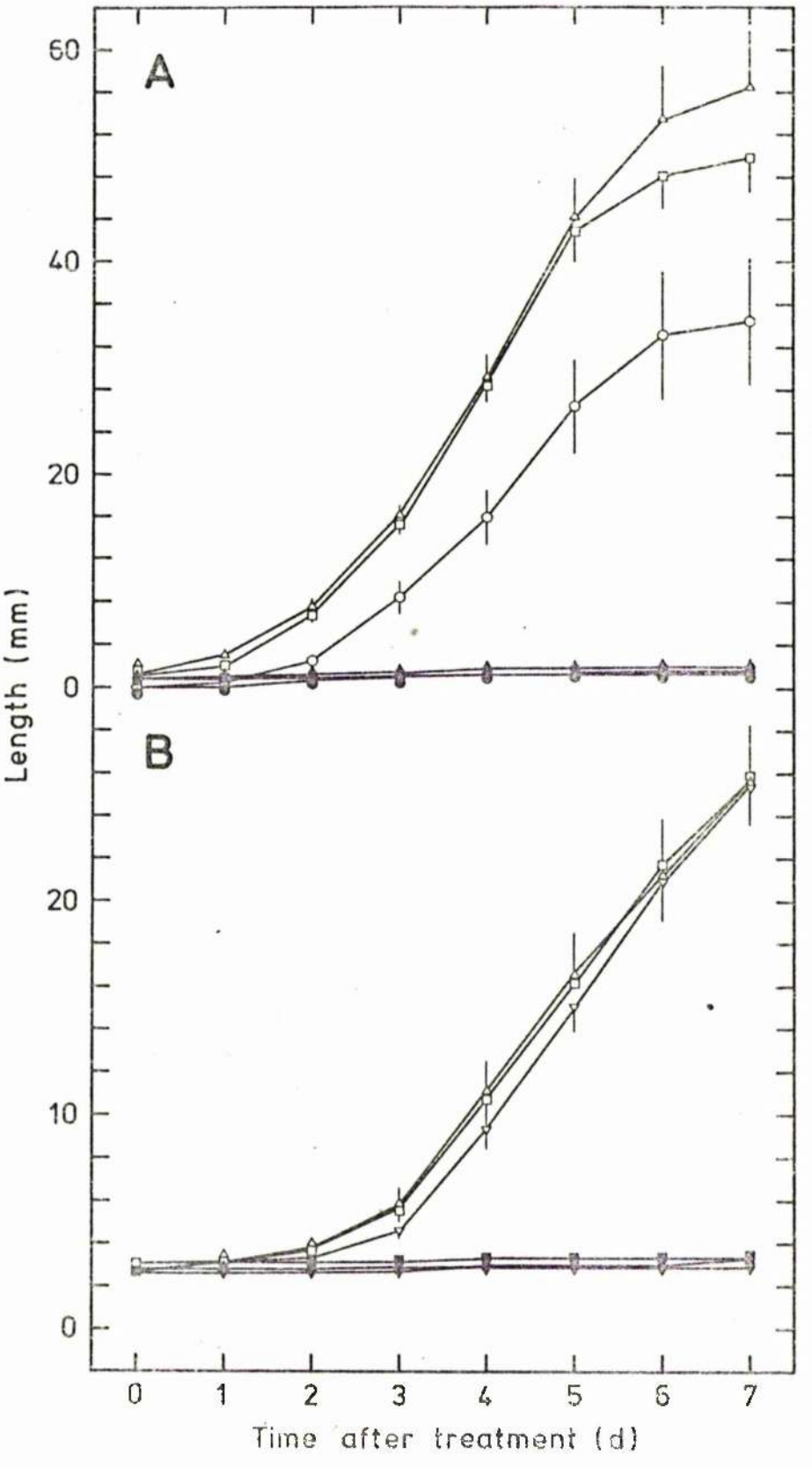
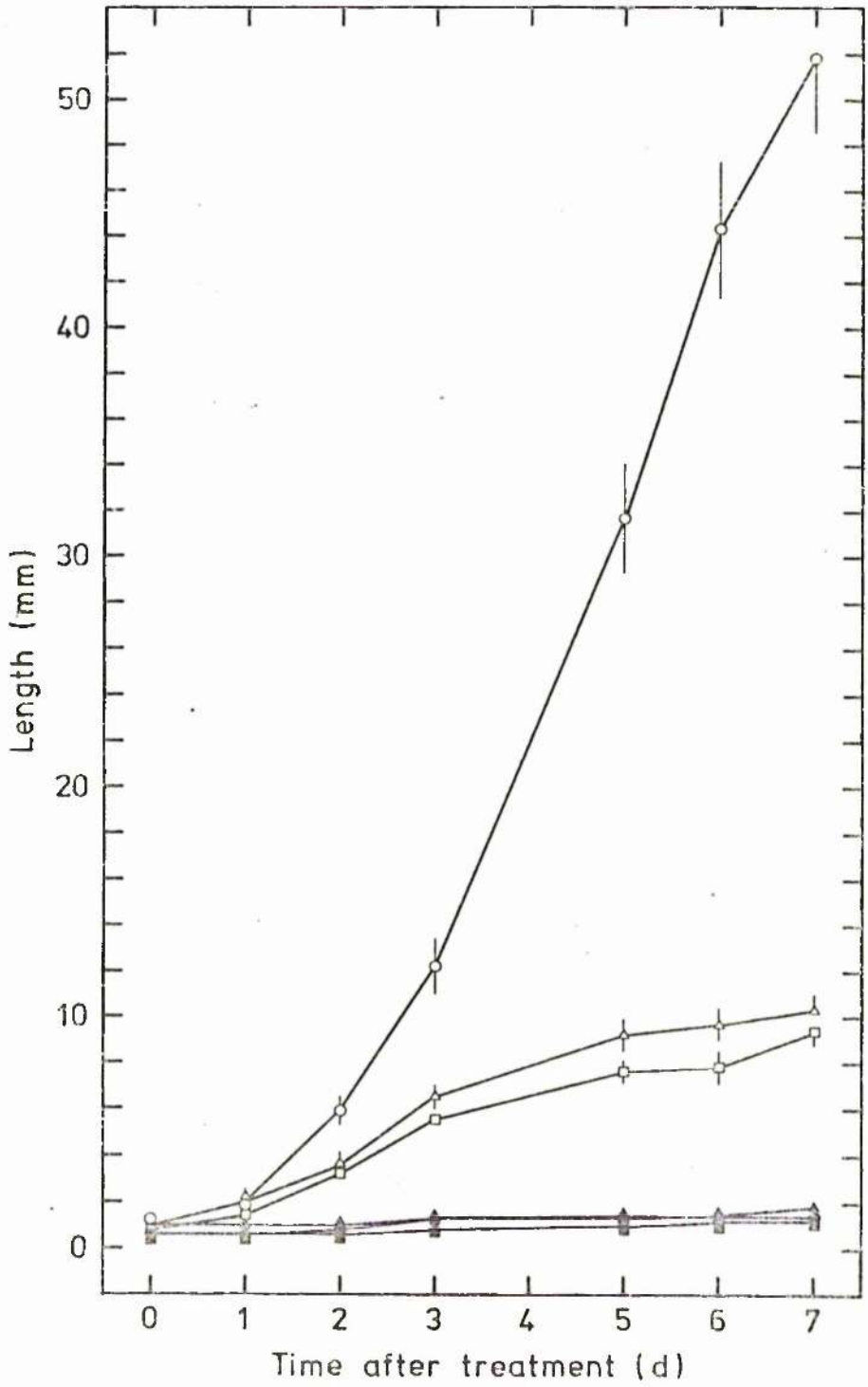

A
B
60
40
20
0
20
10
0
Length (mm)
0
1
2
3
4
5
6
7
Time after treatment (d)

Figure 14 : Growth of primary leaf axillary buds on intact (closed symbols) and decapitated (open symbols) plants with primary leaves intact (●○), with lamina and midrib excised (■□) or completely excised (▲△).

19.1.71 : Ph. vulgaris; 14 d old plants : controlled environment cabinet : decapitation through 2nd internode : 10 plants/treatment; randomized : points = mean ± S.E. of combined length of 1st internodes of primary leaf axillary buds : results confirmed in 3 further experiments.

Mean dry weight (mg) of primary leaf axillary buds and shoot above the 2nd node on the 7th day after treatment:-

Treatment		Weight	
Upper shoot	Primary leaves	Buds	Upper shoot
Intact	Intact	0.7	255.4
	Lamina + midrib excised	0.4	28.1
	Completely excised	0.5	25.0
Decapitated	Intact	57.9	
	Lamina + midrib excised	8.6	
	Completely excised	6.8	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Length (mm)
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Time after treatment (d)

also greatly improved the dry weight of the shoot above the 2nd node on intact plants.

Using older plants decapitated just above the 3rd node it was shown that the presence of the mature 1st trifoliate leaf completely (Figure 15A,B) or almost completely (Figure 15C) nullified the promotory effect of the primary leaves on bud growth. That the promoting influence of the primary leaves on growth extended not only to their own axillary buds (Figure 14) but also to the cotyledon axillary buds (Figure 10A) and to the main shoot (Figure 14) and that the presence of another mature leaf apparently replaced it suggests it to be a general nutritive effect rather than a specific effect on bud growth.

(ii) Trifoliate leaves

The effect of different trifoliate leaves on the growth of the primary leaf axillary buds was tested on plants with no other leaves present bar the mature primary leaves, which were left to allow potentially vigorous outgrowth of their axillary buds. The 2nd trifoliate leaf and the 3rd trifoliate leaf alone, and the 4th trifoliate leaf plus the terminal bud, almost completely prevented the growth of the primary leaf axillary buds over 7 d, while the 1st trifoliate leaf gave a partial inhibition of bud growth for 5 d (Figure 16). The 2nd, 3rd and 4th trifoliate leaves all exhibited considerable growth over the experimental period while the 1st trifoliate leaf was almost fully grown by the beginning of the experiment. While it is clear that a young, growing leaf can on its own virtually completely inhibit the growth of these buds this may not necessarily be the case in intact plants as the leaves grew much more alone than in the presence of the other leaves.

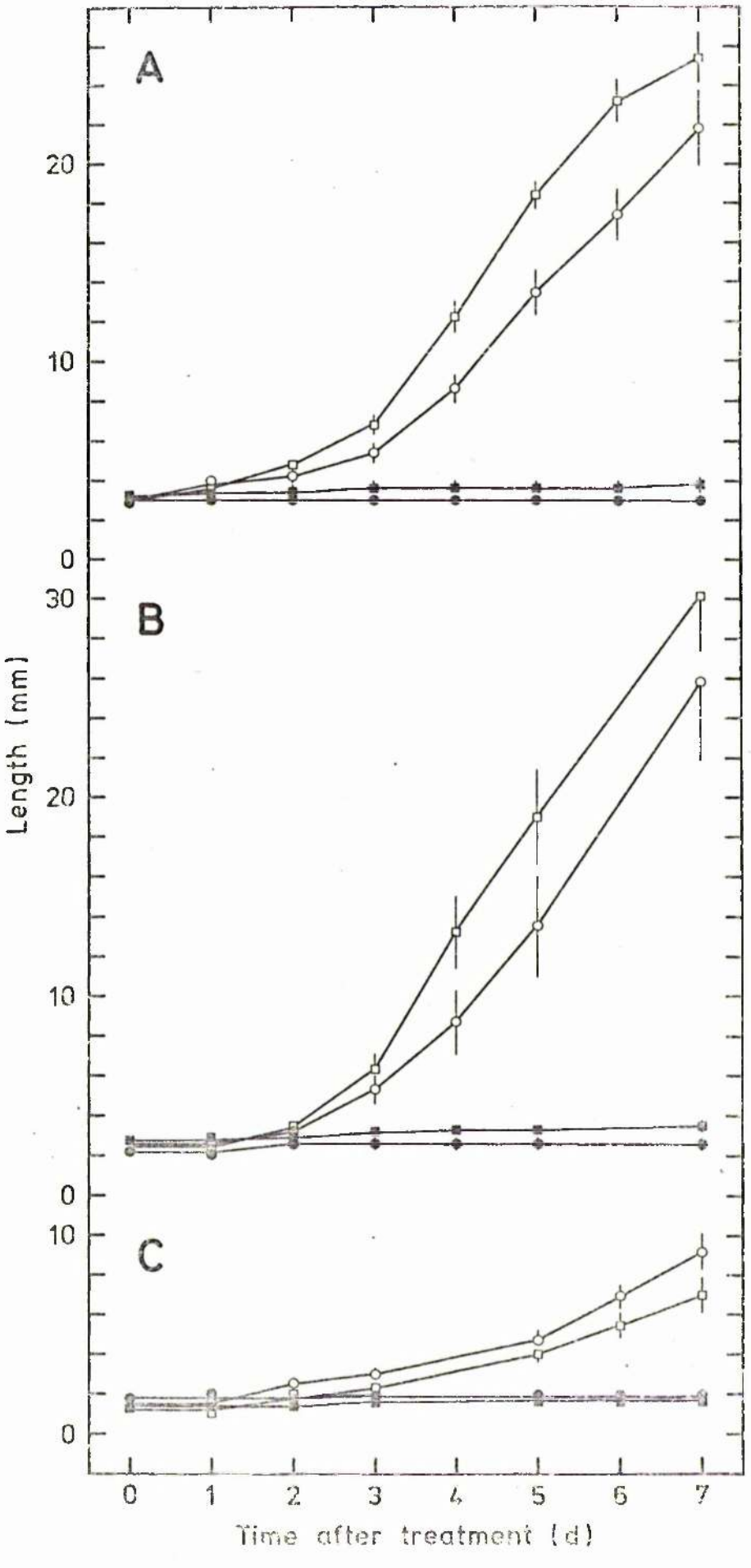
The involvement of the young leaves and the apical bud was further tested by determining the minimal amount of the upper shoot necessary for inhibition of lateral bud growth. Apart from the primary leaves,

Figure 15 : Growth of primary leaf axillary buds on intact (closed symbols) and decapitated (open symbols) plants of Ph. vulgaris with primary leaves intact (●○) or excised (■□).
A,B,C = 3 experiments.

A : 10.5.71 : 21 d old plants, B : 4.10.71 : 21 d old plants, C : 15.12.71 : 23 d old plants : glasshouse : decapitation through 2nd internode : 10 plants/treatment; A randomized, B,C organized : points = mean ± S.E. of combined length of 1st internodes of primary leaf axillary buds.

Mean dry weight (mg) of primary leaf axillary buds on the 7th day after treatment (Experiment A):-

Treatment		Weight
Upper shoot	Primary leaves	
Intact	Intact	1.5
	Excised	3.2
Decapitated	Intact	66.1
	Excised	118.8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Length (mm)
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Time after treatment (d)

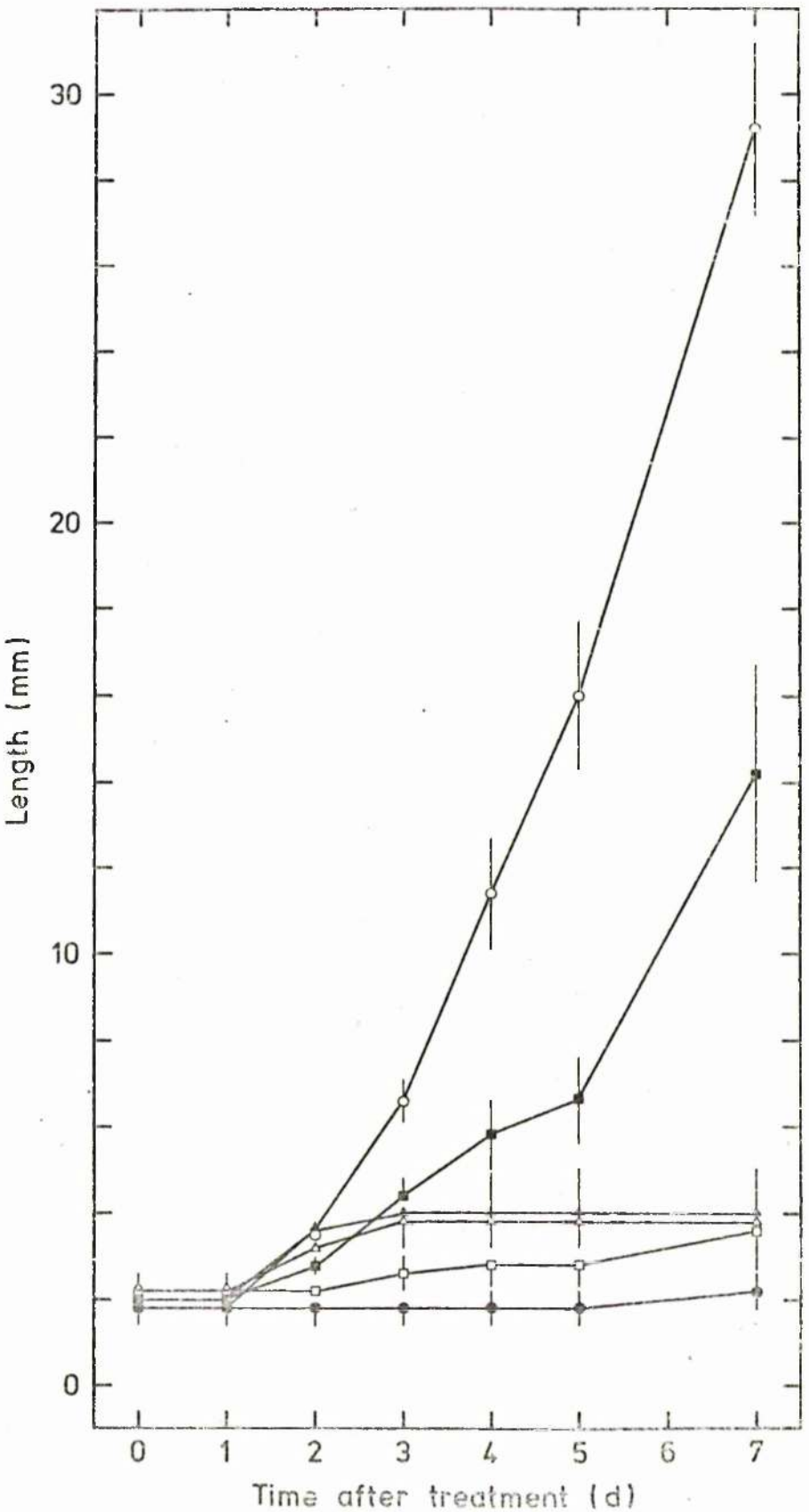
Figure 16 : Growth of primary leaf axillary buds on plants with different trifoliate leaves present.

26.7.71 : Ph. vulgaris ; 21 d old plants : glasshouse : all leaves excised except treatment leaf and primary leaves; all lateral buds excised except primary leaf axillary buds : 10 plants/treatment; organized : points = mean ± S.E. of combined length of 1st internodes of primary leaf axillary buds : results confirmed in 1 further experiment : key:-

Symbol	Remaining trifoliate leaf
●	All
○	None
■	1st
□	2nd
▲	3rd
△	4th (+terminal bud)

Mean length (mm) of terminal leaflet of each trifoliate leaf:-

Trifoliate leaf	Leaves individually			Leaves on intact plants		
	Day 0	Day 7	Increase	Day 0	Day 7	Increase
1st	114.8	119.4	4.6	118.8	123.0	4.2
2nd	61.2	107.2	46.0	66.6	105.0	38.4
3rd	9.2	54.2	45.0	11.6	42.2	30.6
4th	4.0	42.4	38.4	3.4	8.0	4.6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Length (mm)
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6
7
Time after treatment (d)

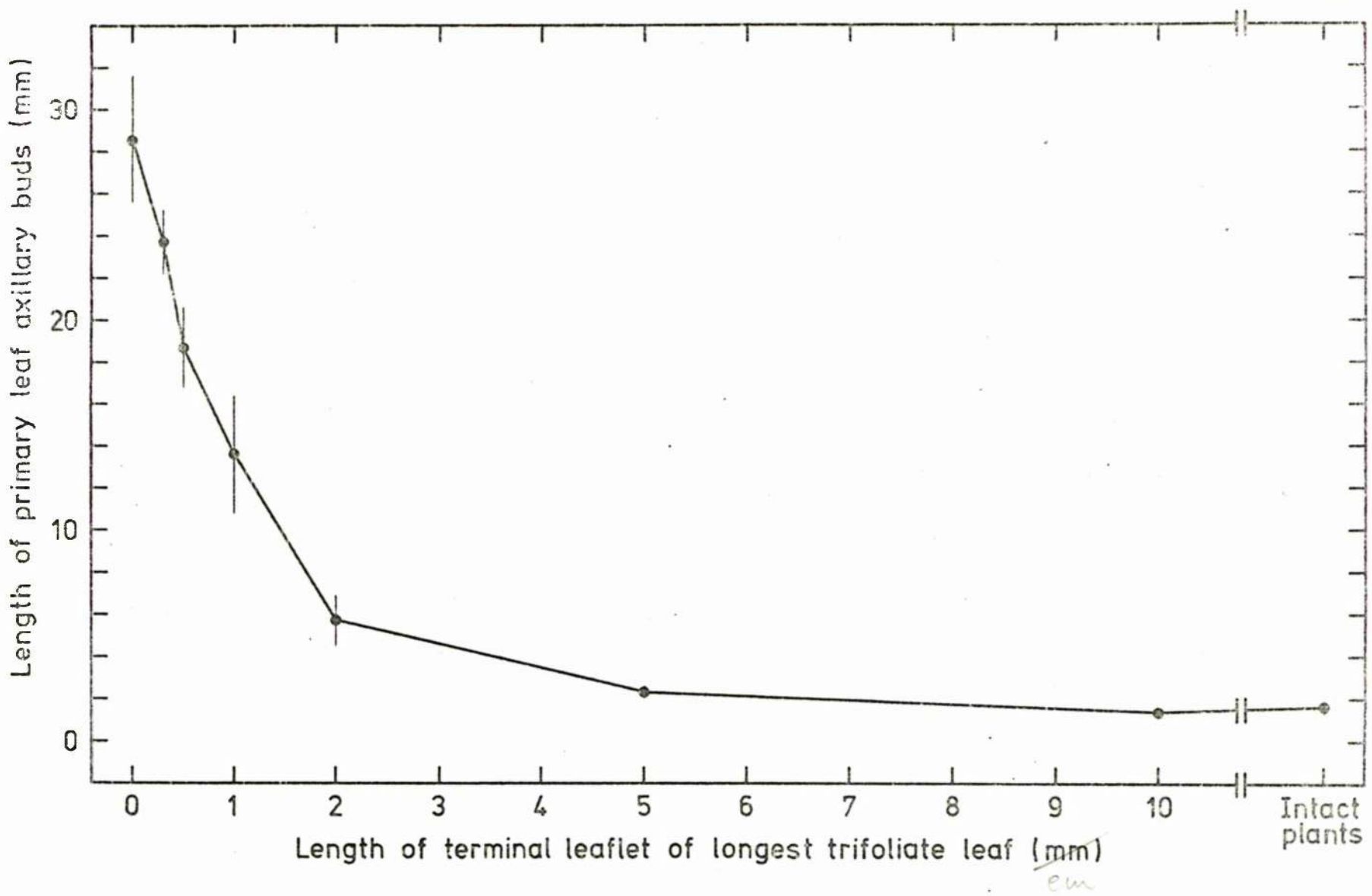
which were mature, all the leaves greater than a certain length were removed from the plant and, over the duration of the experiment, any more leaves that reached that length were also excised. It is clear that the apex itself did not cause any inhibition of the primary leaf axillary buds; it was the young leaves produced from the apex which were the inhibiting organs (Figure 17). Complete inhibition of these buds was not shown unless the upper shoot contained leaves greater than 2 cm long, although some inhibition was produced by leaves less than 5 mm long.

(iii) Polarity of inhibition

The specificity of action of young leaves was tested by observing the effect of a single, young, growing trifoliate leaf on buds below, in and above its own axil (Figure 18). The growth of the bud in the axil below the growing 1st trifoliate leaf was strongly inhibited by that leaf (Figure 18A) while the 1st trifoliate leaf axillary bud (Figure 18B) and the 2nd trifoliate leaf axillary bud (Figure 18C) were only slightly suppressed. These results probably do not reflect different susceptibilities to inhibition of the particular buds used, because similar results were obtained with a different set of buds, using the 2nd trifoliate leaf on slightly older plants (Figure 18D,E,F). It appears then, that a young, expanding leaf is only an effective inhibitor of the growth of lateral buds in leaf axils at lower nodes. This is, indeed, suggested by the observation that a young lateral bud develops to a certain size while in the axil of a leaf which is still growing. It was shown in intact plants (Figure 8A) that the primary leaf axillary buds were increasing in length over a period when the primary leaves were still growing and before the 1st trifoliate leaf reached its most rapid expansion.

Figure 17 : Length of primary leaf axillary buds after 7 d on plants with different sizes of trifoliate leaves present.

4.12.71 : Ph. vulgaris; 19 d old plants : all trifoliate leaves greater than a given length excised throughout the experimental period; primary leaves left intact : 10 plants/treatment; organized : points = mean $\pm$ S.E. of combined length of 1st internodes of primary leaf axillary buds : results confirmed in 2 further experiments.


Length of primary leaf axillary buds (mm)
30
20
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8
9
10
Intact plants
Length of terminal leaflet of longest trifoliate leaf (mm)

Figure 18 : Growth of lateral buds of Ph. vulgaris in presence (●) or absence (○) of 1st (A,B,C) or 2nd (D,E,F) trifoliate leaf.

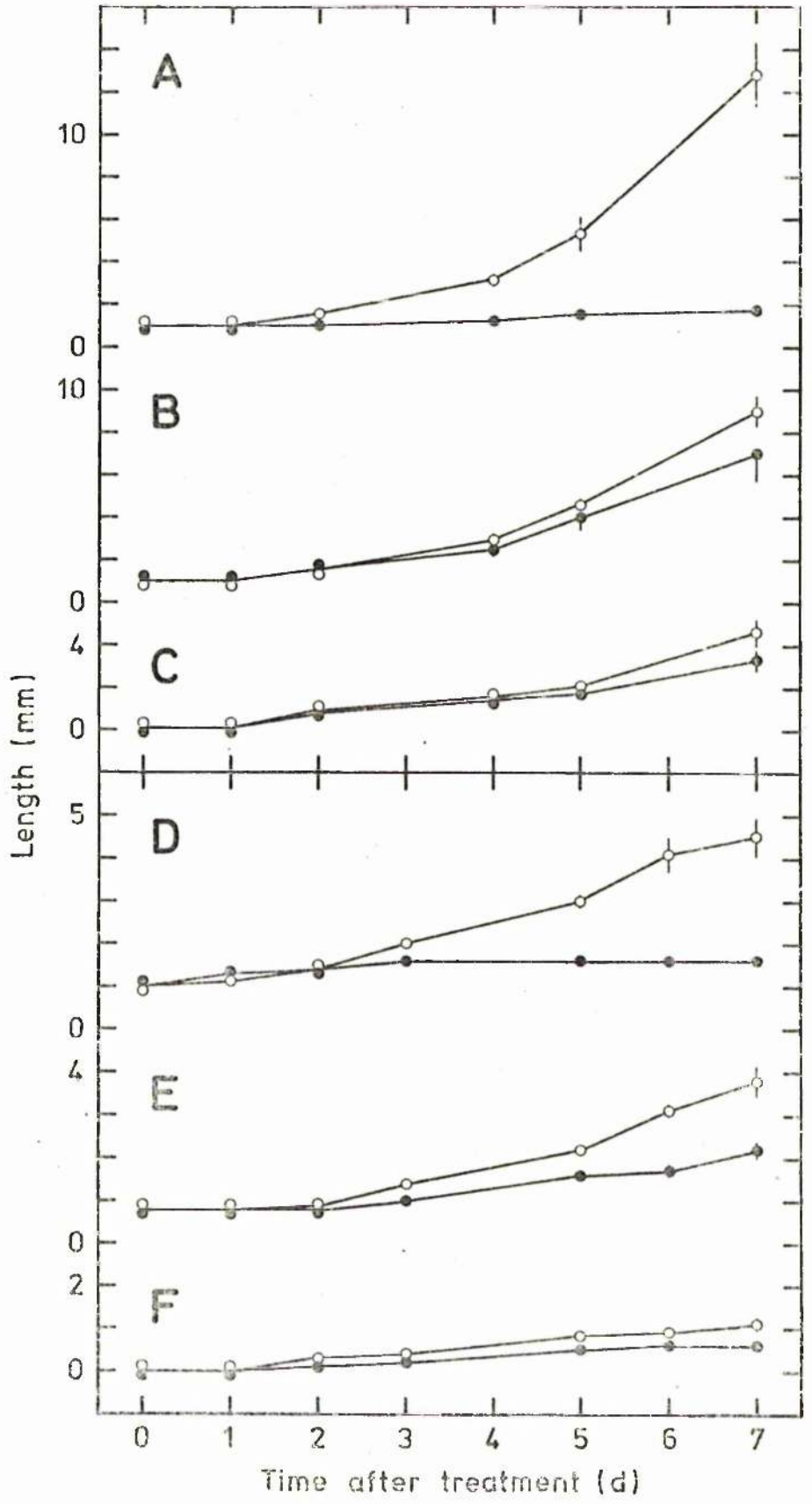
A,B,C : 16.11.71 : 16 d old plants : A = shorter primary leaf, B = 1st trifoliate leaf, C = 2nd trifoliate leaf axillary bud.

D,E,F : 11.1.72 : 22 d old plants : D = 1st trifoliate leaf, E = 2nd trifoliate leaf, F = 3rd trifoliate leaf axillary bud.

Glasshouse : all leaves and buds apart from those specified were excised from plants : 10 plants/treatment; organized : points = mean $\pm$ S.E. of length of 1st internode of bud.

Mean length (mm) of terminal leaflet of 1st (A,B,C) or 2nd (D,E,F) trifoliate leaf:-

	Day 0	Day 7	Increase
A	47.3	124.8	77.5
B	48.4	128.4	80.0
C	49.1	128.1	79.0
D	42.5	79.6	37.1
E	42.4	89.2	46.8
F	41.2	84.4	43.2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Time after treatment (d)

D. Effects of buds

(i) Longitudinal effects

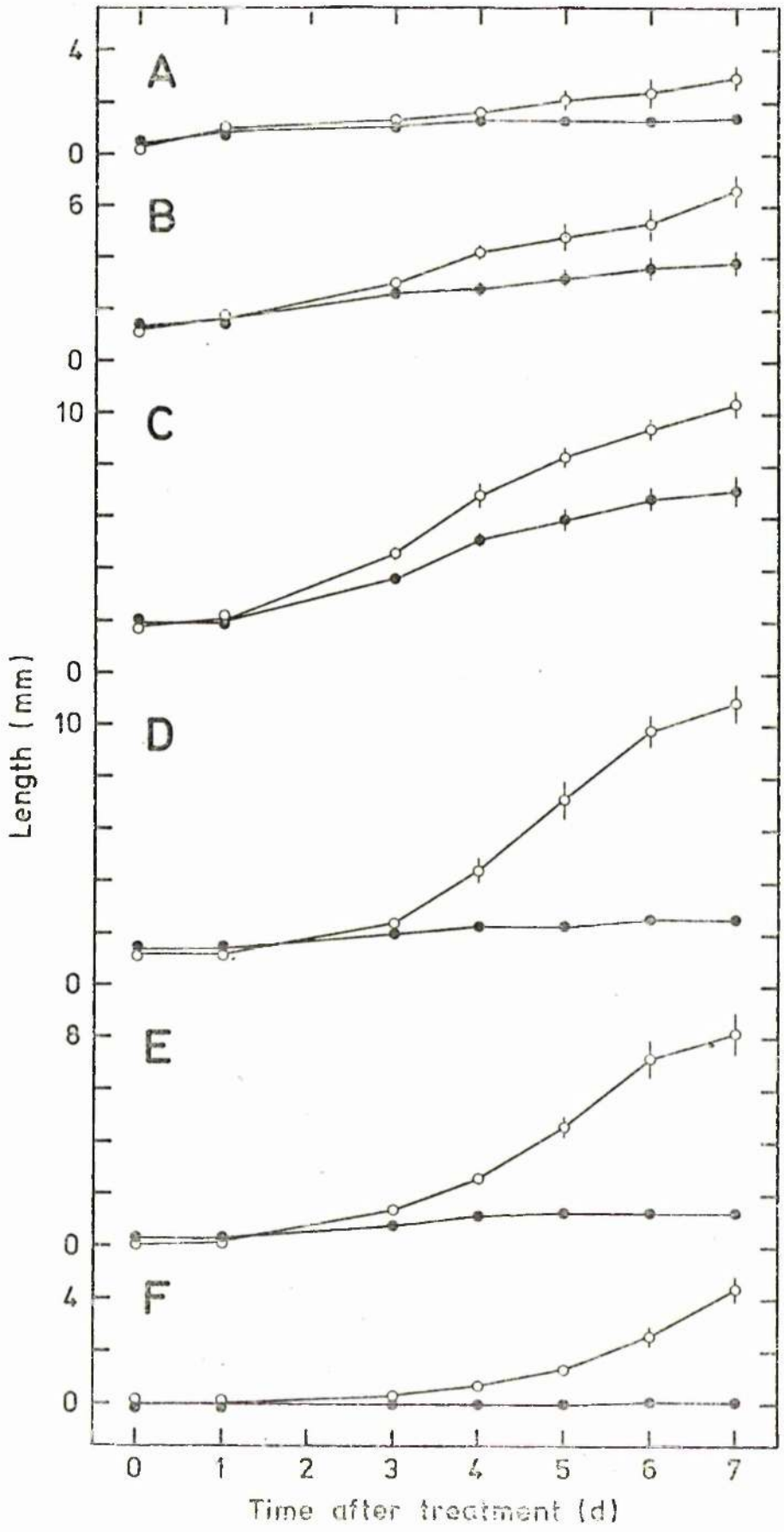
The growth of the buds tested individually on decapitated and defoliated plants (Figure 11A) was also examined in the presence of all the other lateral buds (Figure 19). These other buds very strongly suppressed the growth of the buds in the cotyledon and primary leaf axils, while having a lesser effect on those in the axils of the 1st, 2nd and 3rd trifoliate leaves. Although some of the inhibition of buds by other buds may be the result of competition for nutrients, there was a basipetal polarity of inhibition which was, it seems, similar to that found with young leaves (Figure 18). This was to be expected because the growth of a bud involves a fairly rapid expansion of its 1st trifoliate leaf (Figure 9C) which would soon, presumably, be capable of inhibiting buds lower down the plant.

(ii) Transverse effects

In many of the experiments presented herein on the effects of various factors on bud growth the primary leaf axillary buds have been used as the test buds. It was of interest, therefore, to examine the effects of these unequal buds upon one another. This was carried out with plants decapitated by excision at the top of the 2nd internode (Figure 20A). The growth of the initially longer bud was unaffected by the presence of the shorter bud, but the growth of the shorter bud was considerably suppressed by the longer bud over 7 d after decapitation of the plant. Beyond 14 d after decapitation of the plant many of the shorter shoots were completely inhibited by the longer shoot but could be induced to grow again by removal of the longer shoot (Figure 20B). After a further 2 to 3 weeks some of the shorter shoots were actually dying in the presence of the longer shoot.

Figure 19 : Growth of A 3rd trifoliate leaf, B 2nd trifoliate leaf, C 1st trifoliate leaf, D longer primary leaf, E shorter primary leaf, F cotyledon axillary buds in the presence (●) or absence (○) of other lateral buds on plants decapitated through the 5th internode and completely defoliated.

1.10.71 : Ph. vulgaris; 21 d old plants : glasshouse : plants with either specified bud alone (○) or 3rd, 2nd and 1st trifoliate leaf, primary leaf and cotyledon axillary buds all present (●) : 9 plants/treatment; organized : points = mean $\pm$ S.E. of length of 1st internode of bud : results confirmed in 2 further experiments.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Time after treatment (d)

Figure 20A : Growth of each primary leaf axillary bud in the presence (closed symbols) or absence (open symbols) of the other primary leaf axillary bud on plants decapitated through the 2nd internode.

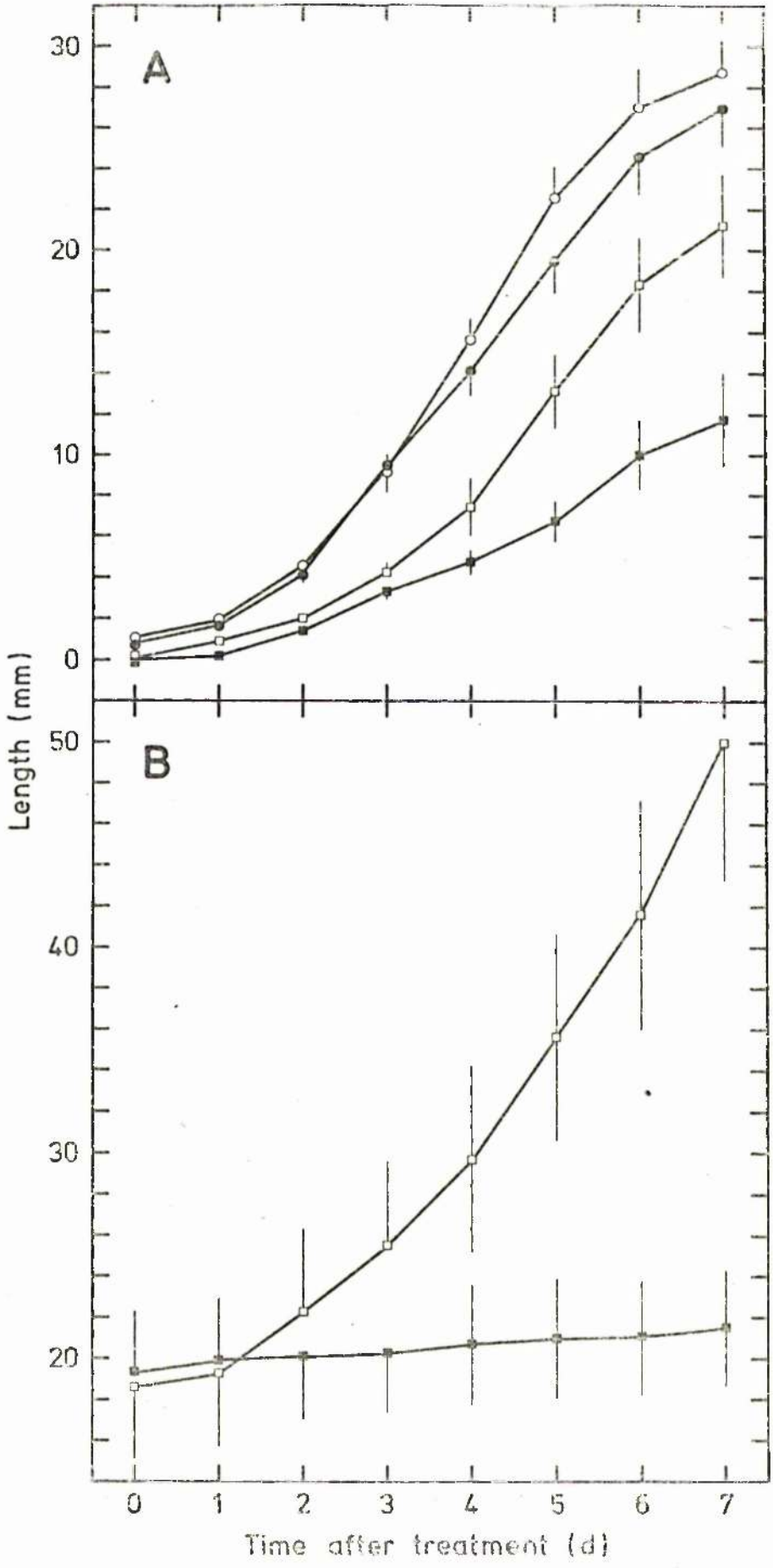
30.3.71 : Ph. vulgaris; 14 d old plants : controlled environment cabinet : 10 plants/treatment; randomized : points = mean ± S.E. of length of 1st internode of bud : results confirmed in 2 further experiments : key:- longer primary leaf axillary bud = ●○
shorter primary leaf axillary bud = ■□

Mean dry weight (mg) of primary leaf axillary buds on the 7th day after treatment:-

Measured primary leaf axillary bud	Other bud Present	Other bud Absent
Longer ..	44.0	63.9
Shorter ..	10.0	38.0

Figure 20B : Growth of shorter primary leaf axillary shoot in the presence (■) or absence (□) of the longer primary leaf axillary shoot on plants decapitated through the 2nd internode 14 d previously.

16.8.71 : Ph. vulgaris : plants decapitated 14 d after sowing : glasshouse : 10 plants/treatment; organized : points = mean ± S.E. of total length of shoot : results confirmed in 2 further experiments.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Length (mm)
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Time after treatment (d)

E. Effects of other organs

(i) Stem internodes

In one experiment, using plants completely defoliated above the 2nd node and on which the primary leaves were beginning to senesce, it was discovered that the presence of 5 stem internodes above the 2nd node improved the outgrowth of the primary leaf axillary buds (Figure 21). This effect of the stem was confirmed on completely defoliated plants decapitated at different nodes above the 2nd node (Table 3A); the more internodes left on the plant, the more growth was shown by the primary leaf axillary buds. In two further experiments, however, the presence of the mature primary leaves was shown not only to induce greater bud growth but also to negate the effect of the stem internodes (Table 3B,C). Bean stems contain photosynthetic green tissue and it may be that their effect on bud growth was nutritive.

