THE ROLE OF CYTOKININS IN THE REGULATION OF

APICAL DOMINANCE IN Pisum sativum

BY

RICHARD ANTHONY KING

Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

in the

Department of Botany, Faculty of Science

University of Natal, Pietermaritzburg

December 1988

PREFACE

I hereby declare that this thesis, submitted for the degree of Doctor of Philosophy in the Faculty of Science, University of Natal, Pietermaritzburg, except where the work of others is acknowledged, is the result of my own investigation.

<div style="text-align: right;">

———————————————

Richard Anthony King

December 1988

</div>

ACKNOWLEDGEMENTS

ABSTRACT

Intact or decapitated Pisum sativum seedlings were used to investigate the correlative inhibition of lateral buds by the shoot apex – a developmental process known as apical dominance. Apical dominance is considered to be regulated by the relative ratios of growth regulators, especially auxin and cytokinin, and resource availability in the plant. This study considered the role of cytokinins in the regulation of correlative inhibition, and was closely linked to theoretical models of auxin and nutrient gradients in the whole plant.

Firstly, the response of all lateral buds on the plant to decapitation of the shoot apex, and the subsequent growth of these buds, was documented. The pattern of lateral bud branching following decapitation, noted to change with increasing age of the plant, was found to be consistent with the view that apically synthesized auxin, moving basipetally, exerts an inhibitory influence on lateral bud growth. Removal of selected lateral buds on decapitated plants which left various combinations of buds to compete indicated that correlation between lateral buds on the same plant was likely an important factor controlling the patterns of lateral bud branching.

Secondly, a quantitative study of the biological responses which result from the application of a cytokinin to a lateral bud were performed. The different abilities of ten cytokinins tested to release lateral buds from dominance paralleled their activity in a soybean callus bioassay, and were likely a result of the intrinsic activities of the cytokinins due to their structure and their subsequent metabolism in the plant following application. A consistent feature of these experiments was the low biological activity of isopentenyladenine in relation to the high activity of zeatin. Further investigation of the role of isopentenyladenine and zeatin in apical dominance control indicated that lateral buds differed in their sensitivity to these two cytokinins. On decapitated plants, lateral buds were more able to

utilize applied isopentenyladenine, and high zeatin concentrations were no longer supraoptimal for growth.

Finally, the metabolism of $[^3H]$ isopentenyladenine in the intact plant or in isolated explants was investigated in an attempt to relate the biosynthesis, transport, interconversion and degradation of cytokinins to the developmental process of apical dominance. Comparison of the uptake and metabolism of $[^3H]$ isopentenyladenine by isolated organs of Pisum sativum indicated that the roots, leaves and stems, but not isolated lateral buds had the capacity to metabolise $[^3H]$ isopentenyladenine to zeatin metabolites. This metabolite activity was very notable in the stem, where it occurred as a gradient and was related to the age of the plant. Auxin was considered to be the factor controlling this distribution of cytokinin metabolic activity in the stem since parallel gradients had been noted in the patterns of lateral bud branching and the response of buds to cytokinin application. Indeed, it was shown that indole-3-acetic acid affected the metabolism of $[^3H]$ isopentenyladenine in isolated stem sections.

These results are discussed in relation to the control of lateral bud growth via the auxin mediated distribution and metabolism of root produced cytokinins in the shoot system, necessary for the release of apical dominance.

CONTENTS

Page

LIST OF TABLES

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

A living plant system typically possesses several growth zones, or lateral meristems, which are interrelated such that the growth of one is interdependent on the growth of others. In this open-ended pattern of development, a plant must possess a system for coordinating and regulating the processes of growth and differentiation. This control of 'form or function' in one part of a plant by another part can be considered as a correlative event.

Such a correlative control system is evident in the phenomenon of apical dominance. In this developmental process, the growing apex appears to correlate the growth of lateral organs on the plant. Removal of the apex by physical means results in the removal of the inhibitory influence and subsequent growth of the lateral organs.

The phenomenon of apical dominance is by no means universal, and may vary in different species from complete dominance by the apex to free branching in the presence of the apex. The degree of dominance is a function of genetic loci, environmental factors and the physiological status of the plant. Apical dominance can mean a complete or nearly complete control of lateral organs by an apex, the dominance of one growing organ by another or the apical influence on the orientation of organs such as shoots and leaves.

The control exerted by apically located organs profoundly affects the ecological interests of the plant. The inhibited lateral organs represent a reserve of meri-

stems which could replace a damaged or lost apical bud. In addition, physical stimuli such as light could dictate the form of the growing plant, allowing it to best exploit the environment. In this way the plant is able to overcome the disadvantages of a sessile growth habit.

The phenomenon of apical dominance has significance in agriculture, as envisaged by the manipulation of plant growth and form by physical or chemical means. Yield-bearing lateral organs may be increased and the shape of ornamental plants may be altered.

The study, and thus the review, are concerned with one aspect of apical dominance - the correlative inhibition of lateral buds by the shoot apex. Particular emphasis will be placed on results obtained from Pisum sativum L. plants - the plants used in this study.

1.2 The release of apical dominance

The events occurring in lateral buds following their release from apical dominance have been documented in an attempt to understand the mechanisms of correlative control operative in the whole plant. However, caution should still be applied to such results since the events being measured may not necessarily be linked to the release mechanism, but may be another manifestation of growth and de- velopment. A description of the early growth of lateral buds will therefore be given in this section without attempting an explanation of the control mechanisms.

All vegetative buds on a plant possess essentially equal developmental potential (PHILLIPS, 1975). The lateral buds in several plant species originate directly

from the growing shoot apex, from which a bud is formed from a group of cells in the axil of a leaf primordium (SUSSEX, 1955). The newly formed buds may either continue to grow slowly, as in the case of plants with incomplete apical dominance such as Phaseolus vulgaris L. (HILLMAN, 1984), or bud development may be arrested, as in Tradescantia paludosa E. Anders and R.E. Woodson which exhibits complete apical dominance (NAYLOR, 1958; BOOKER AND DWIVEDI, 1973). In plants exhibiting weak or incomplete apical dominance, mitosis and cell expansion can occur, while in plants exhibiting complete apical dominance, these processes are arrested at an early stage (NAYLOR, 1958; HILLMAN, 1984). Thus the degree of dominance experienced by the lateral buds of different plants varies, and may also vary with the physiological conditions experienced by one species.

On decapitation (removal of the shoot apex), cytokinin application or any other treatment that removes apical dominance, mitotic activity in the lateral buds resumes after some delay and growth occurs. Rapid changes in bud growth following decapitation have been detected in Glycine max (L.) Merr. (ALI and FLETCHER, 1970), Pisum sativum (WARDLAW and MORTIMER, 1970; NAGAO and RUBINSTEIN, 1976), Cicer arietinum L. (GUERN and USCIATI, 1972) and Bidens pilosus L. (KRAMER, DESBIEZ, GARREC, THELLIER, FOURCY and BOSSY, 1980). Application of a cytokinin to the lateral buds of intact plants may also promote rapid growth (USCIATI, CODACCIONI and GUERN, 1972; PILLAY and RAILTON, 1983), while increasing the nitrogen availability to Linum usitatissimum L. plants resulted in cell division in the lateral buds about 12 hours before growth could be detected (McINTYRE, 1975).

The time period between the removal of dominance and the onset of lateral bud growth - the lag phase, has been considered critical in relating observable growth

to the possible mechanisms controlling the events in the plant. Reported lag phases range from 30 minutes to three to five hours for lateral buds of Phaseolus vulgaris (HALL and HILLMAN, 1975; YEANG and HILLMAN, 1981a), four to eight hours for lateral buds of Pisum sativum (WARDLAW and MORTIMER, 1970) to six hours for lateral buds of Cicer arietinum (USCIATI et al., 1972). In the definitive work on this aspect, NAGAO and RUBINSTEIN (1976) observed lateral bud growth on Pisum sativum plants after a lag phase of six to eight hours following decapitation of, or cytokinin application to, rootless plants. The lag phase will depend on the degree of inhibition imposed by the plant. In Phaseolus vulgaris, which exhibits incomplete apical dominance, increased internodal expansion occurred four hours after decapitation (HILLMAN, 1984) while in Tradescantia paludosa a much longer lag phase was due to a greater degree of bud inhibition (NAYLOR, 1958). The differences in lengths of lateral buds on the same plant are attributed to differences in this lag phase, not rate of growth, and may be related to the transport of auxin in the plant.

The time sequence of resumption of lateral bud growth following removal of apical dominance will also depend on the stage of cell division occurring in the buds. In Cicer arietinum lateral buds, mitosis occurs one hour after removal of dominance with a resumption of bud growth followed by DNA synthesis (GUERN and USCIATI, 1972). The cells in the lateral bud apex are in the G_2 stage, having duplicated their DNA and can thus undergo mitosis without delay. However, in the lateral buds of Tradescantia paludosa, which exhibit a lag phase of up to four days, the cells are in the G_1 stage, and DNA needs to be synthesized before mitosis can occur (NAYLOR, 1958; BOOKER and DWIVEDI, 1973). Increased cell division in lateral buds 24 to 48 hours after removal of the shoot apex has been observed by NAYLOR (1958); BALLARD and WILDMAN (1964); DWIVEDI and NAYLOR (1968); ALI and FLETCHER (1970) and PETERSON

and FLETCHER (1975). In Glycine max (PETERSON and FLETCHER, 1975) and Tradescantia paludosa (NAYLOR, 1958) cell division occurs before measurable growth. However, in Pisum sativum, cell division does not preceed initial growth of lateral buds stimulated by decapitation or cytokinin application, but may accompany growth or occur afterwards (NAGAO and RUBINSTEIN, 1976). In Phaseolus vulgaris, initial growth is due to internodal cell enlargement, with cell division occurring 24 hours later (HILLMAN, 1984).

Biochemical studies relating to events occurring on release from apical dominance are few but indicate that the correlative signal is perceived rapidly after removal of dominance. An increase in potassium ions at the nodes of released Bidens pilosus buds was noted five minutes after decapitation (KRAMER et al., 1980). One of the earliest events occurring after the decapitation of Pisum sativum is the increase of Mg^{2+} - ATPase activity within 30 minutes, indicating an intensification of cellular exchanges (NOUGAREDE, LANDRE and REMBUR, 1983). Increased synthesis and methylation of fatty acids have also been noted shortly after cytokinin application to Nicotiana tabacum (SCHAEFFER and SHARPE, 1971, 1974) and Cicer arietinum (USCIATI, CODACCIONI, RAUTUREAU, MAZLIAK and GUERN, 1974) lateral buds.

The correlative signal thus appears to arrive at the site of primary events very soon after apical dominance is removed. These sites have yet to be located, but could involve specific target cells within the bud (TAMAS, 1987). Differential responses of plants to factors which remove apical dominance may be a result of differences in the structure or location of these putative target cells.

1.3 Early research: A historical perspective

Early research on apical dominance gave rise to two diverse views on the subject: first, that a competition for nutrients by the most actively growing meristems deprived the inhibited buds of factors necessary for growth (Figure 1). This proposal gave way to the second theory that translocatable substances produced in the growing regions of the plant were responsible for the inhibition of lateral buds (Figure 1). These translocatable substances were later regarded as growth regulators and apical dominance control was described as being hormonal. These theories will be reviewed chronologically as they emerged in the literature.

The view that competiton of nutrients was the main factor in apical dominance arose towards the end of the nineteenth century (SACHS, 1874, 1880, 1882; DARWIN, 1881) but was first developed by GOEBEL (1900). He postulated that the apical bud is a sink for nutrients transported along the stem and that lateral buds are inhibited by nutrient deprivation. This view suggests that a given module on the plant enjoys its status at the expense of another module. This theory was investigated in a systematic way by LOEB (1918, 1924). His classic work on Bryophyllum calycinum Salisb. was interpreted as support for the nutrient control theory expressed by Goebel. Loeb established that the inhibitory effect of a piece of stem attached to an isolated leaf on the growth of buds on that leaf was due to a drain of nutrients to the stem tissue. In the intact plant, this drain would be in the direction of the shoot apex. Supporting evidence for this theory was supplied by DORSTAL (1926). Using Scrophularia nodosa L. explants comprising a stem segment, attached pair of leaves and a lateral bud in the leaf axil, he showed that removal of a leaf led to bud growth in its axil. Dorstal claimed that lateral bud growth was repressed due to the leaf acting as a sink for nutrients and water from the bud. His earlier speculations that this influence by the leaf could be due to an inhibitory substance (DORSTAL, 1909) was yet to be reinvestigated.
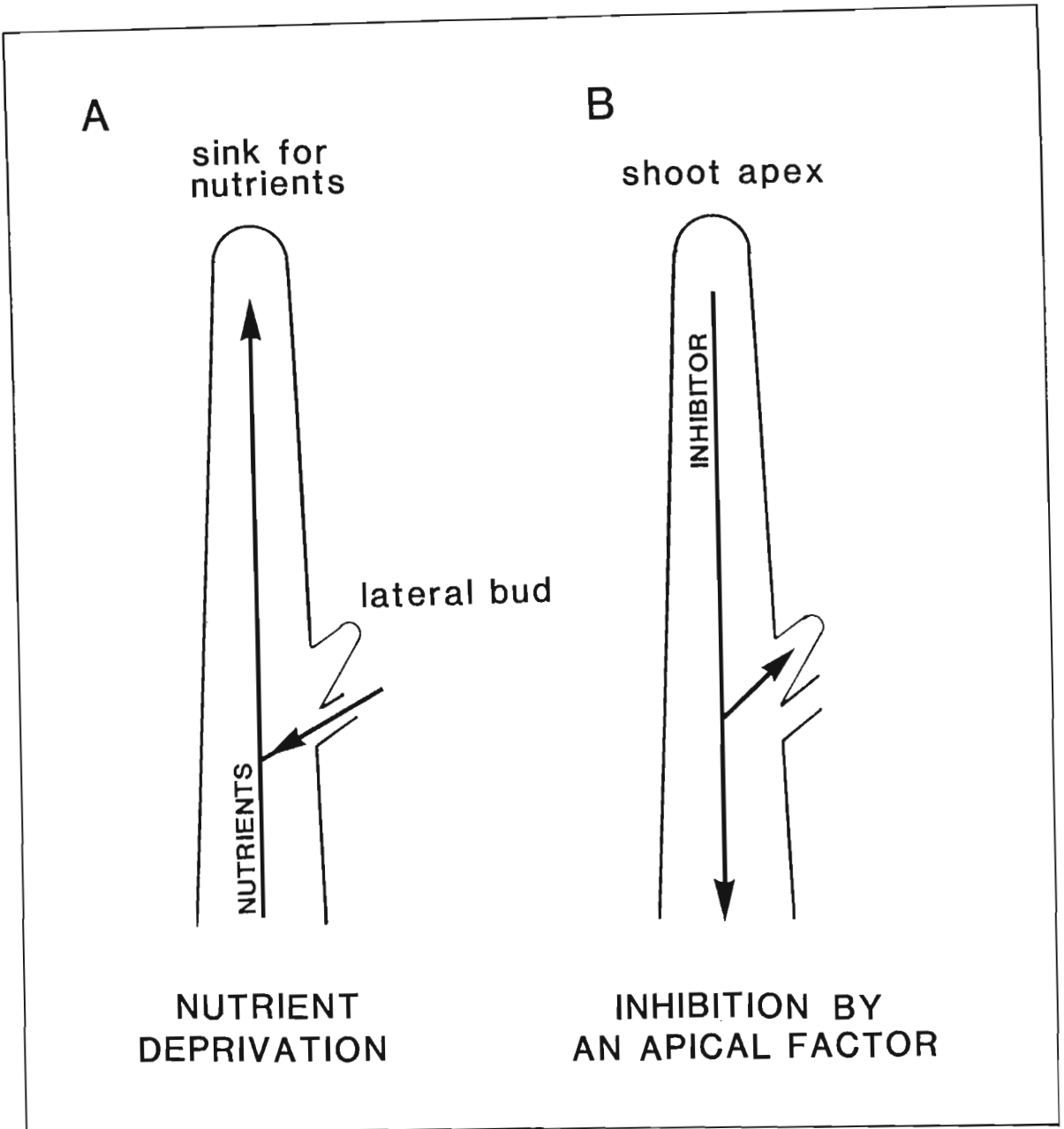
Figure 1 Diagrammatical representation of two early and diverse views on apical dominance. **A,** Competition for nutrients by the most actively growing meristems deprives the inhibited lateral buds of factors necessary for growth and development. **B,** Translocatable substances produced in the growing regions of the plant are responsible for the inhibition of the lateral buds.