(ii) Roots

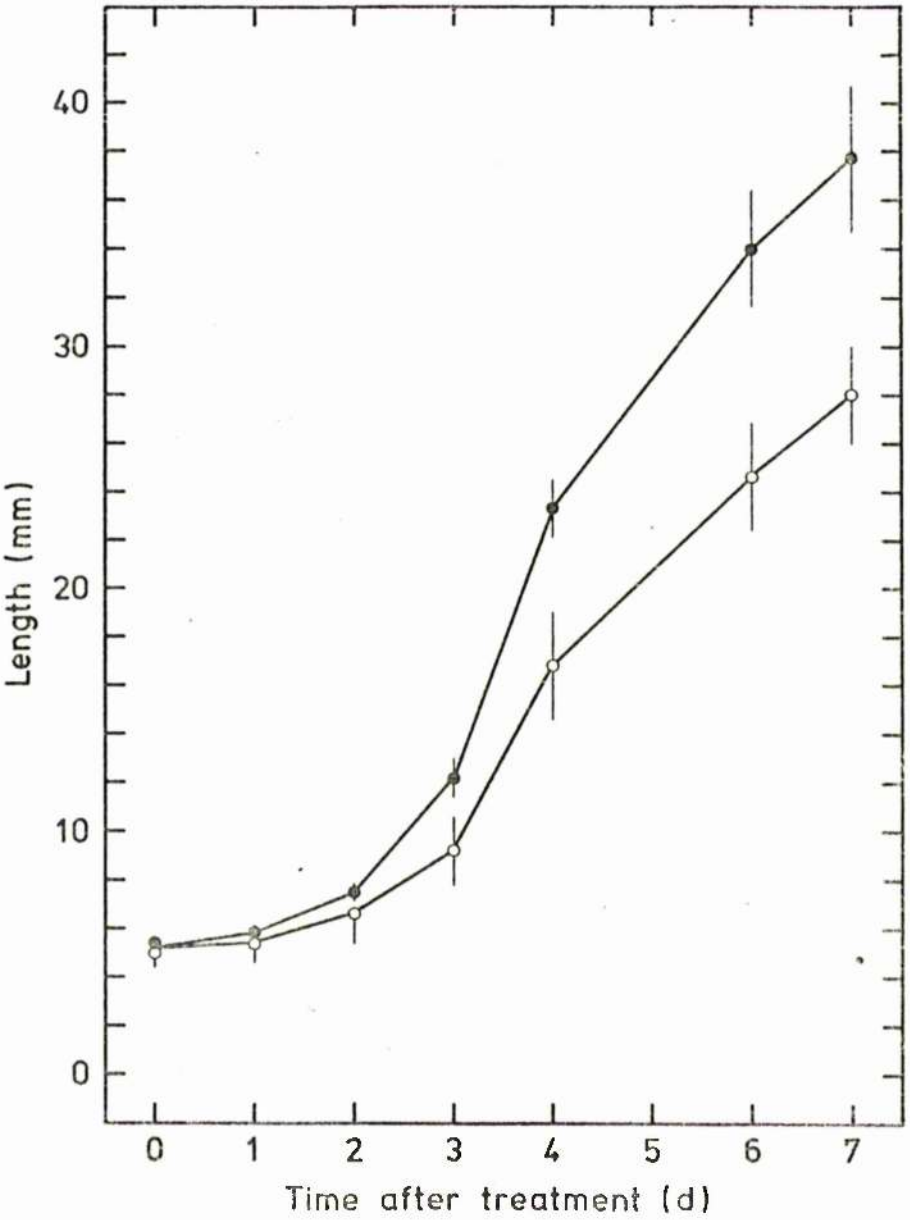
In the one experiment testing the involvement of roots in apical dominance the plants without roots wilted for about 12 h after derooting but subsequently recovered. Derooting had no significant effect on the growth of the primary leaf axillary buds on either intact or decapitated plants (Figure 22). A complete root system does not appear to be important for either the maintenance of apical dominance or the outgrowth of lateral buds in these plants, at least over a short time period. Some regeneration of roots had occurred by the end of the experiment but there was no visible sign of this happening on the 3rd day, by which time the effects of decapitation were clearly evident.

(iii) Cotyledons

In some plant species a cotyledon can inhibit the bud in its own axil (Dostál, 1909) or can control the balance of growth between the two cotyledon axillary buds (Champagnat, 1951). The cotyledons of Phaseolus are exhausted and abscind within 2 to 3 weeks of sowing.

Figure 21 : Growth of primary leaf axillary buds in the presence (●) or absence (○) of the stem above the 2nd node on plants decapitated through the 7th node and with all trifoliate leaves excised.

8.6.71 : Ph. vulgaris : 22 d old plants : glasshouse : 2nd, 3rd, 4th, 5th and 6th stem internodes either present (●) or excised (○) : 5 plants/treatment; organized : points = mean $\pm$ S.E. of combined length of 1st internodes of primary leaf axillary buds.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Length (mm)
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Time after treatment (d)

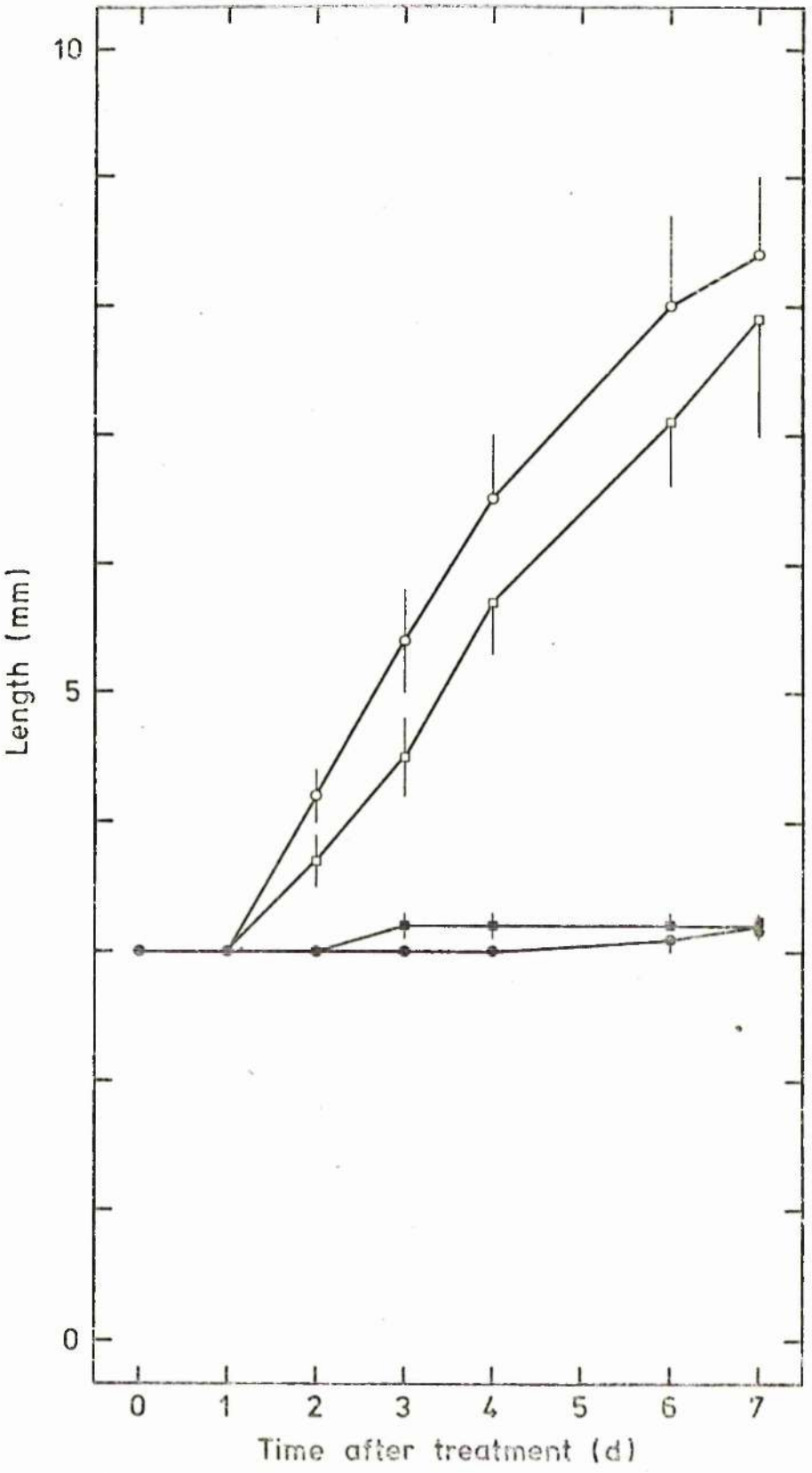
Table 3 : Growth of primary leaf axillary buds on plants defoliated above the 2nd node and decapitated at different nodes.

Internodes present above 2nd node	A Primary leaves removed	B Primary leaves present	C Primary leaves present
None	10.0 ± 1.9	29.6 ± 7.0	30.2 ± 3.0
2nd	11.9 ± 1.8	27.3 ± 3.4	28.5 ± 2.5
2nd + 3rd	14.4 ± 1.8	35.6 ± 6.9	31.2 ± 2.0
2nd + 3rd + 4th	18.6 ± 2.1	27.7 ± 5.3	28.7 ± 2.4

A, 10.1.72; B, 16.12.71; C, 10.1.72 : Ph. vulgaris; 24 d old : glasshouse : 10 plants/treatment; organized : results = mean ± S.E. of combined lengths of 1st internodes of primary leaf axillary buds on 7th day after treatment.

Figure 22 : Growth of primary leaf axillary buds on plants intact (closed symbols) or decapitated (open symbols) and with roots intact (●○) or excised (■□).

17.4.73 : Ph. vulgaris; 22 d old plants; controlled environment cabinet : decapitation through 2nd internode; derooting as described in 'EXPERIMENTAL PROCEDURES' : 10 plants/treatment; randomized : points = mean $\pm$ S.E. of combined length of 1st internodes of primary leaf axillary buds.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Length (mm)
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Time after treatment (d)

Plants were, therefore, selected on which the cotyledons were still fairly full and the cotyledon axillary buds were visible. The plants were decapitated 1 cm above the 1st node and both, one or neither of the cotyledons removed. The excision of one cotyledon slightly reduced the outgrowth of the cotyledon axillary buds while the removal of both cotyledons severely curtailed the growth of these buds (Figure 23A). Presumably this was a nutritive effect: it has already been shown that the presence of mature primary leaves also improved the growth of these buds (Figure 10A). The buds distal and proximal to a single cotyledon showed no difference in their outgrowth (Figure 23B). The cotyledons, then, did not greatly influence the pattern of bud growth but in very young seedlings they may provide the buds with nutrients or growth factors. In 2 to 4 week old plants, which were used in most of the experiments, the cotyledons were almost exhausted or actually abscinded and were not, therefore, a major factor in the present study.

F. Further correlations

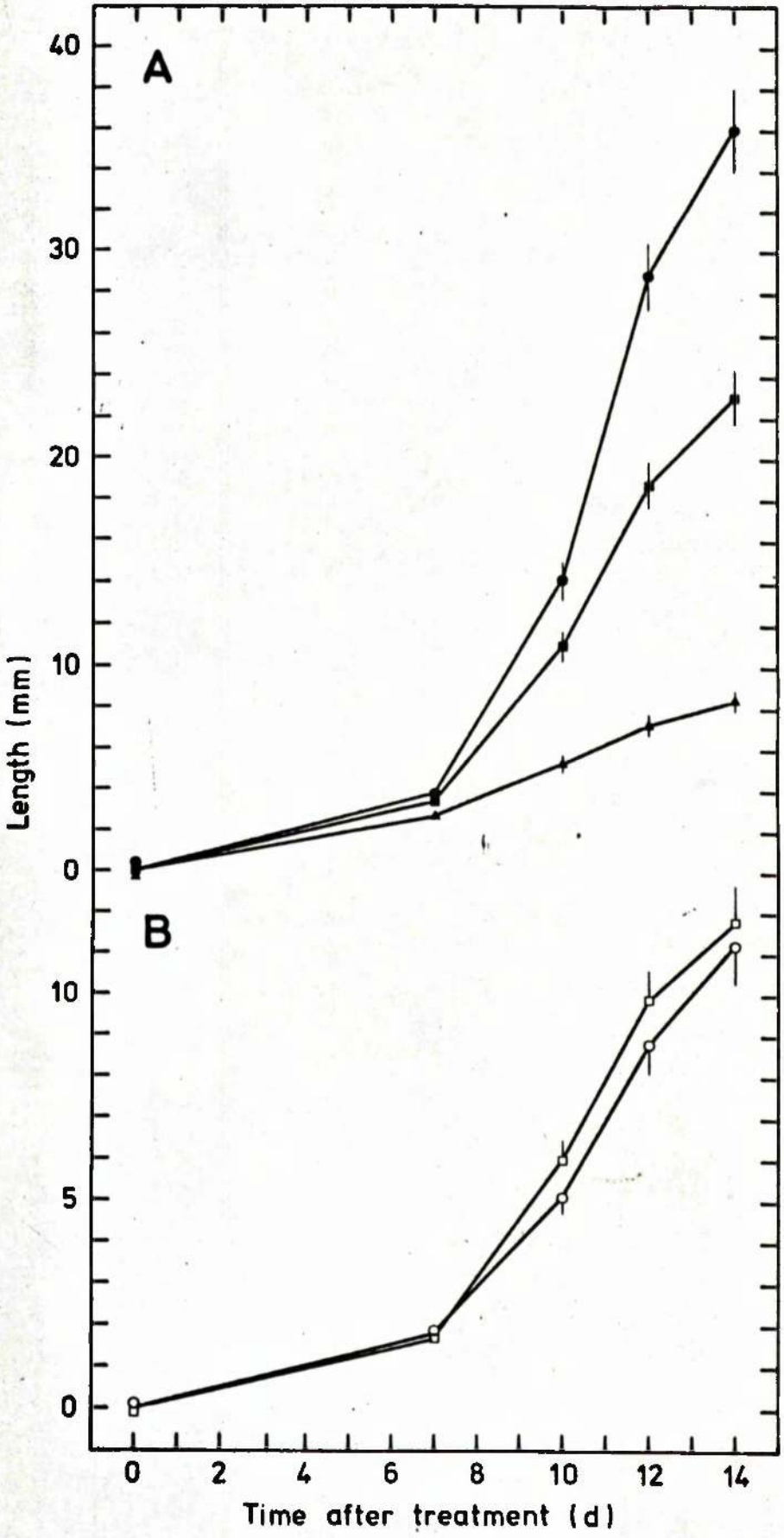
In this research apical dominance has been investigated as manifest in the correlative inhibition of lateral bud growth by the upper parts of the main shoot. This may not be the only aspect of apical dominance in bean plants. During the experiments reported previously it was found that the presence of the upper shoot, as well as inhibiting lateral bud growth, often promoted the senescence of the primary leaf laminae and the abscission of the primary leaf laminae and petioles (Table 4A). The upper shoot and young leaves also stimulated the growth of the stem internodes immediately below and, apparently, of the primary root (Table 4A;2).

There were also other correlative effects. It has already been shown that the presence of young leaves inhibited the growth of other

Figure 23 : A : Growth of cotyledon axillary buds on plants with both (●), one (■) or no (▲) cotyledons present.

B : Growth of cotyledon axillary buds on plants with one cotyledon present : ○ = bud proximal to cotyledon, □ = bud distal to cotyledon.

24.11.71 : Ph. vulgaris; 12 d old plants : glasshouse : plants decapitated 1 cm above 1st node : 25 plants/treatment; randomized : points = mean ± S.E. of A combined total length of cotyledon axillary buds, B total length of each cotyledon axillary bud : results confirmed in 2 further experiments.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Length (mm)
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14
Time after treatment (d)

Table 4A : Effect of upper shoot on senescence, abscission and stem internode extension.

	Intact	Decapitated	+1st leaf	+ 2nd leaf
No. of primary leaves senescing	20	0		
No. of primary leaf laminae abscinded	20	0		
No. of primary leaf petioles abscinded	20	0		
Length of 2nd internode (mm)	65.0 ± 6.5	42.0 ± 6.9	59.6 ± 5.5	70.2 ± 6.1

Table 4B : Effect of roots on senescence and of laminae on abscission.

	Roots present	Roots removed
No. of primary leaves senescing	14	2
	Lamina present	Lamina removed
No. of primary leaf petioles abscinded	0	20

Ph. vulgaris : 14 to 28 d from sowing : controlled environment : decapitation at 3rd node; 1st and 2nd leaves both expanding, each left as only leaf above 2nd node : 10 plants/treatment; randomized : results for length = mean ± S.E.; senescence estimated visually (yellowing of lamina); abscission estimated by lightly tapping lamina or petiole.

leaves on the same plant (Figure 16). The removal of the primary leaf laminae hastened the abscission of their petioles and the presence of roots appeared to stimulate the senescence of the primary leaves (Table 4B).

It seems, therefore, that the inhibition of lateral bud growth is just one aspect of the correlation of growth and development of dwarf french bean seedlings.

2. THE INVOLVEMENT OF INDOLE ACETIC ACID

The involvement of IAA in apical dominance in the dwarf french bean has been tested in 4 different ways. Firstly, the ability of exogenous IAA to replace the upper shoot with respect to inhibition of the lateral buds has been examined. Secondly, the quantity and identity of endogenous auxin found in, and exported from young shoot tips has been investigated by a combination of classical and modern methods. Thirdly, an attempt was made to analyse the involvement of translocation of endogenous auxin in apical dominance by applying IAA transport inhibitors to intact plants. Fourthly, the translocation of radioactively labelled IAA was examined after application to intact and surgically treated plants: this aspect is considered in later sections.

A. Application

Bean plants were decapitated 3 cm above the 2nd node and IAA was applied to the cut stump using 2 techniques.

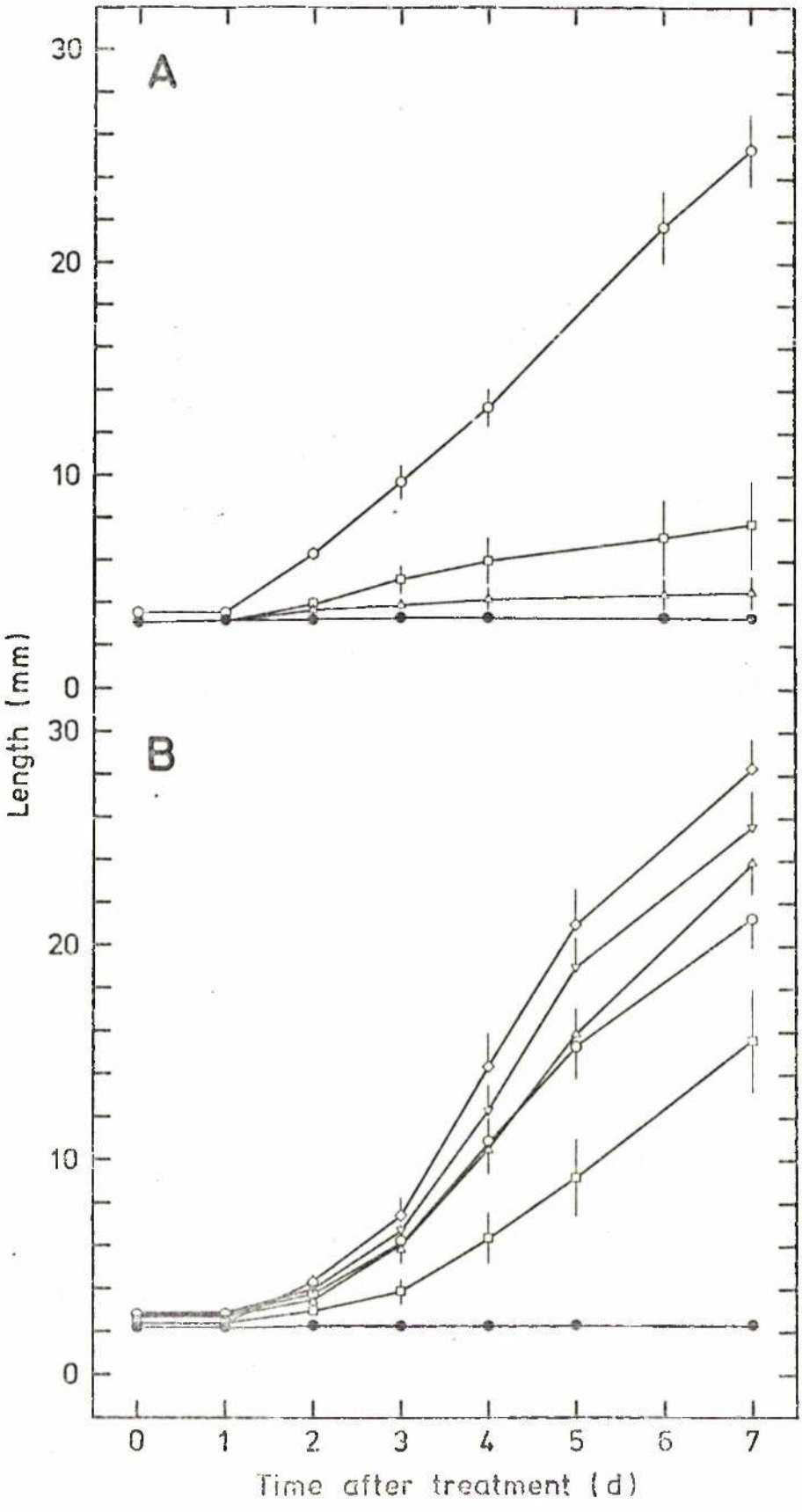
(i) Aqueous solutions

Aqueous solutions of IAA were applied using the reservoir technique of Hillman (1970). It was found that 1 mmol l^{-1} IAA did not completely inhibit the outgrowth of the primary leaf axillary buds (Figure 24). In one experiment (Figure 24A) the incomplete mean inhibition of bud growth by this concentration of IAA represented absolute inhibition of bud growth on 6 out of 10 plants. In the other experiment (Figure 24B) the relatively slight effect of this concentration of IAA included the complete inhibition of bud growth on only 1 plant. Bud growth on 9 out of 10 plants was completely prevented by 5 mmol l^{-1} IAA (Figure 24A) but this solution created some severe side-effects: the internode to which the solution was applied wilted and the nastic movements of the pulvini at both ends of the primary leaf petioles ceased, the laminae and petioles

Figure 24 : Growth of primary leaf axillary buds on plants of Ph. vulgaris intact or decapitated through the 2nd internode 3 cm above the 2nd node with IAA in aqueous solution applied to the cut stump. A,B = 2 experiments.

A : 10.8.71 : 18 d old plants, B : 28.6.71 : 14 d old plants : controlled environment cabinet : 10 plants/treatment; randomized : points = mean $\pm$ S.E. of combined length of 1st internodes of primary leaf axillary buds : slightly inhibitory effect of 1 mmol l^{-1} IAA confirmed in 1 further experiment : key:-

Symbol	Concentration of IAA (mmol l^{-1})	
	A	B
○	0.0	0.0
□	1.0	1.0
△	5.0	0.1
▽		0.01
◇		0.001
●	Intact plants	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Time after treatment (d)

remaining horizontal. Other effects associated with complete inhibition of bud growth by 5 or 1 mmol l^{-1} IAA included swelling around the primary leaf node, with the apparent formation of root initials. Bud growth was not affected by 0.1 mmol l^{-1} IAA, but was promoted slightly by 10 µmol l^{-1} IAA and distinctly by 1 µmol l^{-1} IAA (Figure 24B).

The application of 1 mmol l^{-1} IAA, which could completely prevent bud growth on some plants, was equivalent, assuming complete uptake of the applied solution, to a dosage of 9 µg IAA d^{-1}.

(ii) Lanolin pastes

Complete inhibition of the growth of the primary leaf axillary buds was maintained for 7 d by 1 mg g^{-1} (0.1%) IAA applied in lanolin to the decapitated 2nd internode of 29 and 36 d old plants (Figure 25B,C). This concentration of IAA only partially prevented the outgrowth of these buds on 22 d old plants (Figure 25A). Bud growth was considerably reduced on 22 and 29 d old plants by 0.1 mg g^{-1} (0.01%) IAA: this concentration maintained complete inhibition of bud growth on 36 d old plants for 5 d. An IAA concentration of 0.01 mg g^{-1} (0.001%) produced no inhibition of bud growth whatsoever; indeed, it slightly promoted bud growth on 22 and 29 d old plants. Although the growth of the buds on decapitated control plants was similar for each age of plant used it appears that the buds were increasingly sensitive to inhibition by IAA with increasing age. The virtually complete inhibition of bud growth by 0.1 mg g^{-1} IAA represented the effect of approximately 9 µg IAA applied at the start of the experiment and another 9 µg IAA on the 3rd day (i.e. 3 µg d^{-1}). The higher concentration of IAA was equivalent to 10 times this amount and although this consistently produced powerful inhibition of lateral bud growth it did not cause the side effects apparent with similar quantities of IAA applied as an aqueous solution. Furthermore, the inhibition was reversible: when the lanolin was removed after 7 d the buds exhibited considerable growth over the next 7 d

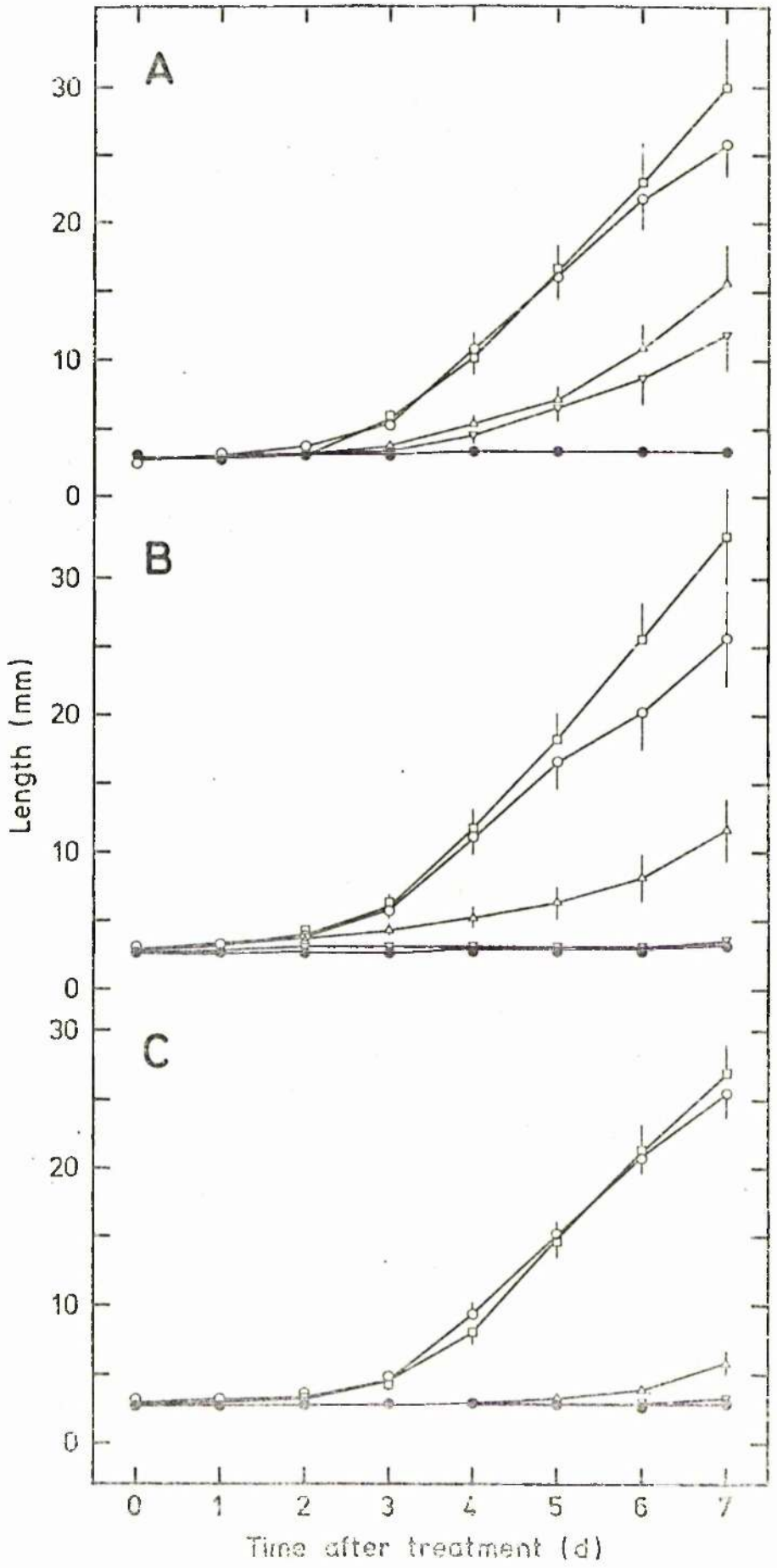
Figure 25 : Growth of primary leaf axillary buds on plants intact or decapitated 3 cm above the 2nd node with IAA in lanolin applied to the cut stump.

10.4.73 : Ph. vulgaris; A 22, B 29, C 36 d old plants : glasshouse : IAA applied in 0.1 ml lanolin on day 0 and day 3 : 10 plants/treatment; organized : points = mean ± S.E. of combined length of 1st internodes of primary leaf axillary buds : powerful inhibition of bud growth by 1 mg g^{-1} IAA in lanolin confirmed in 2 further experiments : key:-

Symbol	Concentration of IAA (mg g^{-1})
○	0.0
□	0.01
△	0.1
▽	1.0
●	Intact plants

Total length ± S.E. (mm) of primary leaf axillary buds on decapitated plants treated with 1 mg g^{-1} IAA until day 7:-

Time after treatment (d)	Age of plants (d) 22	29	36
7	18.0 ± 3.9	5.7 ± 0.4	5.6 ± 0.2
14	60.1 ± 7.2	20.8 ± 5.3	16.3 ± 2.7


A
B
C
30
20
10
0
Length (mm)
0
1
2
3
4
5
6
7
Time after treatment (d)

(Figure 25). Inhibition of bud growth by IAA in lanolin was characteristically accompanied by swelling of the top of the decapitated internode. No concentration of IAA tested induced any obvious elongation growth of the decapitated internode.

B. Extraction

Young shoot tips with the lowest trifoliate leaf between 5 and 30 mm long - previously shown to give slightly less than complete inhibition of lateral bud growth (Figure 17) - were excised from bean plants and extracted with methanol. After purification by acid-base partitioning, the extracts were subjected to successive paper chromatography and substances co-chromatographing with IAA were analysed by bioassay, a chromogenic reaction, g.l.c. and mass spectrometry. The procedures were tested for efficiency with standard IAA and for possible artifacts with methanol only.

(i) Standard IAA

Tissue, weighing 50 g, from mature primary leaves of bean together with 50 µg IAA were subjected to the extraction and partitioning procedures described under 'AUXIN ISOLATION EXPERIMENTS' in 'EXPERIMENTAL PROCEDURES'. The purified extract was loaded onto 2 paper chromatograms which were developed in the acidic solvent (butanol:acetic acid:water::25:5:11). An IAA marker chromatogram tested chromogenically with Ehrlich's reagent showed a mauve area between Rf 0.85 and 0.93, but a small amount of [1-^{14}C]IAA on one of the extract chromatograms was detected by the chromatogram scanner at Rf 0.73 to 0.83. The presence of the plant extract, then, appeared to retard the movement of IAA in this solvent system. In subsequent experiments, therefore, when only 'cold' marker IAA on a separate chromatogram was used, the region just behind the IAA zone was eluted together with the IAA zone from extract chromatograms developed in this solvent system.

The area of each chromatogram between Rf 0.70 and 0.90 was eluted with methanol. One of the eluants (equivalent to 25 µg applied IAA) was loaded onto a paper chromatogram which was developed in the basic solvent (isopropanol:ammonia:water::8:1:1). An IAA marker chromatogram exhibited a bright blue fluorescent area at Rf 0.48 to 0.56 under 254 nm u.v. light but no fluorescence under 350 nm light. The extract chromatogram showed many fluorescent and absorbent areas under u.v. light, including bright blue fluorescence, similar to that produced by IAA, between Rf 0.46 and 0.53 under 254 nm light. [1-^{14}C]IAA on this chromatogram was detected between Rf 0.45 and 0.55. The presence of plant material did not, apparently, affect the movement of IAA in this solvent system. The area of the chromatogram between Rf 0.45 and 0.57 was eluted with methanol and the eluant loaded onto a t.l.c. sheet, alongside 1 µg and 5 µg IAA markers. The chromatogram was developed with the acidic solvent (chloroform:acetic acid::19:1) and sprayed with Ehrlich's reagent. The marker spots of IAA appeared mauve, later becoming blue, at Rf 0.19 to 0.25. The extract gave a similar coloration between Rf 0.18 and 0.26 and was estimated visually by colour intensity and area to contain approximately 12 µg IAA. The recovery of applied IAA by this method was, therefore, approximately 50 percent.

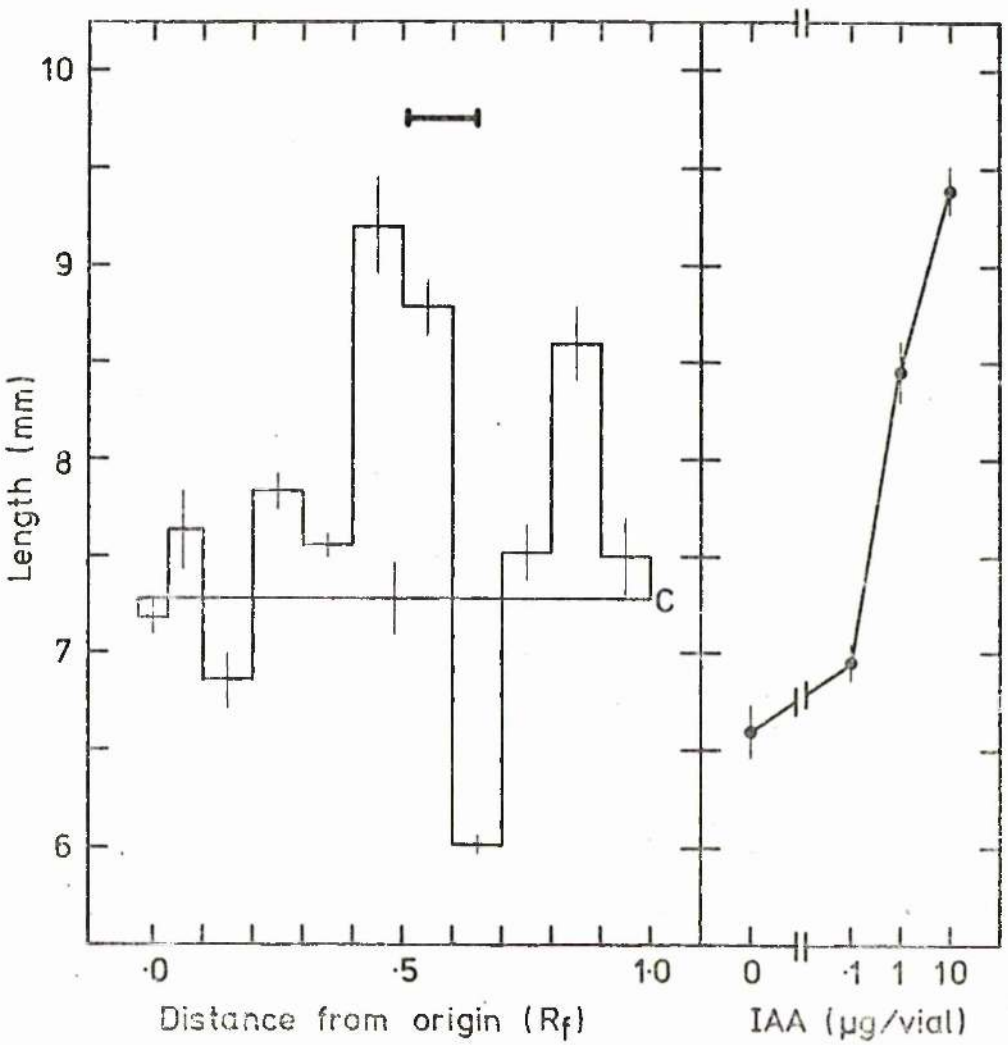
The second eluant (≙25 µg applied IAA) was loaded onto a small paper chromatogram which was developed with the basic solvent. An IAA marker chromatogram showed fluorescence under 254 nm u.v. light at Rf 0.51 to 0.65 and pink over the same area after testing with Ehrlich's reagent. The extract chromatogram exhibited a number of fluorescent and absorbent areas under u.v. light including IAA-like fluorescence at Rf 0.45 to 0.52. This chromatogram was bioassayed with Avena coleoptile segments, revealing the presence of strong growth promoting activity between Rf 0.4 and 0.6 and also some promotion at Rf 0.8 to 0.9 (Figure 26). The major growth activity, which was just behind the

Figure 26 : Bioassay of extract of c. 25 µg standard IAA.

7.8.72 : data = mean ± S.E. of length of Avena coleoptile segments for each segment of chromatogram or each quantity of authentic IAA; horizontal line (C) = control segment of chromatogram from behind the origin; horizontal bar = position of IAA on marker chromatogram detected under u.v. light and chromogenically.

Approximate estimated quantity of growth activity present on chromatogram:-

Rf zone	IAA equivalents (µg)
0.4 to 0.5	6.3
0.5 to 0.6	2.2
0.8 to 0.9	1.4


10
9
8
7
6
Length (mm)
C
·0
·5
1·0
Distance from origin (R_f)
0
·1
1
10
IAA (µg/vial)

position of the marker IAA but centred around the area of IAA-like fluorescence on the extract chromatogram, was equivalent to approximately 8.5 µg IAA. This represents a recovery of approximately 35% of the applied IAA. The growth activity at Rf 0.8 to 0.9 was equivalent to c. 1.4 µg IAA and may represent breakdown of IAA by the extraction and purification procedures, an impurity present in the stock IAA or possibly a substance(s) extracted from the leaf tissue or created by the procedures.

(ii) Solvent blank

The possibility that the procedures adopted for the extraction of shoot tissue might in themselves lead to the occurrence of auxin-like activity or indolic properties was investigated by passing methanol plus distilled water through those procedures. Accordingly, 1 l methanol mixed with 50 ml distilled water was left in darkness at room temperature, and taken through the standard partitioning and chromatographic procedures. Half of the extract, after acidic and basic paper chromatography and acidic t.l.c., gave no colour reaction to Ehrlich's reagent. The other half, after acidic and basic paper chromatography, showed no growth activity in the bioassay (Figure 27).

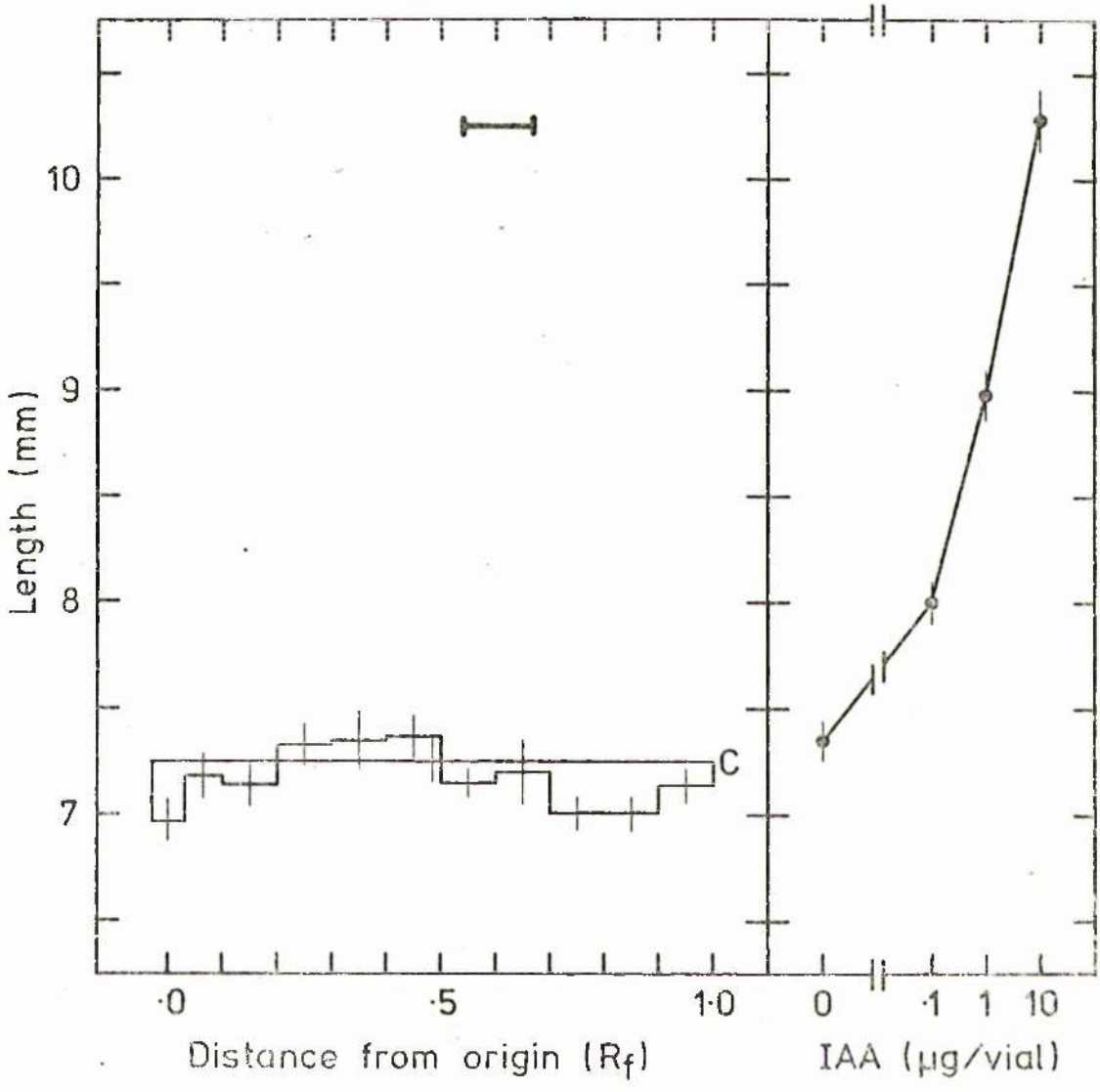
(iii) Young shoot tissue

Young shoot tips excised from batches of 3000 bean plants grown in wooden boxes were extracted with methanol. The extracts from 5 separate batches were purified in similar manner to the standard IAA and solvent blank extracts and were assayed in a number of different ways.

Experiment a. Extract of approximately 2800 shoot tips, weighing 56 g, excised from 12 d old plants, was purified by partitioning and acidic and basic paper chromatography before t.l.c. and testing with Ehrlich's reagent. IAA-like fluoresence was exhibited by the base-developed chromatograms. Half of the extract ($\hat{=}$1400 shoot tips), after acidic t.l.c., exhibited colour estimated visually by area and

Figure 27 : Bioassay of blank extract.

14.8.72 : presentation as in Figure 26.


10
9
8
7
Length (mm)
C
·0
·5
1·0
Distance from origin (R_f)
0
·1
1
10
IAA (µg/vial)

intensity to be equivalent to c. 0.25 µg IAA (Plate 7).

Experiment b. Extract of c. 2800 shoot tips, weighing 45 g, excised from 10 d old plants, was purified by partitioning and acidic and basic paper chromatography. Approximately one-third of the extract (≏930 shoot tips) was inserted by direct probe into the AEI MS12 mass spectrometer. There was no indication of the presence of IAA in the extract. Each remaining one-third of the extract was subjected to t.l.c., one portion in acidic solvent, the other in basic solvent, before testing chromogenically. There was no indication of IAA.

Experiment c. Extract of c. 2800 shoot tips, weighing 65 g, excised from 12 d old plants, was purified by partitioning and acidic and basic paper chromatography. The extract was strip-loaded onto a glass-backed silica gel plate and the chromatogram developed with the basic solvent (methyl acetate:isopropanol:ammonia::9:7:4). There was bright blue fluorescence with the same Rf as an IAA marker but this was visible under both 254 and 350 nm u.v. light. This area was scraped from the t.l.c. plate and extracted with 1 ml methanol for 1 h with occasional shaking. The silica gel was centrifuged out at 3000 rev min^{-1} for 10 min and the extract inserted by direct probe into the AEI MS12 mass spectrometer. There was no evidence for the presence of IAA.

Experiment d. Extract of c. 2800 shoot tips, weighing 45 g, excised from 14 d old bean plants, was purified by partitioning and acidic and basic paper chromatography. One quarter of the extract (≏700 shoot tips) showed no evidence of growth promoting activity in the bioassay (Figure 28): indeed, there was an inhibitory region overlapping the Rf zone of IAA. U.v. fluorescence suggested that the chromatogram may have been overloaded.

The other three-quarters of the extract (≏2100 shoot tips), which had shown IAA-like fluorescence under u.v. light on the base developed paper chromatogram, showed approximately 0.5 µg IAA

Plate 7 : Thin layer chromatogram developed in chloroform:acetic acid::19:1 and sprayed with Ehrlich's reagent : Experiment a. 27.7.72 : left = 0.5 µg IAA; centre = extract from c. 1400 shoot tips; right = 1.0 µg IAA.

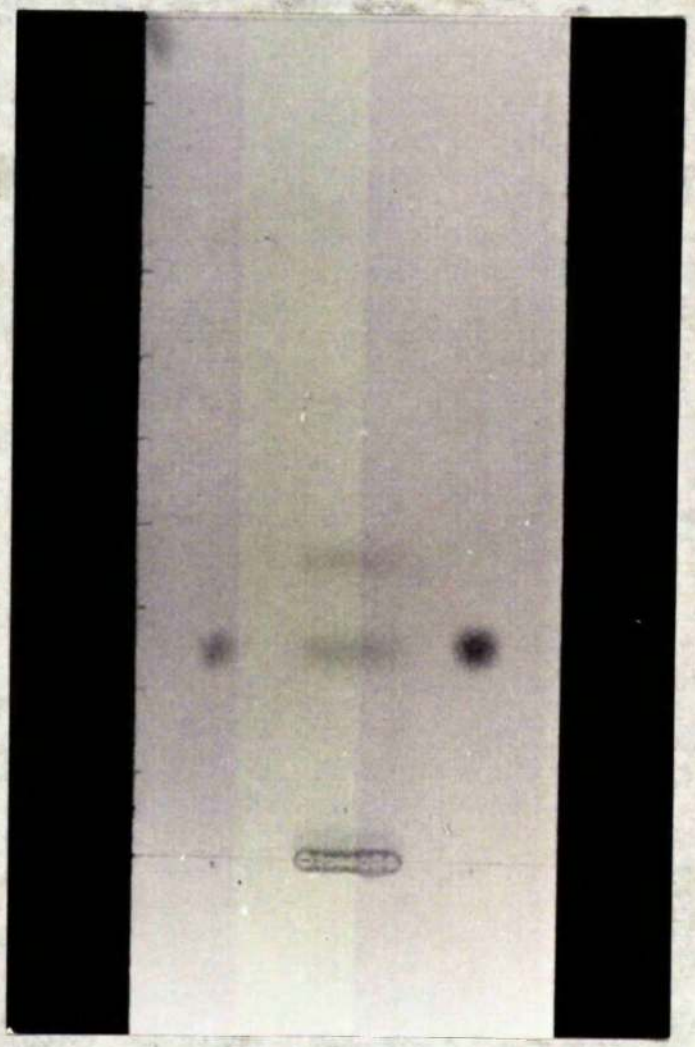


Figure 28 : Bioassay of shoot tip extract : Experiment d.

24.8.72 : presentation as in Figure 26.

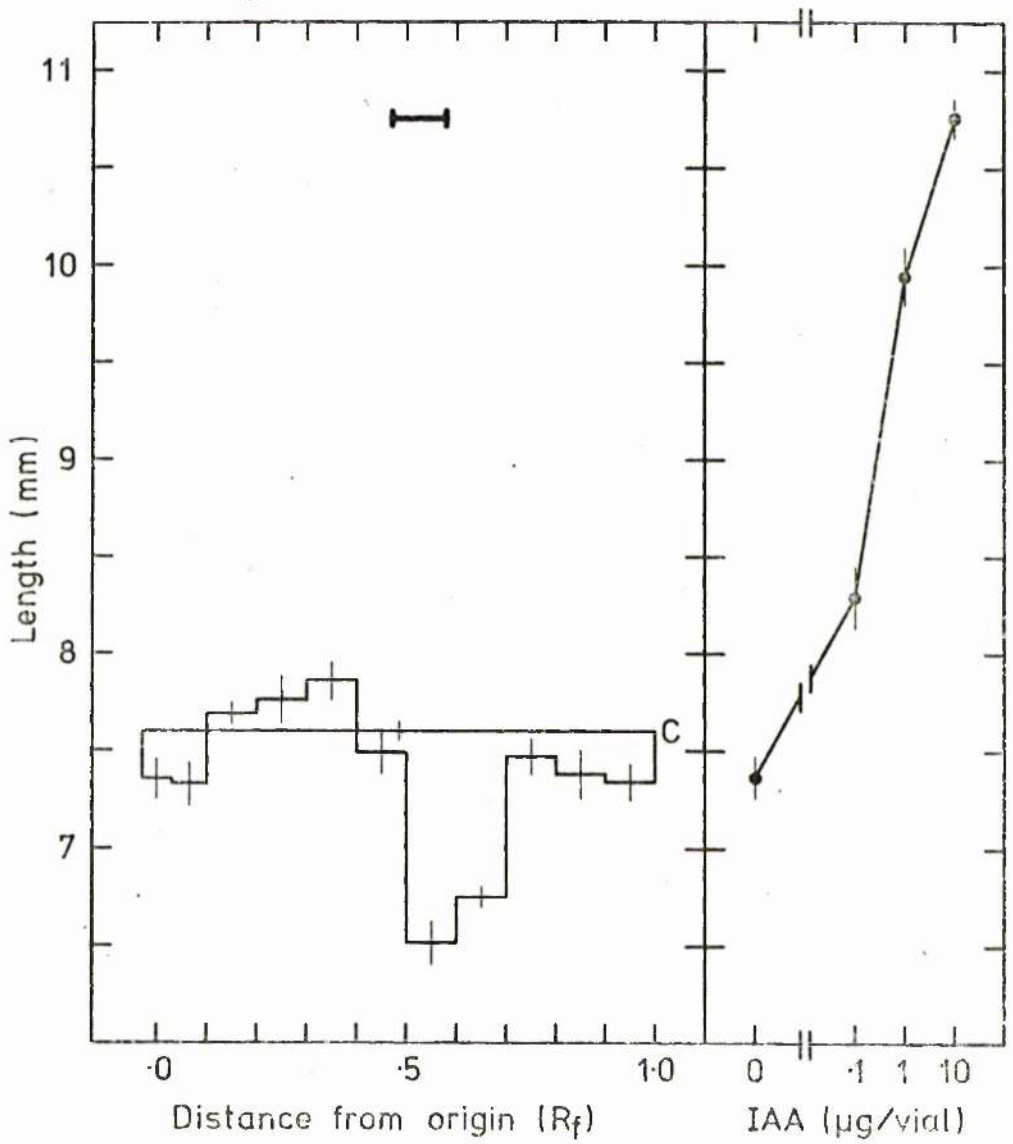
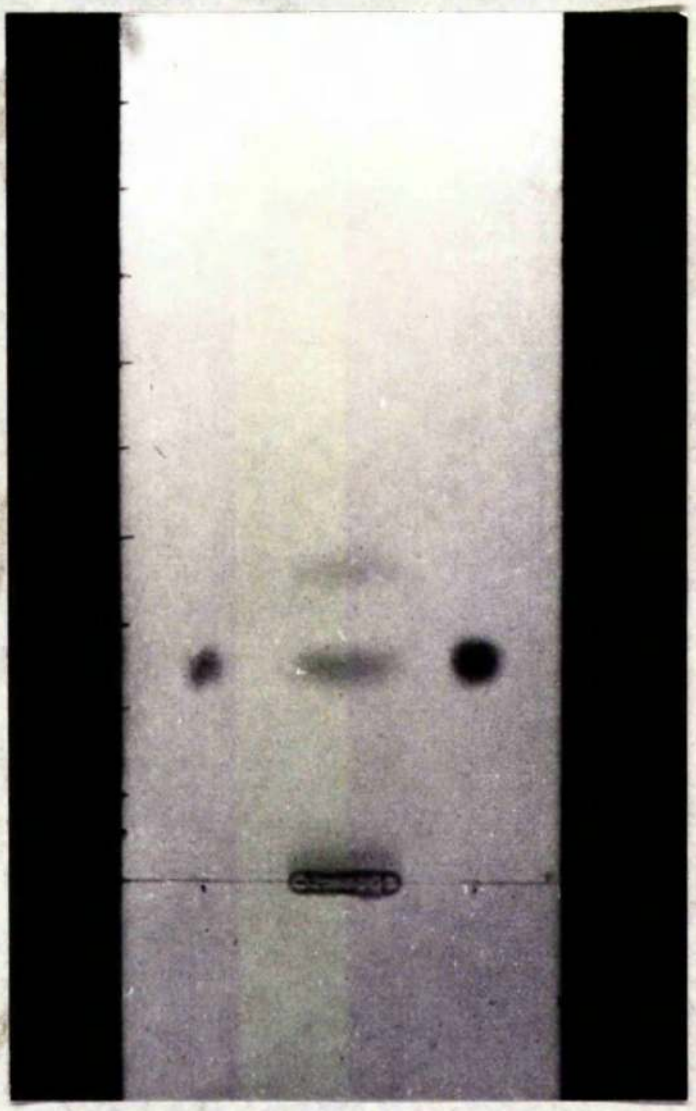

11
10
9
8
7
Length (mm)
C
·0
·5
1·0
Distance from origin (R_f)
0
·1
1
10
IAA (µg/vial)

Plate 8 : Thin layer chromatogram developed in chloroform:acetic acid::19:1 and sprayed with Ehrlich's reagent : Experiment d. 24.8.72 : left = 0.5 µg IAA; centre = extract from c. 2100 shoot tips; right = 1.0 µg IAA.



equivalents after acidic t.l.c. and testing chromogenically (Plate 8).

Experiment e. Extract of c.2700 shoot tips, weighing 68 g, excised from 19 d old plants, was purified by partitioning and acidic and basic paper chromatography. One half of the extract (≏1350 shoot tips), which had shown no IAA-like u.v. fluorescence on the base developed chromatograms, was shown by bioassay to contain growth promoting activity in the region of the chromatogram just behind that of IAA, equivalent to c. 0.4 µg IAA (Figure 29). Furthermore, between Rf 0.8 and 0.9 there was growth promotion equivalent to c. 0.14 µg IAA.

The other half of the extract, after basic t.l.c., was tested chromogenically but gave no evidence for the presence of IAA. The chromatogram may have been overloaded.

(iv) Young shoot tissue, old leaf tissue and standard IAA-adapted method

Experiment f. Approximately 8000 shoot tips, weighing 180 g, were excised from 28 d old soil-grown bean plants and extracted 3 times for 24 h at 2°C with 3 l methanol. Two similar quantities of mature primary leaf tissue from the same plants, one with 400 µg added IAA, were treated similarly. Each extract was filtered through sintered glass and purified by the standard partitioning and acidic and basic paper chromatography procedures, although on a larger scale than previously. On the base-developed paper chromatograms the standard IAA and the shoot tips extracts both exhibited IAA-like u.v. fluorescence.

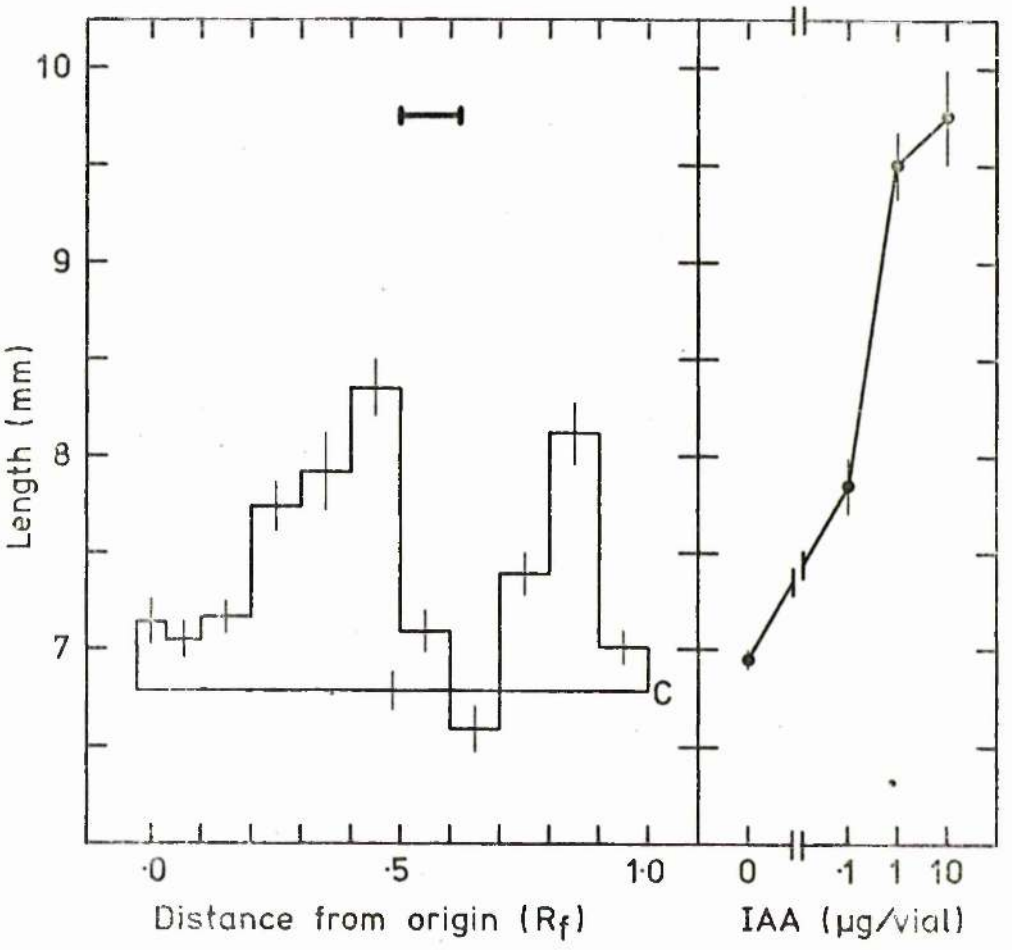
One-twelfth of each extract (≏665 shoot tips or 33 µg added IAA) was bioassayed (Figure 30). Growth promoting activity in the IAA zone equivalent to c. 0.1 µg IAA was demonstrated in the shoot tip extract, together with c. 0.025 µg IAA equivalents at Rf 0.8 to 0.9. There was no growth promoting activity in the old leaf extract. The standard IAA

Figure 29 : Bioassay of shoot tip extract : Experiment e.

7.10.72 : presentation as in Figure 26.

Approximate estimated quantity of growth activity present on chromatogram:-

Rf zone	IAA equivalents (µg)
0.2 to 0.3	0.1
0.3 to 0.4	0.1
0.4 to 0.5	0.2
0.8 to 0.9	0.14


10
9
8
7
Length (mm)
C
·0
·5
1·0
Distance from origin (R_f)
0
·1
1
10
IAA (µg/vial)

extract chromatogram exhibited growth promotion at the IAA region equalling c. 3 µg IAA plus c. 0.7 µg IAA equivalents at Rf 0.8 to 1.0.

One-sixth of each extract (≙1330 shoot tips or 67 µg standard IAA) was tested chromogenically after t.l.c. in acidic solvent (Plate 9). There was no sign of indole activity in the old leaf extract, c. 10 µg IAA equivalents in the standard IAA extract and c. 0.5 µg IAA equivalents in the shoot tip extract.

The remainder of each extract (≙5330 shoot tips or 267 µg IAA) was evaporated to dryness and treated with 50 µl BSA to form the trimethylsilyl derivative of IAA (TMS-IAA). Analysis of 5 µl samples (≙533 shoot tips or 26.7 µg added IAA) by g.l.c. indicated the presence of a large number of substances in each of the extracts (Figure 31). The retention time for the large standard IAA peak under the conditions employed was 9.45 min. After the same time the shoot tip extract showed a small but distinct peak of activity, while the old leaf extract exhibited just a tiny shoulder on a previous peak. Similar traces were also obtained from the chart recorder linked to the g.l.c. at its connection with the AEI MS30 mass spectrometer (Figure 32). The samples were chromatographed under the same conditions as before, but with He carrier gas instead of N_2, and showed a similar retention time for IAA - 9.6 min. The arrowed numbers on these traces show the positions where mass spectra were taken from other aliquots of the same extracts.

Mass spectrometry confirmed that the large g.l.c. peak from the standard IAA extract with a retention time of 9.6 min was indeed IAA (Figure 33). The spectra of both standard TMS-IAA (Figure 33D) and the TMS-IAA extract (Figure 33C) showed large peaks for m/e 73, 202 and 319. The latter represents the molecular ion for TMS-IAA (Figure 34), while the larger peak at m/e 202 represents the major fragmentation product, which has lost COOTMS (117) from the molecular ion by cleavage

Figure 30 : Bioassay of A shoot tip, B primary leaf, C primary leaf + standard IAA extracts : Experiment f.

24.4.73 : presentation as in Figure 26.

Approximate estimated quantities of growth activity present on chromatograms:-

Extract	Rf zone	IAA equivalents (µg)
A	0.3 to 0.4	0.05
	0.4 to 0.5	0.05
	0.8 to 0.9	0.025
C	0.3 to 0.4	0.5
	0.4 to 0.5	2.4
	0.5 to 0.6	0.1
	0.8 to 0.9	0.63
	0.9 to 1.0	0.04

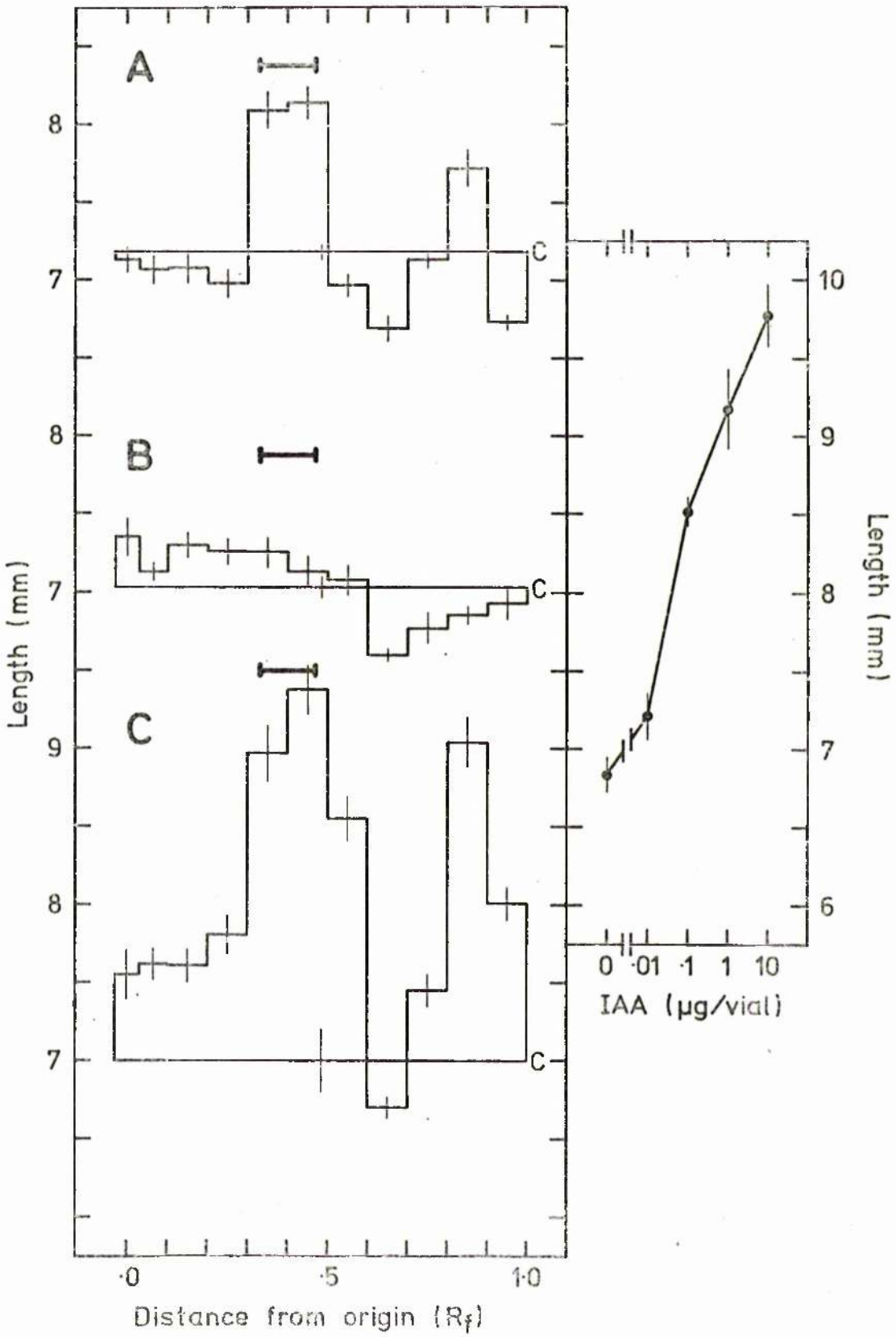

A
B
C
C
C
C
8
7
8
7
9
8
7
Length (mm)
·0
·5
1·0
Distance from origin (R_f)
10
9
8
7
6
Length (mm)
0
·01
·1
1
10
IAA (µg/vial)

Plate 9 : Thin layer chromatograms developed in chloroform:acetic acid::19:1 and sprayed with Ehrlich's reagent : Experiment f. 24.4.73 : A = extract from c. 1330 shoot tips; B = extract from primary leaves; C = extract from primary leaves + c. 67 µg standard IAA.

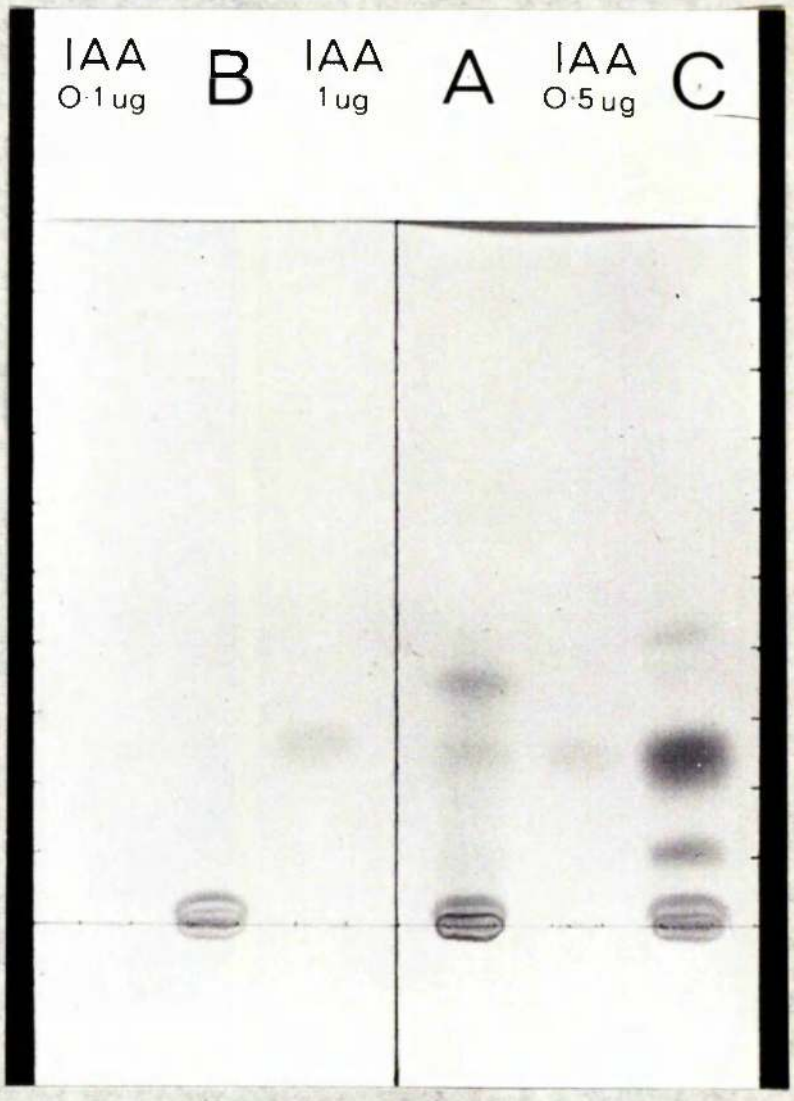


Figure 31 : Gas-liquid chromatography of BSA-treated extracts from A shoot tips, B primary leaves, C primary leaves + standard IAA : Experiment f .

24.4.73 : g.l.c. performed as described in 'EXPERIMENTAL PROCEDURES'; 5 µl samples injected; chart speed = 20 in h^{-1} : traces taken directly from charts produced by gas chromatograph; arrows indicate retention time of TMS-IAA.

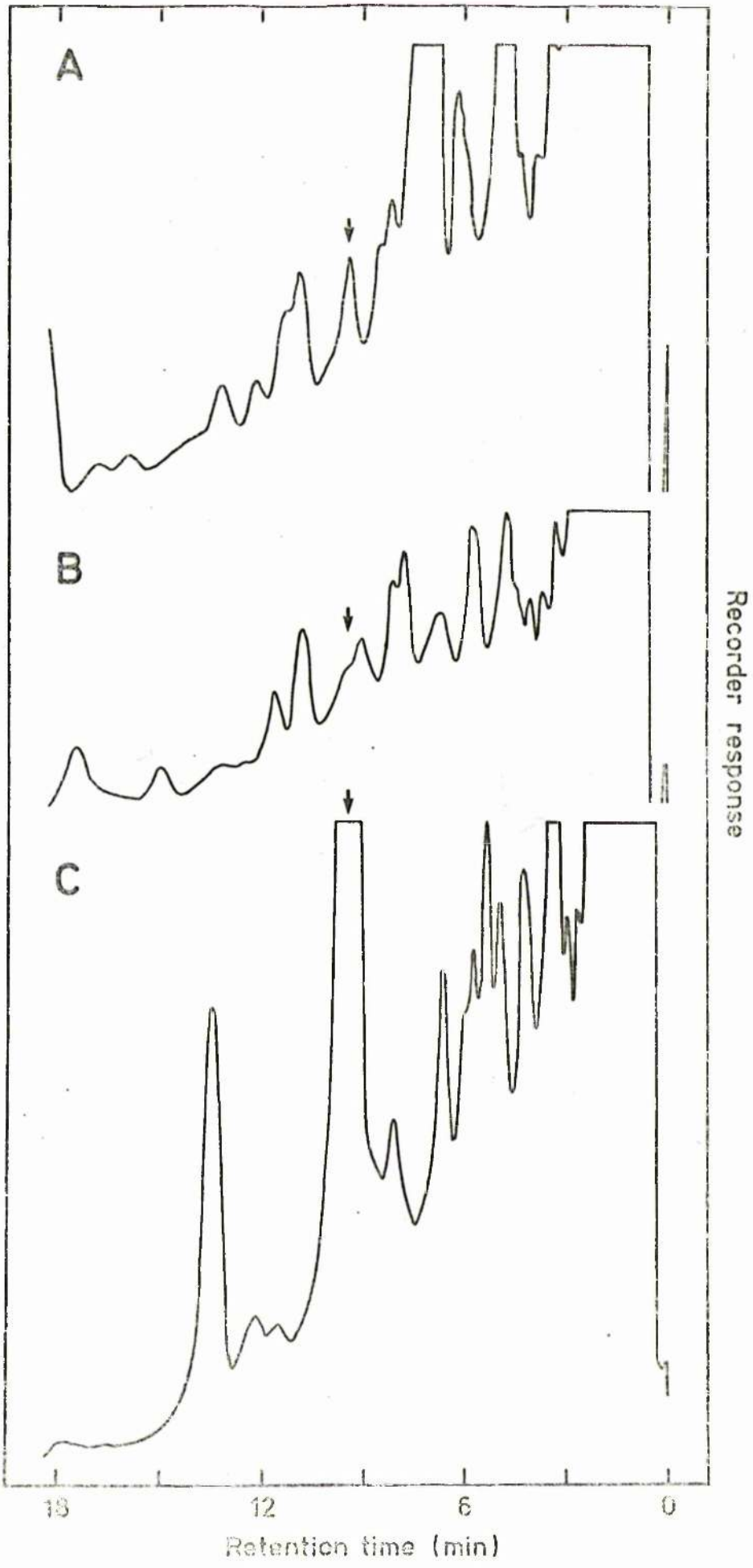

A
B
C
Recorder response
18
12
6
0
Retention time (min)

Figure 32 : Gas-liquid chromatography of BSA-treated extracts from A shoot tips, B primary leaves, C primary leaves + standard IAA : Experiment f :

24.4.73 : g.l.c. performed as described in 'EXPERIMENTAL PROCEDURES' for g.l.c.-mass spectrometry; A 2, B 5, C 2 µl samples injected; chart speed = 1 cm min^{-1} : traces taken directly from charts produced by Servoscribe chart recorder at outlet from gas chromatograph to AEI MS30 mass spectrometer; number arrows indicate position where mass spectra were taken from other aliquots of the same extracts (1 = retention time of TMS-IAA - Figure 33, 2 - Figure 35).

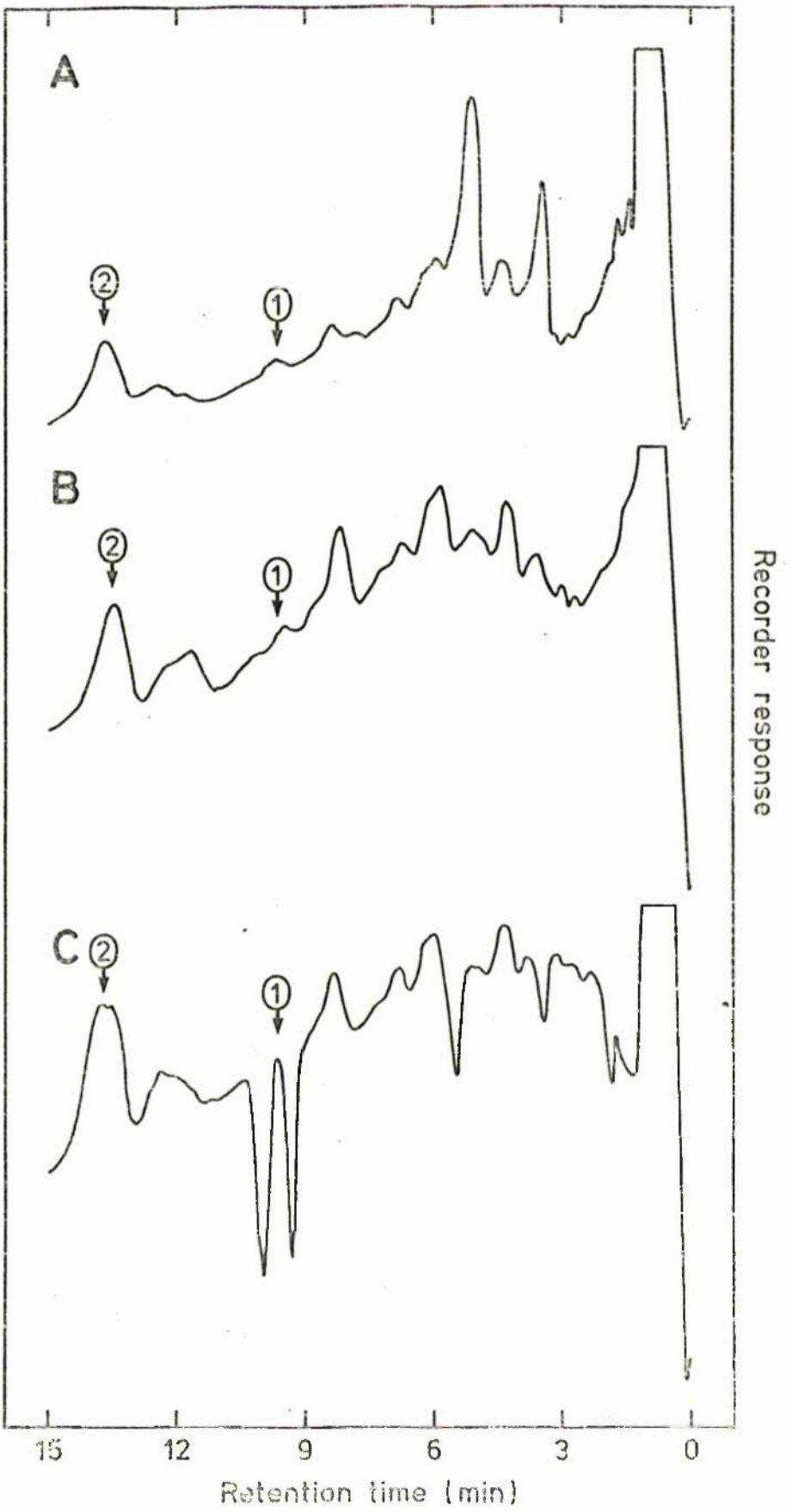

A
B
C
2
1
Recorder response
15
12
9
6
3
0
Retention time (min)

Figure 33 : Mass spectra of peak 1 (Figure 32) or authentic TMS-IAA introduced from the gas chromatograph to the AEI MS30 mass spectrometer : Experiment f.

24.4.73 : A = shoot tip extract, B = primary leaf extract, C = primary leaf + standard IAA extract, D = authentic TMS-IAA : g.l.c.-mass spectrometry performed as described in 'EXPERIMENTAL PROCEDURES'; A, C, D = 2, B = 5 µl samples : largest peak assigned 100% relative abundance and other peaks expressed as a percentage of this peak; peaks which appeared substantially in background spectra were not included.

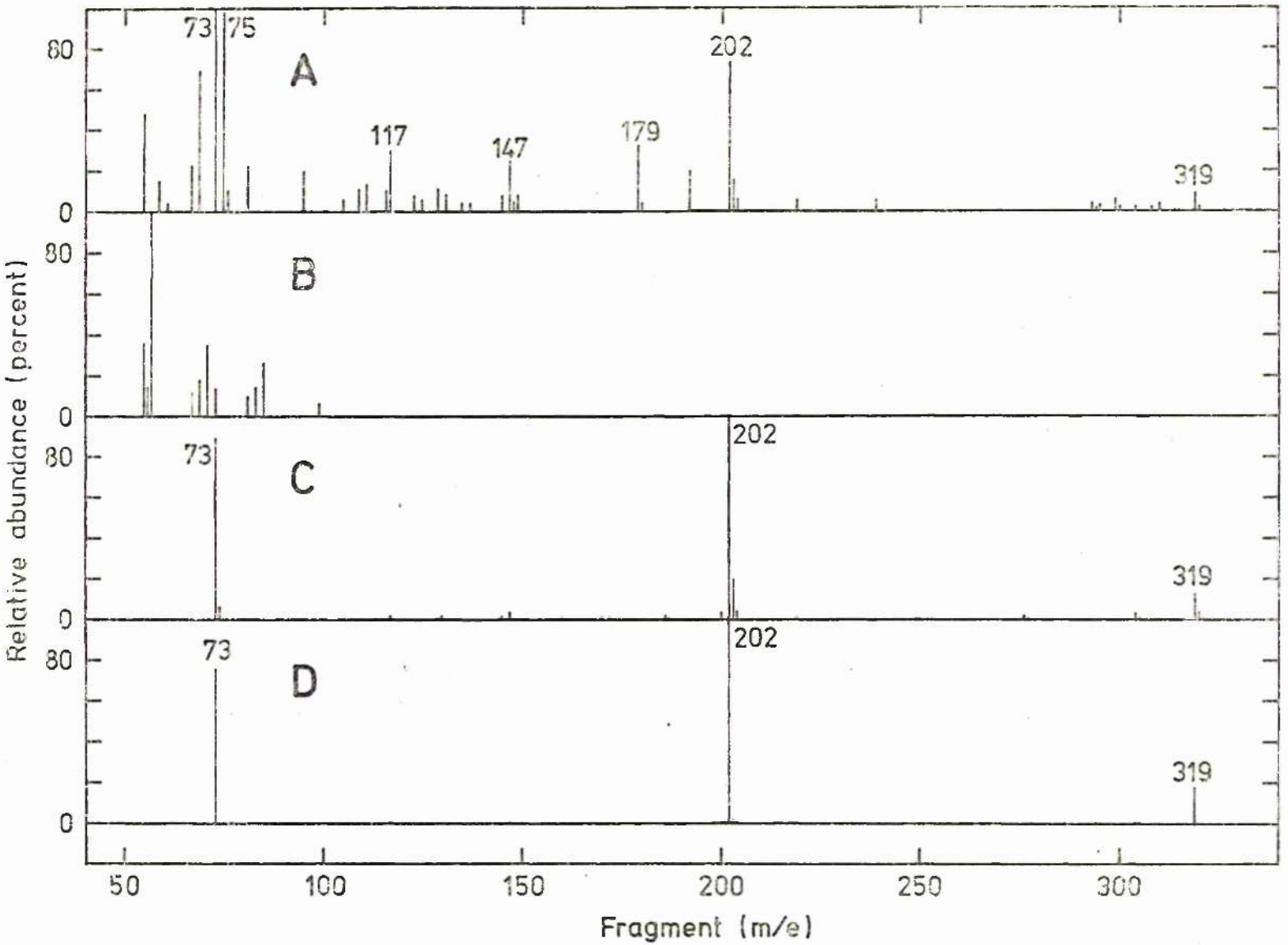

A
B
C
D
73
75
117
147
179
202
319
80
0
Relative abundance (percent)
50
100
150
200
250
300
Fragment (m/e)

Figure 34 : Formation and fragmentation of the trimethylsilyl derivative of indole acetic acid.

A = indole acetic acid (IAA)

B = bis-trimethylsilyl acetamide (BSA)

C = bis-trimethylsilyl indole acetic acid (TMS-IAA) - m/e 319

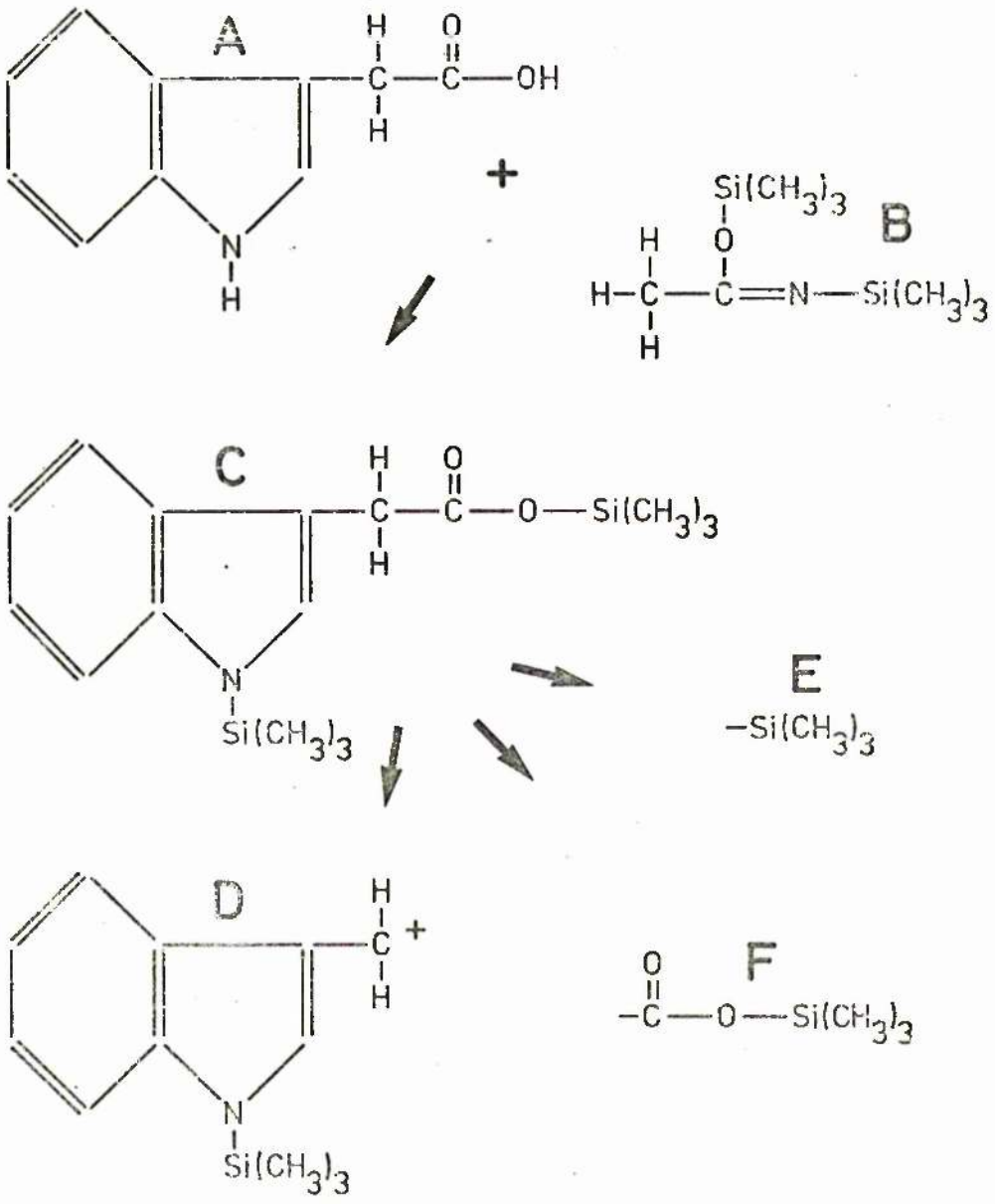
D = m/e 202

E = trimethylsilyl (TMS) - m/e 73

F = COOTMS - m/e 117

A + B → C ,

Upon fragmentation by mass spectrometry, C → D, E, F et al.