During the same era investigators began finding evidence for the hypothesis of a translocatable inhibitor. Earlier, ERRERA (1904) suggested that correlative inhibition might be controlled through 'internal secretions'. Work by LOEB (1917) on segmented leaves did support the idea of a translocatable inhibitor, but did not receive much attention. Two years later, REED and HALMA (1919) published evidence against the nutrient theory of Loeb. They found that a horizontal cut made just above the lateral bud on a Citrus L. sp. shoot was sufficient to allow the bud to grow out. Buds above the cut did not grow, providing clear evidence that the flow of a translocatable inhibitor from above was being disrupted. Further evidence for a translocatable inhibitor was provided by SNOW (1925, 1929) using Phaseolus vulgaris and Vicia faba L. Following the performance of a series of grafting unions between decapitated and intact plants, SNOW (1925) concluded that the inhibitory influence could pass across a protoplasmic discontinuity between two cut surfaces of tissue pressed together. Later, this inhibitory influence was demonstrated to pass across a ringed zone of the stem (SNOW, 1929). While the work of Snow undoubtedly laid the foundations for the acceptance of the growth substance or hormone theory, it was not until the advent of auxin research in the 1930s that a specific substance was related to the apical dominance phenomenon. Significant progress was made by THIMANN and SKOOG (1933, 1934) who demonstrated that auxin was able to replace the apical bud with respect to the inhibition of lateral buds. Agar blocks containing a crude auxin extract from the fungus Rhizopus sinensis were applied to the cut surface of decapitated Vicia faba shoots, preventing lateral bud outgrowth. THIMANN and SKOOG (1934) provided further evidence for the role of auxin in apical dominance by demonstrating the presence of auxin in the apical bud and showing that lateral buds produced auxin only after release from dominance. Most, if not all investigations published since this period have shown that auxin plays a central role in the control of apical dominance. It was not until the 1950s

that a second class of growth regulator, the cytokinins, was implicated in this phenomenon. WICKSON and THIMANN (1958), investigating the antagonism between auxin and cytokinin in the growth of lateral buds, pioneered the concept of an integrated hormonal control of apical dominance.

The early theories of nutrient and hormonal control of apical dominance have since become less exclusive, and control of lateral bud growth may now be considered to be dependent on both the nutritional and hormonal (growth regulator) status of the plant. These two major influences on apical dominance will be critically reviewed.

1.4 Plant nutrition and apical dominance

Early researchers of the apical dominance phenomenon favoured the view that nutrient relations in the plant played an important role in the control of lateral bud growth. Since, ontogenetically, the stem apical meristem is initiated before the lateral meristems, they presumed that the shoot apex would achieve an initial advantage in nutrient competition over the inhibited lateral buds (Figure 2). The shoot apex on the intact plant is an important site of auxin synthesis and a sink for nutrients and metabolites. This status is only reversed by decapitation of the shoot apex or treatment of the lateral buds with a promotive growth regulator. This theory of direct competition for nutrients began to lose favour following the discovery that auxin, and later that cytokinins were implicated in apical dominance control. However, the importance of plant nutrition has again become a focus of consideration, and it is pertinent that this aspect be fully reviewed.
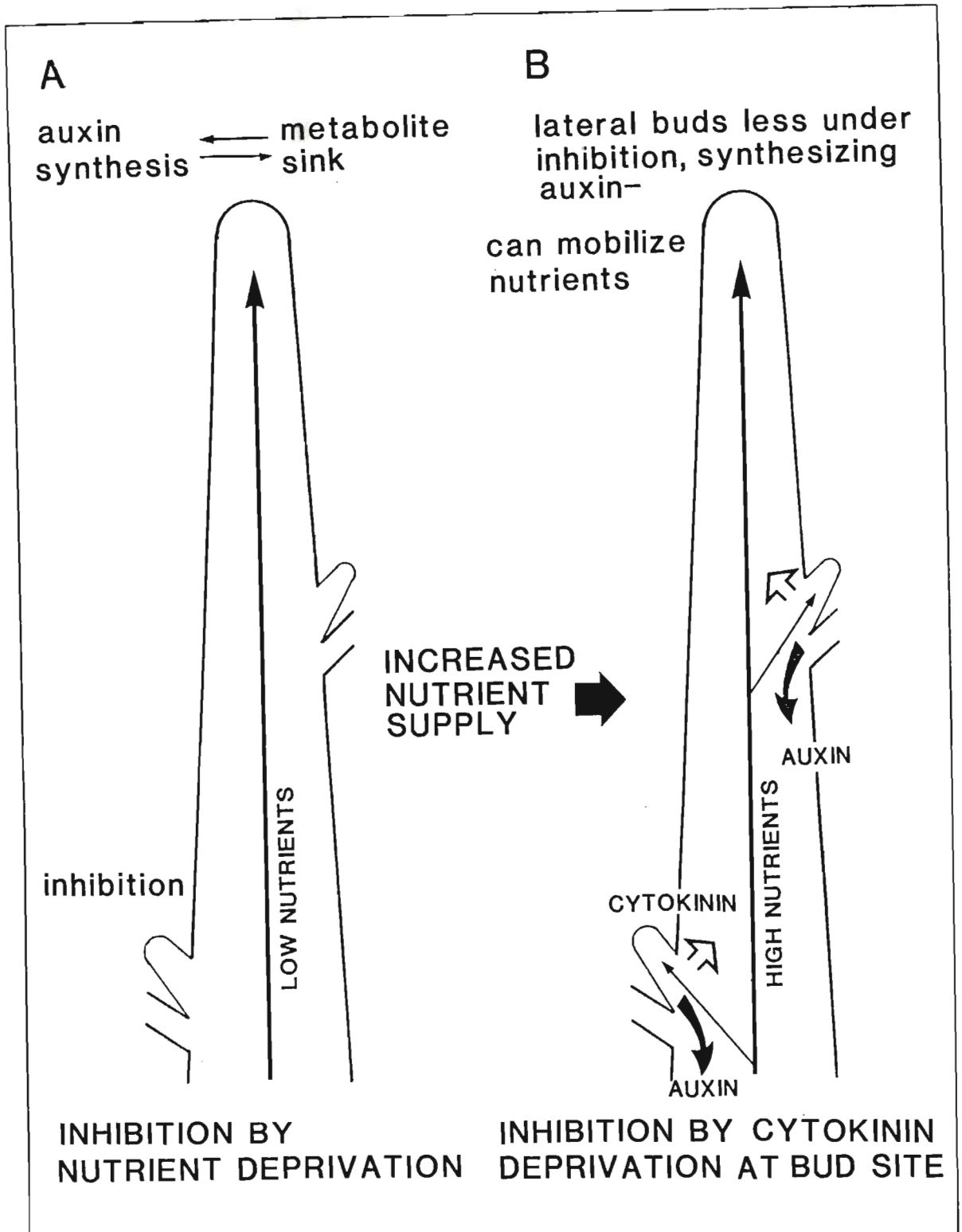
Figure 2 Diagrammatical representation of nutrient relations in the plant considered to influence the expression of apical dominance. A, The inhibitory effect of auxin at the shoot apex is higher on plants under low than high nitrogen nutrition (GREGORY AND VEALE, 1957). B, A high-nitrogen status renders apically supplied auxin ineffective in suppressing lateral bud growth (PALMER AND PHILLIPS, 1963; PHILLIPS, 1968). Cytokinin application to inhibited lateral buds on nutrient-deprived plants can release these buds, but partially inhibited buds on high-nutrient status plants are unaffected by exogenous cytokinin (WAKHLOO, 1970).

The most significant evidence to support the concept of nutritional control have been reports of inhibited lateral bud release following increases in organic and inorganic nutrient availability to the plant. Conversely, suboptimal nutrient availability has been shown to increase the level of lateral bud inhibition. This phenomenon was first noted by MORELAND (1934), who found that decapitated Phaseolus vulgaris plants were very sensitive to experimentally induced differences in nitrogen and carbohydrate supply. Significant evidence was provided by GREGORY and VEALE (1957) who found that under conditions of nitrogen starvation, apical dominance in intact Linum usitatissimum plants was complete, but weakened on increase of the nitrogen levels. Similarly, McINTYRE (1964, 1971a, 1987) found that the growth of lateral buds on the intact rhizomes of Agropyron repens Beauv. were inhibited at low nitrogen levels, but could be induced to grow by increasing the nitrogen availability. These same nutrient effects have been observed with Hordeum distichum (ASPINALL, 1961; FLETCHER and DALE, 1974), Solanum sisymbrifolium Lam. (WAKHLOO, 1970), Coleus blumei Benth. (THIMANN, SACHS and MATHUR, 1971), Phaseolus vulgaris (McINTYRE, 1973) and Triplochiton schleroxylon K. Schum. (LEAKEY and LONGMAN, 1986). Evidence from the above examples suggests that while the nutrient requirement appears to be unspecific, nitrogen appears to be the major nutrient affecting apical dominance. Potassium and phosphorus may have a secondary role. Assimilable carbohydrates have also been implicated in apical dominance. However, failure of direct applications of nutrients to buds to remove or reduce bud inhibition (GOODWIN and CANSFIELD, 1967; CUTTER, 1972a; CUTTER and CHIU, 1972) has been regarded as evidence against a purely nutritional basis of apical dominance control.

Events occurring in the lateral buds following bud release by decapitation suggest a role for nutrient availability. BALLARD and WILDMAN (1964) found that

the mitotic activity of lateral buds in Helianthus annuus L. seedlings was limited by the carbohydrate supply to these buds. Decapitation of the stem produced a rise in bud mitotic activity, which was strongly stimulated by the addition of sucrose to isolated buds and shoots. Sucrose and thiamine have been used to replace the promotive effective of the cotyledons on cotyledonary bud growth of Cicer arietinum (CHAMPAGNAT and DALZON, 1958; HUGON, 1958). Increased inhibition of these buds due to removal of the cotyledons suggested that inhibition was due to nutrient deprivation. However, WARDLAW and MORTIMER (1970) found that inhibited buds of Pisum sativum contained storage starch and concluded that carbohydrate availability did not limit their growth.

The sensitivity of lateral buds to applied growth regulators has also been correlated to nutrient availability in the plant. In Linum usitatissimum, inhibition of the lateral buds by applied auxin to the decapitated stem was higher under low than high nitrogen nutrition (GREGORY and VEALE, 1957). Removal of the cotyledons rendered the cotyledonary buds much more sensitive to the inhibition by auxin (GREGORY and VEALE, 1957). Similarly, in Helianthus annuus (PALMER and PHILLIPS, 1963) and Phaseolus vulgaris (PHILLIPS, 1968) high doses of nitrogen were reported to render the apically supplied auxin ineffective in suppressing lateral bud growth. Complete inhibition of Nicotiana tabacum L. lateral buds, resulting from a low-potassium nutrient status, were released by cytokinin application to the buds (WAKHLOO, 1970), while the partially inhibited buds on 'high-potassium' plants were not affected by exogenous cytokinin. It is possible that higher levels of potassium may have caused an increase in the endogenous cytokinin levels in the plant. The auxin levels of the plant have been shown to rise upon increasing soil-potassium (WAKHLOO, 1965) and nitrate availability (WITT, 1964). It is possible that at low nutrient levels, the meristematic regions of the shoot tip can satisfy their nutrient needs at the expense of the lateral buds, but at

higher nutrient levels, the lateral buds attain the limiting element in quantities sufficient to promote growth regulator synthesis (Figure 2). In any event, endogenous growth regulator levels appear to be affected by nutrient availability to the plant (Figure 2).

Further evidence for the regulatory role of nutrition in apical dominance has been provided by nutrient distribution studies. Decapitation of Pisum sativum plants led to the accumulation of root supplied radiolabelled phosphorus at the base of the plant (NAKAMURA, 1964). In the intact plant and in decapitated plants where the apex had been substituted with auxin, accumulation of phosphorus was anterior. Likewise, radiolabelled phosphorus supplied to a single leaflet on Pisum sativum plants moved to the apex in the intact plant, but to the lateral buds in the decapitated plant (HUSAIN and LINCK, 1966). PHILLIPS (1968), providing auxin to the decapitated epicotyl of Phaseolus vulgaris inhibited the growth of lateral buds and observed the accumulation of nitrogen in the decapitated internode.

These studies are in accordance with the early theory of WENT (1936, 1939), who suggested that under the influence of auxin produced in the shoot apex, nutrients (and probably growth regulators) are preferentially transported to these regions (Figure 3). An alternative theory that auxin may mediate the availability of nutrients by affecting the transport processes in the plant is also plausible (Figure 3). This concept of hormone directed transport will be dealt with fully in section 1.5.2.1 of this review.

Despite all indications to the contrary, PHILLIPS (1968) disregarded this aspect as evidence in favour of the nutrient theory as he found that the total nitrogen, potassium and phosphorus content of the decapitated epicotyl and the lateral buds was less for his auxin treated plants than for the decapitated controls. In
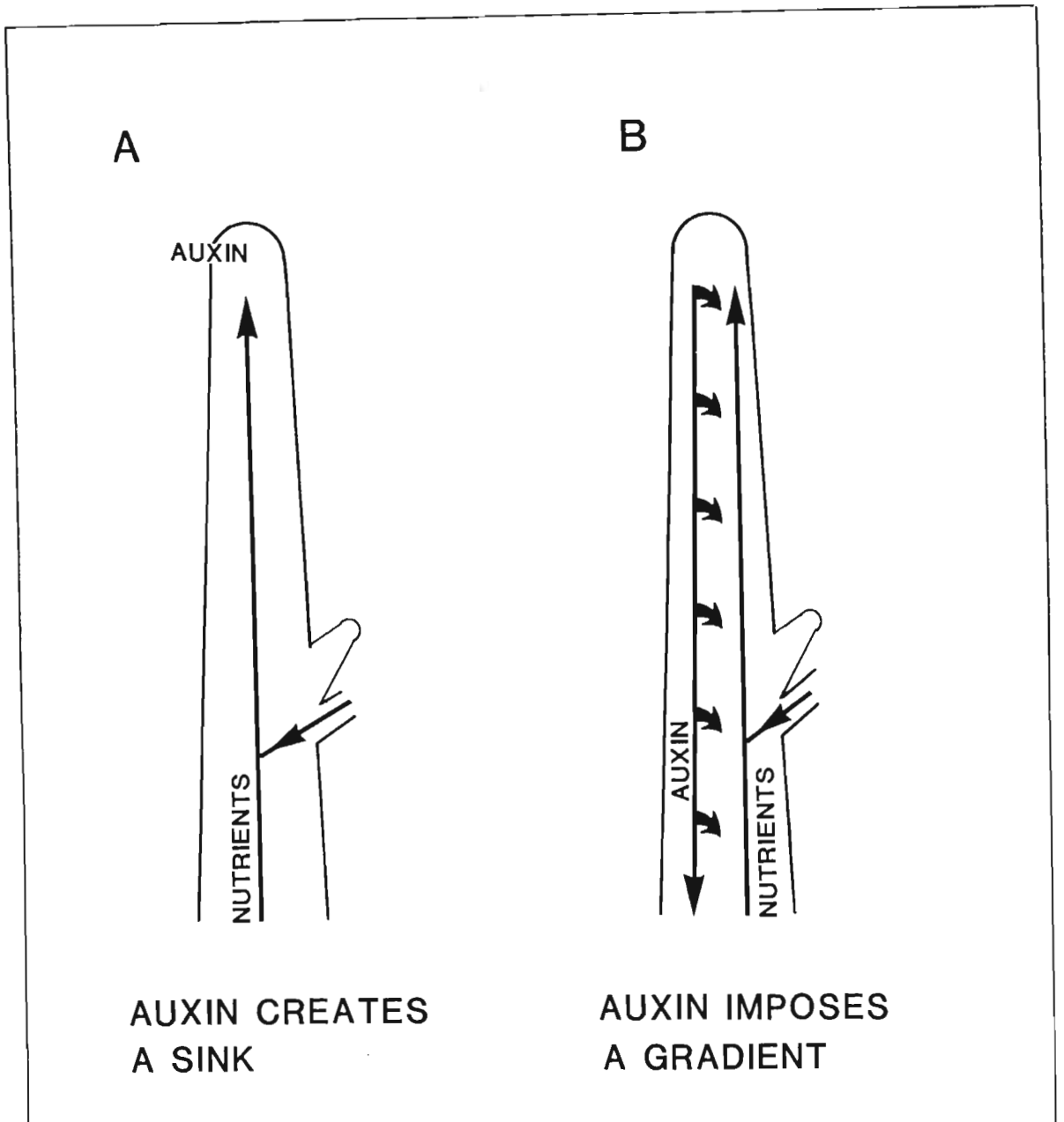
Figure 3 Diagrammatical representation of two hypothetical mechanisms by which apically synthesized auxin might regulate apical dominance via control of nutrient distribution in the plant. **A,** Under the influence of auxin produced in the shoot apex, nutrients (and probably growth regulators) are preferentially transported to this region. **B,** Auxin may mediate the availability of nutrients by affecting the transport processes in the plant.

addition, he observed that the nitrogen, potassium and phosphorus levels in inhibited lateral buds were as high as in the equivalent growing region of lateral buds released by decapitation.

The pattern of lateral bud outgrowth can be correlated to the nutrient distribution in the plant. A definite gradient of protein nitrogen was noted in the stems of Pisum sativum (GALSTON and DALBERG, 1954), being higher at the shoot apex and lower at the base. Similarly, THIMANN and LALORAYA (1960) observed a decline in cytokinin promoted protein synthesis in older (basal) isolated Pisum sativum stem sections. Bud growth on isolated rhizomes of Agropyron repens showed a basipetal gradient of decreasing activity, with more basal buds being strongly inhibited (McINTYRE, 1972). This gradient of bud growth was correlated to a basally decreasing gradient of nitrogen in the stem. Isolating the buds from each other reduced the effect of this apparent gradient. Differential responses of lateral buds to growth regulators and nutrients have been reported by HUSAIN and LINCK (1966), CUTTER (1972b), CUTTER and CHIU (1975) and GOULD, CUTTER, YOUNG and CHARLTON (1987). The growth potential of an inhibited lateral bud is thus influenced by the nutrient status of the bud and its associated stem tissue.

An aspect of correlation inhibition that has received much attention from proponents of the nutrient theory is the inhibition of one shoot by another shoot on the same plant. The shoots may arise at separate nodes or from the same axil. Intershoot competition is regarded to be controlled by an unequal partitioning of nutrients between the vigorously growing shoot and its more inhibited counterpart. Using unequal cotyledonary shoots on decapitated Pisum sativum plants, SNOW (1931, 1932, 1937) observed that defoliation of the one shoot led to its inhibition by the leafy shoot. Similar experiments by CHAMPAGNAT and DALZON (1958) and HUGON (1958) showed that removal of the cotyledons of a plant

increased the inhibition of unequally growing cotyledonary buds. Successful substitution of the cotyledons with sucrose and thiamine (HUGON, 1958) led to the conclusion that inhibition was due to nutrient deprivation. However, SACHS (1966) found that application of nutrients to the senescing shoot on two-shooted Pisum sativum plants could not restore shoot vigour. McINTYRE (1968) suggested that the levels of applied nitrogen used by SACHS (1966) were still too low. SACHS (1966) was able to restore growth to the inhibited senescing shoot with an application of cytokinin, but this was regarded as a mobilization effect (MOTHES, ENGELBRECHT and KULAJEWA, 1959; THIMANN and LALORAYA, 1960). McINTYRE (1968) using Linum usitatissimum provided convincing evidence for the nutritional control of correlative inhibition between lateral shoots. He found that the inhibitory influence of the dominant shoot was inversely related to the nitrogen level in which the plants were grown. Removal of the dominant shoot led to an increase in the nitrogen levels of the inhibited shoot, while the inhibited shoot could be released by increasing the nitrogen supply to the plant.

This inhibition and eventual senescence of the subordinate shoot in a two-shoot plant is likely to be regulated by auxin produced in the apex of the dominant shoot (Figure 4). MORRIS (1977) found that removal of the dominant shoot apex allowed regrowth of the subordinate shoot, while application of exogenous auxin to the decapitated dominant shoot partially restored dominance of the latter (SACHS, 1966). The effect of auxin is therefore indirect, perhaps polarizing the flow of growth regulators (especially cytokinin) and nutrients from the root system thus depriving the weaker shoot. Cytokinin polarization to regions of high auxin activity' has been observed by MORRIS and WINFIELD (1972) and WOOLLEY and WAREING (1972a, 1972b). This view of competition between shoots was not supported by McKEE (1968) who in similar studies revealed that the concentration of amino acids in the inhibited shoot were at least equal to those
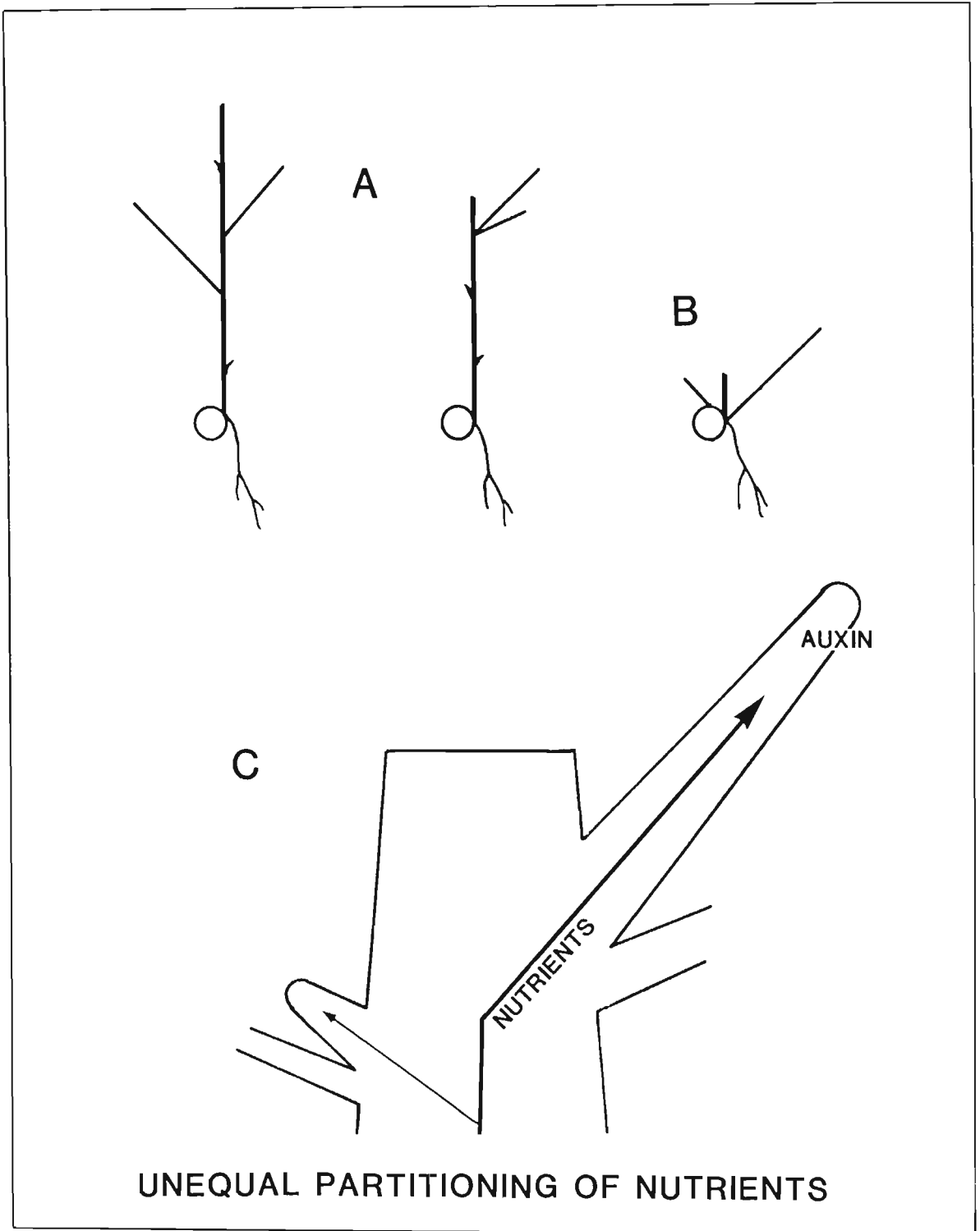
Figure 4 Diagrammatical representation of the correlative inhibition of one shoot by another shoot on the same plant. The shoots may arise at separate nodes (A) or from the same leaf axil (B). Intershoot competition is regarded as being controlled by an unequal partitioning of nutrients between the two shoots. C, Auxin from the apex of the dominant shoot probably inhibits the subordinate shoot indirectly by polarizing resources towards it. The inhibitory influence of a dominant shoot is inversely related to the nutrients available to the plant, and the inhibition of the subordinate shoot can be overcome by increasing the nutrient availability (McINTYRE, 1968).

in the dominant shoot. In addition, CUTTER and CHIU (1972) found that the transmission of the inhibitory influence from the dominant shoot to the inhibited shoot could occur through a graft union of parenchyma cells.

Considerable evidence thus argues that nutrient availability is an important aspect of the control of apical dominance. In particular, the distribution of nutrients in the shoot can be related to gradients of growth potential exhibited by lateral buds. However, factors influencing the availability of nutrients in the plant are probably also responsible for the distribution of certain growth regulators. With this in mind, control of apical dominance must at present be viewed as an interaction between environmental influences governing the extent of apical dominance expressed, and the growth regulator levels in the plant mediating the control mechanism/s.