A
B
C
D
E
F
Si(CH3)3

through the side chain. The peak at m/e 73, found in all the mass spectra, is produced by TMS. The ratio of the height of the peak at m/e 319 to that at m/e 202 was 0.16:1 for the standard IAA and 0.13:1 for the IAA passed through the extraction procedures.

The peak from the shoot tip extract with a retention time of 9.6 min also exhibited a strong mass spectrum for IAA (Figure 33A) with a ratio of peak heights for m/e 319:202 of 0.12:1. Measurement of the peak height at 202 in this spectrum, compared with that in the spectrum from standard IAA (0.06 µg) indicated approximately 0.14 µg IAA to be present in 2 µl of the TMS-extract. This method of measurement, therefore, gave a figure of 3.5 µg IAA extracted from 5330 shoot tips. There was no evidence for the presence of IAA in the old leaf extract from the mass spectrum of the activity in g.l.c. with a retention time of 9.6 min (Figure 33B).

All 3 extracts showed a peak from g.l.c. with a retention time of 13.6 min (Figure 32). On the introduction of this activity from each of the extracts into the mass spectrometer similar spectra were obtained (Figure 35). The major peaks, in addition to that at m/e 73, were at m/e 75, 117, 129, 132, 145 and 313. The standard IAA extract also showed a trace of IAA, with small peaks at m/e 202 and 319. The peak at m/e 117, which appeared to a greater or lesser extent in all the spectra, may represent COOTMS: the other peaks have not been identified.

(v) Summary

It is obvious that there was much variation between individual experiments, despite using similar techniques for each. IAA-like fluorescence under u.v. light was exhibited by 4 out of 6 extracts. The bioassay technique was used in 3 experiments (Table 5A): growth promoting activity which had co-chromatographed with IAA in 2 solvent systems was detected from shoot tip extracts in 2 of these. The amount

Figure 35 : Mass spectra of peak 2 (Figure 32) introduced from the gas chromatograph to the AEI MS30 mass spectrometer : Exporiment f.

24.4.73 : A = shoot tip extract, B = primary leaf extract, C = primary leaf + standard IAA extract : 5 µl samples injected : presentation as in Figure 33.

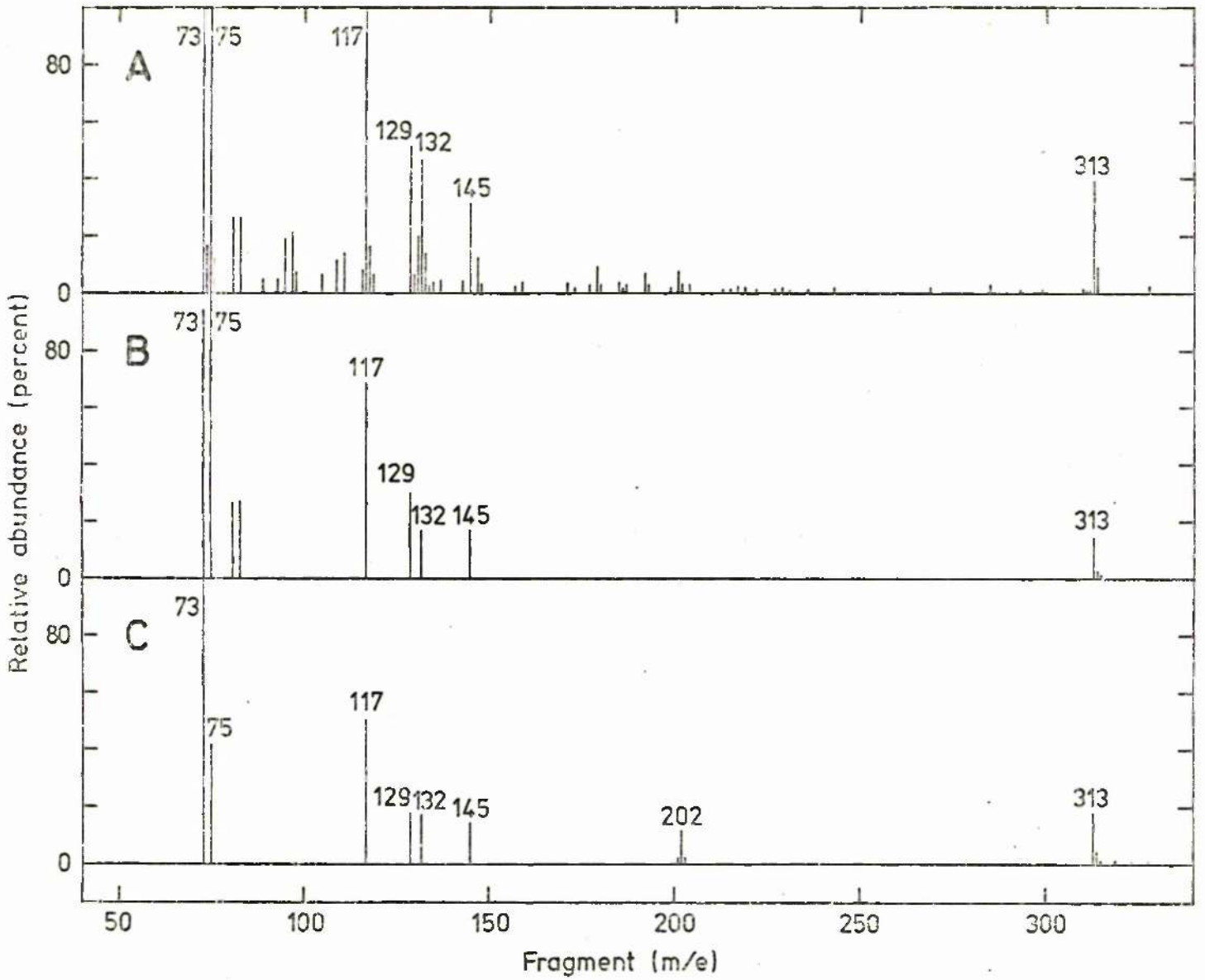

A
B
C
73
75
117
129
132
145
313
202
80
0
Relative abundance (percent)
50
100
150
200
250
300
Fragment (m/e)

Table 5A : Amounts and concentrations of auxin extracted from shoot tips of Ph. vulgaris ; estimated by bioassay.

Expt.	No. of shoot tips	Weight of shoot tips (g)	IAA equivalents (µg)	IAA equivalents (ng/shoot tip)	IAA equivalents (ng/g)	Recovery (%)
d	700	11	0.0			
e	1350	34	0.4	0.30 (0.9)	11.8 (35)	35
f	665	15	0.1	0.15 (1.5)	6.7 (67)	10

Table 5B: Amounts and concentrations of auxin extracted from shoot tips of Ph. vulgaris; estimated by chromogenic assay.

Expt.	No. of shoot tips	Weight of shoot tips (g)	IAA equivalents (µg)	IAA equivalents (ng/shoot tip)	IAA equivalents (ng/g)	Recovery (%)
a	1400	27	0.25	0.18 (0.4)	9.3 (19)	50
b	930	15	0.00			
d	2100	33	0.50	0.24 (0.5)	15.2 (30)	50
e	1350	34	0.00			
f	1330	30	0.50	0.38 (2.5)	16.7 (111)	15

Figures in brackets are estimates corrected for losses during extraction and purification.

of growth activity was estimated, by comparison with IAA standards and calculation to allow for losses during purification, to be in the order of 0.9 to 1.5 ng IAA equivalents from each shoot tip or 35 to 67 ng IAA equivalents g^{-1} fresh weight of tissue.

The chromogenic assay indicated indole activity which had co-chromatographed with IAA in 3 solvent systems in 3 out of 5 extracts (Table 5B). Approximate quantification, allowing for purification losses, suggested that the amount of activity in IAA equivalents was in the order of 0.4 to 2.5 ng from each shoot tip or 19 to 111 ng g^{-1} fresh weight.

G.l.c. - mass spectrometry of the shoot tip extract from Experiment f confirmed the presence of IAA in a quantity equivalent to c. 0.66 ng from each shoot tip (without allowance for purification losses) suggesting that the IAA-like activity detected by bioassay and chromogenic testing was, indeed, mostly due to IAA.

C. Collection in agar

The 'agar-diffusion' technique was used to collect auxin translocated basipetally out of isolated shoot tips similar to those used for auxin extraction. The agar was extracted with methanol or ether and the extract subjected to paper chromatography in 1 or 2 solvent systems before assay with similar techniques to those employed for the shoot tip extracts. Extracts of standard IAA in agar, and of agar alone, were also tested.

(i) Standard IAA

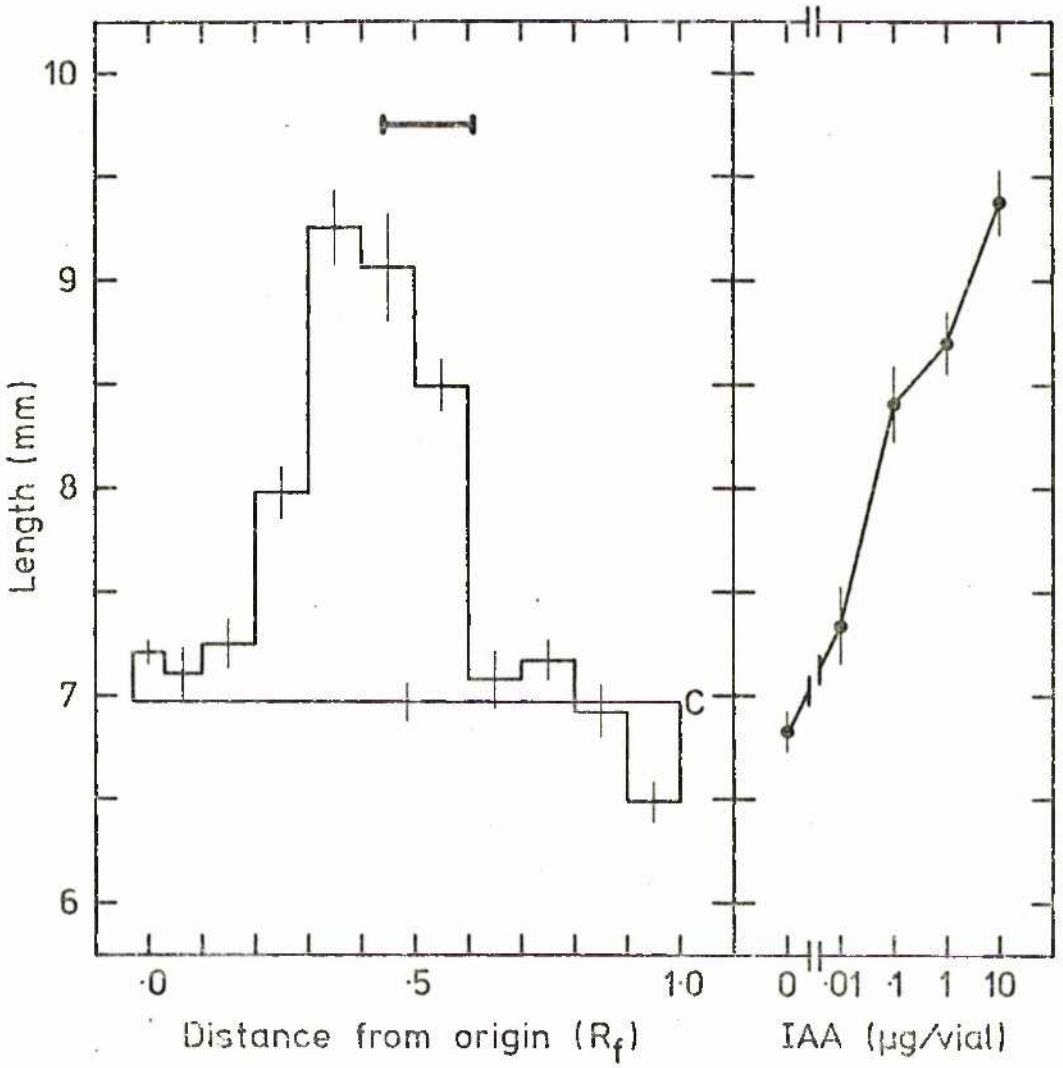
175 µg IAA in 10 ml agar was allowed to stand for 6 h at 25°C under white light before being extracted 3 times with 30 ml methanol. The methanol was evaporated under vacuum and the residue redissolved in 3 x 0.5 ml methanol. The extract was loaded onto a small paper chromatogram which was developed with the basic solvent and bioassayed with Avena coleoptile segments (Figure 36). Growth activity equivalent to c. 10.7 µg IAA was detected in the IAA Rf zone of the chromatogram: this represented a recovery of only 6% of the applied IAA.

Figure 36 : Bioassay of extract from agar containing 175 µg standard IAA.

9.3.73 : presentation as in Figure 26.

Approximate estimated quantity of growth activity present on chromatogram:-

Rf zone	IAA equivalents (µg)
0.3 to 0.4	7
0.4 to 0.5	3.5
0.5 to 0.6	0.2


10
9
8
7
6
Length (mm)
C
·0
·5
1·0
Distance from origin (R_f)
0
·01
·1
1
10
IAA (µg/vial)

(ii) Young shoot tips

Experiment a. 95 shoot tips were excised from 18 d old plants and left on 3.6 ml agar for 6 h. The methanolic extract of the agar was subjected to paper chromatography in acidic followed by basic solvent prior to bioassay (Figure 37). The extract contained growth promoting activity, equivalent to 0.25 µg IAA, which had co-chromatographed with marker IAA in both solvent systems. There was also strongly inhibitory activity near the solvent front of the chromatogram. The chromatography paper used in this experiment was not prewashed in methanol and there was a yellow coloration near the solvent front which may have arisen from the paper itself, and may have been associated with the inhibition.

Experiment b. 88 shoot tips from 14 d old plants were left for 6 h on 2.4 ml agar. Another 2.4 ml agar without shoot tips was treated similarly as a control. The methanolic extract of each portion of agar was chromatographed in acidic then basic solvent and bioassayed (Figure 38). There was no significant growth activity in either extract.

Experiment c. 70 shoot tips, weighing 10 g, from 20 d old plants were left for 6 h on 4.8 ml agar, alongside 4.8 ml control agar. Methanolic extracts of each portion of agar were chromatographed in basic solvent and bioassayed (Figure 39). The agar control chromatogram (Figure 39B) exhibited some growth promoting activity at Rf 0.5 to 0.7. The shoot tip agar chromatogram (Figure 39A) showed this activity as well as c. 0.14 µg IAA equivalents between Rf 0.3 and 0.5. By subtracting the values of coleoptile length for the control agar chromatogram from those for the shoot tip agar chromatogram, a narrow band of growth promoting activity, co-chromatographing with IAA, was indicated (Figure 39C).

Experiment d. 94 shoot tips, weighing 4.5 g, from 18 d old plants were left on 4.0 ml agar for 6 h, alongside 4.0 ml control agar. Bioassay of the methanolic extracts, after chromatography in

Figure 37 : Bioassay of extract from agar after collection from shoot tips : Experiment a.

3.3.72 : presentation as in Figure 26.

Approximate estimated quantity of growth activity present on chromatogram:-

Rf zone	IAA equivalents (µg)
0.5 to 0.6	0.22
0.6 to 0.7	0.03

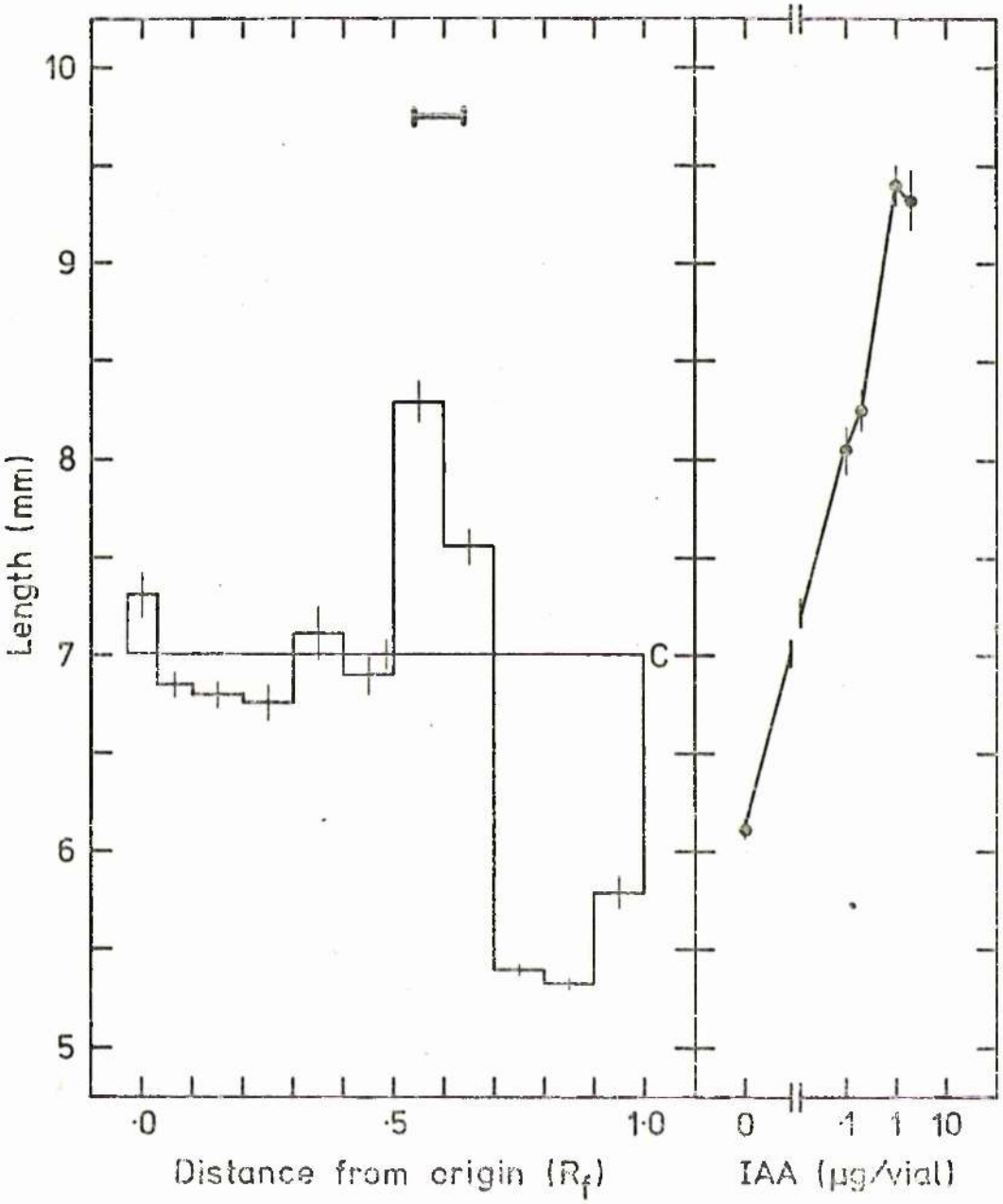

10
9
8
7
6
5
Length (mm)
C
·0
·5
1·0
Distance from origin (R_f)
0
·1
1
10
IAA (µg/vial)

Figure 38 : Bioassay of extracts from A agar after collection from shoot tips, B plain agar : Experiment b.

1.5.72 : presentation as in Figure 26.

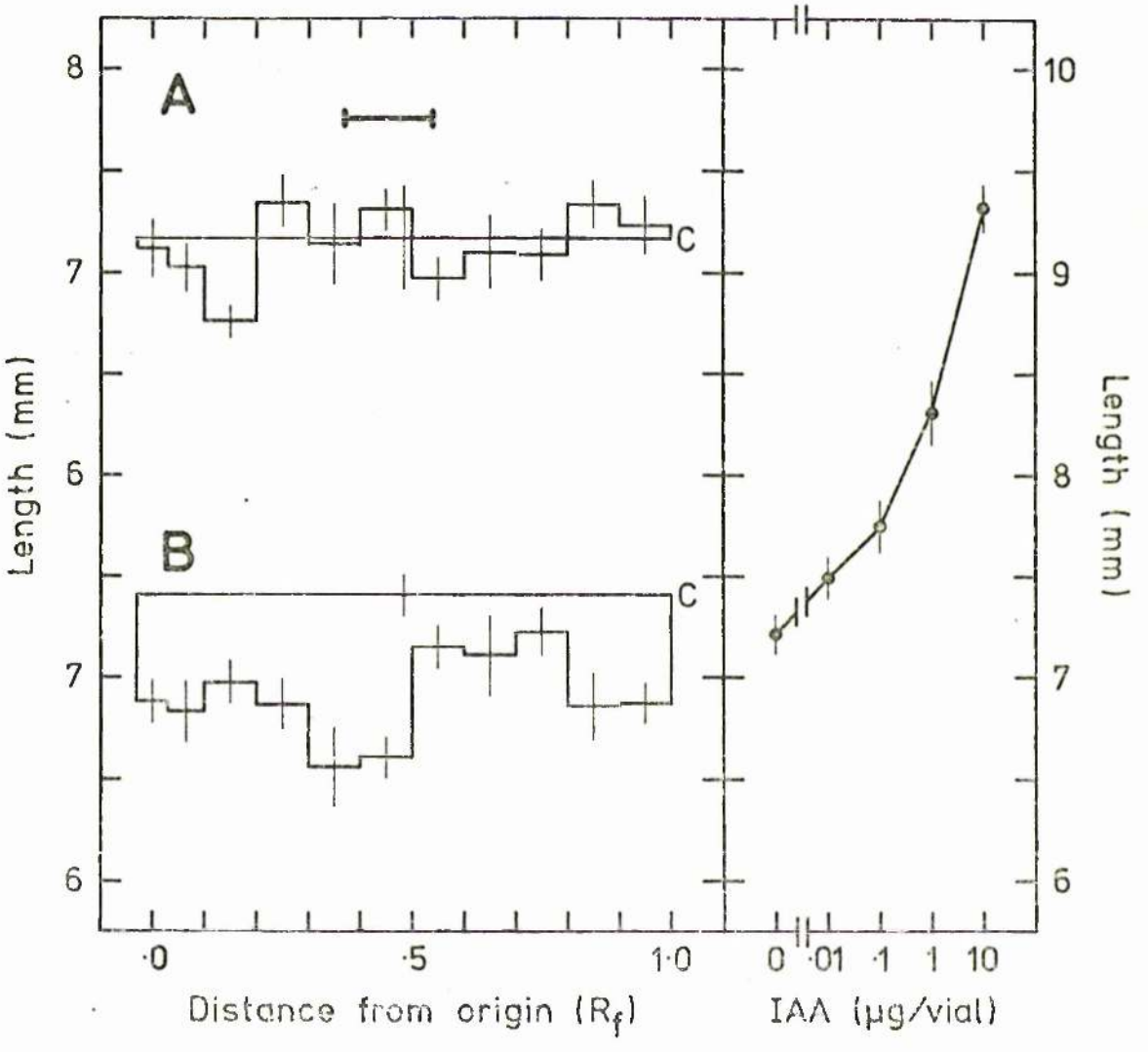
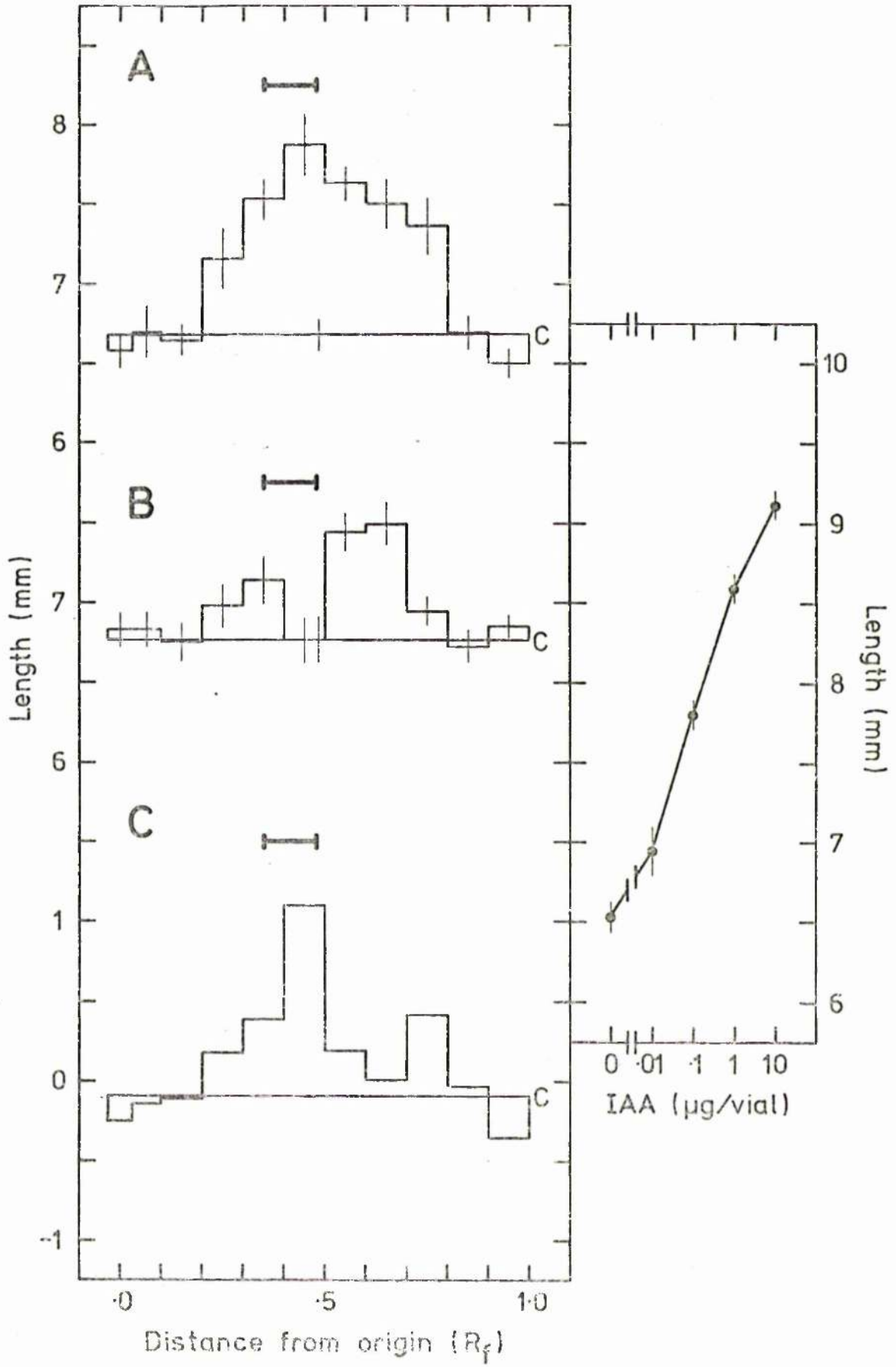

A
B
C
C
8
7
6
7
6
10
9
8
7
6
Length (mm)
Length (mm)
·0
·5
1·0
0
·01
·1
1
10
Distance from origin (R_f)
IAA (µg/vial)

Figure 39 : Bioassay of extracts from A agar after collection from shoot tips, B plain agar : Experiment b.

4.3.73 : presentation as in Figure 26 : C = A-B.

Approximate estimated quantity of growth activity present on chromatogram of extract A :-

Rf zone	IAA equivalents (µg)
0.3 to 0.4	0.03
0.4 to 0.5	0.11


A
B
C
C
C
C
8
7
6
7
6
1
0
-1
·0
·5
1·0
Length (mm)
Distance from origin (R_f)
10
9
8
7
6
Length (mm)
0
·01
·1
1
10
IAA (µg/vial)

the basic solvent, again indicated the presence of growth activity from the agar at Rf 0.5 to 0.7 and of growth promotion from shoot tips, co-chromatographing with IAA and equivalent to 0.45 µg IAA (Figure 40).

Experiment e. Approximately 500 shoot tips, weighing 24 g, from 21 d old plants grown in wooden boxes were left for 6 to 8 h on 22 ml agar, alongside 22 ml control agar. One half of each portion of agar was extracted with methanol, the other half with ether. The extracts were chromatographed in acidic then basic solvent and bioassayed (Figure 41). All the extracts exhibited growth promoting activity near the IAA zone, but there was little evidence for activity collected from the shoot tips.

Experiment f. Approximately 2400 shoot tips, weighing 130 g, from 22 d old soil-grown plants were left on 400 ml agar for 6 to 8 h, alongside 400 ml control agar. The methanolic extract of each portion of agar was divided into 2 portions. One half (≏1200 shoot tips) was chromatographed twice in the basic solvent and bioassay (Figure 42) indicated the shoot tips to have produced c. 8.5 µg IAA equivalents of growth activity co-chromatographing with IAA. The other half (≏1200 shoot tips) was subjected to paper chromatography in the basic solvent followed by t.l.c. in the acidic solvent, before testing with Ehrlich's reagent. Markers of 0.5 and 1.0 µg IAA showed clearly as mauve areas at Rf 0.18 to 0.23; on the same chromatogram there was no coloration from the control agar extract. On another chromatogram developed simultaneously in the same solvent 0.05 and 0.1 µg IAA markers were not detected chromogenically but there was an area the same colour as IAA, equivalent to c. 1 µg IAA, from the shoot tip agar extract. This region was at Rf 0.15 to 0.19 (just behind the Rf of IAA on the other chromatogram).

(iii) Young shoot tips - adapted method

Experiment g. Approximately 6000 shoot tips, weighing

Figure 40 : Bioassay of extracts from A agar after collection from shoot tips, B plain agar : Experiment d.

9.3.73 : presentation as in Figure 26 : C = A-B.

Approximate estimated quantity of growth activity present on chromatogram of extract A:-

Rf zone	IAA equivalents (µg)
0.3 to 0.4	0.05
0.4 to 0.5	0.4

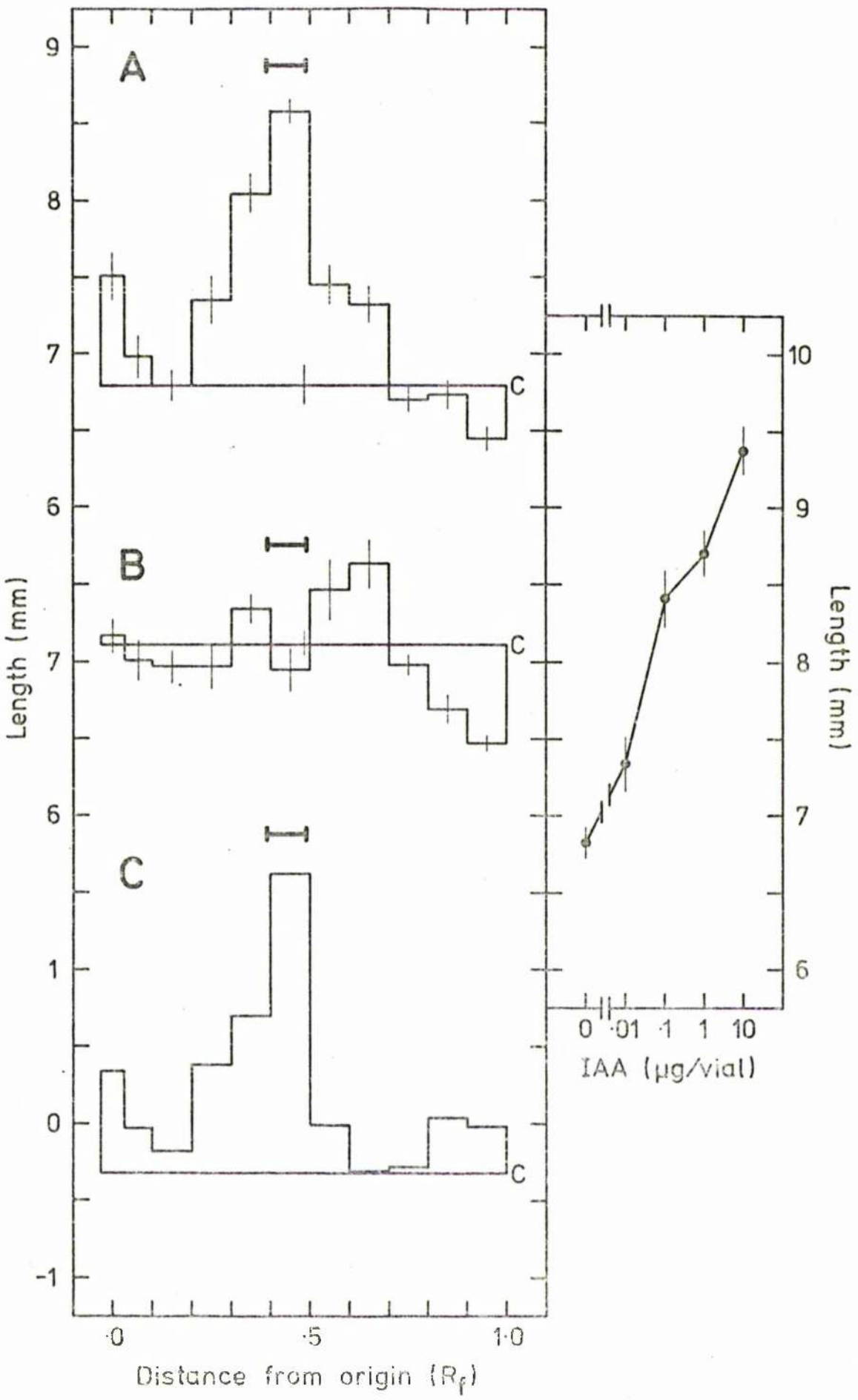

A
B
C
C
C
C
9
8
7
6
7
6
1
0
-1
Length (mm)
·0
·5
1·0
Distance from origin (R_f)
10
9
8
7
6
Length (mm)
0
·01
·1
1
10
IAA (µg/vial)

Figure 41 : Bioassay of methanol extract from A agar after collection from shoot tips, B plain agar and of ether extract from C agar after collection from shoot tips, D plain agar; Experiment e.

13.3.72 : presentation as in Figure 26.

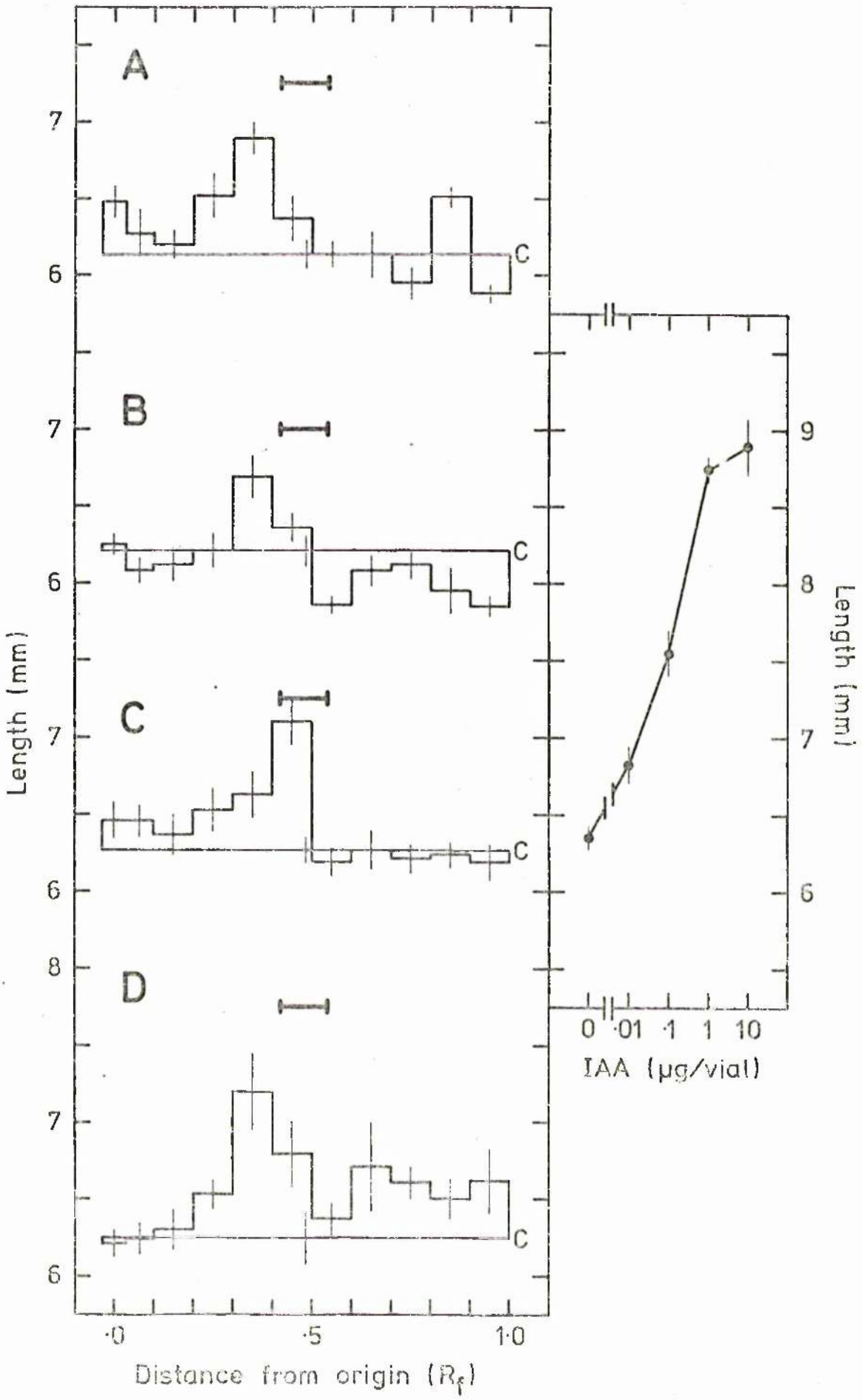
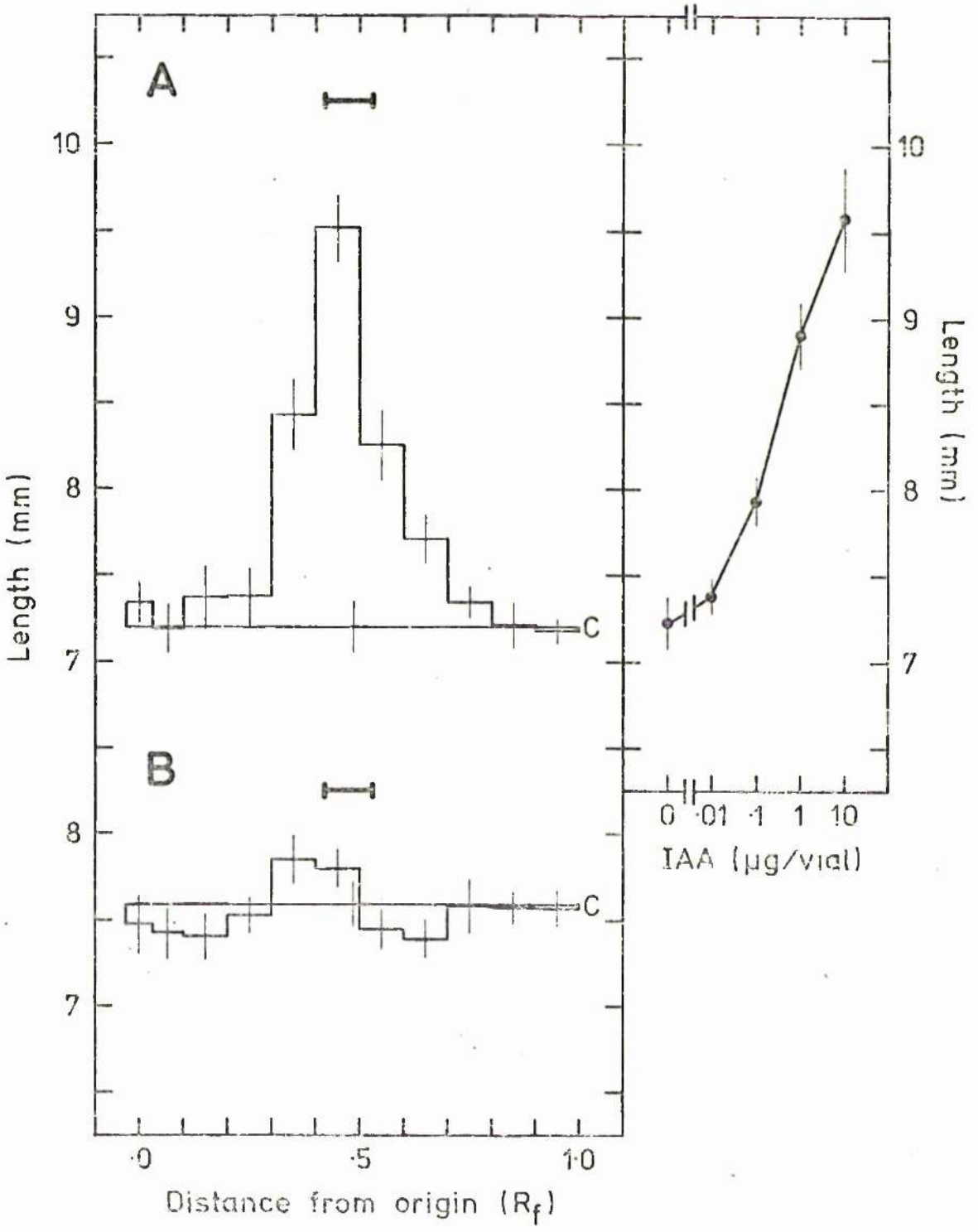

A
B
C
D
C
Length (mm)
7
6
8
9
·0
·5
1·0
Distance from origin (R_f)
0 ·01 ·1 1 10
IAA (µg/vial)

Figure 42 : Bioassay of extract from A agar after collection from shoot tips, B plain agar : Experiment f.

22.3.73 : presentation as in Figure 26.

Approximate estimated quantity of growth activity present on chromatogram of Extract A:-

Rf zone	IAA equivalents (ug)
0.3 to 0.4	0.3
0.4 to 0.5	8.0
0.5 to 0.6	0.2


A
B
C
C
10
9
8
7
8
7
Length (mm)
Length (mm)
·0
·5
1·0
Distance from origin (R_f)
0
·01
·1
1
10
IAA (µg/vial)

170 g, were excised from 17 d old soil-grown plants and left on 600 ml agar for 6 to 8 h. The agar was extracted at 2°C with 3 portions of diethyl ether which was subsequently stored at -15°C. The extract was decanted while < 0°C and filtered through sintered glass before evaporation of the ether. The residue was redissolved in methanol, paper chromatographed in basic, acidic and basic solvents and introduced by direct probe into the AEI MS12 mass spectrometer. The characteristic peaks of IAA at m/e 175 and 130 (Jamieson and Hutzinger, 1970) were not present in the spectra of the extract although they were evident in a spectrum obtained from a sample of authentic IAA.