1.5 Growth regulators and apical dominance

The role of the five classes of growth regulators - auxins, cytokinins, gibberellins, ethylene and abscisic acid in apical dominance control will be reviewed separately. This does not imply that each regulator acts independently. On the contrary, much evidence exists to suggest that these regulators interact with each other in the control of lateral bud growth. Where possible, this will be indicated. Special attention will be paid to the role of auxins and cytokinins. While cytokinins are the main growth regulator researched in this study, a review of the auxins will provide much of the theoretical knowledge on which to base an interpretation of cytokinin action. Until relevant, the growth regulators will be referred to by their classes and not specifically.

1.5.1 Auxin and cytokinin transport in relation to apical dominance

Endogenous hormone patterns, resulting from the biosynthesis, translocation and metabolism of the various growth regulators (BRUINSMA, 1980) coordinate development in plants. This development is the result of a controlled distribution of growth substances between the root and the shoot. In apical dominance, a polarity of the distribution of the controlling factor is recognisable externally from the patterns of bud growth following decapitation. Apical dominance appears to be governed by a complex set of reactions based on the balance of growth regulators transported from the shoot apex and the roots (WICKSON and THIMANN, 1960). HARRISON and KAUFMAN (1984) suggested that the relative amounts of auxin and cytokinin transported to and metabolised at the lateral bud site provide an explanation of how the cytokinin : auxin ratio regulates lateral bud release. A review of both auxin and cytokinin transport and distribution in the whole plant will thus be undertaken in an attempt to relate these growth regulators to the mechanism of apical dominance control.

1.5.1.1 Auxin transport

Auxin transport and auxin action in relation to apical dominance are likely to be intimately related. The transport properties of auxin have been shown to alter following decapitation of the shoot apex (VARDAR and KALDEWAY, 1972). Thus, the transport capacity for auxin may be an important component in determining the level of apical dominance in the plant (BRENNER, WOLLEY, SJUT and SALERNO, 1987).

The transport of auxin has received considerable attention and has been reviewed by McCREADY (1966) and GOLDSMITH (1968, 1977). Auxins synthesized in the meristematic tissues of the shoot apex and young developing leaves are transported in a predominantly basipetal direction towards the roots of the plant.

The polar transport of auxins (a manifestation of the existence of polarity in each individual cell) occurs in tissues of the vascular strands. The exact mechanism is not yet understood. Properties of auxin transport most likely to affect apical dominance are listed below.

1) Auxin transport in aerial organs is predominantly, but not exclusively basipetal although it is largely so (JACOBS, 1950; WICKSON and THIMANN, 1960; MORRIS, 1977; HILLMAN, YEANG and FAIRHURST, 1985). Acropetal transport has been determined for Coleus blumei (LEOPOLD and GUERNSEY, 1953).

2) The physiological age of tissue influences auxin transport and metabolism (JACOBS, 1950; GALSTON and DALBERG, 1954; KALDEWAY, 1965; PILET, 1967; VEEN, 1969). In general, the young and actively growing anterior stem tissue transports auxin more effectively than older basal stem tissue (LEOPOLD and GUERNSEY, 1953; LEOPOLD and LAM, 1962; JACOBS, 1970; ZAJACZKOWSKI and WODZICKI, 1978). The decline in transport intensity down the stem will result in a differential auxin gradient in the shoot.

3) The proximity of the stem tissue to the shoot apex and the condition of this apex (vegetative or flowering) play an important role in determining the polarity of auxin transport (LEOPOLD and GUERNSEY, 1953). In Coleus blumei, a decline in the auxin polarity gradient caused by flower initiation may be involved in apical dominance control (LEOPOLD and GUERNSEY, 1953).

4) The transport properties for auxin in the stem are altered following decapitation of the shoot apex - a treatment which removes apical dominance (LEOPOLD and LAM, 1962; VARDAR and KALDEWAY, 1972). Decapitation

of the shoot apex of Pisum sativum resulted in a reduction of radiolabelled auxin transported down the stem (WICKSON and THIMANN, 1960). Decapitation of the dominant shoot in the two-shooted Pisum sativum plant led to the resumption of inhibited basipetal auxin transport in the subordinate shoot (MORRIS, 1977).

5) The transport of auxins in the shoot is affected by exogenous applications of growth regulators. Cytokinin applied to Phaseolus vulgaris plants increased the rate of basipetal auxin transport from donor blocks at the decapitated shoot stump (BLACK and OSBORNE, 1965). Gibberellins applied with auxin to the cut stump of decapitated Pisum sativum plants increased the concentration of auxin function some distance from the apical site, possibly by increasing auxin transport (JACOBS and CASE, 1965).

The differential transport of auxin in the shoot as a consequence of the age and physiological status of the tissue results in a polar gradient of auxin in the shoot system. Considering the central role of auxin in apical dominance control and the fact that treatments resulting in the removal of dominance also affect auxin transport, the redistribution of auxin in the shoot following decapitation is likely to be of major importance. The auxin relationships in Pisum sativum have been thoroughly investigated by SCOTT and BRIGGS (1960, 1962). In Pisum sativum, the apical bud appeared to be the only source of diffusable 'free' auxin. In this study, SCOTT and BRIGGS (1960, 1962) determined both the total 'free' auxin content in the tissues by extraction and the 'free' auxin content by diffusion. A detailed analysis of endogenous auxin revealed a linear basipetal decrease in diffusable auxin within the growing region, but a decrease in extractable auxin occurred only in the more mature basal tissue (Figure 5). This decline of 'free' auxin towards the base of the intact shoot may be attributed to oxidation
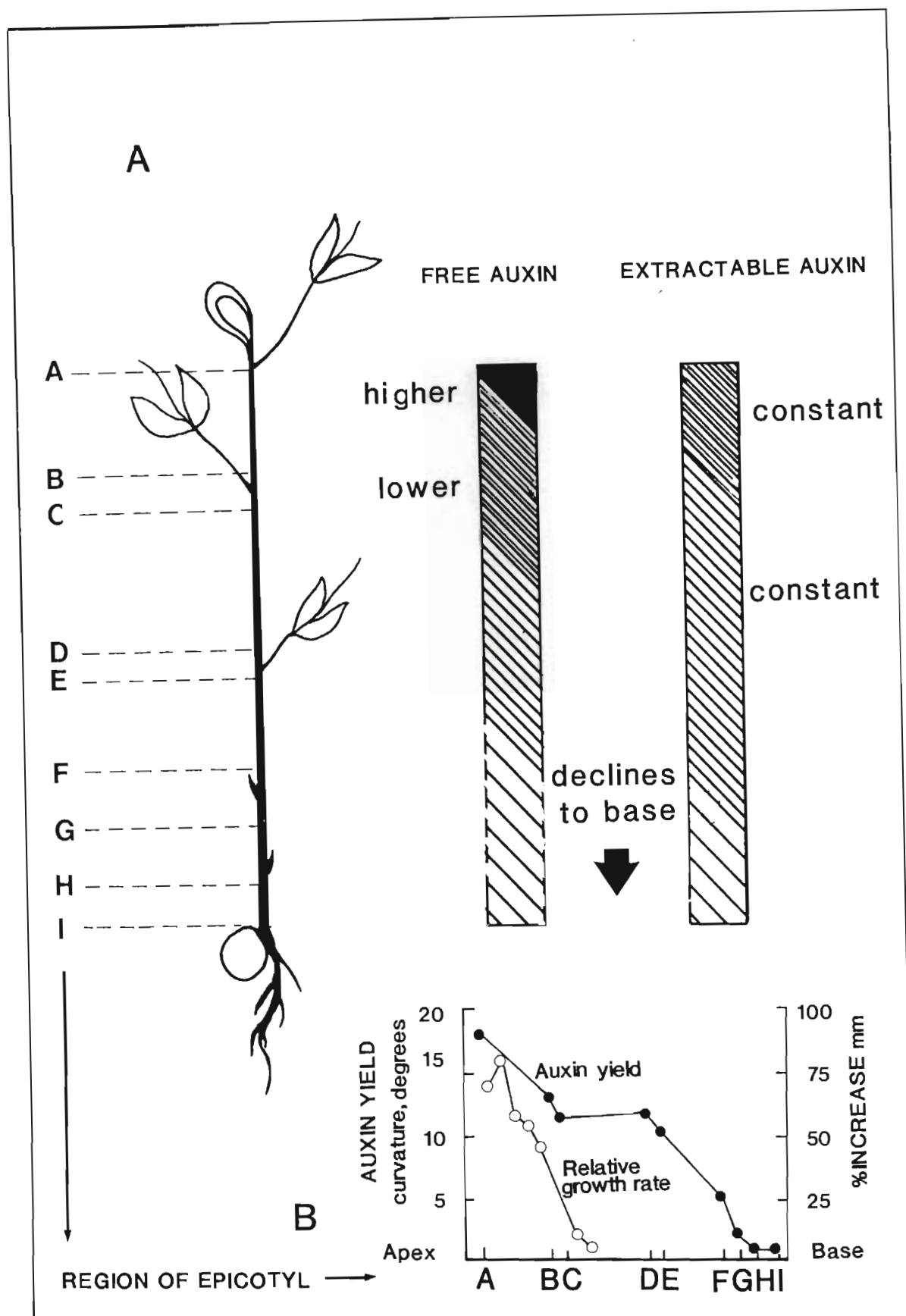
Figure 5 The auxin relationships in _Pisum sativum_ as determined by SCOTT AND BRIGGS (1960). **A,** A detailed analysis of endogenous auxin in the plant revealed a linear basipetal decrease in diffusable auxin within the growing region, but a decrease in extractable auxin only in basal tissue. **B,** A comparison of the distribution of diffusable auxin and growth in the _Pisum sativum_ shoot.

(GALSTON and DALBERG, 1954), conversion to 3-indoleacetylaspartic acid (ANDREAE and VAN YSSELSTEIN, 1956) or the formation of an auxin-protein complex (WILDMAN and GORDON, 1942; GORDON, 1946; SIEGEL and GALSTON, 1953). This would explain why the concentration of auxin applied to the decapitated stump of plants used in donor / receiver experiments is not proportional to the auxin recovered (GOLDSMITH and THIMANN, 1962). Even so, a certain amount of auxin, if applied to the intact shoot apex or decapitated stump does reach the root systems as unmetabolized auxin (MORRIS, BRIANT and THOMPSON, 1969; ROWNTREE and MORRIS, 1979; NONHEBEL, HILLMAN, et al., 1985). Of significance is that this basipetally translocated auxin does not enter the lateral buds of the intact plant (EVERAT-BOURBOULOUX and BONNEMAIN, 1980).

Following decapitation, it would be expected that the gradient of auxin would fall first at the anterior end of the shoot. However, distribution studies of auxin following decapitation of Pisum sativum revealed that extractable auxin declined first at the apical region of the shoot (SCOTT and BRIGGS, 1960) (Figure 6). This led to the proposition that a static 'free' auxin pool (GOLDSMITH and THIMANN, 1962) and diffusable 'free' auxin are interchangeable in the shoot system, with the auxin moving down the transport system being gradually replaced by the static 'free' auxin in the shoot tissues (SCOTT and BRIGGS, 1962).

The differential gradients of auxin in the shoot system could thus be envisaged as a controlling factor in apical dominance. Distinct changes in auxin distribution following decapitation may provide a correlative signal by influencing the metabolism and ditribution of growth regulators and nutrients necessary for lateral bud release. The events occurring in the stem tissue, while indirect, assume a greater importance than the direct inhibition of the lateral buds by auxin.
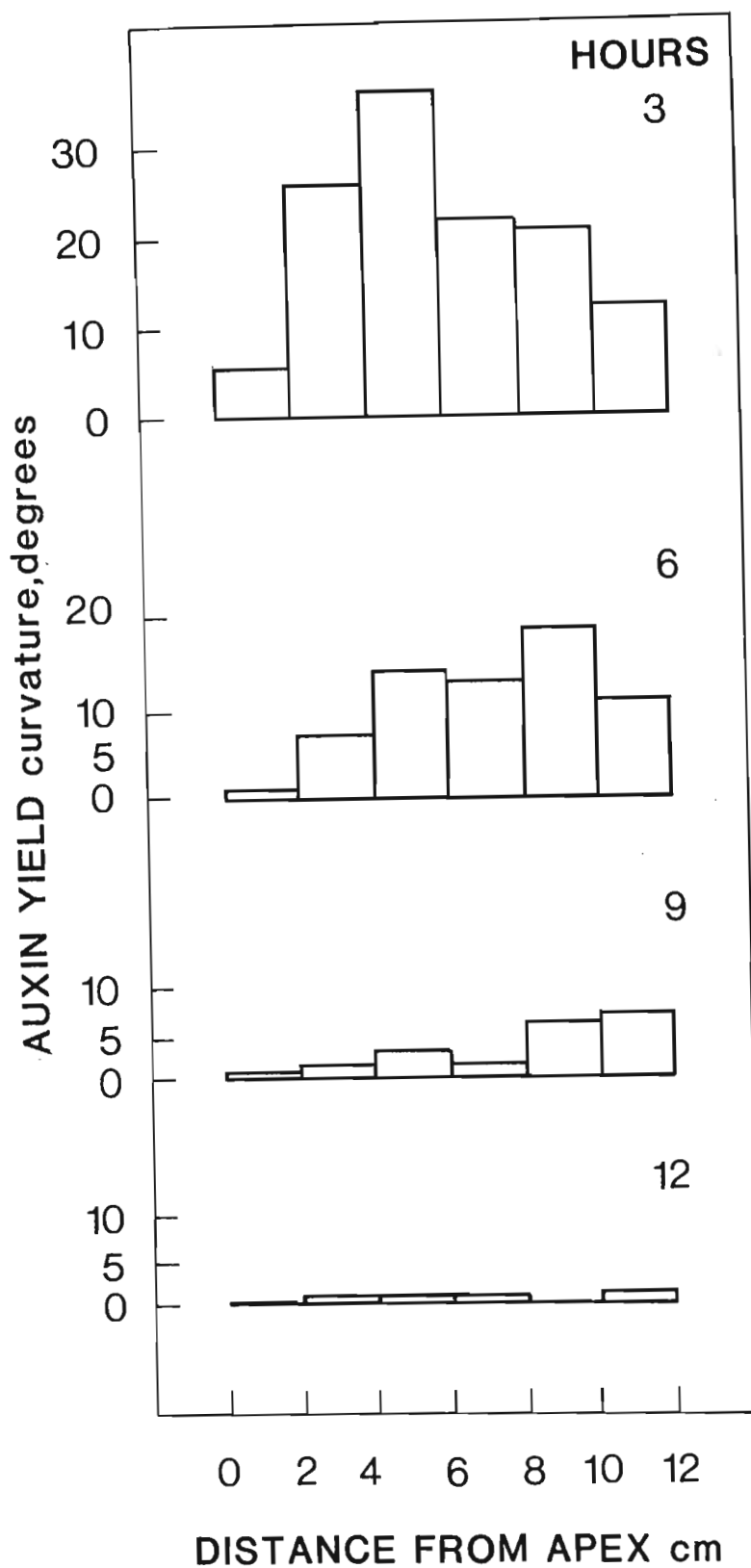
Figure 6 The distribution of extractable auxin in each of 20 millimetre <u>Pisum</u> <u>sativum</u> epicotyl sections at various times following excision of the shoot apex (SCOTT AND BRIGGS, 1960). The auxin declined first at the apical region of the shoot.

1.5.1.2 Cytokinin transport

The ability of direct cytokinin applications to release inhibited lateral buds from apical dominance (WICKSON and THIMANN, 1958), together with an observed mobilization of cytokinins by released lateral buds (MORRIS and WINFIELD, 1972) has led to the suggestion that inhibited buds are deprived of an adequate supply of cytokinins (PHILLIPS, 1975). PHILLIPS (1969a) suggested that a possible mechanism for the maintenance of apical dominance in the intact plant was via the control of cytokinin distribution. He postulated that cytokinins produced in the roots and transported to the shoot are monopolized by the apex, thus preventing the outgrowth of lateral buds. Support for this hypothesis has come from PHILLIPS (1968), MORRIS and WINFIELD (1972) and WOOLLEY and WAREING (1972a, 1972b). The patterns of cytokinin distribution and their modulation of flow through the plant could therefore be of great importance in determining the development of lateral buds.

Considerable evidence suggests that cytokinins are synthesized in the roots (SHORT and TORREY, 1972; FELDMAN, 1975, 1979; VAN STADEN and DAVEY, 1979) and are exported to the shoot system via the xylem (KENDE, 1964, 1965; NITSCH and NITSCH, 1965; CARR and BURROWS, 1966; BURROWS and CARR, 1969). However, recent evidence has indicated that cytokinin biosynthesis may not be limited to the roots of plants (CHEN and PETSCHOW, 1978a; WANG and WAREING, 1979; KODA and OKAZAWA, 1980; EINSET, 1984; CHEN, ERTL, LEISNER and CHANG, 1985). Possible alternative sites of synthesis include the cambium, lateral buds, seeds and fruits (VAN STADEN and DAVEY, 1979).

The transport of cytokinins in the whole plant is more complex than that of auxins. Cytokinins may be transported acropetally in the xylem and bidirectionally in the phloem (VAN STADEN and DAVEY, 1979) - a phenomenon resulting in

circulatory transport in the plant. In addition, cytokinins can move laterally between the xylem and phloem (VAN STADEN and DAVEY, 1981; JAMESON, ZHANG and LETHAM, 1985) and may be modified as they are transported from the root to the shoot (SCOTT and HORGAN, 1984). The cytokinin levels in the plant may fluctuate depending on the stage of plant development, the season and prevailing environmental conditions (VAN STADEN and DAVEY, 1979).

The cytokinins zeatin and zeatin riboside appear to be the major translocatable forms of cytokinin in the xylem (HEWETT and WAREING, 1973; GORDON, LETHAM and PARKER, 1974; HENSON and WAREING, 1976; SATTELMACHER and MARSCHNER, 1978; VAN STADEN and DAVEY, 1979; DAVIES, HORGAN, HEALD and McGAW, 1986). Zeatin glucoside has also been recovered in lesser amounts (DAVIES, et al., 1986). WIGHTMAN, SCHNEIDER and THIMANN (1980) cited unpublished results on the identification of zeatin, zeatin riboside, isopentenyladenine and isopentenyladenosine in Pisum sativum roots, while FORSYTH and VAN STADEN (1981) identified zeatin, zeatin riboside and possibly isopentenyladenine in Pisum sativum lateral roots. Pisum sativum root nodules have been shown to contain zeatin, zeatin riboside, zeatin riboside-5-monophosphate, isopentenyladenine and isopentenyladenosine (SYONO, NEWCOMB and TORREY, 1976; SYONO and TORREY, 1976), but it is not known if these cytokinins are transported out of the nodules.

Studies on the translocation of applied cytokinins in relation to apical dominance have indicated different cytokinin transport properties in plants. The effects of different cytokinins on lateral bud growth are thus not directly comparable. In experiments using radioactive cytokinins, zeatin (GORDON, et al., 1974) and kinetin (MORRIS, 1981) applied to the roots of plants were taken up but most of the radioactivity remained in the root system. In contrast, zeatin riboside

(JAMESON, et al., 1985), benzyladenine (SETH and WAREING, 1964; BIONI and CANCIANI, 1984; HARRISON and KAUFMAN, 1984) and zeatin (DAVEY and VAN STADEN, 1981), when applied directly into the xylem of plants are well translocated to all parts of the plant. Kinetin is not as readily transported (SETH and WAREING, 1964), but its uptake and translocation may be enhanced by simultaneous applications of gibberellins and auxin (CHANG and GOODIN, 1974). These results reflect differences in the uptake and mobility of synthetic and endogenous cytokinins, as well as differences in plant and tissue types.

The polarity of cytokinin transport may also differ depending on the cytokinin in question. Transport of exogenous cytokinins may be basipetal or acropetal from their site of application, depending on the cytokinin, plant and tissue type used. In general, endogenous cytokinins move acropetally in the xylem (LOEFFLER and VAN OVERBEEK, 1964; KENDE, 1964, 1965; CARR and BURROWS, 1966; HUTTON and VAN STADEN, 1984). The translocation of cytokinins applied to the upper part of the shoot system, or to isolated stem or petiole segments will take place in the living tissues (OSBORNE and BLACK, 1964; BLACK and OSBORNE, 1965; SETH, DAVIES and WAREING, 1966; HUTTON and VAN STADEN, 1984) and may exhibit polarity.

Evidence for a controlling role of cytokinin transport in apical dominance has come from studies on the translocation of applied radiolabelled cytokinins to intact and decapitated plants. Cytokinins applied to the roots or stem bases of intact plants were shown to move in an acropetal direction to regions of high meristematic activity such as the shoot apex or developing fruits (MORRIS and WINFIELD, 1972; CHANG and GOODIN, 1974; PROCHAZKA, SCHRAUDOLF and SONKA, 1977; PROCHAZKA, NAVRATILOVA and BLAZKOVA, 1983) (Figure 7). If applied directly to the shoot apex, the cytokinin was not transported from
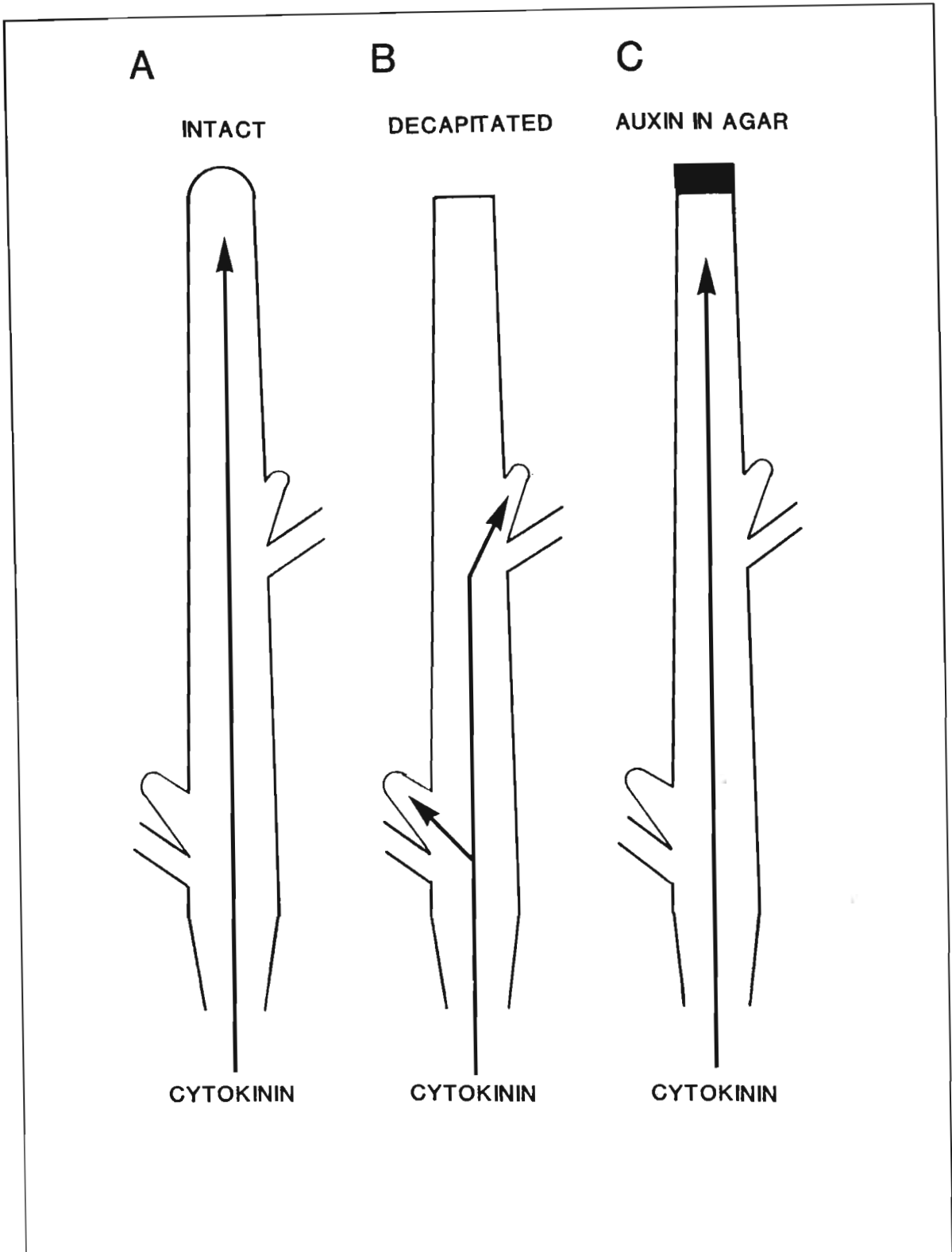
Figure 7   Diagrammatical representation of the translocation of cytokinins applied to the roots of intact or decapitated plants. **A,** In the intact plant, cyto-kinins move in an acropetal direction to the shoot apex or developing fruits. **B,** Decapitation of the shoot apex results in a change in translocation of the cytokinin towards the lateral buds released from apical dominance. **C,** Auxin applied to the cut stump will substitute for the shoot apex in polarizing the flow of applied cytokinins to this region.

the site of application (MORRIS and WINFIELD, 1972). In addition, auxin applied to the cut stump of the decapitated plant could substitute for the shoot apex in polarizing the flow of applied cytokinins to this region (MORRIS and WINFIELD, 1972; CHANG and GOODIN, 1974).

If the plant is decapitated at the time of cytokinin application, a marked change in translocation of the cytokinin occurs towards the lateral buds released from dominance (GUERN and SADORGE, 1967; PILET, 1968; MORRIS and WINFIELD, 1972; PROCHAZKA, et al., 1977; PROCHAZKA, et al., 1983; PROCHAZKA and JACOBS, 1984) (Figure 7). It appears that the translocation of applied cytokinins in decapitated plants occurs towards the more dominant lateral buds. Thus, kinetin applied to the upper internode of decapitated Pisum sativum plants moved to the lower, faster growing lateral buds (MORRIS and WINFIELD, 1972), while benzyladenine applied to decapitated Cicer arietinum seedlings was polarized towards the most actively growing lateral buds, whether these were above or below the point of cytokinin application (GUERN and SADORGE, 1967). These studies indicate that auxin polarizes the flow of cytokinin to regions of high meristematic activity and growth. Removal of the shoot apex some time (8 - 24 hours) before cytokinin application led to a significant increase in the amount of cytokinin recovered from the lateral buds (MORRIS and WINFIELD, 1972). This indicates the establishment of a sink or cytokinin polarizing effect. Since acropetal transport of cytokinin occurs predominantly in the xylem, the effect of auxin is likely to be a 'sink' or mobilizing effect and not an effect on the transport mechanism.

The above studies raise the question of whether the cytokinins diverted to the lateral buds after decapitation are themselves responsible for initiating the out-

growth of these buds, or move to these buds after growth has started. In herbaceous plants, observable growth of lateral buds begins four to six hours after decapitation of the shoot apex (WARDLAW and MORTIMER, 1970; NAGAO and RUBINSTEIN, 1975) (Figure 8). However, studies on the events induced by decapitation or cytokinin application have indicated that cytological and biochemical changes leading to bud growth begin as early as 30 minutes after treatment (USCIATI, et al., 1972; HALL and HILLMAN, 1975; NOUGAREDE, et al., 1983) (Figure 8). PROCHAZKA, et al. (1983), observing a significantly high accumulation of root applied benzyladenine in the lateral buds only after 6 - 12 hours following decapitation and cytokinin application, concluded that root derived cytokinins are probably not the primary correlative signal, but contribute to growth. Similarly, RUBINSTEIN and NAGAO (1976) observed a delayed accumulation of root applied cytokinins in the lateral buds of decapitated plants. Further experiments by PROCHAZKA and JACOBS (1984) showed that application of benzyladenine to the roots of Pisum sativum 10 hours prior to decapitation resulted in more intense growth of the lateral buds than of the lateral buds on plants treated with the cytokinin and decapitated simultaneously. Entry of the benzyladenine into the lateral buds occurred as early as four hours after decapitation of the pretreated plants, but only after 10 hours after decapitation of the untreated plants. These authors then claimed that cytokinins in the root and/or their actual metabolites may participate in the release of lateral buds from apical dominance. A composite time sequence of cytokinin redistribution and events induced by decapitation or cytokinin application to the lateral buds of a hypothetical plant summarizes the above evidence (Figure 8).