Experiment h. Approximately 2500 shoot tips, weighing 60 g, were excised from 18 d old soil-grown plants and left on 300 ml agar for 6 to 8 h, together with 300 ml control agar. Each portion of agar was extracted with ether as in Experiment g and the extract paper chromatographed in the basic solvent. One quarter of each extract ($\simeq$625 shoot tips) was then chromatographed on a t.l.c. sheet in acidic solvent and tested chromogenically. Although 0.5 and 1.0 µg IAA markers were clearly visible there was no colour reaction from either extract. Another quarter of each extract ($\simeq$625 shoot tips) was introduced directly into a vial for bioassay; there was no significant growth activity in either extract (Table 6). The remaining half of the shoot tip agar extract ($\simeq$1250 shoot tips) was dried under OFN, treated with BSA and analysed by g.l.c. (Figure 43). There was no evidence for the presence of IAA in the extract.

(iv) Summary

As in the extraction experiments, there was much variation between individual experiments. Unlike extracts, however, agar collections from shoot tips never exhibited IAA-like fluorescence under u.v. light on base-developed chromatograms. In 4 out of 7 experiments auxin activity in the Avena coleoptile straight growth assay, equalling

Table 6 : Bioassay of standard quantities of IAA and extracts of agar with and without shoot tips of Ph. vulgaris

IAA (µg)	Length of coleoptiles (mm)
0.0	6.9 ± 0.1
0.1	7.8 ± 0.1
1.0	8.3 ± 0.1
10.0	9.3 ± 0.1
Extract	
+ shoot tips	7.2 ± 0.1
- shoot tips	7.3 ± 0.1

Experiment h : 29.3.73 : c. 625 shoot tips : results = mean length 10 Avena coleoptile segments ± S.E.

Figure 43 : Gas-liquid chromatography of BSA-treated A authentic IAA, B extract from agar after collection from shoot tips : Experiment h.

29.3.73 : details as in Figure 31.

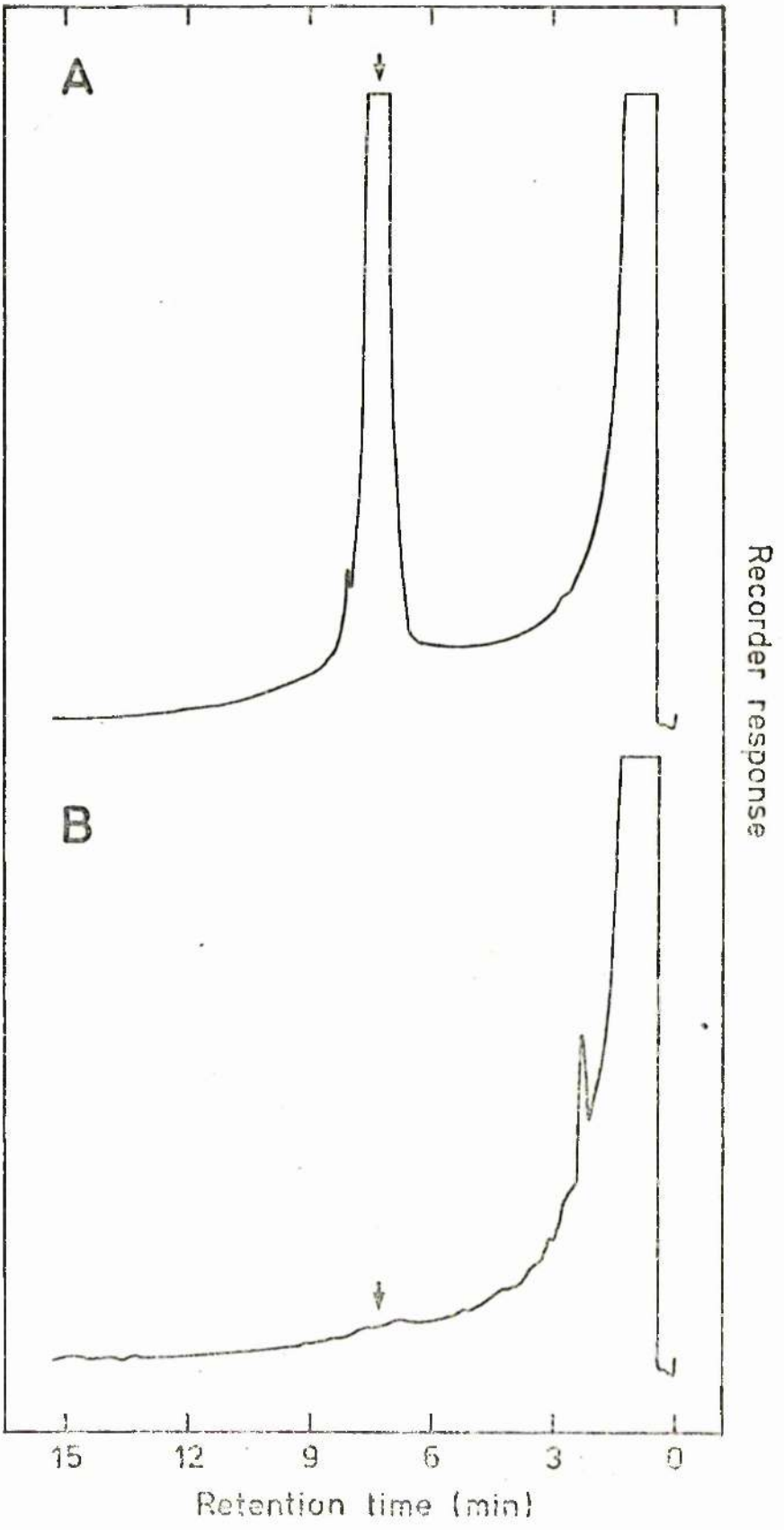

A
B
Recorder response
15
12
9
6
3
0
Retention time (min)

Table 7 : Amounts and concentrations of auxin collected in agar from shoot tips of Ph. vulgaris; estimated by bioassay.

Expt.	No. of shoot tips	Weight of shoot tips (g)	Time (h)	IAA equivalents (µg)	IAA equivalents (ng/shoot tip)	IAA equivalents (ng/shoot tip/d)
a	95		6	0.25	2.6	10.4
b	88		6	0.00		
c	70	10	6	0.14	2.0	8.0
d	94	4.5	6	0.45	4.8	19.2
e	c.250	48	6 to 8	0.00		
f	c.1200	65	6 to 8	8.50	7.1	24.1
h	625	15	6 to 8	0.00		

2 to 7 ng IAA equivalents from each shoot tip and co-chromatographing with IAA in at least one solvent system, was shown by agar collections from shoot tips (Table 7). Even without allowance for losses during extraction and purification this quantity was greater than that extracted directly from shoot tips.

On one occasion chromogenic indication of indole activity equivalent to c. 0.8 ng IAA from each shoot tip was obtained by collection in agar. Mass spectromety and g.l.c. of agar collections from shoot tips gave no evidence for IAA.

Without allowing for losses during extraction and purification, and assuming a steady rate of transfer of auxin into the agar, it was calculated that each shoot tip was producing approximately 8 to 25 ng d^{-1} IAA equivalents. At least 100 times this amount of IAA in lanolin was required to replace the upper shoot with respect to inhibition of lateral bud growth on decapitated bean plants (see 'THE INVOLVEMENT OF INDOLE ACETIC ACID, Application').

D. Application of inhibitors of auxin transport

In order to investigate the possible involvement of endogenous auxin translocation in apical dominance, the auxin transport inhibitors 2,3,5-triiodobenzoic acid (TIBA) and a morphactin (CFM) were used. They were applied in lanolin as a ring around the 2nd internode of approximately 3 week old plants. The ring was c. 2 cm long, contained 0.25 ml lanolin and was replaced on the 2nd and 4th days after the original application.

In the first experiment (Figure 44) it was found that the primary leaf axillary buds, which showed some growth on control plants, extended considerably more in the presence of 0.1 to 10 mg g^{-1} TIBA or 5 to 100 µg g^{-1} CFM. It was also discovered, however, that these substances caused the abscission of many of these buds (Figure 44C,D) as well as of other buds, leaves and internodes from the plants. Confirmatory experiments (Figure 45), with plants on which control buds grew little, suggested

Figure 44 : A, B Growth and C, D abscission of primary leaf axillary buds on intact plants treated with A, C TIBA or B,D CFM.

9.2.72 : Ph. vulgaris; 23 d old plants : controlled environment cabinet : 10 plants/treatment; randomized : growth data = mean ± S.E. of combined length of 1st internodes of primary leaf axillary buds; abscission data = number of buds abscinded from 10 plants; growth plots expressed as dashed lines when some buds abscinded and terminated when buds abscinded from more than 5 plants in that treatment : results confirmed in 1 further experiment : key:-

Symbol	Concentration of chemical	
	TIBA (mg g^{-1})	CFM (µg g^{-1})
●	0.0	0
■	0.1	5
▲	1.0	50
▼	10.0	100

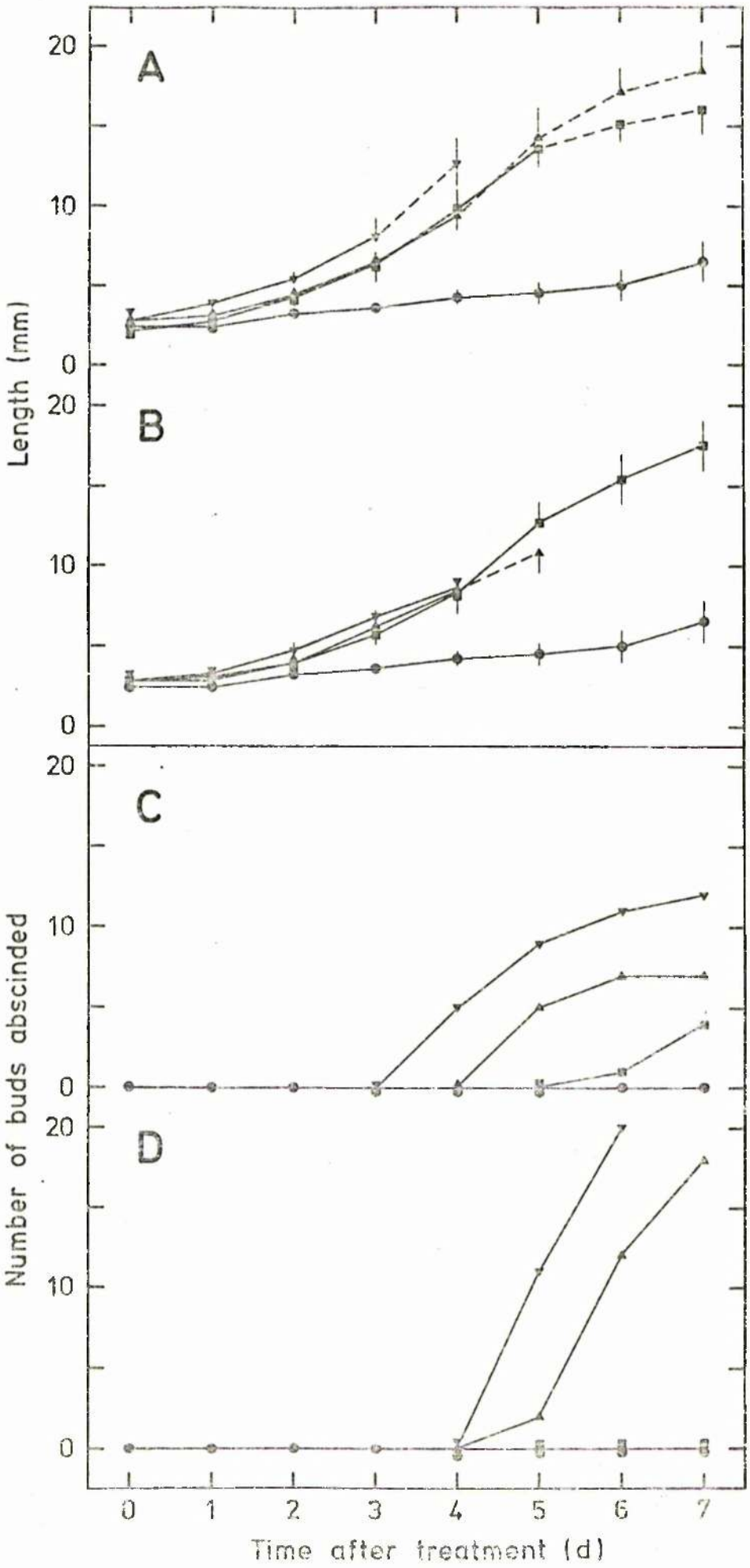
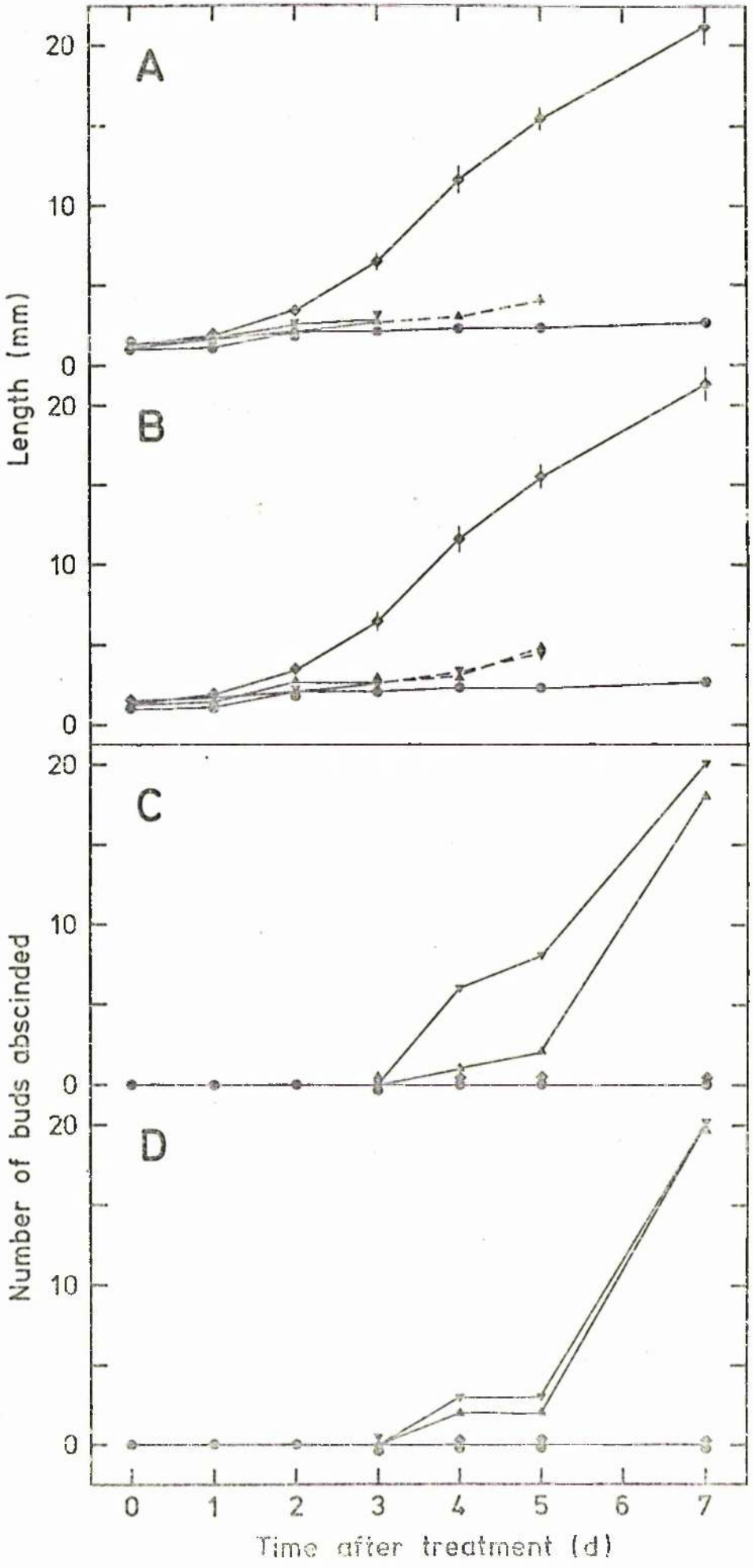

A
B
C
D
20
10
0
Length (mm)
Number of buds abscinded
0
1
2
3
4
5
6
7
Time after treatment (d)

Figure 45 : A, B Growth and C,D abscission of primary leaf axillary buds on intact plants treated with A,C TIBA or B,D CPM and plants decapitated through the 2nd internode.

20.2.72 : details as in Figure 44 : key:-

Symbol	Concentration of chemical	
	TIBA (mg g^{-1})	CPM (µg g^{-1})
●	0	0
▲	1	50
▼	10	100
◆	Decapitated plants	


A
B
C
D
20
10
0
Length (mm)
Number of buds abscinded
0
1
2
3
4
5
6
7
Time after treatment (d)

that there was only limited outgrowth of the primary leaf axillary buds before they abscinded in response to TIBA and CFM.

The effects of these chemicals were subsequently investigated on the growth (Figure 46) and abscission (Figure 47) of all parts of the shoot of these plants. TIBA (1 mg g^{-1}) and CFM (50 µg g^{-1}) had no effect on the 3rd internode, which had almost ceased growth at the start of the experiment (Figure 46, 47A). The growth of the 4th internode was retarded 2 d after application of either of these chemicals; this internode later abscinded from a few of the treated plants. The growth of the 5th internode was more seriously retarded by TIBA and CFM; after 5 d abscission through this internode had occurred on almost all treated plants. These substances also caused within 5 d complete abscission through the 6th internode, which was just beginning its phase of growth. Occasionally abscission of this internode occurred in control plants with plain lanolin applied. With a similar pattern, TIBA and CFM partially inhibited the growth of the 2nd trifoliate leaf, causing occasional abscission, but rapidly led to the abscission of the younger 3rd and 4th trifoliate leaves (Figure 46, 47B). The mature primary and 1st trifoliate leaves were apparently unaffected by these treatments. The promotion by these compounds of the growth and subsequent abscission of the primary leaf axillary buds, which were below the point of application of the chemicals, was confirmed (Figure 46, 47C). TIBA and CFM did not affect the growth of the 1st and 2nd trifoliate leaf axillary buds, which were above the point of chemical application, but, nevertheless, caused their abscission. On plants left longer than a week after treatment the cotyledon axillary buds and the secondary axillary buds at other nodes often showed an initial outgrowth followed by their abscission.

The abscission of leaves and leaflets occurred by the formation of premature abscission zones at the pulvini, where abscission zones are

Figure 46 : Growth of A stem internodes, B trifoliate leaves, C lateral buds on intact plants treated with 1 mg g^{-1} TIBA (■), 50 µg g^{-1} CPM (▲) or control lanolin (●).

4.3.72 : Ph. vulgaris; 22 d old plants : controlled environment cabinet : 10 plants/treatment; randomized : points = mean increase over length on day 0; A = length of 3rd, 4th, 5th and 6th internodes, B = length of terminal leaflet of 2nd, 3rd and 4th trifoliate leaves, C = length of 1st internode of longer (LB) and shorter (SB) primary leaf axillary buds, 1st and 2nd trifoliate leaf axillary buds; plots for each organ expressed as dashed lines when abscission of that organ occurred on any of the plants and terminated when abscission of that organ occurred on more than 5 plants : results confirmed in 2 further experiments.

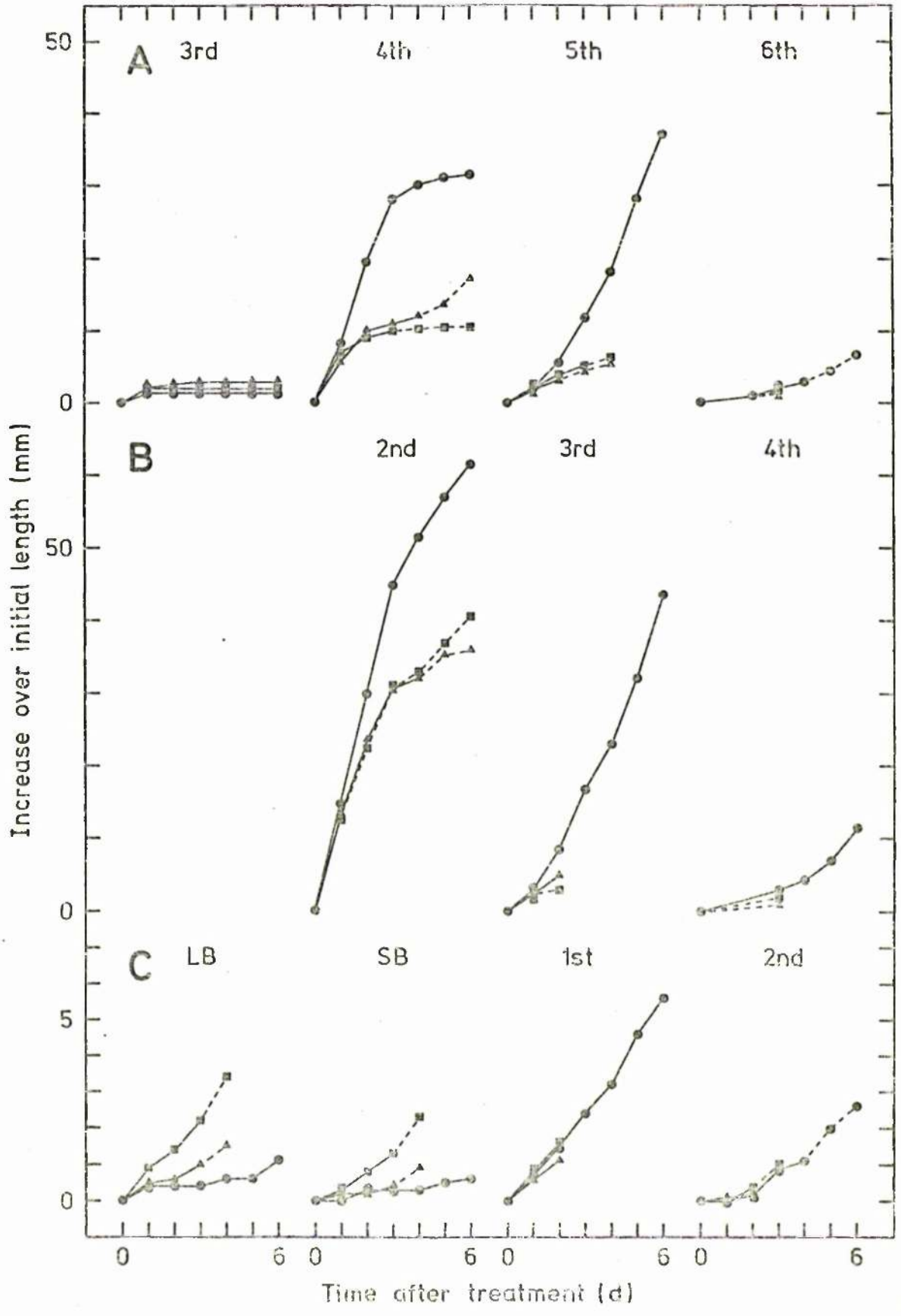
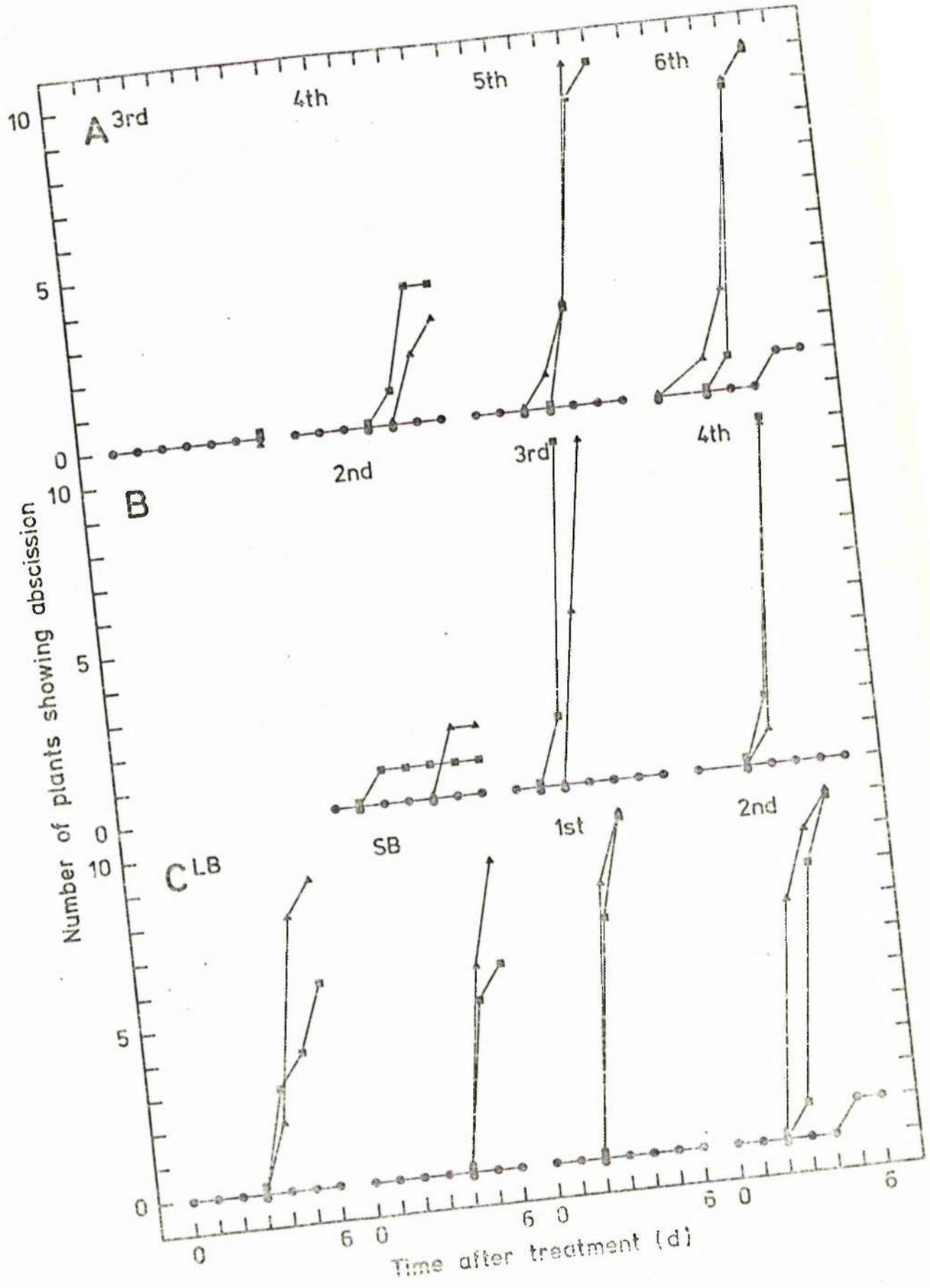

A
3rd
4th
5th
6th
B
2nd
3rd
4th
C
LB
SB
1st
2nd
50
0
50
0
5
0
0
6
0
6
0
6
0
6
Increase over initial length (mm)
Time after treatment (d)

Figure 47 : Abscission of A stem internodes, B trifoliate leaves, C lateral buds from intact plants treated with 1 mg g^{-1} TIBA (■), 50 µg g^{-1} CFM (▲) or control lanolin (●).

Experimental details as in Figure 46 : points = number of each organ abscinded from 10 plants; A = abscission of 3rd, 4th, 5th and 6th internodes, B = abscission of 2nd, 3rd and 4th trifoliate leaves, C = abscission through 1st internode of longer (LB) and shorter (SB) primary leaf axillary buds, 1st and 2nd trifoliate leaf axillary buds : results confirmed in 2 further experiments.


A
3rd
4th
5th
6th
B
2nd
3rd
4th
C
SB
1st
2nd
LB
10
5
0
Number of plants showing abscission
0
6
0
6
0
6
Time after treatment (d)

normally formed during senescence (Brown and Addicott, 1950; Webster, 1968). Abscission of the buds and stem occurred in growing internodes by the formation of adventitious abscission layers which have been described by Whiting and Murray (1948). Other symptoms of treatment with TIBA described by these authors were evident in both TIBA and CFM treated plants in these experiments. The partially expanded 2nd trifoliate leaf exhibited a downward curvature of the tip of each of its leaflets; their margins were also rolled downward and inward, and their laminae showed a 'pebbled' upper surface. Before their abscission the younger leaves, both on the main shoot and on the lateral buds, showed marked epinasty and a silvery colour from hairs on the laminae. There were no obvious signs of senescence.

Application of TIBA or CFM in lanolin as a ring around the stump of the 2nd internode of decapitated plants did affect the primary leaf axillary buds, but less drastically than on intact plants. There was some variation between experiments (Figure 48). In the first experiment (Figure 48A) the growth of the primary leaf axillary buds on decapitation was scarcely affected by TIBA or CFM, although abscission layers were formed through the 1st internodes of 3 of the buds. Surprisingly, some abscission occurred through the 2nd or 3rd internodes of 3 or 4 of the buds on plants of each of the decapitated control treatments; 8 buds showed these abscission zones on both TIBA and CFM treated plants. In the second experiment (Figure 48B), using slightly lower concentrations of the IAA transport inhibitors, CFM slightly inhibited the growth of the primary leaf axillary buds, while TIBA had little influence. Both substances induced abscission of a few buds. In the third experiment (Figure 48C), peculiarly, TIBA and CFM treated plants exhibited bud growth similar to that of decapitated plants without lanolin; plants with plain lanolin applied, however, showed much greater bud growth. TIBA induced an abscission layer on just one bud while CFM caused

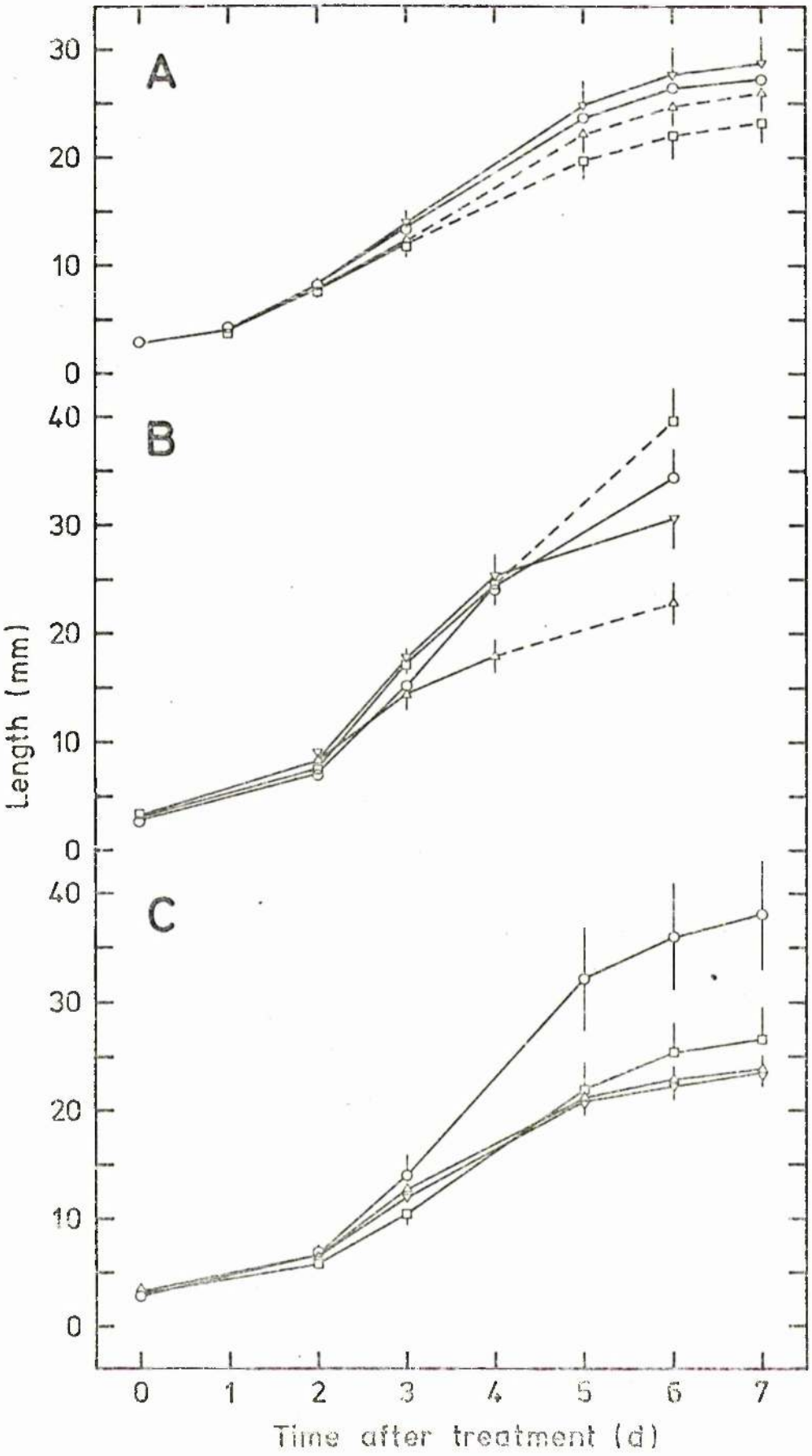
Figure 48 : Growth of primary leaf axillary buds on decapitated plants of Ph. vulgaris treated with no lanolin (▽), control lanolin (○), TIBA (□) or CFM (△) in lanolin.

A,B,C = 3 experiments.

A : 3.3.72 : 21 d old plants, B : 13.5.72 : 19 d old plants, C : 31.5.72 : 23 d old plants : controlled environment cabinet : A = 10 mg g^{-1} TIBA, 100 µg g^{-1} CFM; B,C = 1 mg g^{-1} TIBA, 50 µg g^{-1} CFM : 10 plants/treatment; randomized : points = mean ± S.E. of combined length of 1st internodes of primary leaf axillary buds.

Number of buds abscinded through either the 1st internode or other internodes on the final day of each experiment:-

Treatment	A		B		C	
	1st	Others	1st	Others	1st	Others
No lanolin	0	4	0	0	0	0
Control	0	3	0	0	0	0
TIBA	3	8	2	2	0	1
CFM	3	8	2	7	0	14


A
B
C
Length (mm)
Time after treatment (d)
0
10
20
30
40
0
1
2
3
4
5
6
7

abscission of most of the buds through the 2nd or 3rd internode.

TIBA and CFM in all the experiments induced epinasty and occasional abscission of the expanding young leaves on the outgrowing buds. In general, TIBA and CFM both had little effect on the growth of buds on decapitated plants over a period of one week but they were capable of inducing adventitious abscission layer formation through the growing internodes of these buds.

3. THE TRANSLOCATION AND METABOLISM OF APPLIED GROWTH REGULATORS

In view of the possible roles of the growth regulators IAA, ABA and GA and of phloem translocation in apical dominance, the location and chromatographic properties of radioactivity were studied after application of [^{14}C]IAA, [^{14}C]ABA, [^{14}C]GA and [^{14}C]sucrose to intact bean plants of similar size and age (2 to 3 weeks) to those used for the growth and auxin experiments. The radiochemicals were applied in blocks of agar at concentrations selected to give approximately equal radioactivity in each block for each substance. Application was made to the abraded surface of either a mature primary leaf or a young, expanding first trifoliate leaf of the size (terminal leaflet 3 to 6 cm long) that can alone completely inhibit lateral bud growth (Figure 16).

After 24 h incubation with radioactivity the plants were divided into donor leaf, upper shoot, lower shoot and roots. For quantitative assay of radioactivity by liquid scintillation spectrometry these portions were further segmented, while for qualitative assay they were chopped and extracted with methanol, and the extracts paper chromatographed.

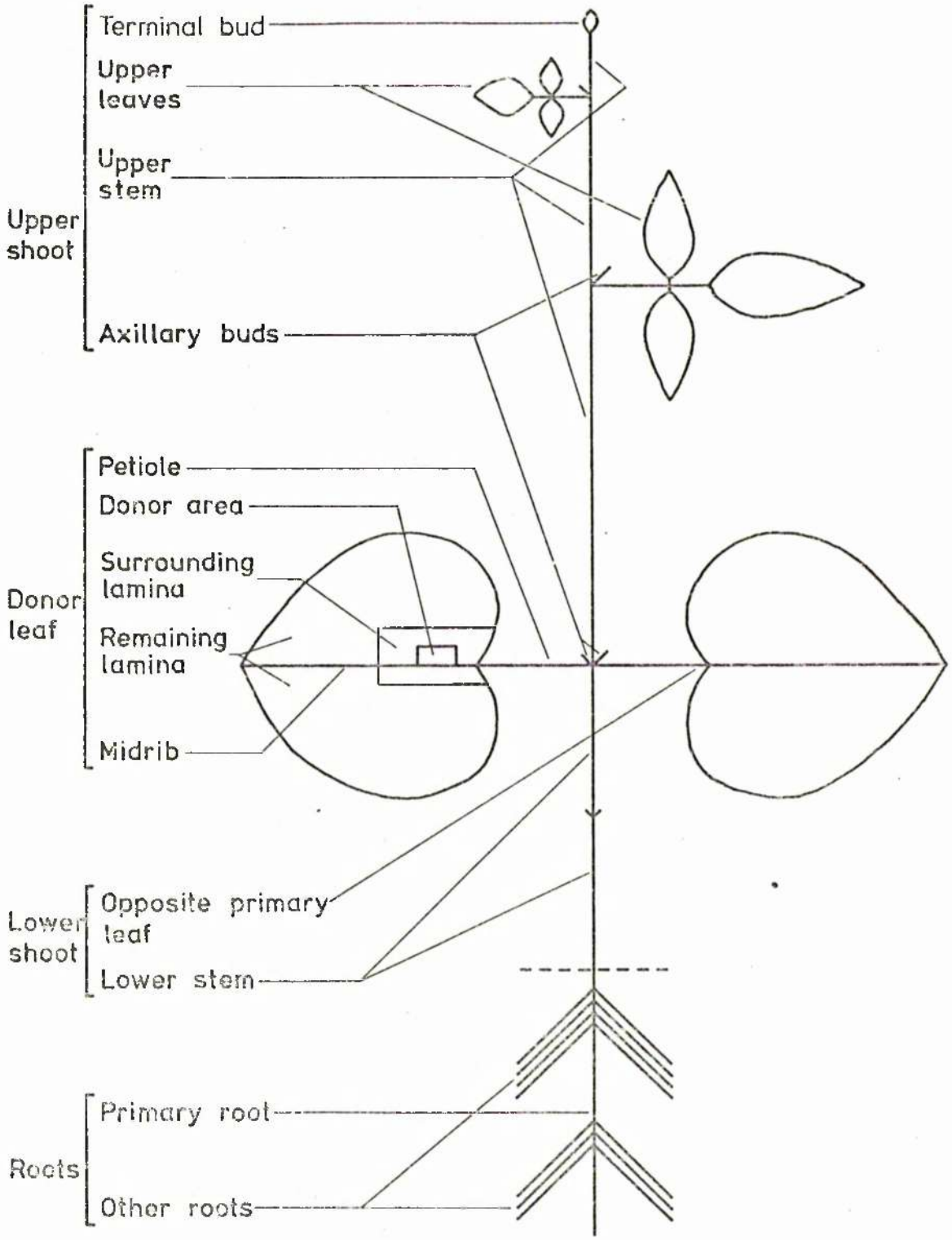
A. Application to a mature primary leaf

(i) Quantitative assay

Of the donor leaf, usually the lamina under the donor block (donor area), the midrib and the petiole only were assayed, but sometimes the lamina immediately around the donor area, and occasionally the rest of the lamina, were included (Figure 49). The whole of the upper shoot, lower stem and roots, but only the midrib and petiole of the opposite primary leaf, were harvested.

The uptake of all the chemicals after 24 h was always greater than 90%, usually greater than 99% (Table 8). The recovery of applied radioactivity, calculated as a percentage of the ^{14}C in unused donor blocks, was very variable between experiments; in general recovery was greatest

Figure 49 : Diagrammatic representation of an approximately 2 week old plant of Ph. vulgaris with primary leaf application of radioactivity.


Terminal bud
Upper leaves
Upper stem
Upper shoot
Axillary buds
Petiole
Donor area
Surrounding lamina
Donor leaf
Remaining lamina
Midrib
Opposite primary leaf
Lower shoot
Lower stem
Primary root
Roots
Other roots

from [^{14}C]GA and [^{14}C]ABA, less from [^{14}C]IAA and least from [^{14}C]sucrose. Because of the differing quantities of radioactivity recovered, the ^{14}C in each segment of each plant was calculated as a percentage of the total radioactivity in that plant.

After the application of [U-^{14}C]sucrose (Table 8A) only 25 to 34% of the radioactivity had remained in the donor area. Most of the radioactivity detected elsewhere had moved out of the donor leaf and was distributed fairly evenly (with some variation between experiments) between the upper shoot, lower shoot and roots. Most of the radioactivity in the upper shoot was in the 1st and 2nd trifoliate leaves, some in the stem and little in the terminal or lateral buds. Little radioactivity was detected in the midrib and petiole of the opposite primary leaf. Of the radioactivity in the roots, nearly all was in the lateral and adventitious roots rather than in the primary root. In three experiments (e,f,g) the lamina immediately round the donor area was assayed and shown to contain only a little radioactivity, while in Experiment g the rest of the lamina contained no detectable radioactivity.

When [1-^{14}C] or [2-^{14}C]IAA were applied (Table 8B) over 90% of the radioactivity was in the donor leaf, most being in the donor area. In two experiments (c,d) the lamina immediately around the donor area was harvested: although there was some radioactivity in this region, including it did not, apparently, markedly affect the pattern of results for the rest of the plant. In Experiment c it was shown that the rest of the lamina contained no detectable radioactivity. Of the 3 to 10% radioactivity found outside the donor leaf, slightly more was, generally, situated in the lower shoot compared with the upper shoot and the roots. As with [^{14}C]sucrose, little radioactivity was detected in the terminal or lateral buds, the midrib and petiole of the opposite primary leaf or the primary root. There was no obvious difference between the patterns of ^{14}C distribution from [1-^{14}C]IAA (Experiments

a,b,c) or [2-^{14}C]IAA (Experiments b,d).

After application of [2-^{14}C]ABA (Table 8C) to the primary leaf, 76 to 86% of the ^{14}C had remained in the donor leaf, 70 to 80% in the donor area. Most of the 14 to 24% radioactivity translocated from the donor leaf was situated in the upper shoot, mainly in the 1st and 2nd trifoliate leaves. Again relatively little radioactivity was detected in the terminal or lateral buds, the midrib and petiole of the opposite primary leaf or the primary root. Hocking (1973), similarly applying [^{14}C]ABA to a primary leaf of 15 d old bean plants, found only 10% of the radioactivity that had moved away from the donor area in the rest of the donor leaf - i.e., if 20 to 30% of the radioactivity in the plant had moved out of the donor area (cf. Table 8) only 2 to 3% was in the rest of the donor leaf. The pattern of results obtained, then, would not have been markedly affected by not harvesting the rest of the donor leaf.