The experiments reviewed above have suggested that cytokinins from the roots may correspond with a factor limiting the sustained outgrowth of lateral buds. However, one cannot conclude that cytokinin synthesized de novo in roots represent
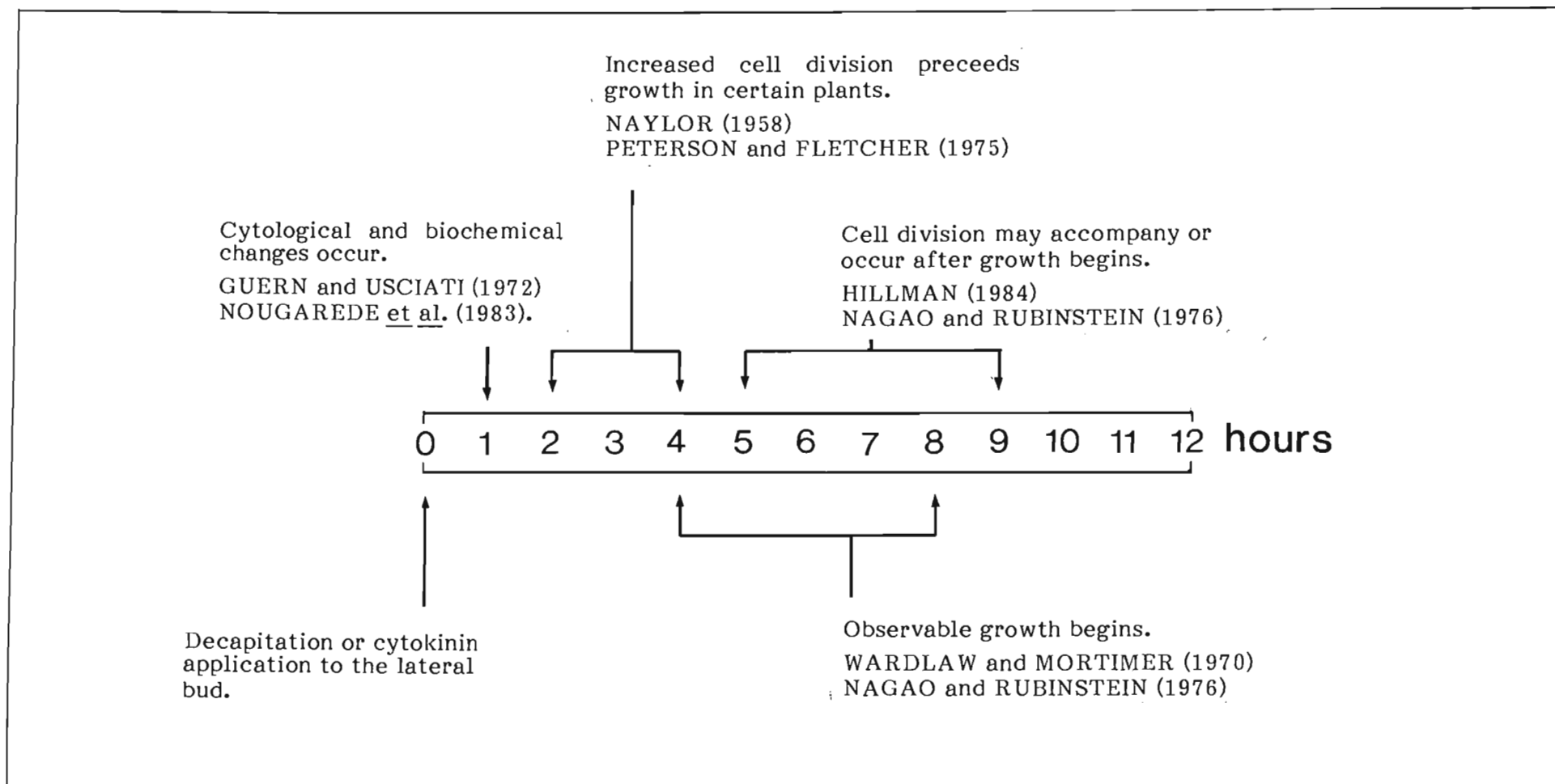
Figure 8 A hypothetical time sequence of cytokinin redistribution and events induced by decapitation or cytokinin application to the lateral buds of a plant. The results have been derived from various studies using different plants and cultivation conditions.

the only source of cytokinins for growing lateral buds. Outgrowth of lateral buds following decapitation have been observed on rootless shoots (KENDE and SITTON, 1967; WOOLLEY and WAREING, 1972a; PETERSON and FLETCHER, 1975; NAGAO and RUBINSTEIN, 1975; WANG and WAREING, 1979). Contrary to these reports, WENT and BONNER (1943) and LOEFFLER and VAN OVERBEEK (1964) found that lateral buds on decapitated plants remained inhibited in the absence of a root system.

It thus seems unlikely that the primary control of apical dominance resides in a mechanism of auxin mediated availability of root cytokinins. Such a mechanism would imply that cytokinins are no more nor less important than nutrients and water. Instead, the nature and quantity of cytokinins at the bud site and associated stem tissue are likely to prove more important regarding an immediate bud response to decapitation. In this regard, the cytokinin status of the plant as determined by the translocation and distribution of cytokinins in the whole plant assumes a new importance.

1.5.2 The role of growth regulators in apical dominance

1.5.2.1 Auxin

There is now little doubt that a primary component of apical dominance control is the synthesis of auxin by the shoot apex and young growing leaves and its transport down the stem. In the previous section, the transport properties of auxin were shown to alter following decapitation of the shoot apex, as well as being affected by factors relating to the physiological state of the plant. A polar gradient of auxin in the shoot system was noted as a result of this modulation of transport. Thus, since auxin is probably responsible for the direct or indirect inhibition of lateral bud growth, redistribution of auxin in the shoot following decapitation is likely to be an important controlling event.

Further support for a controlling role of endogenous auxin transport has come from studies using auxin transport inhibitors. The application of 2, 3, 5-triiodobenzoic acid (TIBA) (PANIGRAHI and AUDUS, 1966), morphactins (KRELLE and LIBBERT, 1968) and 1-(2-carboxyphenyl)-3-phenylpropane-1,3-dione (CPD) PATRICK and STEAINS, 1987) effectively released lateral buds on intact plants basipetal to their point of application. Similar results were observed by LITTLE (1970) and SCHNEIDER (1970). Since TIBA and morphactins are specific inhibitors of auxin transport (NIEDERGANG-KAMEIN and LEOPOLD, 1957; THOMSON and LEOPOLD, 1974), it would appear that auxin movement is necessary to inhibit lateral bud growth.

Apart from studies on auxin transport, experiments with exogenous auxin have associated the high levels of auxin at the shoot apex with the inhibitory effect. THIMANN and SKOOG (1933, 1934) found that applications of exogenous auxins or diffusates from Vicia faba shoot apices could substitute for the apex as an inhibitor of lateral bud growth. Similar results were obtained by THIMANN, et al. (1971) and WHITE, MEDLOW, HILLMAN and WILKINS (1975). However, HILLMAN (1986) recently claimed that these results are not strictly valid. There is a vast discrepancy between endogenous levels of auxin at the shoot apex and the amounts of exogenous auxin required to sustain bud inhibition (JACOBS, DANIELSON, HURST and ADAMS, 1959; WHITE, et al., 1975). GUERN and USCIATI (1972) suggested that these discrepancies are probably due to differences in the mode of cytokinin application, and the size and state of development of the lateral buds. In such experiments, auxin degredation at the cut surface and modifications of auxin transport might influence the amount of auxin reaching the lateral bud. Thus it is not surprising that higher concentrations of auxin are needed to sustain bud inhibition.

The mechanism by which apical auxin or exogenous auxin applied to a cut stump inhibits the growth of lateral buds remains unclear. The theory that inhibited

lateral buds are deficient in nutrients, water and/or essential growth regulators would suggest that auxin stimulates assimilate transport indirectly through increasing sink activity (HALL and HILLMAN, 1975; MORRIS, 1982) and/or by direct action on some phloem transport processes (PATRICK, 1982) (Figure 3). The former possibility was discussed previously in the section on nutrient relations - proposing that by activation of a local sink activity at the growing shoot apex, the lateral buds are deprived of resources. The latter theory proposes that a direct effect of auxin on the transport system directs the metabolites towards regions of high auxin concentration. This phenomenon of hormone directed transport has been reviewed by PHILLIPS (1975) and PATRICK (1982). Auxin applied to the stump of decapitated plants has been shown to direct the accumulation of radiolabelled sucrose (BOOTH, MOORBY, DAVIES, JONES and WAREING, 1962; BOWEN and WAREING, 1971) and radiolabelled phosphorus (SETH and WAREING, 1964; DAVIES and WAREING, 1965; HUSAIN and LINCK, 1966) to the site of auxin application. Application of the auxin transport inhibitor TIBA was also able to prevent phosphorus accumulation (DAVIES and WAREING, 1965), indicating that the ability of auxin to direct transport seems to be related to its basipetal transport. Since it is unlikely that activation of a metabolic demand could occur immediately at a site of auxin application or at a lateral bud following decapitation, the role of hormone directed transport in apical dominance is probably significant. PATRICK and STEAINS (1987) found that their results of auxin promoted transport of metabolities in the stems of Phaseolus vulgaris were more consistent with a remote action of auxin.

The role of auxin in apical dominance discussed above is conspicuous by its lack of interactions with other growth regulators. Since we know of the antagonism between auxin and cytokinin in apical dominance (WICKSON and THIMANN, 1958), it is likely that other mechanisms in addition to an auxin mediated distribution of nutrients and growth regulators are operative. The theory of

lateral bud inhibition by the presence of an inhibitory factor at the lateral bud has been considered and will be dealt with in greater detail later. If auxin were this factor, it would have to be near or in the lateral bud in order to exert its inhibitory effect. Such a direct effect was doubted by HILLMAN (1986) who cited results by HILLMAN, MATH and MEDLOW (1977) showing that the auxin content of lateral buds increased two-fold in 24 hours following decapitation of the shoot apex. Similarly, the auxin content of Avena sativa L. tiller buds released from dominance was greater than those still inhibited (HARRISON and KAUFMAN, 1984). However, this is to be expected because inhibited lateral buds contain low levels of auxin (WAREING and PHILLIPS, 1981), since their ability to synthesize auxin is inhibited. On decapitation, these rapidly growing shoots begin producing auxin, hence the observed increase. This indicates that the inhibitory effect of auxin occurs in the stem and not the lateral buds them-selves. In the previous section on auxin transport and distribution, it was noted that a decline in stem auxin occurring six hours after decapitation of the shoot apex correlated with a 6 - 10 hour lag phase before the onset of growth in Pisum sativum (WARDLAW and MORTIMER, 1970). A linear relationship between applied auxin and the degree of inhibition of bud growth in stem sections of Pisum sativum was noted by WICKSON and THIMANN (1960). In addition, LIBBERT (1964) found that the levels of diffusable auxin from the stem just above an inhibited bud were greater than those from stems of plants decapitated two days earlier, while JACOBS and CASE (1965) observed high concentrations of stem auxin in the immediate vicinity of the inhibited bud.

The above discussion strongly suggests a role of auxin in the stem, and not in the bud itself. SNOW (1937), demonstrating that auxin could not move up into the lateral buds from the downward flow, suggested that the buds were inhibited by some other mobile compound. It is thus likely that auxin does not act directly

on the lateral bud, but that the inhibition is mediated by a process initiated in the stem (Figure 9). It follows that this mediated inhibition could occur via the interaction with another growth regulator.

1.5.2.2 Cytokinins

The discovery of a second class of growth regulators, the cytokinins in the 1950s added impetus to the search for a control mechanism of apical dominance. The natural cytokinins are adenine derivatives capable of stimulating cell division in plants. Together with the synthetic cytokinins discovered, these growth regulators are capable of evoking a large spectrum of physiological responses. Interest in the role of cytokinins in lateral bud growth was generated by early in vitro studies on Nicotiana tabacum pith cultures, which demonstrated that auxin, and adenine or a cytokinin were required for bud formation (SKOOG and TSUI, 1948; SKOOG and MILLER, 1957). Following this, WICKSON and THIMANN (1958) pioneered further research with their now classic investigation into the antagonism between kinetin and auxin in apical dominance.

The purpose of this section will be to review the effects of cytokinins on apical dominance and to speculate on their role in the mechanisms of control. The role of cytokinin distribution and transport dealt with in the previous section will be recapped for the sake of clarity. The early hypothesis of PHILLIPS (1969a, 1975) suggested that inhibited lateral buds are deprived of an adequate supply of cytokinins, possibly via the auxin mediated control of cytokinin distribution. Support for this hypothesis has been based largely on the results of distribution studies of root cytokinins in intact and decapitated plants. Auxin does polarize the flow of cytokinins to regions of high meristematic activity and growth. However, the question central to the role of cytokinins in apical dominance remains - are cytokinins diverted to the lateral buds after decapitation responsible for
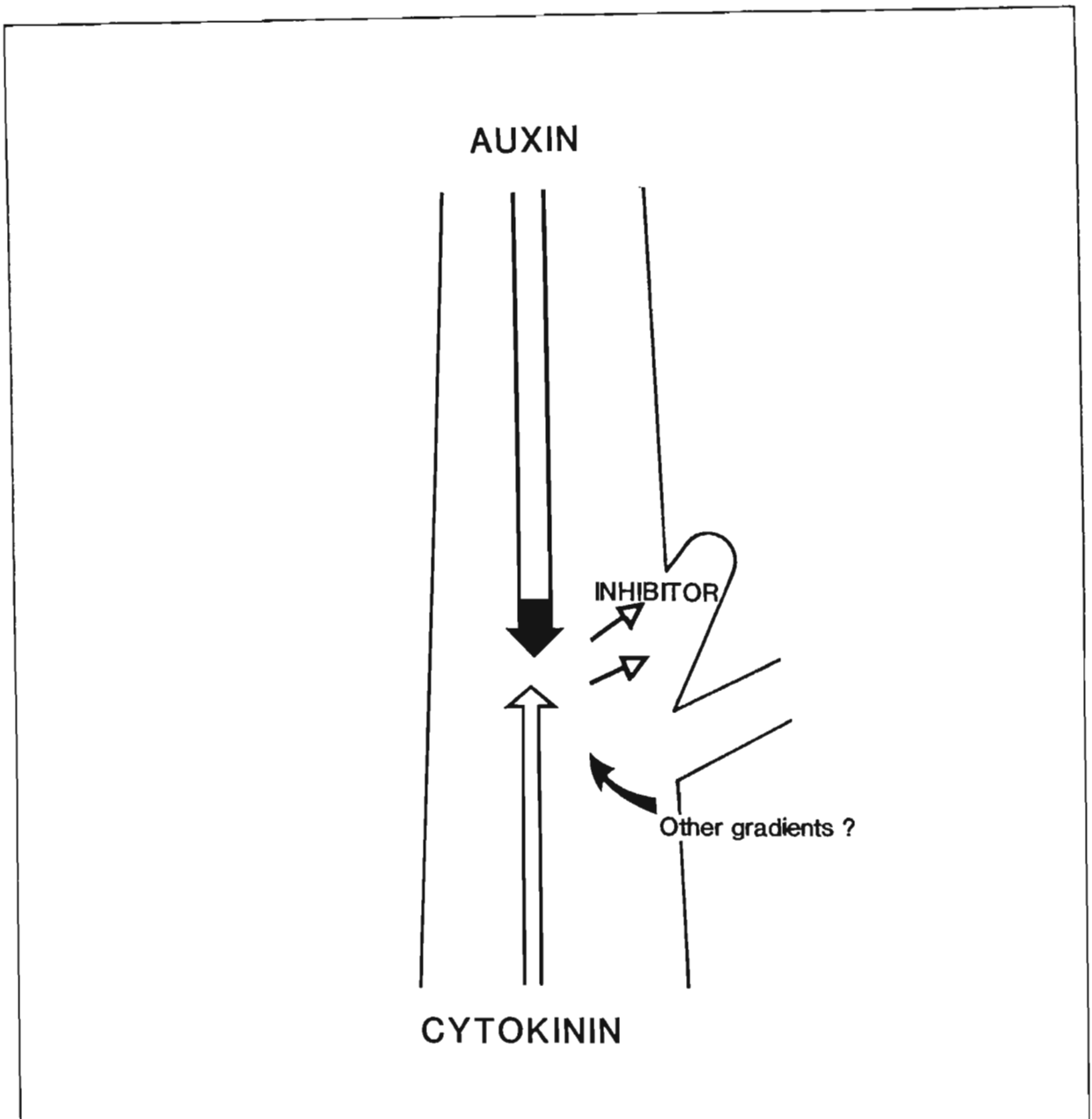
Figure 9 Diagrammatical representation of a hypothetical mechanism of lateral bud inhibition mediated by auxin. Auxin moving basipetally does not enter the inhibited lateral bud itself, but initiates a mobile inhibitory compound in the stem which acts directly on the lateral bud.

initiating the growth of these buds, or do they move to these buds after growth has started? Results of experiments on rootless plants (WANG and WAREING, 1979) and the discrepancies between the rate of cytokinin transport and lateral bud growth (HALL and HILLMAN, 1975; NAGAO and RUBINSTEIN, 1975) are not in accordance with the cytokinin deprivation theory. It thus seems unlikely that the primary control of apical dominance resides in a mechanism of auxin mediated availability of root cytokinins. Auxin may still, indirectly, control the availability of specific cytokinins at the bud site or stem node by influencing the gradient of cytokinin distribution in the plant, and/or by modulating cytokinin biosynthesis and metabolism.

The release of lateral buds from apical dominance may be achieved by the application of a wide range of cytokinin-active adenine derivatives. A range of cytokinins and their effects on lateral buds are listed in Table 1. The ability of cytokinins to promote branching, and thus the yield of such crops as soybean (ALI and FLETCHER, 1971), tomato (AUNG, 1984), apples (ELFVING, 1985), strawberries (BRAUN and KENDER, 1985) and roses (CARPENTER and RODRIGUEZ, 1971) have prompted extensive study on this phenomenon. However, the concentration, mode of application and frequency of application of cytokinins have varied in reports to the extent that an overall interpretation of this effect becomes difficult. Release of lateral buds may be achieved by a direct application of a cytokinin to the inhibited lateral bud (ALI and FLETCHER, 1971; PILLAY and RAILTON, 1983) usually in the manner described by SACHS and THIMANN (1967). Foliar spray applications of the cytokinin to the whole plant have also been effective in releasing inhibited lateral buds (CARPENTER and RODRIGUEZ, 1971; ELFVING, 1985). Cytokinin applications to the roots have resulted in the release of lateral buds on Pisum sativum (KENDE and SITTON, 1967) but not on Phaseolus vulgaris plants (SHEIN and JACKSON, 1971, 1972; FIELD and JACKSON, 1975a).

TABLE 1. A range of cytokinins capable of releasing the lateral buds of a wide range of plants from apical dominance

Plant	Cytokinin	Mode of Application	Reference
Nicotiana tabacum	Benzyladenine	Direct to the lateral buds	SCHAEFFER and SHARPE (1969)
Glycine max			ALI and FLETCHER (1971)
Cicer arietinum			USCIATI et al. (1972)
Pisum sativum			PILLAY and RAILTON (1983)
Euphorbia pulcherrima (Klotz) Wild			SEMENIUK and GRIESBACH (1985)
Hygrophila R. Br. sp.		to the base of excised shoots	CUTTER and CHIU (1975)
Pisum sativum			NAGAO and RUBINSTEIN (1975, 1976)
Rosa (L.) Tourn. sp.		as a foliar spray	CARPENTER and RODRIGUEZ (1971)
Frageria X ananassa L.			BRAUN and KENDER (1985)
Malus X domestica Borkh.			ELFVING (1985)
Lycopersicon esculentum Mill.		direct to the cotyledonary bud	AUNG and BYRNE (1978)
Pisum sativum	Kinetin	direct to the lateral bud	SACHS and THIMANN (1964)
Lycopersicon esculentum			CATALANO and HILL (1969)
Solanum sisymbrifolium			WAKHLOC (1970)
Pisum sativum		to isolated stem segments	WICKSON and THIMANN (1958)
Pisum sativum		to the base of excised shoots	WICKSON and THIMANN (1958)
Pisum sativum	Zeatin	direct to the lateral bud	PILLAY and RAILTON (1983)
Lycopersicon esculentum		direct to the cotyledonary bud	AUNG (1984)

Cytokinins may also be effective in overcoming apical dominance when applied to lateral bud-bearing stem segments in solution (WICKSON and THIMANN, 1958; PETERSON and FLETCHER, 1975). These results would suggest that endogenous cytokinins are limiting in inhibited buds.

However, such a simplistic explanation cannot account for the complex nature of control apparently under the influence of a varied and interacting set of growth regulators. Cytokinins were able to release lateral buds on isolated stem segments in solution, but when similar lateral buds were removed and cultured in vitro, cytokinin application could not effect their release (PETERSON and FLETCHER, 1975). Therefore some other factor, possibly in the stem, may be necessary for lateral bud growth. This is more likely considering the temporary nature of the lateral bud release by cytokinins. In many cases, the stimulatory effect of cytokinins on bud growth lasted for only a few days in intact plants and were not prolonged by multiple applications (ALI and FLETCHER, 1970, 1971; SACHS and THIMANN, 1964, 1967; RUBINSTEIN and NAGAO, 1976). Subsequent applications of gibberellins or auxins to the released buds have been successful in maintaining lateral bud growth (SACHS and THIMANN, 1967). SACHS and THIMANN (1964, 1967) and ALI and FLETCHER (1971) suggested a sequential relationship between cytokinins as cell division factors and auxins or gibberellins as elongation factors in the control of lateral bud growth.

The growth of lateral buds released by cytokinin application is seldom as great as that of buds released by decapitation of the shoot apex (SACHS and THIMANN, 1967) suggesting a continued effect of auxin in the presence of the shoot apex. The antagonism between auxin and cytokinin has been well documented (WICKSON and THIMANN, 1958). Cytokinins can overcome the dominance induced by auxin application to Lycopersicon esculentum plants (CATALANO and HILL, 1969).

The interaction between applied cytokinin and an endogenous auxin gradient was manifest by the patterns of bud growth following application of an artificial auxin gradient to decapitated shoots of a Hygrophila sp. supplied with cytokinin (CUTTER and CHIU, 1975). These results point to a controlling role of auxin in the ability of cytokinins to release lateral buds from apical dominance.

The specificity of the cytokinin used to release lateral buds may be important. The activity of different cytokinins in promoting growth of lateral buds has been investigated and correlated with their potency in promoting bud formation in tobacco callus cultures (SKOOG and ABDUL GHANI, 1981). They noted a wide range of activities with some cytokinins being ineffective and others effective in releasing lateral buds from apical dominance. PILLAY and RAILTON (1983) found that while the cytokinins benzyladenine and zeatin could affect a complete release when applied to the lateral buds of Pisum sativum, kinetin and isopentenyladenine were not effective. They suggested the possibility that inhibited lateral buds are unable to efficiently hydroxylate isopentenyladenine to zeatin. Isopentenyladenine is the immediate precursor of zeatin in some systems (MIURA and MILLER, 1969; MIURA and HALL, 1973). In addition, experiments with N-formyl hydroxyaminoacetate (hadacidin), an inhibitor of purine biosynthesis (SHIGEURA and GORDON, 1962) have shown that this compound can inhibit the growth of lateral buds on decapitated plants, and that this effect can be reversed by cytokinins (LEE, KESSLER and THIMANN, 1974; TUCKER, 1977a). The inhibitory effect could not be reversed by adenine, which also could not release lateral buds from dominance in the system of PILLAY and RAILTON (1983). This could indicate a control at the level of cytokinin biosynthesis after adenine is incorporated into the cytokinin biosynthetic pathway.

The above results contribute an important corollary to the hypothesis of PHILLIPS (1975), namely that apical dominance may be maintained by a regulation of the

specificity of the cytokinin arriving at the bud site (Figure 10). In line with this concept, SACHS and THIMANN (1967) declared that a decline in auxin supply from the shoot apex may enable the lateral buds to commence cytokinin synthesis. However, this would imply that once growing, a dominant shoot would need no external supply of cytokinin. Evidence reviewed in this section has indicated that this is not so. Thus a factor in stem tissue, or the stem tissue itself appears to be implicated. It is likely that the factor regulating this postulated control mechanism could be auxin moving basipetally from the shoot apex (Figure 10). Inexplicably, this concept of apically controlled specificity of cytokinin action at the site of the lateral buds has never been investigated further.

A comparison of events occurring in lateral buds released by decapitation or cytokinin application indicates that cytokinins are necessary for the initiation of some biochemical events prior to mitosis and growth in the lateral buds. Cytokinin application to inhibited buds of Cicer arietinum resulted in a stimulation of cell division after one hour which was preceded by cell elongation after another hour (GUERN and USCIATI, 1972). In addition, benzyladenine pretreatment resulted in earlier and greater incorporation of applied radiolabelled thymidine (SCHAEFFER and SHARPE, 1970) or uridine (USCIATI, et al., 1972) into the RNA of Nicotiana tabacum or Cicer arietinum lateral buds respectively. In Pisum sativum removal of the shoot apex did not lead to an increase in the sensitivity of lateral buds to applied benzyladenine, but high benzyladenine concentrations were no longer supraoptimal for growth on decapitated plants when compared with growth on intact plants (NAGAO and RUBINSTEIN, 1975). These authors argued that new events are initiated by decapitation which cannot be brought about by applications of an exogenous cytokinin, and that other factors besides cytokinins are limiting for bud growth. The significance of this has already been discussed.
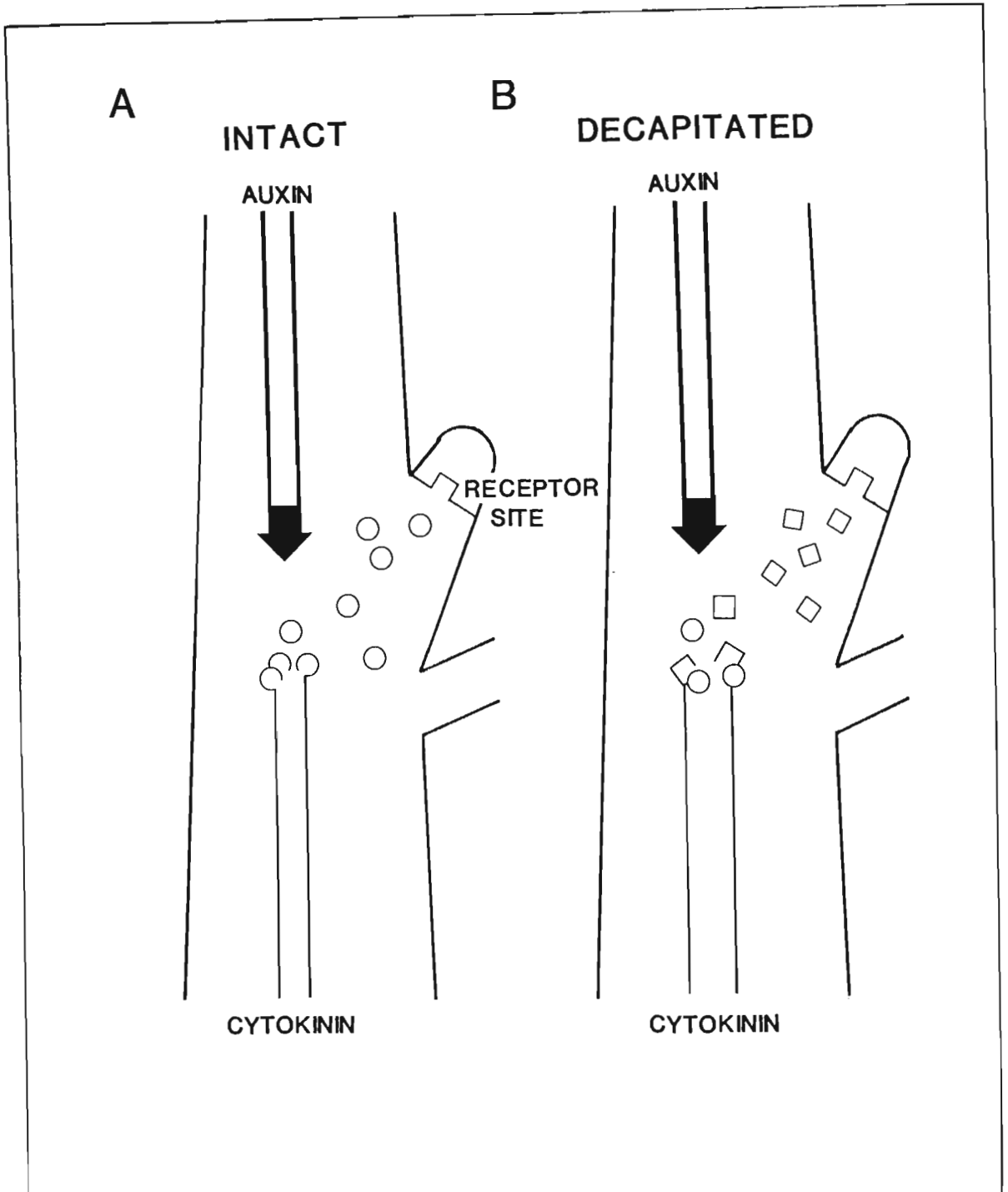
Figure 10 Diagrammatical representation of a hypothetical interaction between auxin and cytokinin controlling lateral bud inhibition. A, In the intact plant, the ratio of auxin : cytokinin is unfavourable for the promotion of events at the lateral bud site leading to bud release. Cytokinin biosynthetic potential in this region may be suppressed, or the lateral buds may lack the capacity to utilize the particular cytokinins prevalent in the shoot. B, Decapitation results in a lowering of the auxin levels, so that the auxin : cytokinin ratio is favourable for cytokinin biosynthesis at this lateral bud site. Alternatively, cytokinins are metabolised to more usable forms.