In two of the experiments (e,f) when approximately 10^4 d min^{-1} radioactivity in [8-methylene-^{14}C]GA were donated to the primary leaf (Table 8D) only 0.5 to 1.5% of the ^{14}C was outside the donor leaf, although as much as 11 to 17% had moved away from the donor area. When 10^5 d min^{-1} radioactivity in [^{14}C]GA were applied (Experiment g) about 4% of the radioactivity was detected outside the donor leaf, with very little of this ^{14}C in the roots. Again a high percentage of radioactivity was located in the lamina immediately surrounding the donor area. According to the results of Experiment f, however, despite the location of much of the radioactivity in the lamina around the donor area, none was detected in the rest of the lamina.

The distribution of ^{14}C from [^{14}C]sucrose showed the pattern expected from transport with the assimilate flow from an exporting leaf (Rabideau and Burr, 1945) the radioactivity being situated in the growth centres and in the transport pathways to these centres. Non-

Table 8. Distribution of radioactivity in plants of Ph. vulgaris after application of A [^{14}C]sucrose, B [^{14}C]IAA, C [^{14}C]ABA, D [^{14}C]GA to a mature primary leaf.

$$\text{Recovery} = \frac{\text{Radioactivity in plant + donor block}}{\text{Radioactivity in unused donor block}} \times 100$$

$$\text{Uptake} = \frac{\text{Radioactivity in plant}}{\text{Radioactivity in plant + donor block}} \times 100$$

$$\text{Other figures} = \frac{\text{Radioactivity in plant segment}}{\text{Radioactivity in plant}} \times 100$$

The results for each chemical are presented as a mean for the plants in each experiment and also as an overall mean ± S.E. for the plants in all the experiments combined.

Experimental details:-

Experiment	Date	Age of plants (d)
a	13. 1.72	15
b	31. 1.72	21
c	21. 2.72	17
d	7.10.72	19
e	30.11.72	17
f	17.12.72	21
g	20.10.72	20

Table 8A : [^{14}C]sucrose

Experiment	a	b	e	f	g	Overall mean
No. of plants	4	4	5	5	5	23
Recovery	58.2	30.1	30.3	16.0	30.5	32.1 ± 3.0
Uptake	99.1	99.4	99.3	99.1	97.0	98.7 ± 0.5
Donor area	29.9	25.1	34.0	31.9	30.6	30.6 ± 1.4
Surrounding lamina			0.4	0.4	0.9	
Remaining lamina				0.0		
Midrib + petiole	9.7	4.3	3.8	1.8	4.2	4.6 ± 0.6
DONOR LEAF	39.6	29.4	38.2	34.1	35.7	35.5 ± 1.5
Terminal bud	0.6	1.0	1.3	0.1	0.6	0.7 ± 0.1
1st and 2nd trifoliate leaves	9.3	17.4	7.7	12.5	26.5	14.8 ± 1.6
Upper stem	7.9	14.5	3.9	4.5	7.6	7.4 ± 1.0
Lateral buds	0.7	0.7	0.6	0.8	0.4	0.6 ± 0.1
UPPER SHOOT	18.5	33.7	13.5	17.9	35.1	23.5 ± 2.2
Opposite midrib + petiole	0.1	0.1	0.0	0.0	0.2	0.1 ± 0.0
Lower stem	27.2	19.1	22.7	18.9	18.8	21.2 ± 1.3
LOWER SHOOT	27.3	19.2	22.7	18.9	19.0	21.2 ± 1.3
Primary root	1.2	2.2	1.1	0.5	0.8	1.1 ± 0.2
Other roots	13.4	15.6	24.5	28.6	9.4	18.6 ± 1.8
ROOTS	14.6	17.8	25.6	29.1	10.2	19.7 ± 1.7
Transported from donor leaf	60.4	70.6	61.8	65.9	64.3	64.5 ± 1.5
Transported from donor area	70.1	74.9	66.0	68.1	69.4	69.4 ± 1.4

Table 8B : [^{14}C]IAA

	[1-^{14}C]IAA			[2-^{14}C]IAA		
Experiment	a	c	b	b	d	Overall mean
No. of plants	4	5	4	4	5	22
Recovery	57.0		50.0	57.0	17.4	43.7 ± 4.7
Uptake	99.4	99.7	99.4	98.8	90.4	97.6 ± 0.9
Donor area	80.6	70.1	90.6	91.8	89.5	84.1 ± 2.3
Surrounding lamina		7.1			4.7	
Remaining lamina		0.0				
Midrib + petiole	12.3	13.5	4.6	3.1	2.1	7.2 ± 1.5
DONOR LEAF	92.9	90.7	95.2	94.9	96.3	94.0 ± 0.6
Terminal bud	0.0	0.0	0.1	0.1	0.0	0.0 ± 0.0
1st and 2nd trifoliate leaves	0.0	0.3	0.9	0.5	0.5	0.5 ± 0.1
Upper stem	0.8	1.7	0.5	0.8	0.3	0.8 ± 0.1
Lateral buds	0.5	0.0	0.2	0.3	0.0	0.2 ± 0.0
UPPER SHOOT	1.3	2.0	1.6	1.7	0.9	1.5 ± 0.5
Opposite midrib + petiole	0.1	0.0	0.1	0.1	0.0	0.0 ± 0.0
Lower stem	4.7	5.9	1.3	1.9	1.8	3.2 ± 0.5
LOWER SHOOT	4.8	5.9	1.4	1.9	1.8	3.2 ± 0.5
Primary root	0.2	0.9	0.3	0.3	0.0	0.3 ± 0.2
Other roots	0.8	0.5	1.5	1.3	1.0	1.0 ± 0.1
ROOTS	1.0	1.4	1.8	1.5	1.0	1.3 ± 0.2
Transported from donor leaf	7.1	9.3	4.8	5.1	3.7	6.0 ± 0.6
Transported from donor area	19.4	29.9	9.4	8.2	10.5	15.9 ± 2.3

Table 8C, D : [^{14}C]ABA and [^{14}C]GA

	[^{14}C]ABA			[^{14}C]GA			
Experiment	a	b	Overall mean	e	f	g	Overall mean
No. of plants	4	4	8	5	5	5	15
Recovery	91.0	58.4	74.7 ± 6.9		63.7	60.8	62.3 ± 2.6
Uptake	99.3	99.0	99.1 ± 0.2	99.3	99.8	94.7	97.6 ± 1.0
Donor area	79.7	70.2	75.0 ± 3.6	83.1	88.5	69.5	80.4 ± 2.5
Surrounding lamina				15.2	8.9	24.6	
Remaining lamina					0.0		
Midrib + petiole	6.1	6.2	6.2 ± 1.4	1.0	1.1	1.8	1.3 ± 0.2
DONOR LEAF	85.8	76.4	81.1 ± 2.6	99.3	98.5	96.0	97.9 ± 0.4
Terminal bud	0.8	1.0	0.9 ± 0.1	0.1	0.1	0.1	0.1 ± 0.0
1st and 2nd trifoliate leaves	6.9	10.3	8.6 ± 1.0	0.1	0.2	0.9	0.4 ± 0.1
Upper stem	1.9	2.9	2.4 ± 0.4	0.0	0.1	0.5	0.2 ± 0.1
Lateral buds	0.4	0.5	0.4 ± 0.0	0.0	0.1	0.1	0.1 ± 0.0
UPPER SHOOT	9.9	14.8	12.3 ± 1.4	0.2	0.5	1.5	0.7 ± 0.2
Opposite midrib + petiole	0.0	0.2	0.1 ± 0.0	0.0	0.0	0.0	0.0 ± 0.0
Lower stem	3.2	5.2	4.2 ± 0.7	0.4	0.8	2.4	1.2 ± 0.3
LOWER SHOOT	3.2	5.4	4.3 ± 0.7	0.4	0.8	2.4	1.2 ± 0.3
Primary root	0.2	0.9	0.6 ± 0.2	0.0	0.1	0.0	0.0 ± 0.0
Other roots	0.9	2.5	1.7 ± 0.4	0.1	0.1	0.2	0.1 ± 0.0
ROOTS	1.1	3.4	2.3 ± 0.6	0.1	0.2	0.2	0.1 ± 0.0
Transported from donor leaf	14.2	23.6	18.9 ± 2.6	0.7	1.5	4.1	2.1 ± 0.4
Transported from donor area	20.3	29.8	25.0 ± 3.6	16.9	11.5	30.5	19.6 ± 2.5

Table 9 : Radioactivity supplied to, and recovered from, plants of Ph. vulgaris after application of A[^{14}C]sucrose, B [^{14}C]IAA, C [^{14}C]ABA, D [^{14}C]GA to a mature primary leaf.

A	[^{14}C]sucrose				
Experiment	a	b	e	f	g
Concentration in donor block (µmol l^{-1})	0.250	0.250	0.125	0.125	0.125
^{14}C in donor block (d min^{-1})	12619	16203	8099	8146	8529
^{14}C recovered (d min^{-1})	7345	4884	2451	1307	2600
^{14}C transported from donor leaf (d min^{-1})	4375	3404	1499	853	1622
Amount transported from donor leaf (pmol)	3.3	2.6	1.1	0.6	1.2

B	[1-^{14}C]IAA		[2-^{14}C]IAA		
Experiment	a	c	b	b	d
Concentration in donor block (µmol l^{-1})	1.5	1.5	1.5	1.5	1.5
^{14}C in donor block (d min^{-1})	10898		11899	12185	12930
^{14}C recovered (d min^{-1})	6214	7615	5951	6946	2256
^{14}C transported from donor leaf (d min^{-1})	414	701	285	349	78
Amount transported from donor leaf (pmol)	3.3	5.5	2.3	2.8	0.6

C, D	[^{14}C]ABA			[^{14}C]GA	
Experiment	a	b	e	f	g
Concentration in donor block (µmol l^{-1})	10	10	50	50	500
^{14}C in donor block (d min^{-1})	11874	15345		11450	102140
^{14}C recovered (d min^{-1})	10802	8959	5759	7298	62081
^{14}C transported from donor leaf (d min^{-1})	1515	2001	39	106	2320
Amount transported from donor leaf (pmol)	57.2	75.5	11.5	31.2	682.4

growing regions, like the buds, mature leaves and tap root contained little radioactivity. Although the other radiochemicals exhibited a smaller percentage of radioactivity moved out of the donor leaf, in terms of actual amounts of substance moved their translocation was equal to or greater than that of sucrose (Table 9). Their pattern of distribution was similar to that of sucrose, although ^{14}C from [^{14}C]ABA was concentrated more in the upper shoot. ^{14}C from ^{14}C[GA] apparently moved better within the leaf than from the other chemicals but only a small proportion moved out of the donor leaf.

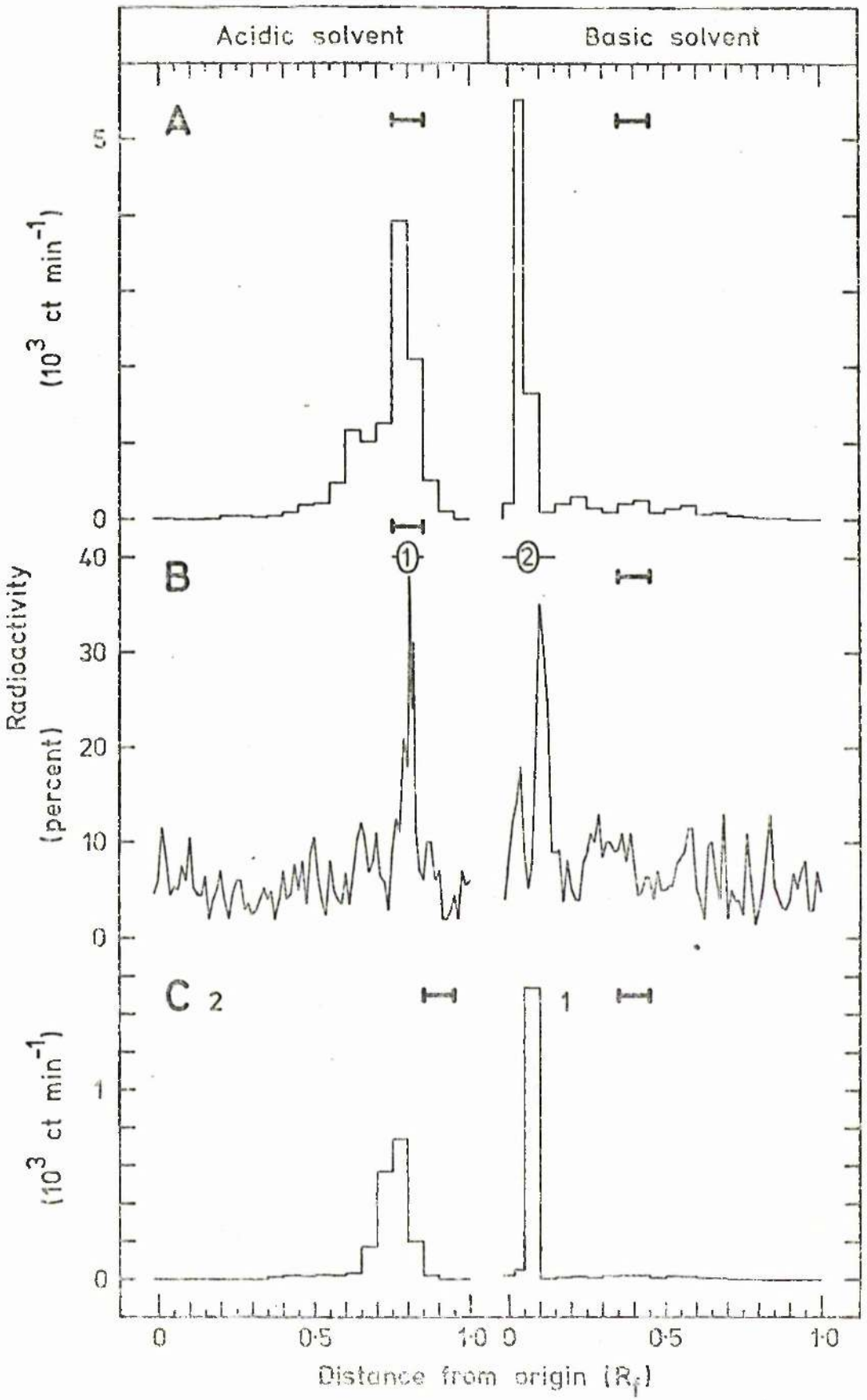
(ii) Qualitative assay

In two experiments 10 µmol l^{-1} [1-^{14}C]IAA was applied in the standard manner to a primary leaf of 10 plants. Of the donor leaves the donor area, the lamina immediately around the donor area, the midrib and the petiole only were extracted (see Figure 49). The upper shoots, lower stems and roots were each extracted separately and the opposite primary leaf was not harvested. The extracts were chromatographed on paper in both acidic (butanol:acetic acid:water::25:5:11) and basic (isopropanol:ammonia:water::8:1:1) solvents.

In the first experiment, chromatograms of the donor leaf extract assayed by liquid scintillation spectrometry (Figure 50A) exhibited 2 zones of radioactivity, one of which corresponded with IAA, when developed with the acidic solvent, but only 1 zone, which was not IAA, on development with the basic solvent. Part of the same extract was similarly chromatographed but assayed using the chromatogram scanner (Figure 50B). A similar pattern emerged, although only the peak corresponding to IAA was detected on the acid-developed chromatogram, while 2 peaks appeared between the origin and Rf 0.14 of the base-developed chromatogram. The numbered region of each chromatogram was eluted and re-chromatographed on the other solvent system (Figure 50C), the results showing the radioactivity in the donor leaf extract to be almost all

Figure 50 : Chromatography of extract from donor leaf after application of [1-^{14}C]IAA to a primary leaf of 18 d old plants of Ph. vulgaris.

A assayed by liquid scintillation spectrometry, B assayed by chromatogram scanner, C numbered zones from B rechromatographed in opposite solvent system and assayed by liquid scintillation spectrometry : horizontal bar indicates position of IAA.


Acidic solvent
Basic solvent
A
B
C
1
2
5
0
40
30
20
10
0
1
0
(10³ ct min⁻¹)
Radioactivity
(percent)
(10³ ct min⁻¹)
0
0·5
1·0
0
0·5
1·0
Distance from origin (Rf)

associated with a single chromatographic moiety, which was not IAA. In the second experiment the donor leaf extract showed a similar major peak on each chromatogram but also small amounts of a substance cochromatographing with IAA, and at least one other substance (Figure 51A). The numbered zones were eluted and rechromatographed on the other solvent system (Figure 51B). Again the major peaks (1 and 2) were chromatographically indistinct: the peaks corresponding to IAA (3 and 4) contained mainly one chromatographic moiety, which may have been IAA, as well as at least one other substance. Similar rechromatography showed that stock IAA remained chromatographically pure during this process.

Liquid scintillation assay of the upper shoot extract from both experiments indicated very little IAA (Figure 52A). Near the origin of the chromatogram in each solvent there was one major zone of radioactivity which did not correspond with either IAA or the main radioactive moiety in the donor leaf extract. These zones were also dominant on chromatograms of both lower shoot and root extracts (Figure 52B,C). There may have been a small amount of IAA in the lower shoot extract from the first experiment but this was not detected in the second experiment (Figure 52B). The root extracts showed no sign of IAA and contained at least one substance other than the one common to shoot and root extracts.

Hocking (1973) demonstrated that donor leaf extracts after primary leaf application of [1-^{14}C] and [2-^{14}C]IAA had similar chromatographic properties.

IAA appeared to be almost completely metabolized to one other substance in mature primary leaves. There was little evidence for either IAA or this metabolite in the rest of the plant.

Hocking (1973) performed chromatographic analyses of extracts made 24 h after similar application of [^{14}C]ABA to a primary leaf of 24 d old bean plants. His results suggested that although a small amount of the

Figure 51 : Chromatography of extract from donor leaf after application of [1-^{14}C]IAA to a primary leaf of 18 d old plants of Ph. vulgaris.

A assayed by chromatogram scanner, B numbered zones from A rechromatographed in opposite solvent system and assayed by liquid scintillation spectrometry : horizontal bar indicates position of IAA.

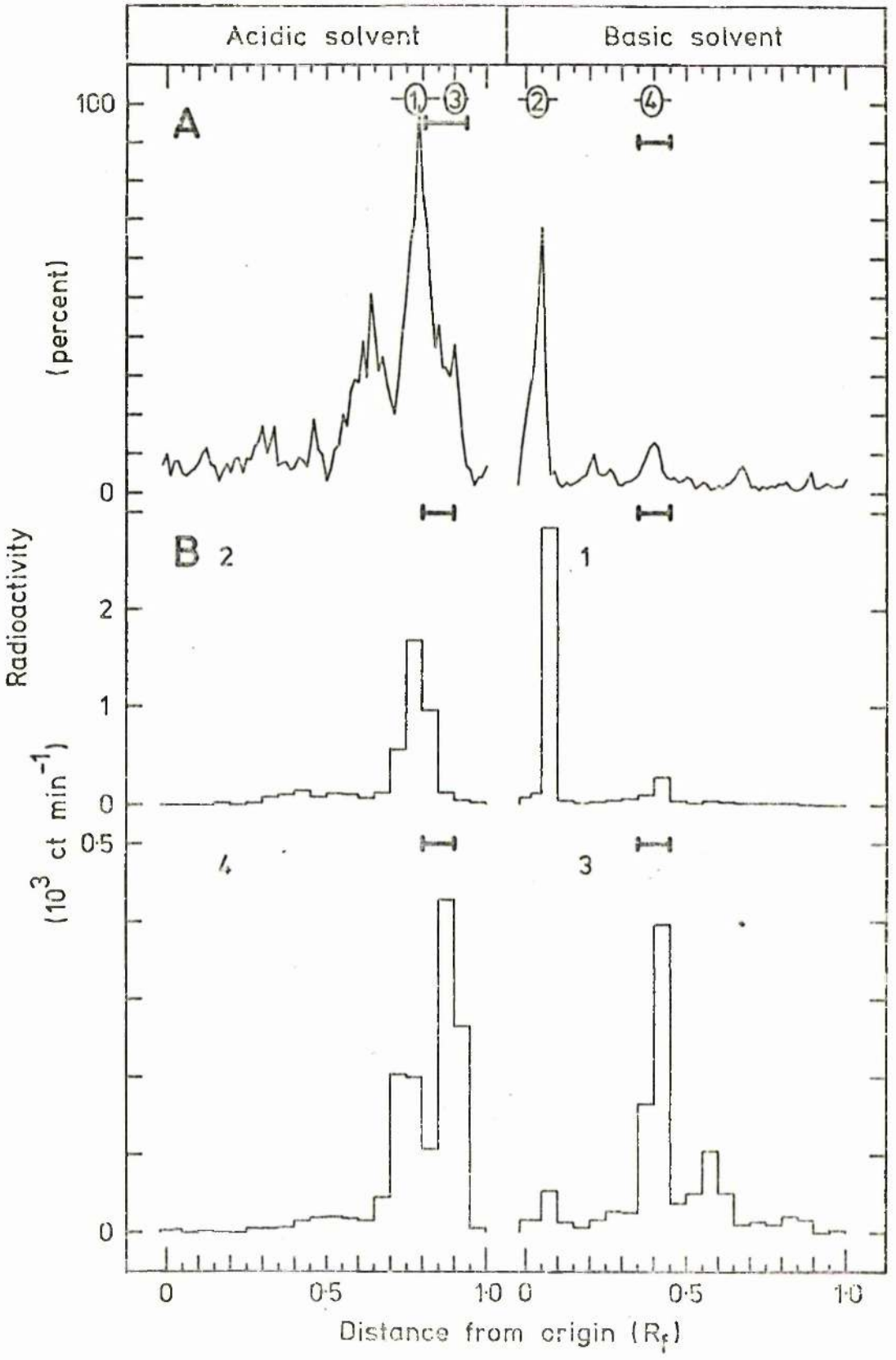

Acidic solvent
Basic solvent
A
B
100
0
(percent)
Radioactivity
(10³ ct min⁻¹)
2
1
0
0·5
0
1
2
3
4
0
0·5
1·0
0
0·5
1·0
Distance from origin (Rf)

Figure 52 : Chromatography of extracts from A upper shoot, B lower shoot, C roots after application of [1-^{14}C]IAA to a primary leaf of 18 d old plants of Ph. vulgaris.

Assayed by liquid scintillation spectrometry : horizontal bar indicates position of IAA : data of 1 experiment presented; results confirmed in 1 further experiment.

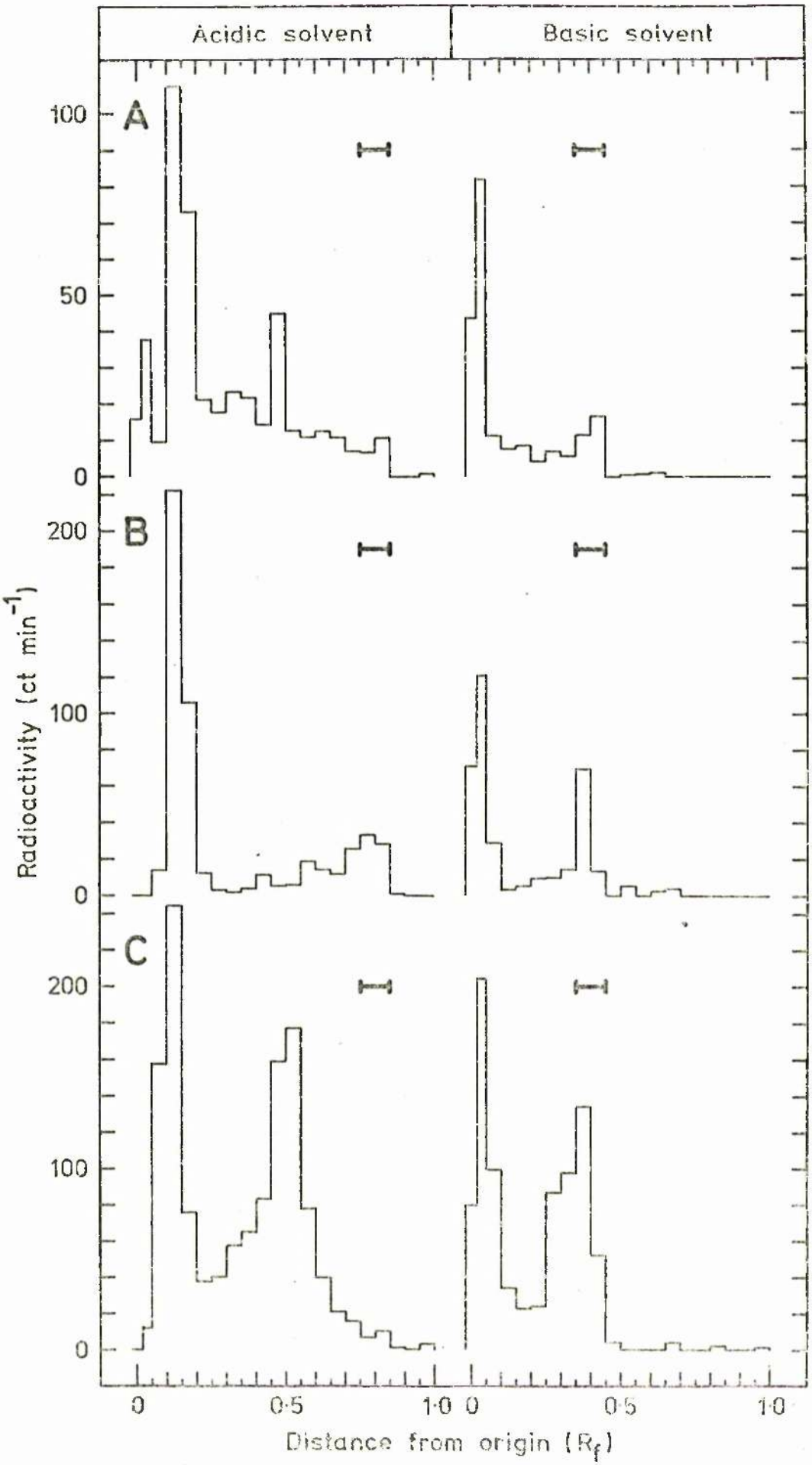
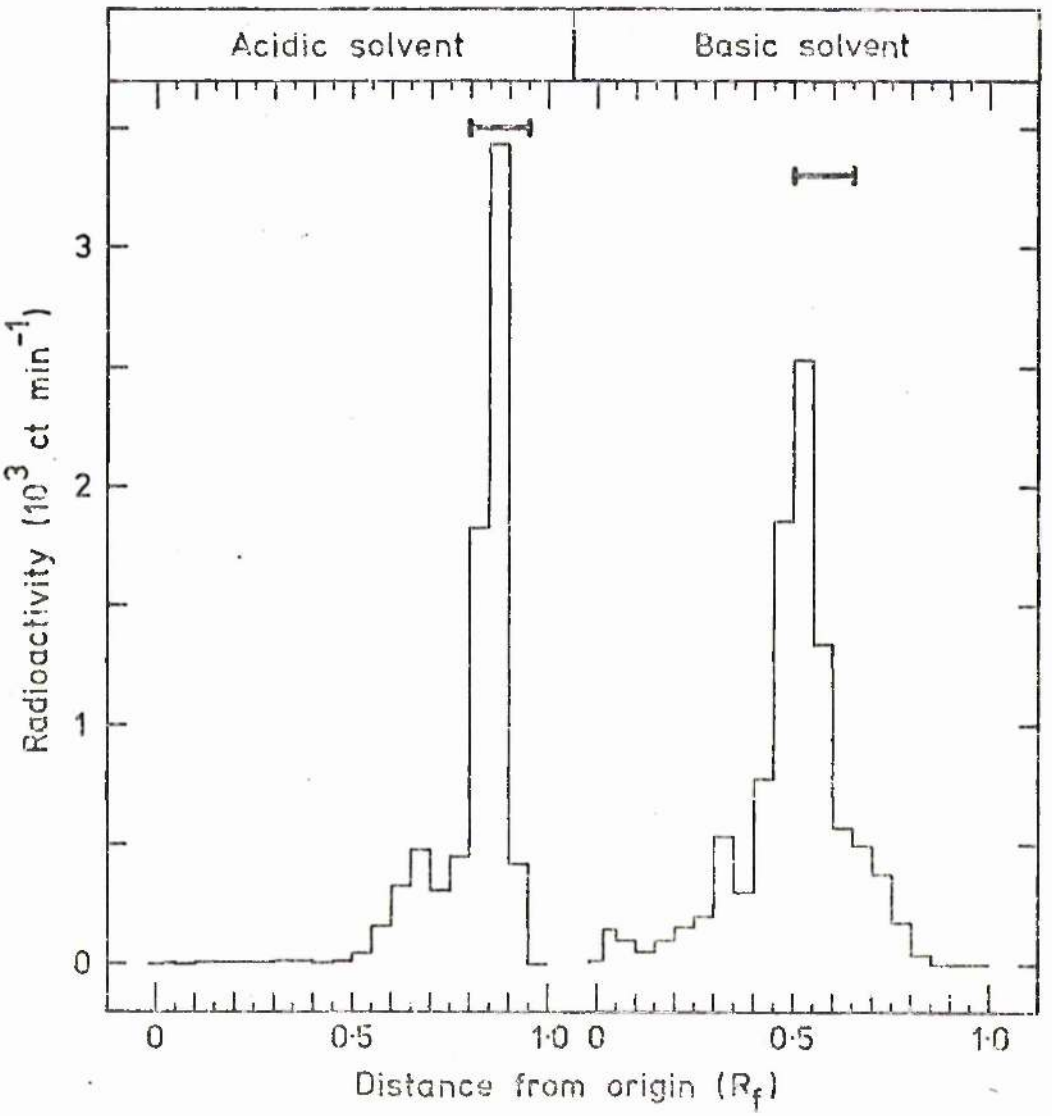

Acidic solvent
Basic solvent
A
B
C
100
50
0
200
100
0
200
100
0
Radioactivity (ct min^{-1})
0
0·5
1·0
0
0·5
1·0
Distance from origin (R_f)

Figure 53 : Chromatography of extract from donor leaf after application of [^{14}C]GA to a primary leaf of 18 d old plants of Ph. vulgaris.

Assayed by liquid scintillation spectrometry : horizontal bar indicates position of GA : results confirmed in 1 further experiment.


Acidic solvent
Basic solvent
Radioactivity (10^3 ct min^{-1})
3
2
1
0
0
0·5
1·0
0
0·5
1·0
Distance from origin (R_f)

translocated radioactivity may have still been associated with ABA, most of the ^{14}C was in one moiety chromatographically distinct from ABA.

Extracts of primary donor leaves after application of [^{14}C]GA showed most of the radioactivity to be associated with a substance chromatographically indistinct from GA, although there was a small amount of at least one other substance (Figure 53).

B. Application to a young first trifoliate leaf

(i) Quantitative assay

The whole donor leaf, upper shoot and roots, and all of the lower shoot apart from the primary leaf laminae were assayed (Figure 54).

Recovery of applied radioactivity was again very variable (Table 10) while, excepting Experiment d, uptake of radioactivity into the plant was again greater than 95% after 24 h. When [^{14}C]sucrose, [^{14}C]ABA or [^{14}C]GA were applied to the 1st trifoliate leaf over 99% of the radioactivity taken into the plant remained in the donor leaf, most of this ^{14}C being in the donor leaflet (Table 10A,C,D). Only from [^{14}C]ABA in Experiment a was considerable radioactivity located outside the donor leaf and this result was not confirmed in two other experiments (b,c).

When [^{14}C]IAA was applied, however, although most of the radioactivity remained in the donor leaf, 2 to 5% was situated in other parts of the plant (Table 10B). Most of the transported radioactivity was found in the lower shoot, although variable amounts of ^{14}C were in the upper shoot and lateral roots. There was no significant radioactivity in the primary leaf axillary buds.

The movement of radioactivity away from the donor area was variable (Table 10). The donor leaf was always expanding during the incubation period and the radiochemicals did not obviously alter this growth (Table 11).

(ii) Qualitative assay

In two experiments 10 μmol l^{-1} [1-^{14}C]IAA was applied in the

Figure 54 : Diagrammatic representation of an approximately 2 week old plant of Ph. vulgaris with 1st trifoliate leaf application of radioactivity.

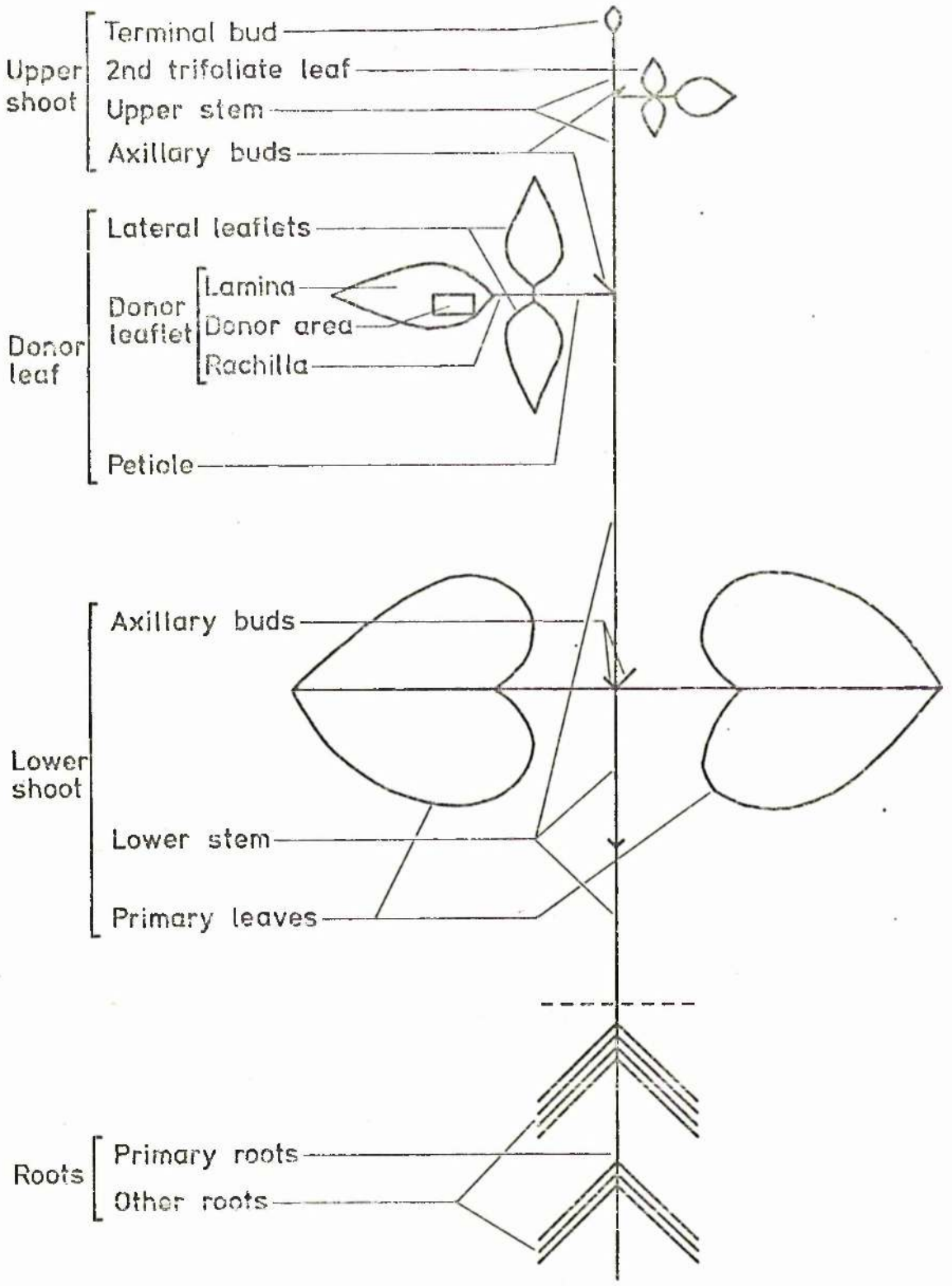

Upper shoot
Terminal bud
2nd trifoliate leaf
Upper stem
Axillary buds
Donor leaf
Lateral leaflets
Donor leaflet
Lamina
Donor area
Rachilla
Petiole
Lower shoot
Axillary buds
Lower stem
Primary leaves
Roots
Primary roots
Other roots

Table 10 : Distribution of radioactivity in plants of Ph. vulgaris after application of A [^{14}C]sucrose, B [^{14}C]IAA, C [^{14}C]ABA, D [^{14}C]GA to a young first trifoliate leaf.

$$\text{Recovery} = \frac{\text{Radioactivity in plant + donor block}}{\text{Radioactivity in unused donor block}} \times 100$$

$$\text{Uptake} = \frac{\text{Radioactivity in plant}}{\text{Radioactivity in plant + donor block}} \times 100$$

$$\text{Other figures} = \frac{\text{Radioactivity in plant segment}}{\text{Radioactivity in plant}} \times 100$$

The results for each chemical are presented as a mean for the plants in each experiment and also as an overall mean ± S.E. for the plants in all the experiments combined.

Experimental details:-

Experiment	Date	Age of plants (d)
a	3.4.72	14
b	22.5.72	14
c	5.6.72	14
d	26.1.73	18

Table 10 A, B : [^{14}C]sucrose and [^{14}C]IAA

	[^{14}C]sucrose				[1-^{14}C]IAA (a) and [2-^{14}C]IAA (b,c)			
Experiment	a	b	d	Overall mean	a	b	c	Overall mean
No. of plants	5	5	5	15	5	5	3	13
Recovery	56.1	74.8	23.8	51.6 ± 6.2	85.6	41.3	38.0	57.6 ± 6.6
Uptake	99.7	97.5	77.8	93.8 ± 4.2	99.0	98.4	95.5	98.0 ± 0.5
Donor leaflet	97.1	99.6	99.5	98.7 ± 0.5	93.3	94.0	97.0	94.4 ± 0.9
Lateral leaflets	1.1	0.3	0.0	0.5 ± 0.2	0.6	0.0	0.0	0.2 ± 0.2
Petiole	1.4	0.0	0.2	0.5 ± 0.3	1.8	1.2	0.5	1.3 ± 0.4
DONOR LEAF	99.6	99.9	99.7	99.7 ± 0.1	95.7	95.2	97.6	96.0 ± 0.7
UPPER SHOOT	0.0	0.0	0.1	0.0 ± 0.0	0.8	0.3	0.1	0.5 ± 0.2
Primary midrib + petiole					0.0	0.0	0.2	0.2 ± 0.0
Lower stem					3.0	2.9	0.8	2.4 ± 0.5
Primary leaf axillary buds					0.2	0.0	0.0	0.1 ± 0.1
LOWER SHOOT	0.2	0.0	0.2	0.2 ± 0.1	3.2	2.9	1.0	2.6 ± 0.5
Primary root					0.0	0.2	0.2	0.1 ± 0.1
Other roots					0.3	1.4	1.1	0.9 ± 0.2
ROOTS	0.2	0.0	0.0	0.1 ± 0.0	0.3	1.6	1.3	1.0 ± 0.3
Transported from donor leaf	0.4	0.1	0.3	0.3 ± 0.1	4.3	4.8	2.4	4.0 ± 0.7
Transported from donor area	42.2	20.4	8.8	23.8 ± 5.1	35.4	23.2	16.0	26.2 ± 3.7

Table 10 C, D : [^{14}C]ABA and [^{14}C]GA

	[^{14}C]ABA				[^{14}C]GA
Experiment	a	b	c	Overall mean	d
No. of plants	5	5	5	13	5
Recovery	103.1	80.8	62.5	85.2 ± 5.1	40.6 ± 1.6
Uptake	99.0	98.5	96.0	98.1 ± 0.4	69.0 ± 3.6
Donor leaflet	95.6	97.1	100.0	97.2 ± 1.1	97.3 ± 1.1
Lateral leaflets	3.1	2.6	0.0	2.2 ± 0.9	2.6 ± 1.1
Petiole	0.2	0.0	0.0	0.1 ± 0.1	0.0 ± 0.0
DONOR LEAF	98.9	99.7	100.0	99.5 ± 0.3	99.9 ± 0.1
UPPER SHOOT	0.1	0.0	0.0	0.0 ± 0.0	0.0 ± 0.0
LOWER SHOOT	0.7	0.1	0.0	0.3 ± 0.2	0.0 ± 0.0
ROOTS	0.3	0.2	0.0	0.2 ± 0.1	0.0 ± 0.0
Transported from donor leaf	1.1	0.3	0.0	0.5 ± 0.3	0.1 ± 0.1
Transported from donor area	41.1	31.0	26.1	33.8 ± 5.1	29.3 ± 2.5

Table 11 : Radioactivity supplied to, and recovered from plants of Ph. vulgaris after application of A [^{14}C]sucrose, B [^{14}C]IAA, C [^{14}C]ABA, D [^{14}C]GA to a young first trifoliate leaf (also growth of terminal leaflet of donor leaf).

A	[^{14}C]sucrose		
Experiment	a	b	d
Concentration in donor block (µmol l^{-1})	0.250	0.250	0.125
^{14}C in donor block (d min^{-1})	13375	7164	9539
^{14}C recovered (d min^{-1})	7500	5362	2273
^{14}C transpc:ted from donor leaf (d min^{-1})	28	5	6
Length of donor leaflet at application (mm)	44.4	39.2	37.6
Length of donor leaflet at harvest (mm)	54.0	48.0	49.0

B	[^{14}C]IAA		
Experiment	a	b	c
Concentration in donor block (µmol l^{-1})	1.5	1.5	1.5
^{14}C in donor block (d min^{-1})	6336	8971	14403
^{14}C recovered (d min^{-1})	5422	3708	5474
^{14}C transported from donor leaf (d min^{-1})	227	177	137
Length of donor leaflet at application (mm)	46.4	41.2	33.7
Length of donor leaflet at harvest (mm)	52.6	50.2	44.7

C, D	[^{14}C]ABA			[^{14}C]GA
Experiment	a	b	c	d
Concentration in donor block (µmol l^{-1})	10	5	5	50
^{14}C in donor block (d min^{-1})	9789	5840	7445	12965
^{14}C recovered (d min^{-1})	10094	4721	4654	5270
^{14}C transported from donor leaf (d min^{-1})	99	14	1	2
Length of donor leaflet at application (mm)	43.8	41.4	34.0	37.2
Length of donor leaflet at harvest (mm)	51.6	50.6	43.3	56.4

standard manner to the 1st trifoliate leaf of 10 plants. After 24 h the donor leaves, upper shoots, lower stems and roots were extracted (see Figure 54) and the extracts chromatographed. In both experiments the extracts showed similar chromatographic properties to those after primary leaf donation of [1-^{14}C]IAA. The donor leaf extract exhibited a main peak near the origin of the base-developed chromatogram assayed by liquid scintillation spectrometry (Figure 55A) or with the chromatogram scanner (Figure 55B). There was a peak cochromatographing with IAA and at least one other peak on both solvent systems. Elution and rechromatography of the numbered zones of each chromatogram (Figure 55C) indicated most of the radioactivity in these zones to be associated with two moieties, one identical to the main substance in primary donor leaf extracts after [1-^{14}C]IAA application, the other similar to IAA. There was little radioactivity in the upper shoot and only small amounts in the lower shoot and roots (Figure 56). There was enough ^{14}C in the latter two regions, however, to indicate the presence of at least two substances in each. One of these substances was apparently the same for both regions. Neither of them was IAA. The chromatographic patterns of these extracts were very similar to those of the same regions after primary leaf application of [1-^{14}C]IAA (Figure 52).

When [^{14}C]GA was applied to a young 1st trifoliate leaf 2 main peaks of radioactivity were evident after chromatography in each solvent system (Figure 57). One of these peaks cochromatographed with GA and the other was similar, but in greater quantity, to the second peak of activity found in primary donor leaf extracts after [^{14}C]GA application (Figure 53).

C. Summary

When applied to a mature leaf radioactivity from all the substances was distributed around the plants, especially in the growing regions,

Figure 55 : Chromatography of extract from donor leaf after application of [1-^{14}C]IAA to a 1st trifoliate leaf of 18 d old plants of Ph. vulgaris.

A assayed by liquid scintillation spectrometry, B assayed by chromatogram scanner, C numbered zones from B rechromatographed in opposite solvent system and assayed by liquid scintillation spectrometry : horizontal bar indicates position of IAA : results confirmed in 1 further experiment.

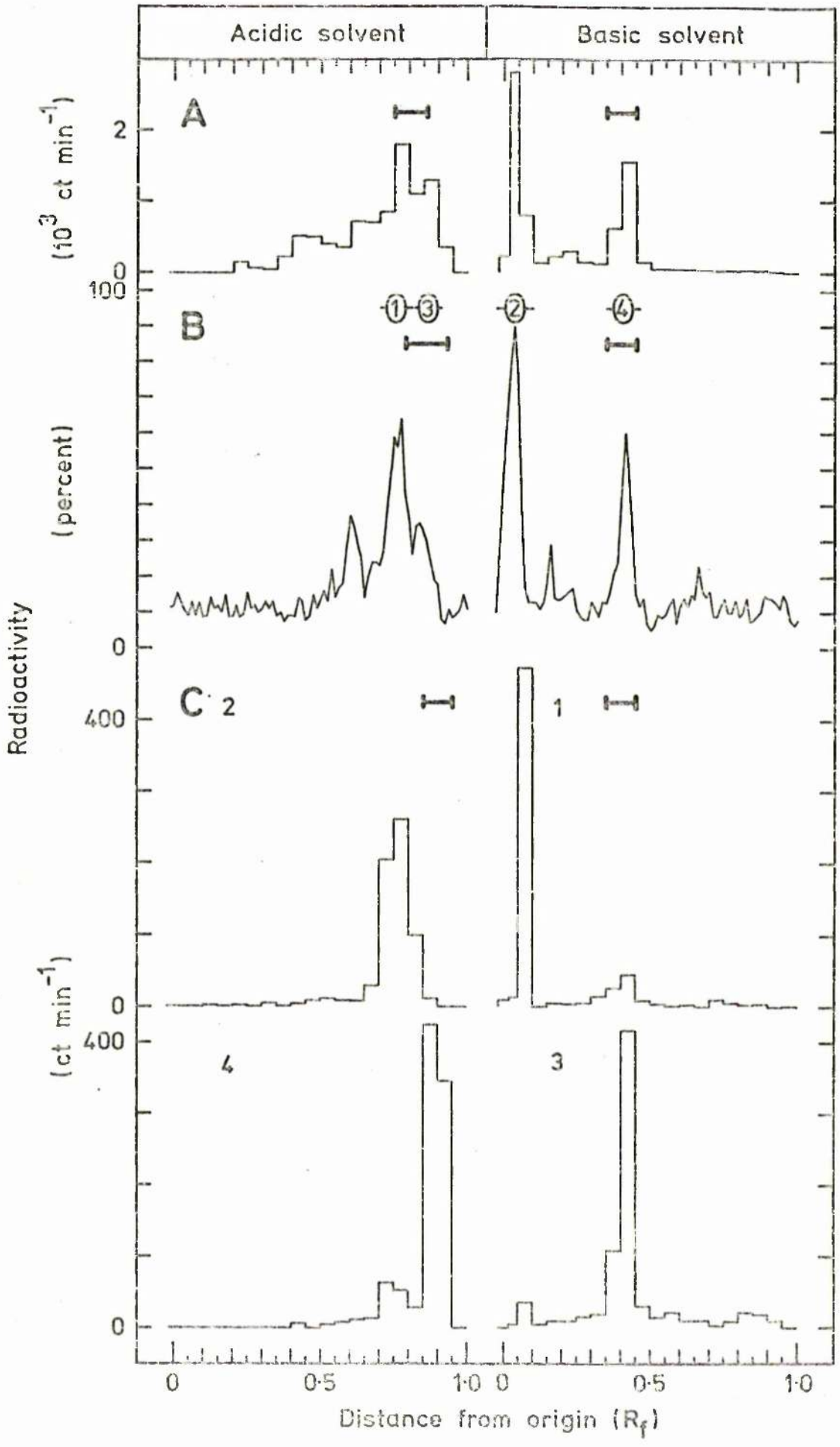

Acidic solvent
Basic solvent
A
B
C
2
1
4
3
Radioactivity
(10³ ct min⁻¹)
(percent)
(ct min⁻¹)
2
0
100
0
400
0
400
0
0
0·5
1·0
0
0·5
1·0
Distance from origin (Rf)

Figure 56 : Chromatography of extracts from A upper shoot, B lower shoot, C roots after application of [1-^{14}C]IAA to a 1st trifoliate leaf of 18 d old plants of Ph. vulgaris.

Assayed by liquid scintillation spectrometry : horizontal bar indicates position of IAA : results confirmed in 1 further experiment.

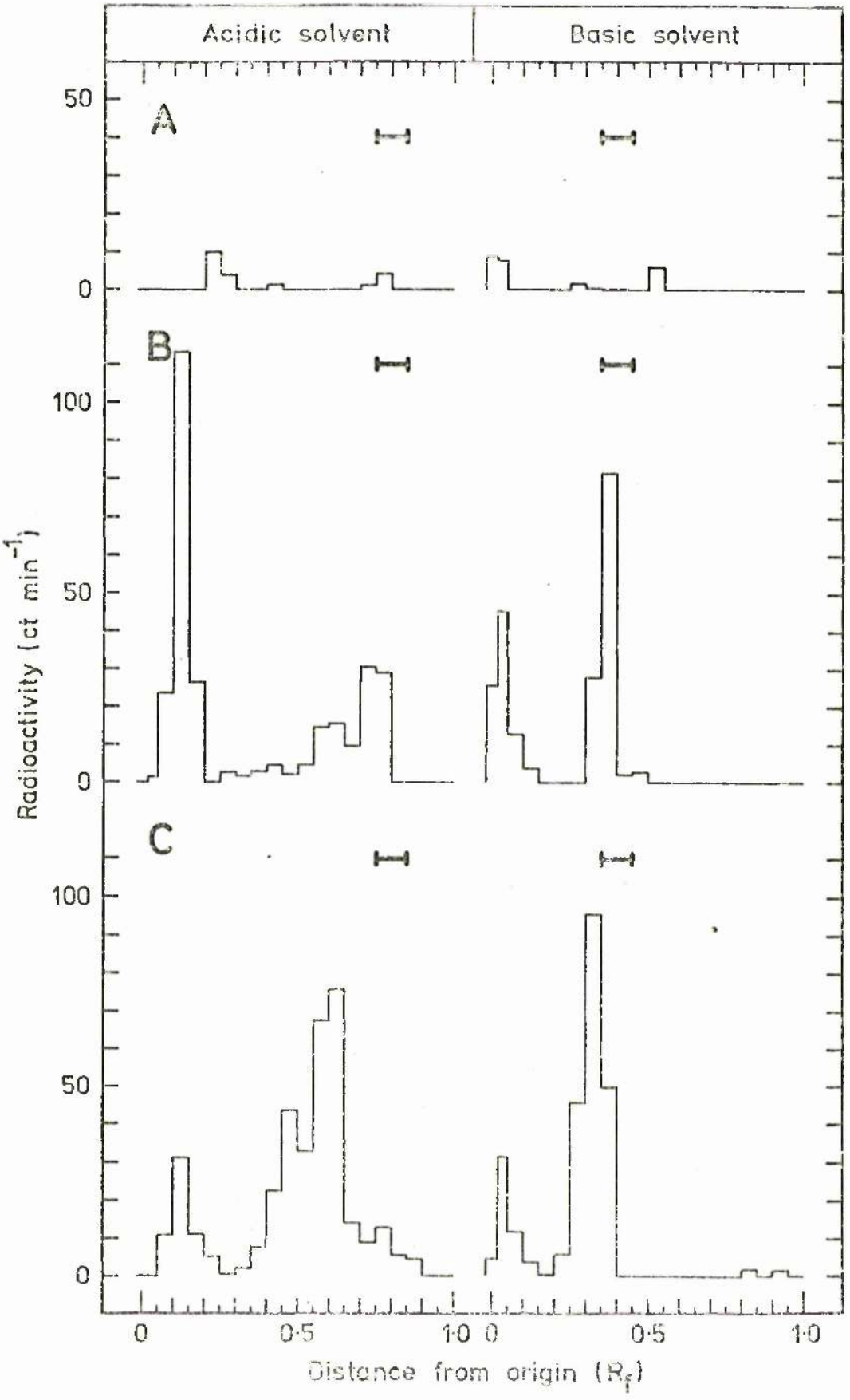
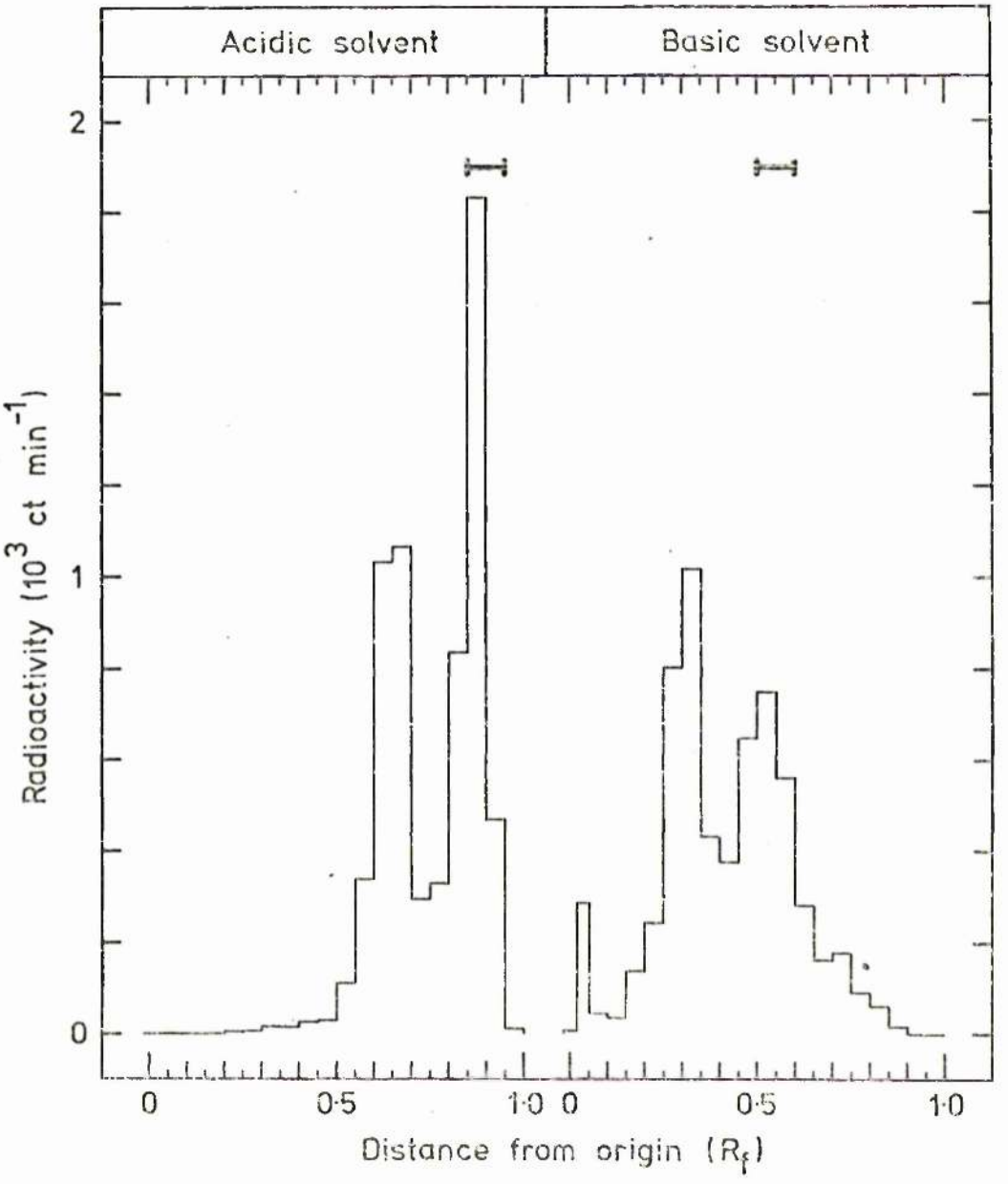

Acidic solvent
Basic solvent
A
B
C
50
0
100
50
0
100
50
0
Radioactivity (ct min^{-1})
0
0·5
1·0
0
0·5
1·0
Distance from origin (R_f)

Figure 57 : Chromatography of extract from donor leaf after application of [^{14}C]GA to a 1st trifoliate leaf of 18 d old plants of Ph. vulgaris.

Assayed by liquid scintillation spectrometry : horizontal bar indicates position of GA ; results confirmed in 1 further experiment.


Acidic solvent
Basic solvent
2
1
0
Radioactivity (10^3 ct min^{-1})
0
0·5
1·0
0
0·5
1·0
Distance from origin (R_f)

although in percentage terms only ^{14}C from sucrose was efficiently transported. Over the 24 h incubation period there was apparently extensive metabolism of IAA and considerable change of ABA although GA seemed more stable.

On the other hand, only radioactivity from IAA was transported out of a young, growing leaf although after 24 h this ^{14}C did not appear to be associated with IAA. Again there was considerable metabolism of IAA and some of GA. The translocation of some part of the IAA molecule out of the young leaf was presumably taking place against a strong flow of assimilates into that leaf.

4. THE MECHANISMS OF APICAL DOMINANCE

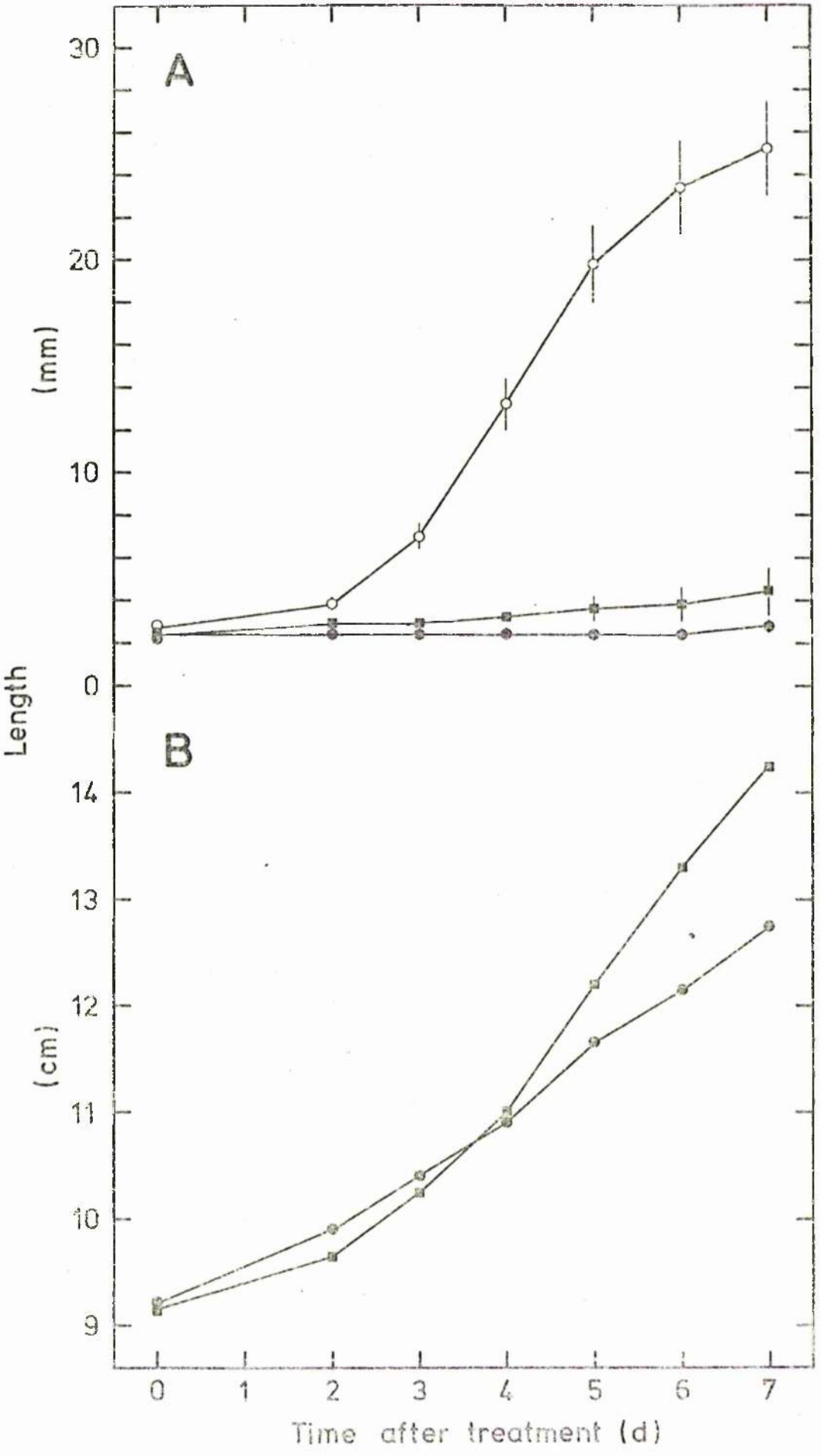
Much of the early work on the mechanism of apical dominance was performed by Snow (1925 to 1940), who used surgical techniques to elucidate the pathways and patterns of correlative inhibition, thereby providing an insight into the possibility of various proposed mechanisms. Although much of Snow's work suffered from poor experimental design it still provides some of the best arguments both against the 'direct auxin' and 'nutrient diversion' theories and for the existence of a specific correlative inhibitor. Consequently, some of Snow's experiments on the pathways through which inhibition of lateral bud growth can occur have been re-examined. The translocation of [^{14}C] sucrose and [^{14}C]IAA has been investigated in relation to one of these pathways. The vascularization of the primary leaf axillary buds has also been examined to test whether restriction of vascular development could be a cause of correlative inhibition.

A. Routes of inhibition

Snow (1925) claimed to have shown that the upper shoot of *Phaseolus* could inhibit the growth of the cotyledon axillary buds through a zone of the epicotyl which had had all tissues outside the xylem removed, although he did not present any bud measurements. Similar removal of the tissues outside the xylem of a 2 cm length of 2nd internode (see Plate 3) was confirmed to have little effect on the inhibition of growth of the primary leaf axillary buds by the upper shoot (Figure 58A). This 'ringing' treatment did not greatly affect the growth of the upper shoot either (Figure 58B). Snow (1925) also claimed to have shown that inhibition of the cotyledon axillary buds of *Phaseolus* could pass through the pith, but his results were not very convincing and difficulty has been found in repeating the experiments in this study. Snow (1925) also performed an experiment to show inhibition of the cotyledon

Figure 58 : Growth of A primary leaf axillary buds, B upper shoot on plants intact (●), surgically ringed around the 2nd internode (■) or decapitated through the 2nd internode (○).

28.4.72 : Ph. vulgaris; 18 d old plants : glasshouse : 10 plants/ treatment; organized : points = mean ± S.E. of A combined length of 1st internodes of primary leaf axillary buds, B length from 2nd node to terminal bud of main shoot : results confirmed in 6 further experiments.


A
B
30
20
10
0
14
13
12
11
10
9
(mm)
(cm)
Length
0
1
2
3
4
5
6
7
Time after treatment (d)

axillary buds acting through the xylem. To test the validity of this experiment a slightly different technique to that of Snow was used. Plants were ringed, one half of the ringed region was excised for a length of c. 1 cm and the pith scraped from the middle of the other half. Partial inhibition of the growth of the primary leaf axillary buds through this length of xylem was demonstrated (Figure 59).

Harvey (1920) showed that killing a zone of the epicotyl of Phaseolus by steaming allowed the cotyledon axillary buds to grow out although the main shoot above this 'steam girdle' continued to grow. This result has been confirmed - steam girdling the 2nd internode of bean plants (see Plate 4) allowed the primary leaf axillary buds to grow out as well as decapitation through the 2nd internode (Figure 60A). The main shoot of the girdled plants continued to grow, although much more slowly than on untreated plants (Figure 60B).

After showing that inhibition could pass a protoplasmic discontinuity, Snow (1925) concluded that a water-soluble substance was at least partially responsible for apical dominance. Although this conclusion seemed to have been justified when Thimann and Skoog (1933) demonstrated the effect of auxin on lateral bud growth, Snow's experiment has proved difficult to confirm in this study.

Snow (1931a) carried out some experiments with pea to show that inhibition of bud growth by the upper shoot was stronger with increasing distance of the bud from the inhibiting organ. Similar types of experiments have been conducted with both bean and pea plants. 25 to 30 d old bean plants were decapitated and defoliated and the effect of a single remaining young trifoliate leaf upon two different buds below was examined (a different set of plants was used for each bud). In one experiment (Figure 61A) the 5th trifoliate leaf strongly inhibited both the 1st and 3rd trifoliate leaf axillary buds. The results were analysed by the method of Snow (1931a) to allow for the difference

Figure 59 : Growth of primary leaf axillary buds on plants intact (●), surgically ringed through the 2nd internode (■), surgically ringed through the 2nd internode with pith removed from the ringed zone (▲) or decapitated through the 2nd internode (○).

4.3.73 : Ph. vulgaris; 27 d old plants : glasshouse : 10 plants/treatment; organized : points = mean $\pm$ S.E. of combined length of 1st internodes of primary leaf axillary buds.

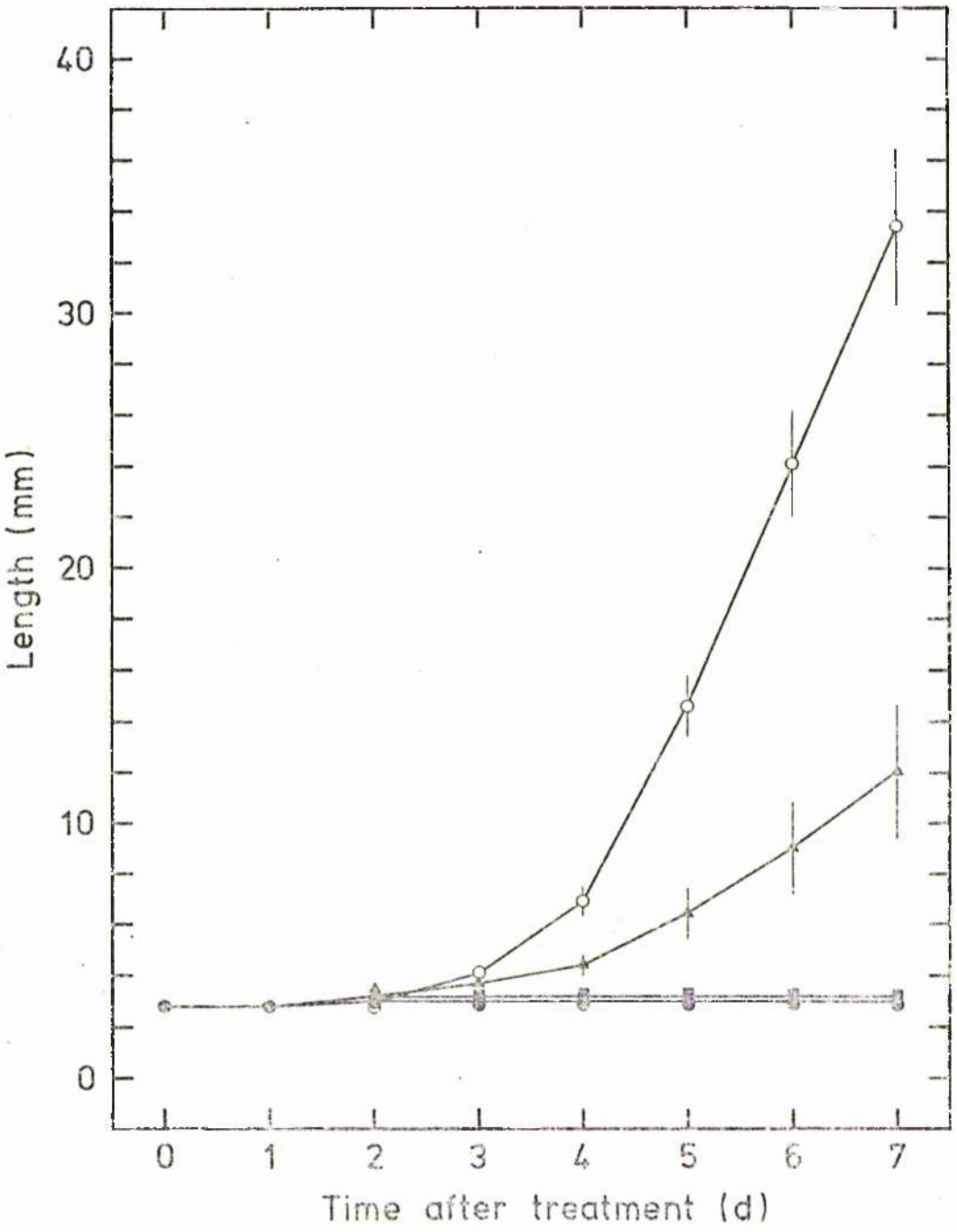
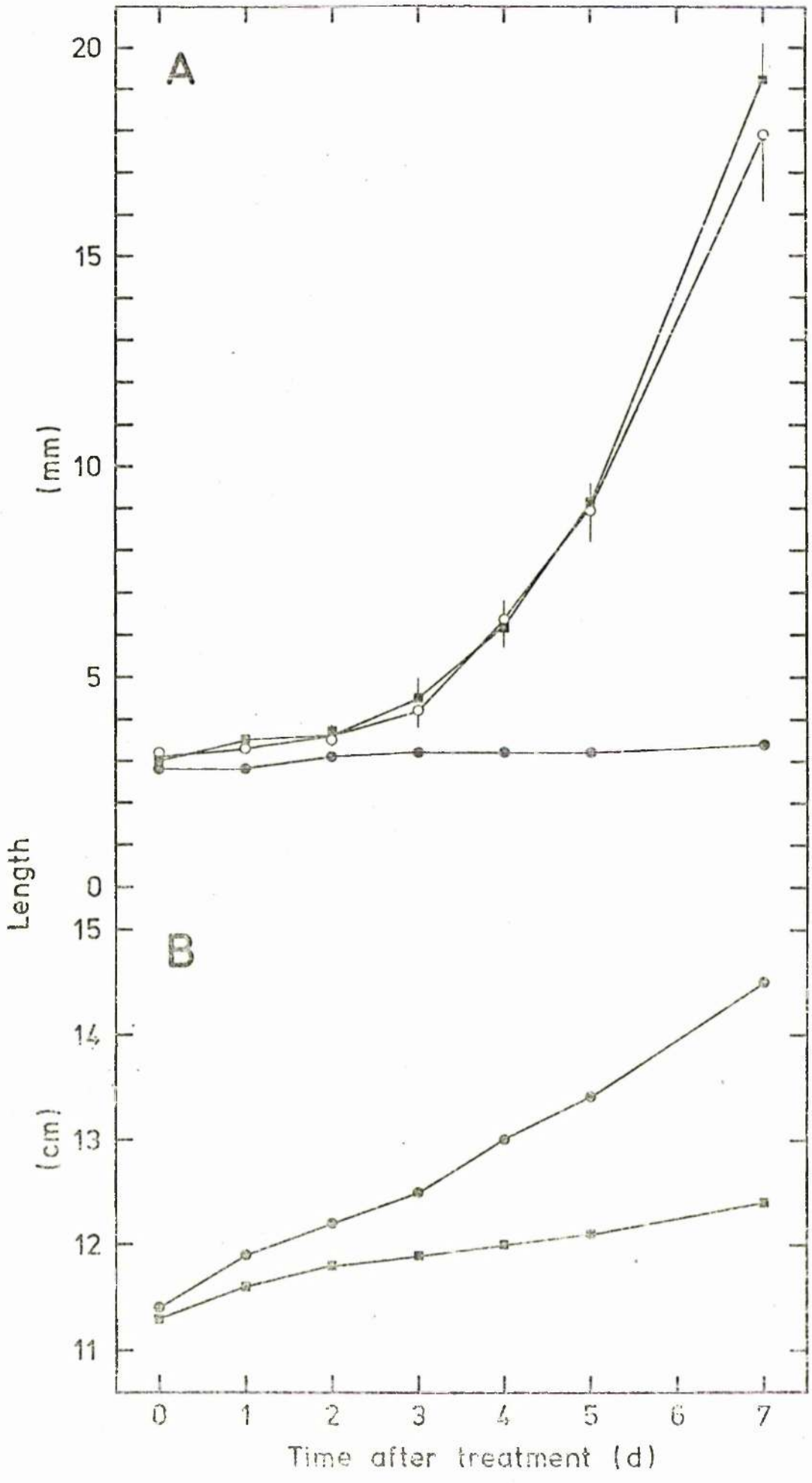

40
30
20
10
0
Length (mm)
0
1
2
3
4
5
6
7
Time after treatment (d)

Figure 60 : Growth of A primary leaf axillary buds, B upper shoot on plants intact (●), steam girdled around the 2nd internode (■) or decapitated through the 2nd internode (○).

23.10.72 : Ph. vulgaris; 24 d old plants : glasshouse : 10 plants/treatment; organized : points = mean ± S.E. of A combined length of 1st internodes of primary leaf axillary buds, B length from 2nd node to terminal bud of main shoot : results confirmed in 5 further experiments.


A
B
Length
(mm)
(cm)
20
15
10
5
0
15
14
13
12
11
0
1
2
3
4
5
6
7
Time after treatment (d)

between buds in their growth on completely defoliated plants: the bud lengths in the presence of the inhibiting leaf were compared at times when each bud had reached a certain length on completely defoliated plants. This method revealed little difference in the strength of inhibition despite the distance from the inhibiting leaf to the 1st trifoliate leaf axillary bud being 2 to 3 times that to the 3rd trifoliate leaf axillary bud. In a second experiment again strong, but not complete, inhibition of both 1st and 2nd trifoliate leaf axillary buds by the 4th leaf occurred and there was no evidence for the increase of inhibition with distance (Figure 61B). Similar experiments were performed with plants that had been decapitated just above the 2nd node and the primary leaf axillary buds allowed to grow to form 'two-shoot plants'. The effect of the 3rd leaf of the longer shoot of otherwise decapitated and defoliated plants upon the growth of the 1st and 2nd trifoliate leaf axillary bud on each shoot was investigated (all the buds were on the same set of plants). Although in two experiments the 3rd leaf of the longer shoot partially inhibited the growth of the buds on both shoots, there was no distinct evidence for an increase of inhibition with distance, either down the longer shoot or up into the shorter shoot (Figure 62). There was no obvious evidence, therefore, for a 'distance effect' in beans.

Similar experiments were also performed using normal and two-shoot plants of the pea variety used by Snow (1931a), Pisum sativum var. 'Thomas Laxton'. With otherwise defoliated normal pea plants a single growing pinna of the 5th leaf inhibited the 1st leaf axillary bud much more than the 3rd leaf axillary bud (Figure 63), confirming the results of Snow (1931a). In two experiments with two-shoot peas, created by decapitating pea plants just above the cotyledons and allowing the cotyledon axillary buds to grow out into shoots, no evidence for a 'distance effect' was found. A single growing pinna of the 3rd leaf

Figure 61 : Growth of 1st (●○) and A 3rd, B 2nd (■□) trifoliate leaf axillary buds on plants of Ph. vulgaris with A 5th, B 4th trifoliate leaf present (closed symbols) or absent (open symbols). A,B = 2 experiments.

A : 29.6.71 : 25 d old plants, B : 8.9.71 : 30 d old plants : glasshouse : plants decapitated through 7th internode; all other trifoliate leaves and axillary buds excised : 10 plants/ treatment; organized : points = mean ± S.E. of length of 1st internode of bud.

Analysis of bud lengths by the method of Snow (1931a) and distance of each bud from inhibiting leaf:-

Experiment	Trifoliate axillary bud	Length on completely defoliated plants (mm)	Day	Length on plants with inhibiting leaf (mm)	Distance of bud from inhibiting leaf (mm)	
					Day 0	Day 7
A	1st	12.3	4	5.9	157	209
	3rd	12.4	7	4.6	45	93
B	1st	9.2	5	2.4	234	248
	2nd	9.2	6	3.1	138	149

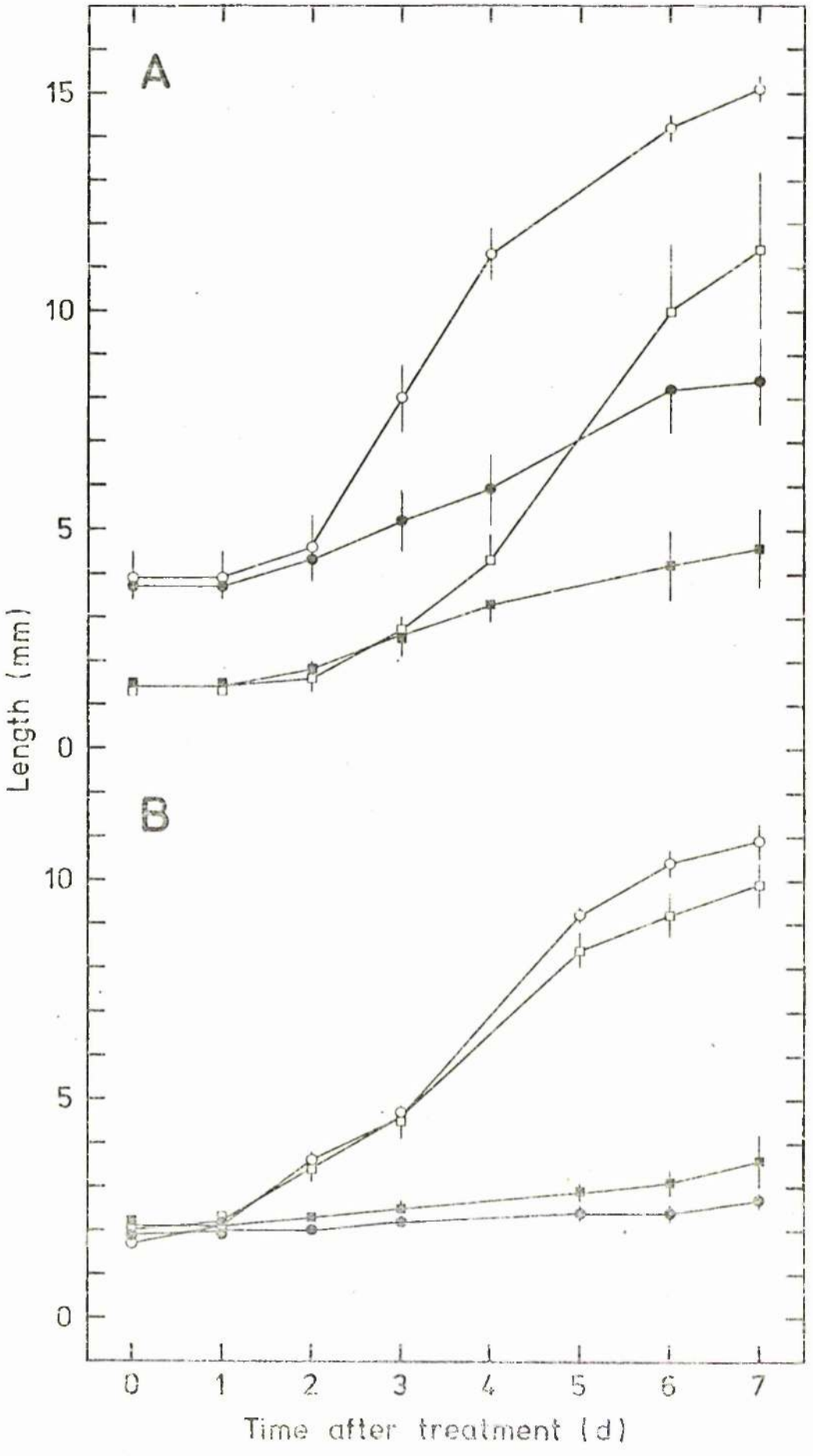

A
B
15
10
5
0
10
5
0
Length (mm)
0
1
2
3
4
5
6
7
Time after treatment (d)

Figure 62 : Growth of buds of A 2nd, B 1st trifoliate leaf of longer shoot, C 1st, D 2nd trifoliate leaf of shorter shoot of 'two-shoot plants' in the presence (●) or absence (○) of the 3rd trifoliate leaf on the longer shoot.

31.5.71 : Ph. vulgaris : glasshouse : 'two-shoot plants' 14 d after decapitation through the 2nd internode; longer shoot decapitated through 4th internode, shorter shoot decapitated through 3rd internode; all leaves bar 3rd trifoliate leaf excised : 8 plants/treatment; organized ; points = mean $\pm$ S.E. of length of 1st internode of bud : results confirmed in 1 further experiment.

Analysis of bud lengths by the method of Snow (1931a) and distance of each bud from inhibiting leaf:-

Shoot	Trifoliate axillary bud	Length on completely defoliated plants (mm)	Day	Length on plants with inhibiting leaf (mm)	Distance of bud from inhibiting leaf (mm)	
					Day 0	Day 7
Longer	3rd	9.0	5	5.5	50	67
	1st	9.0	3.5	6.0	110	127
Shorter	1st	9.0	4.5	6.6	216	233

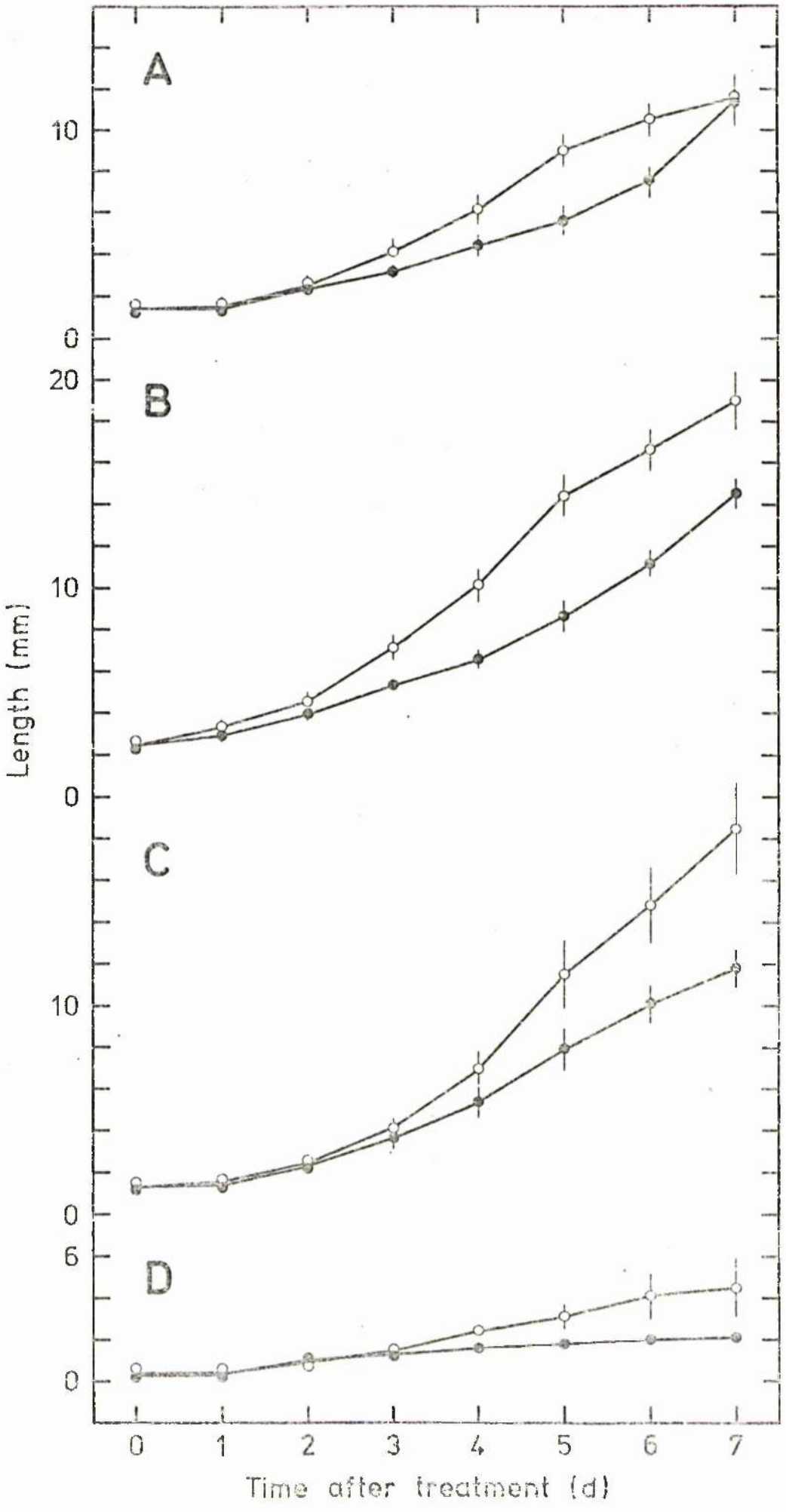

A
B
C
D
10
0
20
10
0
10
0
6
0
Length (mm)
0
1
2
3
4
5
6
7
Time after treatment (d)

Figure 63 : Growth of 1st (● ○) and 3rd (■ □) leaf axillary buds on plants with one pinna of 5th leaf present (closed symbols) or absent (open symbols).

10.2.73 : P. sativum; 15 d old plants : glasshouse : plants decapitated through 6th internode; all other leaves and axillary buds excised : 10 plants/treatment; organized : points = mean ± S.E. of length from base to apex of each bud : results confirmed in 2 further experiments.

Analysis of bud lengths by the method of Snow (1931a) and distance of each bud from inhibiting leaf:-

Leaf axillary bud	Length on completely defoliated plants (mm)	Day	Length on plants with inhibiting pinna (mm)	Distance of bud from inhibiting pinna (mm)	
				Day 0	Day 7
1st	17.7	7	10.3	105	118
3rd	17.7	6.5	14.0	59	69

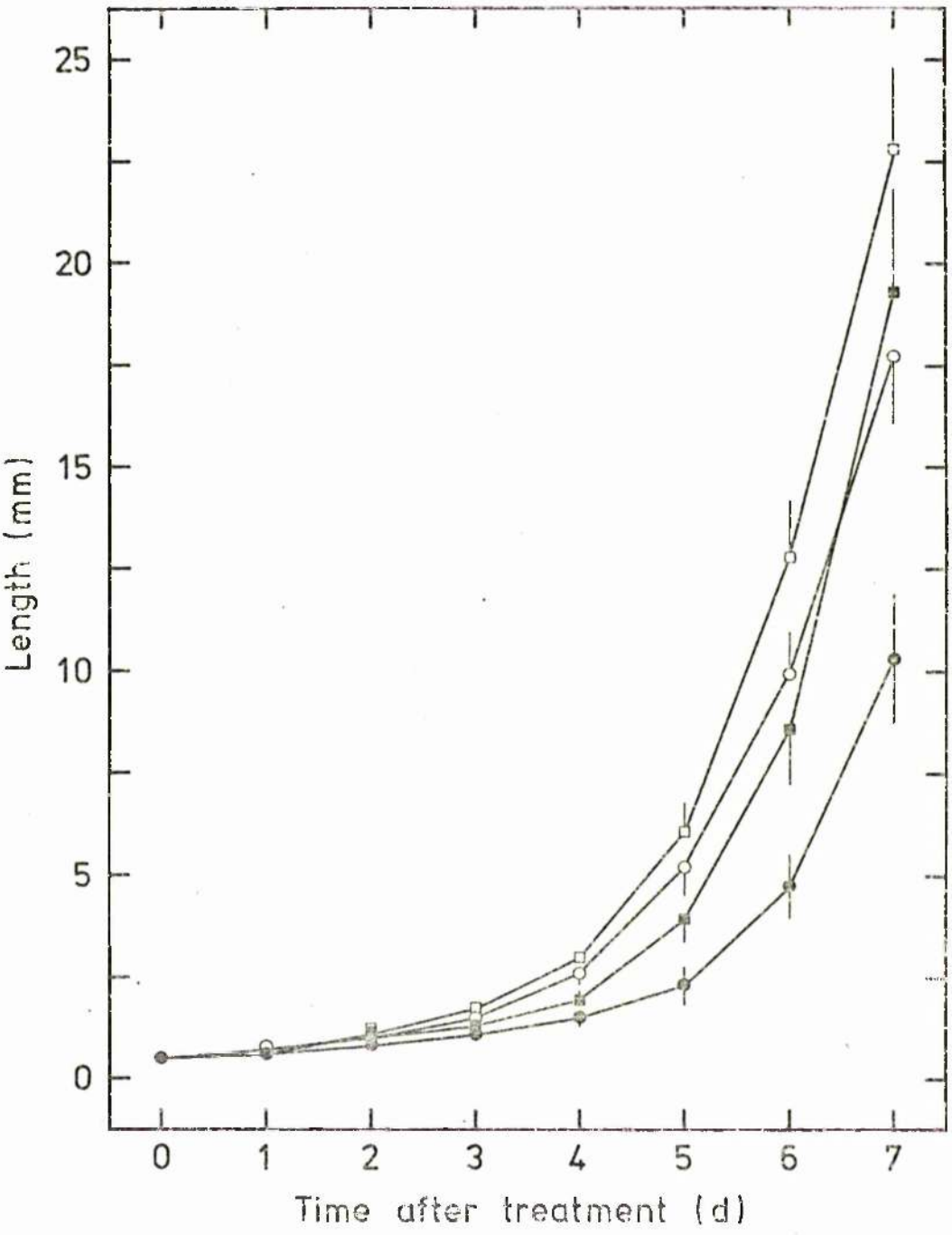
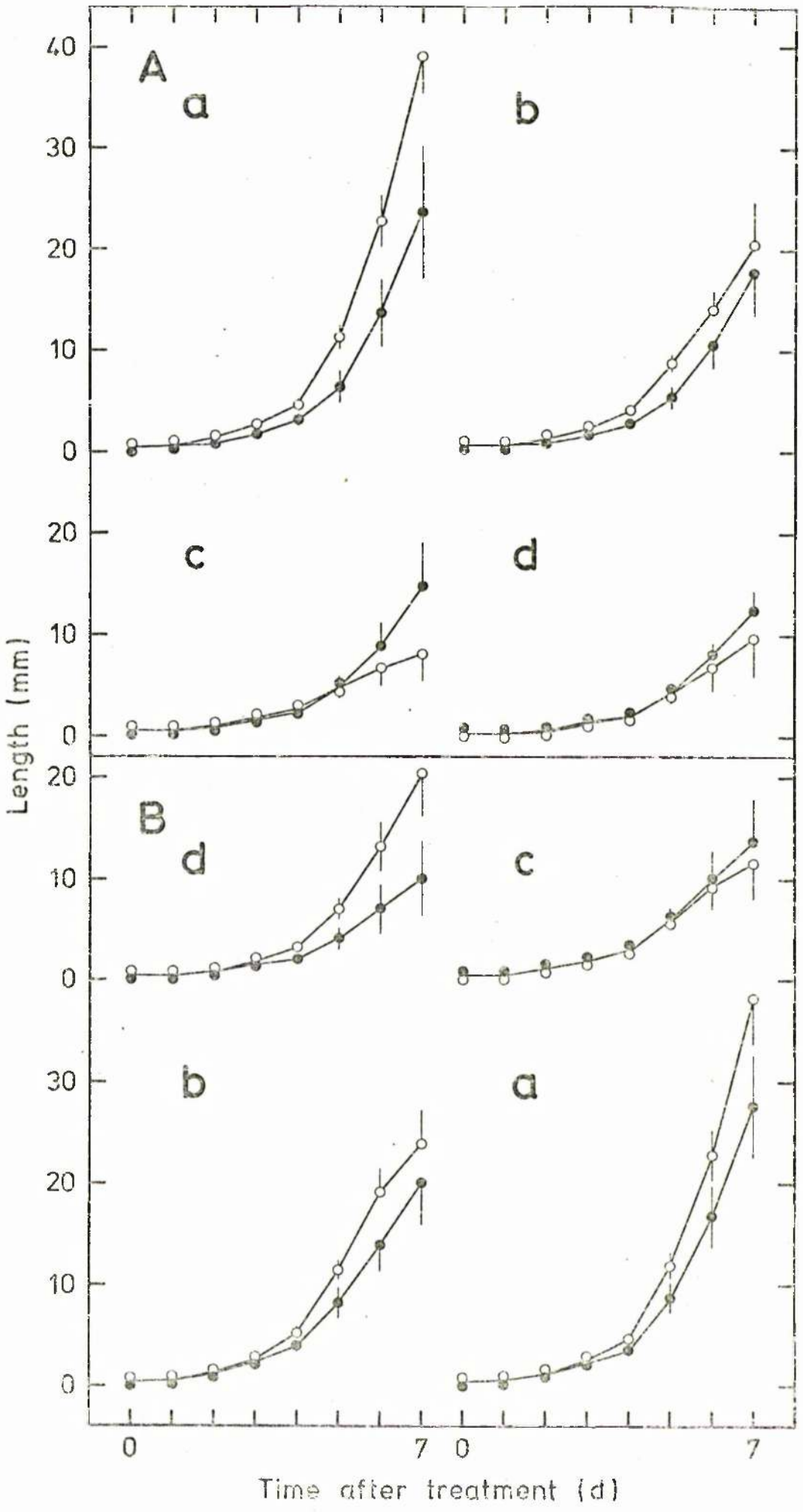

25
20
15
10
5
0
Length (mm)
0
1
2
3
4
5
6
7
Time after treatment (d)

Figure 64 : Growth of buds in axils of a 2nd, b 1st leaf of longer shoot, c 1st, d 2nd leaf of shorter shoot of two-shoot plants in the presence (●) or absence (○) of a single pinna of the 3rd leaf on the A longer, B shorter shoot.

4.3.73 : P. sativum : glasshouse : two-shoot plants 15 d after decapitation through 1st internode : A longer shoot decapitated through 4th internode, shorter shoot decapitated through 3rd internode; B vice versa; 1st and 2nd leaves (mature) of each shoot left intact : 10 plants/treatment; organized : points = mean ± S.E. of length from base to apex of each bud.

Analysis of bud lengths by the method of Snow (1931a):-

Experiment	Shoot	Leaf axillary bud	Length on plants without inhibiting leaf (mm)	Day	Length on plants with inhibiting leaf (mm)
A	Longer	2nd	8.0	4.5	4.7
		1st	8.7	5	5.4
	Shorter	1st	8.1	7	14.7
		2nd	8.3	6.5	10.3
B	Shorter	2nd	11.5	5.75	6.3
		1st	11.5	7	13.7
	Longer	1st	11.4	5	8.2
		2nd	11.9	5	8.6

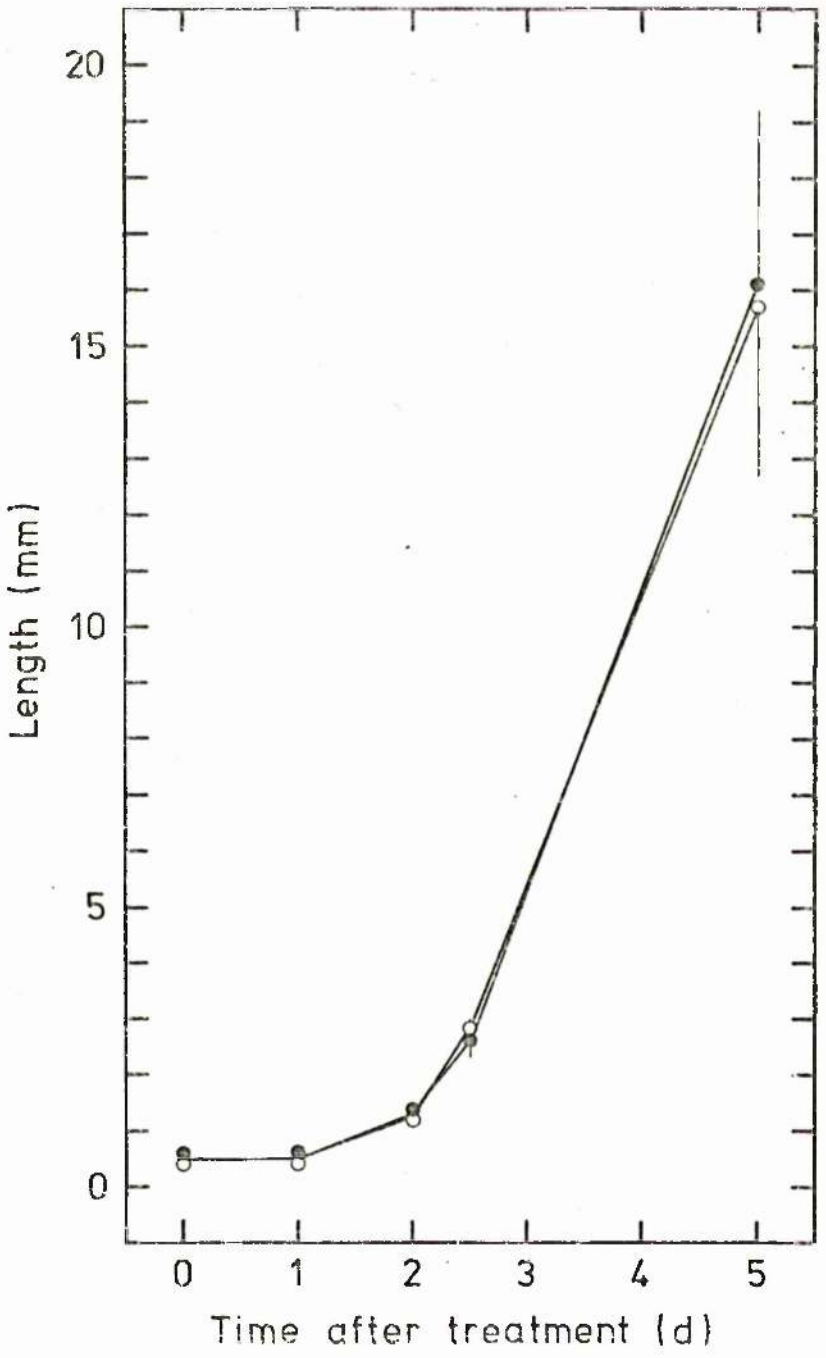

A
a
b
c
d
B
d
c
b
a
40
30
20
10
0
20
10
0
20
10
0
30
20
10
0
0
7
0
7
Length (mm)
Time after treatment (d)

on the longer shoot of otherwise decapitated and defoliated two-shoot plants inhibited the growth of the two lowest buds on the longer shoot but not of those on the shorter shoot (Figure 64A). A single growing pinna of the 3rd leaf of the shorter shoot inhibited the growth of the 2nd leaf axillary buds on the longer and shorter shoots and partially prevented the growth of the 1st leaf axillary bud on the longer shoot (Figure 64B). In neither experiment was there evidence for an increase of inhibition with distance, even within the shoot subtending the inhibiting leaf. The 'distance effect' found by Snow (1931a), confirmed for normal pea plants may, in fact, have been a characteristic of the particular buds used for these experiments.

Snow (1931b to 1939) continued his investigation with a series of experiments on the inhibition by auxin of shoot growth, which may not have been relevant to bud growth. The final experiment of his study (Snow, 1940) purported to show that inhibition of lateral bud growth could travel downwards, upwards, through a protoplasmic discontinuity and downwards again. Incorrect controls were used, however, and poor replication also. Pairs of two-shoot pea plants were prepared as described by Snow (1940). One shoot of each plant was decapitated above the 2nd leaf axil and the bud in this axil was removed. The epidermis and part of the cortex were stripped from one side of the 2nd internode of both of these shoots, which were bound together with the stripped sides facing each other and vaselined, thus forming a moist graft connection between the plants. The second shoot of one of the plants was excised and the effect of the second shoot of the other plant upon the growth of the 1st leaf axillary bud on the remaining shoot of the first plant was examined. No indication was found of inhibition acting through the graft region (Figure 65).

Figure 65 : Growth of axillary bud in presence (●) or absence (○) of a shoot on another plant connected to the first by a protoplasmic discontinuity as described by Snow (1940).

29.1.73 : P. sativum; pairs of 'two-shoot plants' 13 d after decapitation through 1st internode : controlled environment cabinet : 10 plants/treatment; randomized : points = mean ± S.E. of length from base to apex of each bud : results confirmed in 1 further experiment.


20
15
10
5
0
Length (mm)
0
1
2
3
4
5
Time after treatment (d)

B. Translocation in ringed and steam girdled plants

One of the most interesting of Snow's experiments which have been confirmed was that involving the transmission of inhibition of lateral bud growth through a ringed zone - a length of stem internode from which the tissues outside the xylem, including the phloem, had been removed. Snow (1925) claimed that the phloem and cambium had been completely removed and that no regeneration of vascular tissues occurred for at least a week after treatment. Transverse sections of the ringed zone have been cut and compared with the intact and steam girdled regions. The effectiveness of phloem removal has been tested using [^{14}C]sucrose as a tracer for phloem transport. Furthermore, the possible involvement of IAA translocation in apical dominance has been investigated by studying the movement of radioactivity from [^{14}C]IAA applied to a young leaf of intact, ringed and steam girdled plants.

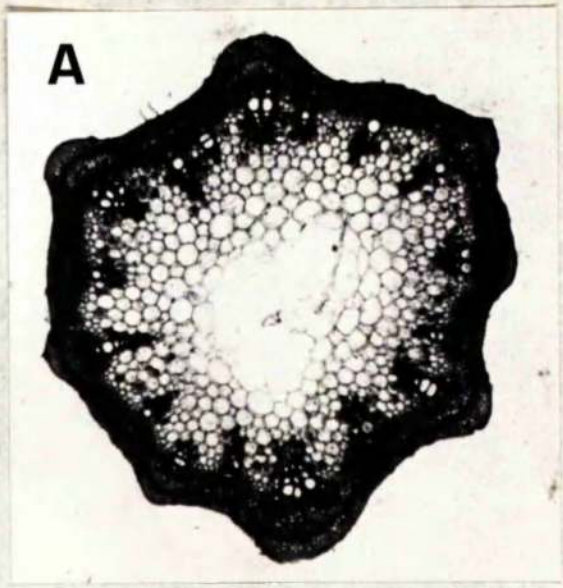
(i) Histology

Microscopic examination of transverse sections of intact and ringed internodes (Plate 10A,B) indicated that the ringing may not have been complete. In every section of ringed internode examined there was a small amount of tissue remaining in a small arc outside the xylem. There was never any sign, however, of tissue regeneration from remaining cambium, nor was it certain whether remaining tissue was continuous through the ringed zone. In sections of steam girdled tissue, which appeared dead and was difficult to section, the xylem vessels were still clearly visible (Plate 10C). Testing internode segments with 2,3,5-triphenyltetrazolium chloride (TTC) showed that intact and ringed tissue was still living (Plate 11A,B) while steam girdled tissue was probably dead (Plate 11C).

(ii) Translocation of [^{14}C]sucrose

[^{14}C]sucrose was applied in the standard manner to a mature primary leaf of intact, ringed and steam girdled plants. After 24 h

Plate 10 : Transverse sections of A intact, B surgically ringed, C steam girdled internodes.



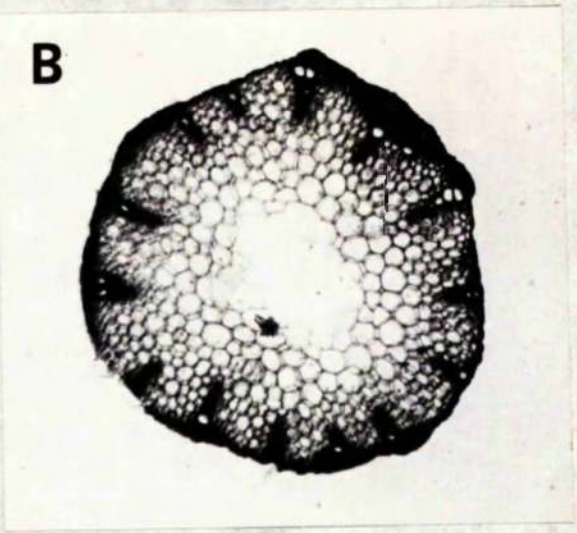
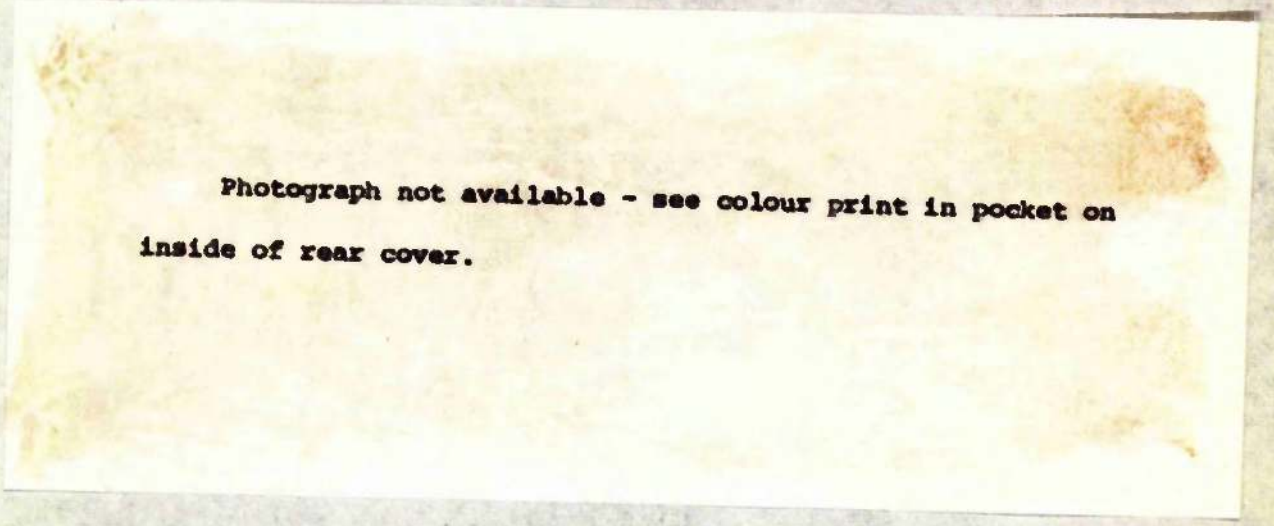


Plate 11 : Segments of A intact, B surgically ringed, C steam girdled internodes after treatment with TTC.



incubation the donor area, midrib and petiole of the donor leaf, the lower stem and the upper shoot (excepting the mature 1st trifoliate leaf) were harvested, segmented and assayed by liquid scintillation spectrometry. As shown previously, in intact plants radioactivity moved out of the donor leaf into both the lower stem and the upper shoot (Table 12A). In both ringed plants, in which inhibition of bud growth was maintained and steam girdled plants, which showed bud outgrowth, no significant ^{14}C was detected in the upper shoot, although ^{14}C had moved out of the donor leaf into the lower stem (Table 12B,C).

(iii) Translocation of [^{14}C]IAA

[1-^{14}C]IAA was applied in the standard manner to the young, growing 2nd trifoliate leaf of intact, ringed and steam girdled plants. In two experiments not enough ^{14}C moved from the donor leaf to be detectable below the treated region. In one experiment, however, radioactivity was detected in the lower stem of both intact and ringed plants but not of steam girdled plants (Table 13A,B,C). In control plants [^{14}C]sucrose had not moved out of the 2nd trifoliate leaf (Table 13D). It appeared, therefore, that some part of the IAA molecule could pass through a ringed zone, but not a steam girdled region.

C. Vascularization of buds

Cleared longitudinal sections of inhibited primary leaf axillary buds excised from intact plants showed distinct vascular connections to the main shoot (Plate 12).

Table 12 : Distribution of radioactivity in shoots of Ph. vulgaris after application of [^{14}C]sucrose to a mature primary leaf of A intact, B surgically ringed, C steam girdled plants.

Treatment	A Intact	B Ringed	C Girdled
No. of plants	10	8	7
Shoot above treated zone	191.6 ± 36.1	1.6 ± 1.2	0.3 ± 0.3
Shoot below treated zone	87.9 ± 21.7	143.0 ± 26.2	348.7 ± 53.3
Donor leaf	526.3 ± 68.3	491.1 ± 71.3	404.2 ± 57.4
Length of primary leaf axillary buds (mm)	3.0 ± 0.0	3.5 ± 0.4	17.9 ± 3.0

9.3.73 and 23.4.73 : 25 d old plants : 0.125 µmol l^{-1} [^{14}C]sucrose applied 3 d after treatment : 24 h incubation : results = mean d min^{-1} ± S.E. for plants from two experiments.

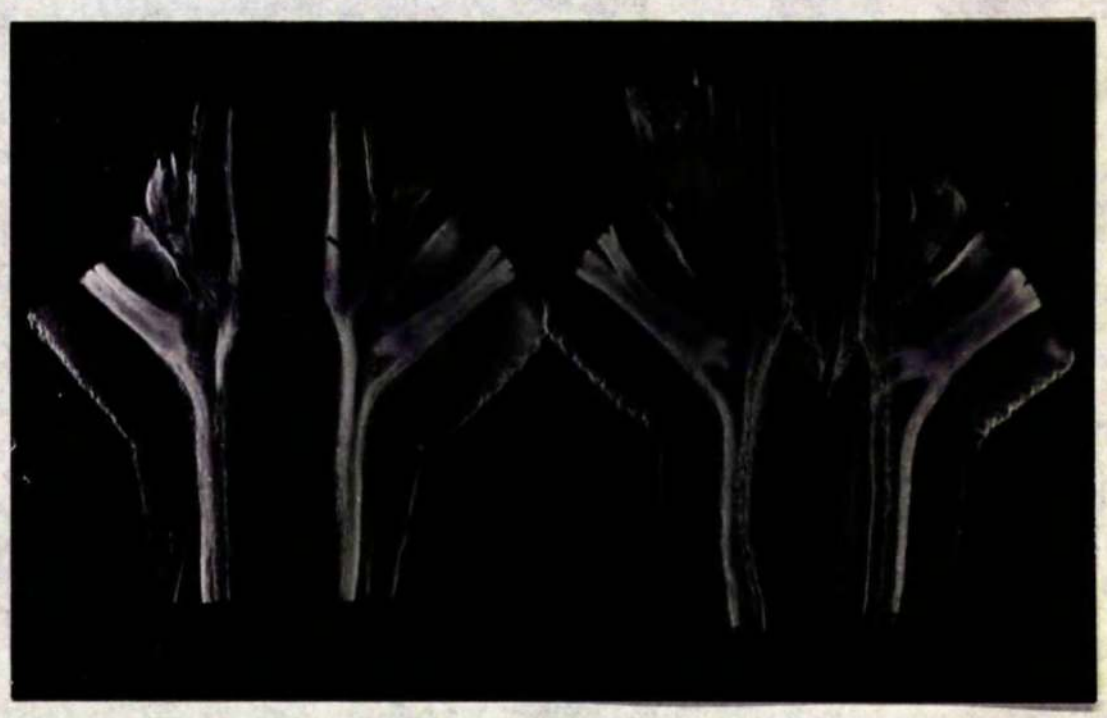
Table 13. Distribution of radioactivity in shoots of Ph. vulgaris after application of [1-^{14}C]IAA or [^{14}C]sucrose to a young second trifoliate leaf of A,D intact, B surgically ringed, C steam girdled plants.

	[1-^{14}C]IAA			[^{14}C]sucrose
Treatment	A Intact	B Ringed	C Girdled	D Intact
No. of plants	5	5	3	5
Donor leaf	818.4 ± 132.1	831.2 ± 119.7	1000.7 ± 29.4	2607.2 ± 119.6
Shoot above treated zone	36.8 ± 2.7	108.9 ± 8.1	229.5 ± 26.5	0.4 ± 0.4
Shoot below treated zone	61.9 ± 10.1	46.1 ± 8.2	2.2 ± 1.3	1.6 ± 1.6
Length of primary leaf axillary buds (mm)	3.0 ± 0.0	4.0 ± 0.8	18.3 ± 0.9	3.0 ± 0.0

20.3.73 : 22 d old plants : 1.0 µmol l^{-1} [^{14}C]IAA or 0.125 µmol l^{-1} [^{14}C]sucrose applied 3 d after treatment : 24 h incubation : results = mean d min^{-1} ± S.E.

Plate 12 : Longitudinal sections through 2nd nodes from intact plants.

Sections cleared with lactic acid : arrows indicate vascular connections between axillary buds and main stem.



DISCUSSION

Correlative inhibition of lateral bud growth has been examined in Phaseolus vulgaris, a legume which is a popular test plant for studies of apical dominance and other processes. The investigation began with an appraisal of the influence of various parts of the plant upon lateral bud growth. Firstly, the pattern of growth of intact plants was briefly analysed. In the shoot there was a sequential movement of maximal elongation acropetally from internode to internode and from leaf to leaf, while root growth was initially associated with the primary root but subsequently transferred to the adventitious and lateral roots. Dry weight measurements gave a similar pattern to those of length, although dry matter accumulation of stem internodes continued after their elongation growth had ceased, and was presumably associated with secondary growth. It can be inferred from the results that the plants began photoassimilation and nutrient uptake between 8 and 14 days after sowing, having obtained their nutrition from the cotyledons until that time. On the 8th day after sowing the primary leaf laminae were greater than 5 cm long and probably exporting assimilates (Köcher and Leonard, 1971).

The tissues of the shoot arise from an apical meristem, which together with the recently formed stem and leaf tissue above the uppermost unfolded leaf constitute the 'apical' or 'terminal' bud. A meristem is also formed in the axil of each leaf and gives rise to a 'lateral' or 'axillary' bud analagous and apparently similar to the apical bud. On intact plants the cotyledon axillary buds grew to less than 1 mm in length in 19 d. The primary leaf axillary buds, however, developed considerably over a short period when the internode and leaf above were growing: the growth of these buds subsequently declined.

Removal of the growing upper shoot just above the 2nd node led to a redistribution of growth, mainly to the primary leaf axillary buds but

also partially to other regions. Growth of a shoot, either leading or lateral, consists of complex sequences of cell division, extension and differentiation. One of the most obvious products of these processes is the increase in size of the young tissues of the shoot. Measurements of length indicated the outgrowth of the primary leaf axillary buds after removal of the main shoot to involve initially extension of the 1st internode and expansion of the 1st leaf: each of the buds exhibited similar qualitative responses. It was further established that correlative inhibition of these buds occurred under both glasshouse and controlled environment conditions. Changes in environment can alter apical dominance but the responses usually occur over a long time period (e.g. Nakamura, 1965 ; Tucker and Mansfield, 1972). The lack of far-red light in the controlled environment cabinet (see Figure 1), for instance, known to reduce apical dominance in Xanthium (Tucker and Mansfield, 1972), appeared to be ineffective over a week with Phaseolus.

The point of decapitation of the plant markedly influenced the pattern of bud outgrowth: in general on any particular plant the uppermost of the buds that had completed their early phase of growth (see Figure 8A) exhibited the greatest extension and any particular bud grew more strongly when more of the shoot was removed from above it. An examination of the growth of each bud individually on completely defoliated plants indicated that the inherent capacity of different buds to grow may vary, although their position in relation to stem internodes and roots, or to the environment, may influence their growth. The poor growth of the cotyledon and 3rd trifoliate leaf axillary buds (Figure 11) may have been due to their small initial size, although the shorter primary leaf axillary bud was often of a similar initial length and generally exhibited better growth. Another possibility is that growth capacity of lateral buds might vary with age. The primary leaf axillary buds, however, were found to grow out vigorously on decapitated plants up to 5 weeks old and there was no

consistent effect of age upon this growth.

Whatever the factors affecting the pattern of bud growth the upper shoot was clearly a powerful influence. Removal of the terminal bud alone did not markedly affect lateral bud growth over a period of 7 days. Mature leaves improved the growth of buds released from apical dominance but did not influence lateral bud growth on intact plants. It was assumed that the promotive influence of mature leaves was a nutritive one and the primary leaves were maintained intact during most experiments to allow potentially vigorous outgrowth of lateral buds. The report by Weiskopf (1927) that mature leaves of legumes inhibited lateral bud growth was not confirmed for Pisum by Snow (1929b) or for Phaseolus in this study. It has been suggested that mature leaves might be the source of inhibitor-precursors for correlative inhibition (Libbert, 1955b) in which case it might be expected that removal of the only mature leaves on a plant would reduce correlative inhibition. This role of mature leaves, however, need not require their continuous presence: furthermore, roots have been implicated as an alternative source of inhibitor-precursors (Libbert, 1955b).

The major inhibiting influence exerted upon the lateral buds was shown to originate in the young, growing leaves, some of which were capable of completely inhibiting the growth of the primary leaf axillary buds in the absence of any other young leaves. This confirms the results of Weiskopf (1927) for Phaseolus and Snow (1929b) for Pisum. It was further demonstrated that inhibition was exerted by a young leaf with a basipetal polarity of action, in confirmation of previous observations (see Thimann, 1939). Growing lateral buds inhibited other lateral buds, also with a basipetal polarity and presumably by virtue of their young, growing leaves. The polarity of action of young leaves and buds tends to discount a purely 'nutritive' theory of apical dominance, although some of the inhibition may be a result of compensatory growth effects.

The generally better growth of the upper buds on decapitated or defoliated plants would be expected on the basis of a primarily downward-acting inhibition.

Upon removal of the main shoot above the 2nd node both the buds in the primary leaf axils grew out initially but after 4 days the growth of the shorter bud became retarded by the presence of the longer. After 2 weeks many plants exhibited complete inhibition of the shorter shoot and although this inhibition could be alleviated by removal of the longer shoot, without this removal many of the shorter shoots eventually died. This effect was noted by Mogk (1913) who claimed that two such growing shoots of equal length could actually kill each other. Further investigations by Snow (1931b ; 1937) with Pisum revealed that one of two equal shoots could be rendered more susceptible to inhibition by darkening or by excision of its young leaves and that this susceptibility could be overcome by applying indole acetic acid to the darkened or defoliated shoot. This indication that auxin levels in inhibited shoots were suboptimal was interpreted by Snow (1937) as opposing the 'direct' theory of auxin action in apical dominance. Although inhibited intact shoots (as in this study) can be induced to grow by direct application of IAA (Libbert, 1954a ; Sachs and Thimann, 1967) lateral buds have not been released from correlative inhibition by such application (Thimann, 1937 ; Sachs and Thimann, 1967). Although the inhibition of shoots does resemble the inhibition of buds in some respects (e.g. Sachs 1966), the death of inhibited shoots of both Phaseolus (this study) and Pisum (Sachs, 1966) occurs within 3 to 4 weeks of the onset of inhibition whereas inhibited buds survive for much longer periods. These features may be indicative of basic physiological differences between the natural correlative inhibition of lateral bud growth and the inhibition of larger shoots: this point will be considered later in relation to the development of the correlative inhibitor theory.

Stem internodes remaining on defoliated plants furthered the outgrowth of lateral buds, this effect being masked by the presence of mature leaves which also promoted bud growth. It was assumed that the effect of the stem was a nutritive one - in the absence of other photosynthetic tissue the green stem could supply a significant supply of assimilate to the buds. This function of stem tissue would presumably be of less importance in intact plants and one cannot ascribe any regulatory role in apical dominance to the stem on the basis of these results. Nevertheless, the stem serves a very important function as the pathway for transmission of inhibition from the young leaves to the lateral buds; this and other possible roles for the stem will be discussed later.

In a single experiment it was demonstrated that an intact root system was required, at least over 7 days, neither for the maintenance of correlative inhibition, nor for the outgrowth of lateral buds. Although perhaps indicative that the continuous presence of roots may not be essential for inhibition or bud growth this experiment, which requires confirmation, does not rule out the possibility that root-produced inhibitor-precursors (Libbert, 1955b) or bud growth factors (Went, 1939) are involved in apical dominance, for enough of these may have accumulated in the stem before root excision. Furthermore, mature leaves may act as an alternative source of inhibitor-precursors (Libbert, 1955b). Certainly in plants other than Phaseolus root excision can influence correlative phenomena in the shoot (e.g. Goebel, 1908; Libbert, 1955b; Woolley and Wareing, 1972) and environmental effects on roots can influence shoot growth and may be mediated by an effect of roots on the hormone physiology of the shoot (e.g. Phillips, 1964a; b; Reid and Crozier, 1971; Railton and Reid, 1973).

In Phaseolus the only function of the cotyledons as regards bud growth appeared to be a nutritive one, promoting the growth of cotyledon

axillary buds released from inhibition by the upper shoot. This is in marked contrast to other species in which cotyledons can either inhibit the growth of their axillary buds or influence the balance of growth between two cotyledon axillary buds (e.g. Dostal, 1909; Champagnat, 1951). Most of the experiments in this investigation were conducted with 2 to 4 week old plants, in which the cotyledons were almost exhausted or actually abscinded (see Figure 8E) and therefore could not contribute to apical dominance.

The correlative effects of the upper shoot and mature leaves on abscission are explainable in terms of auxin physiology, although other factors may be involved (see Carns, 1966). Correlative senescence may also be induced by auxin (Sachs, 1966). The inhibitory effect of young leaves on the growth of other young leaves parallels that demonstrated for Coleus and may be a compensatory growth effect independent of auxin physiology (Jacobs and Bullwinkel, 1953). The apparent stimulation of senescence of the primary leaves by the presence of roots was more surprising and the experiment obviously needs confirmation. Roots have been shown to delay senescence of isolated leaves of Phaseolus (Chibnall, 1954) and this effect has been ascribed to root-produced cytokinins (Sitton et al., 1967). The opposite effect of roots on intact leaves of Phaseolus could conceivably be explained in terms of competition for nutrients, removal of both the upper shoot and roots releasing nutrients to the remaining leaves. If this were the case, however, root excision might also have been expected to boost the growth of the primary leaf axillary buds on decapitated plants, but this did not occur.

The preliminary growth studies have indicated that the correlative inhibition of lateral bud growth in Phaseolus is caused by young, rapidly expanding leaves, either on the main shoot or on outgrowing lateral shoots. Although the stem must play a role as the link between young leaves and lateral buds, other parts of the plant only influenced

bud growth quantitatively or not at all, rather than qualitatively. The investigation, therefore, continued as an examination of the influence of young leaves upon lateral bud growth, an inhibitory effect which acted powerfully in a basipetal direction. This well-known polarity of inhibition (see Thimann, 1939) is obviously difficult to explain in terms of compensatory growth on the basis of the 'nutritive' theory and the discovery of the possible involvement of auxin in correlative inhibition (Thimann and Skoog, 1933; 1934) has led to the general acceptance of the controlling influence of the basipetal transport of apically produced indole acetic acid in apical dominance. In view of the doubts surrounding this concept (see 'GENERAL INTRODUCTION') the involvement of IAA in apical dominance in Phaseolus was re-examined.

Two methods were employed to introduce IAA to decapitated bean plants through the cut surface of the upper remaining internode. Continuously supplied aqueous solutions only inhibited lateral bud growth at concentrations greater than 0.1 mmol l^{-1} IAA and the 5 mmol l^{-1} IAA required to completely prevent bud growth on all plants was associated with severe side-effects. Complete inhibition of bud growth by 1 or 5 mmol l^{-1} IAA was always accompanied by swelling around the primary leaf node with apparent formation of root initials in this region. No inhibition of bud growth was produced by 0.1 mmol l^{-1} IAA, a result which is in accordance with the finding of Hillman (1970), using the same technique and the same species, that 25 ppm (0.14 mmol l^{-1}) IAA exerted little influence on bud growth unless applied in combination with certain other growth substances. An essentially similar method of application was employed by Skoog and Thimann (1934) who claimed that complete inhibition of bud growth of Pisum could be maintained by aqueous IAA without any increase in either length or thickness of the internode of application. Scott and Pritchard (1968) on the other hand, required 150 ppm (c. 1 mmol l^{-1}) IAA to completely prevent bud growth in Pisum: this concentration did induce

growth of the internode of application and was just short of toxic to the plants. The relevance, therefore, of inhibition of lateral bud growth by such high concentrations of IAA to natural correlative inhibition is extremely doubtful, although, of course, it cannot be certain how much IAA actually enters the plant.

The application of IAA in lanolin paste, as used by Laibach (1933) and subsequently employed routinely by many workers, gave a completely different pattern of results. Complete inhibition of bud growth could be maintained on 29 and 36 d old plants of Phaseolus by 1 mg g^{-1} IAA, although only partial inhibition was found with 22 d old plants. Although 1 mg g^{-1} (1000 ppm or c. 5 mmol l^{-1}) was as great a concentration as those aqueous solutions which produced apparently toxic effects, there was no sign of toxicity; neither was the region around the 2nd node swollen. Inhibition was, however, characteristically accompanied by swelling at the region of IAA application, as demonstrated by Laibach (1933) for Vicia and subsequently confirmed by MacQuarrie (1965) with Pisum. Removal of the applied IAA led to subsequent outgrowth of the inhibited buds and this method of IAA application to decapitated plants appeared to more nearly simulate natural correlative inhibition than did aqueous application.

The sensitivity of the primary leaf axillary buds to inhibition by both 1 and 0.1 mg g^{-1} IAA increased with age from 22 to 36 d, although the growth of these buds on decapitated control plants did not vary. Although little evidence was found for age influencing the pattern of bud growth on decapitated plants (Figure 12; 13), it is possible that differential sensitivities of buds to inhibition on intact plants could be explained in terms of changes with age in susceptibility to auxin. Such an effect, for instance, could explain the early growth of the primary leaf axillary buds despite the presence of a growing leaf above them (Figure 8).

Powerful inhibition of lateral bud growth by 1 mg g^{-1} (0.1%) IAA in lanolin applied to decapitated plants of Phaseolus, as shown in this

study, has been reported previously (Phillips, 1968; 1971a). Jacobs et al. (1959) and Shein and Jackson (1972), however, have reported 1% IAA in lanolin to exert little or no effect on lateral bud growth of Coleus and Phaseolus respectively. The lack of effect in Coleus may have been because the plants were showing only a little apical dominance anyway and this slight inhibition could have been due to a compensatory growth effect. Under different environmental conditions 1% IAA in lanolin can inhibit bud growth in Coleus (Thimann et al., 1971). The lack of effect of 1% IAA in the experiments of Shein and Jackson (1972) is more difficult to explain: these workers were also using Ph. vulgaris var. 'Canadian Wonder' in a glasshouse and although they did not apply IAA to the 2nd internode they tested a number of points of application just above the 3rd node and there is no reason to think that this slight difference would lead to a complete loss of activity. Although Shein and Jackson did not quote plant age, the plants they used included a mature 1st trifoliate leaf and so were probably well over 21 d old. A possible explanation is that Shein and Jackson applied hormones in anhydrous rather than hydrous lanolin. It is quite possible that the lack of an aqueous medium reduced entry of IAA into the plant, although other workers have elicited effects from IAA in anhydrous lanolin (e.g, Catalano and Hill, 1969) and Shein and Jackson (1972) themselves obtained effects from similarly applied gibberellic acid and kinetin.

The first of Jacobs' (1959) requirements for the demonstration of involvement of a substance in a physiological process is that the substance should be present in the organism. There is an abundance of evidence for the existence of IAA-like activity in both extracts and 'diffusates' of shoot tips of green plants, but this evidence is based on non-specific methods of identification (see Scott and Jacobs, 1964). Recently, the technique of mass spectrometry has been employed, in conjunction with classical methods, for the conclusive identification of IAA in Citrus fruits (Igoshi et al., 1971) and Zea coleoptile tips (Greenwood et al.,