A further effect not yet discussed is the ability of applied cytokinin to increase the number of lateral buds on a shoot. Kinetin applied to the upper epicotyl of Pisum sativum resulted in significantly more buds on the plant (SCOTT and PRITCHARD, 1968), while cytokinins play a key role in the initiation of adventitious buds in Torenia fournieri Lind. stem segments (TANIMOTO and HARADA, 1984). Studies of moss development, reviewed by BOPP (1980) have also indicated that cytokinins are responsible for the induction of bud formation on the moss caulonema. Cytokinin treatment of the moss resulted in an accumulation of membrane-bound calcium in the prospective bud region (HEPLER and WAYNE, 1985), indicating that the effect of cytokinin may be mediated by calcium. TAMAS (1987) has questioned whether lateral bud growth release by cytokinins could be achieved by reorientating auxin transport and cell differentiation in the lateral bud region. The role of calcium in cell polarity (SCHNEPF, 1986) has been reviewed in relation to auxin transport by TAMAS (1987).

There thus appears to be strong evidence that cytokinins are a primary controlling factor in apical dominance. Their effect is likely to be mediated, either directly or indirectly by an interaction with apically produced auxin. In this respect, the distribution of auxin and cytokinins in the plant are of great significance. If cytokinins are the primary correlative signal at the lateral bud site, qualitative changes in the specificity of the cytokinins following decapitation are likely to be more important than a redistribution of root cytokinins, yet the latter may still be important in maintaining growth.

1.5.2.3 Gibberellins

The gibberellins are potent stimulators of growth in many plant systems (LANG, 1970). Their exact role in plant growth and development is extremely complex,

since gibberellins occur in many parts of the plant, at different levels and as different types. The great number of gibberellins do not have the same biological activity per se, and there exist qualitative and quantitative changes related to genotype, developmental stage and environmental influences (SPONSEL, 1985).

There is little evidence to suggest that direct application of a gibberellin to inhibited lateral buds can release these buds from apical dominance. Reports by MARTH, AUDIA and MITCHELL (1956), CATALANO and HILL (1969) and ALI and FLETCHER (1970) claim that gibberellins released lateral buds from apical control, but their evidence is questionable. Gibberellins do have a positive effect on the extension of buds released from apical dominance. Applications of gibberellin increased the length of lateral buds following apex removal, but not the number of lateral buds (BRIAN, HEMMING and RADLEY, 1955; KATO, 1958; WICKSON and THIMANN, 1958). Gibberellins may also stimulate bud growth in isolated stem sections (SCOTT and PRITCHARD, 1968; KATSUMI and IKEO, 1970). Lateral buds of Pisum sativum, released by cytokinin application, continued to grow only with subsequent gibberellin treatment (SACHS and THIMANN, 1964). This suggests that gibberellins may have a role in the immediate post-release phase of lateral bud growth.

The gibberellins are known to interact with other growth regulators in the control of apical dominance. A synergistic effect between gibberellins and auxins was shown to enhance the inhibitory action of auxin (JACOBS and CASE, 1965; SCOTT, CASE and JACOBS, 1967; PHILLIPS, 1969b). Applications of gibberellins to the shoot apex increased the growth rate of the apex and accentuated the degree of apical dominance (BRIAN, HEMMING and LOWE, 1959) probably by increasing the apex mobilizing effect (MARTIN, 1987). The antagonism of the inhibitory effect of auxin by gibberellins has been reported by KATO (1958) and PHILLIPS (1971a, 1971b).

Gibberellins appear to promote the effects of cytokinins on bud growth. When gibberellins were applied together with benzyladenine, growth was much enhanced (ALI and FLETCHER, 1970). If gibberellin application was delayed after benzyladenine treatment, the growth effect was greater (ALI and FLETCHER, 1971). Contrary to these and similar results by SACHS and THIMANN (1964) and CATALANO and HILL (1969), gibberellins prevented the benzyladenine-induced bud release of Cyperus alternifolius L. (FISHER, 1971).

An interaction between ethylene action and gibberellins is also indicated. Ethylene evolution resulting from shoot inversion appears to induce the release of apical dominance in Pharbitis nil L. (PRASAD and CLINE, 1987). Apical dominance release can also be prevented by promoting elongation of the inverted shoot via treatment with gibberellin (PRASAD and CLINE, 1987). A synergistic effect was observed when $Ag\ NO_3$, an inhibitor of ethylene action, was applied with the gibberellin (PRASAD and CLINE, 1987).

Correlations between gibberellin distribution, endogenous levels of gibberellins and apical dominance remain inconclusive. Roots are the probable source of gibberellins (JONES and PHILLIPS, 1966), but this class of growth regulator occurs in high levels in the apical tissues (THOMAS, 1972). In Pisum sativum, bud inhibition on rootless plants could be restored by application of gibberellin to the base of the plant (SEBANEK, 1972) indicating a possible controlling role of root gibberellins. However, in studies of the translocation of labelled and unlabelled gibberellin applied to the roots of decapitated plants, the gibberellin was noted to arrive at the lateral buds very late and in negligible amounts (THOMAS, 1972; PROCHAZKA and JACOBS, 1984).

Gibberellin action thus appears to be effected by auxins, cytokinins and ethylene, and therefore its effects in apical dominance are likely to be modulated by other

growth regulators. While it is very likely involved in shoot growth, it does not appear to play a primary role in the release of lateral buds from dominance.

1.5.2.4 Ethylene

Ethylene is a potent plant growth regulator synthesized in all plant cells as a gas (ABELES, 1985). The production of ethylene in the plant is increased by auxin - suggesting that ethylene so produced might play a role in auxin action. Thus ethylene may be an important secondary controlling factor in apical dominance.

Lateral bud growth on decapitated plants has been inhibited by applications of ethylene (BURG and BURG, 1968; YEANG and HILLMAN, 1981b; BLAKE, REID and ROOD, 1983), the ethylene precursor 1-aminocyclo-propane-1-carboxylic acid (BLAKE et al., 1983) or 2-chloroethylphosphonic acid (ethephon), a synthetic ethylene releasing agent (YEANG and HILLMAN, 1982). Conversely, treatments that remove ethylene may enhance lateral bud growth, especially in decapitated plants (BURG and BURG, 1968; BLAKE et al., 1983). However, aminoethoxyvinyl glycine treatment (YEANG and HILLMAN, 1982), CO_2 and hypobaric conditions (BURG and BURG, 1967) - all known to eliminate ethylene action, did not result in the removal of apical dominance in treated plants. It is possible that these treatments may inhibit other growth responses in addition to ethylene action.

Ethylene evolution has been shown to decrease following decapitation of the shoot apex and during initial lateral bud growth (ABELES and RUBINSTEIN, 1964; YEANG and HILLMAN, 1982). In some cases, this ethylene evolution has occurred in both nodal and internodal stem sections (YEANG and HILLMAN, 1982) while in others decapitation did not reduce ethylene production at the nodes (ABELES

and RUBINSTEIN, 1964; BURG and BURG, 1968). YEANG and HILLMAN (1981b) found that internal ethylene concentrations of Pisum sativum were increased by treatments that induced or prevented lateral bud growth.

Following decapitation, auxin treatment of the plant may lead to increased ethylene production which can be correlated to lateral bud inhibition (BLAKE et al., 1983). Again, this effect may be noted only in the internodal tissue (YEANG and HILLMAN, 1982) or the nodal tissue (ABELES, 1966). These discrepancies together with the fact that a continuous supply of auxin was necessary for ethylene evolution to prevail (BURG and BURG, 1968) led YEANG and HILLMAN (1982) to conclude that auxin does not sustain the inhibition of bud growth in decapitated plants via auxin induced ethylene. This hypothesis, first proposed by BURG and BURG (1968) is supported by BLAKE et al. (1983).

The interaction of ethylene and apically produced auxin in apical dominance does however seem likely. Ethylene can inhibit the polar basipetal transport of auxin (MORGAN and GAUSMAN, 1966; ALI and FLETCHER, 1971) as well as auxin biosynthesis (ERNEST and VALDOVINOS, 1971).

HILLMAN et al. (1985) favoured the hypothesis that ethylene may act by suppressing the growth of the shoot apical region, thus releasing lateral buds from dominance by decreasing auxin synthesis and/or lessening the apical mobilizing effect. Physical constriction of the apical bud or enclosure of the apical bud in an atmosphere of high ethylene concentration resulted in a rapid outgrowth of the lateral buds (HILLMAN and YEANG, 1979). This release of inhibited buds occurred after ethylene treatment to the whole shoot (HALL, TRUCHELUT, LEINWEBER and HERRERO, 1957; CATCHPOLE and HILLMAN, 1976).

Cytokinins, known promotors of cell division, have been shown to overcome the inhibitory effect of ethylene on lateral bud growth in decapitated plants (BURG and BURG, 1968). Ethylene is known to inhibit cell division in apical meristems (APELBAUM and BURG, 1972a, 1972b), perhaps by antagonising DNA synthesis (APELBAUM, SFAKIOTAKIS and DILLEY, 1974). It is therefore not surprising that continuous exposure of intact plants to ethylene inhibited growth of the main apex, but did not bring about an outgrowth of lateral buds (BURG, 1973).

The mechanism by which ethylene may exert an effect on apical dominance appears complex, but is likely to include an interaction with apically produced auxins. The ethylene effects confirm that any factor which affects the synthesis, transport, distribution and action of endogenous growth regulators is likely to influence the degree of apical dominance in the plant.

1.5.2.5 Abscisic acid

The theory of lateral bud inhibition by the presence of inhibiting factors in the plant achieved credibility following demonstrations by SNOW (1937) that the inhibitory influence, but not auxin, could move from the apical bud to the lateral buds. Later, DORFFLING (1964, 1965, 1966) noted a decline in the levels of several inhibitory fractions from lateral buds of Pisum sativum and Acer pseudoplatanus L. following their release from apical dominance. With the identification of abscisic acid as a natural plant growth inhibitory regulator, many investigators were eager to ascribe a controlling role to this hormone in apical dominance. However, much of the evidence related to abscisic acid and apical dominance remains inconclusive and contradictory. While abscisic acid has been implicated in a number of plant development processes, MARTIN (1987) has cautioned on the uncertainty that still exists with regard to the control of lateral bud growth by this growth regulator.

The results of exogenous abscisic acid application on lateral bud growth are mixed, possibly due to differences in the methods of application and plants used. Applications of abscisic acid to the stem, roots or buds of decapitated plants has resulted in the inhibition of lateral bud growth (ADDICOTT and LYON, 1969; ARNEY and MITCHELL, 1969; HILLMAN, 1970; SEBANEK, 1973; BELLANDI and DORFFLING, 1974; WHITE and MANSFIELD, 1977; HARTUNG and FUNGER, 1981; NOUGAREDE, RONDET, LANDRE and REMBUR, 1987). However, the wide range of concentrations necessary to impose inhibition and the differing degree of inhibition achieved illustrate the variation of the lateral bud response. Under certain conditions, abscisic acid has a promotive effect on lateral bud growth, even after apex removal (HILLMAN, 1970; BELLANDI and DORFFLING, 1974; HARTUNG and STEIGERWALD, 1977; HARTUNG and FUNGER, 1981).

The levels of abscisic acid are reported to be higher in inhibited lateral buds than in their associated stem and petiole tissue (ARNEY and MITCHELL, 1969; TUCKER and MANSFIELD, 1973; DORFFLING, 1976; EVERAT-BOURBOULOUX, 1987). On decapitation of the plant, the levels of abscisic acid have been shown to decline in the lateral buds (TUCKER and MANSFIELD, 1972, 1973; KNOX and WAREING, 1984; EVERAT-BOURBOULOUX and CHARNAY, 1982; ZIESLI'N and KHAYAT, 1983). However, it is largely agreed that the decline of abscisic acid in lateral buds following decapitation is a result of bud growth, not a cause of it. In Phaseolus vulgaris the decline in abscisic acid occurs only after bud release (TAMAS, OZBUN, WALLACE, POWELL and ENGELS, 1979). The levels of abscisic-like substances in the bud on decapitated and intact plants have been found to be similar when calculated on a concentration for fresh mass basis (DORFFLING, 1976; WHITE and MANSFIELD, 1977).

Despite these conflicting results, TUCKER (1977a, 1977b, 1977c, 1978) and EVERAT-BOURBOULOUX (1987) have noted an inverse correlation between

the levels of abscisic acid and growth of lateral buds. Working with two cultivars of Lycopersicon esculentum, TUCKER (1978) noted that the cultivar with strong apical dominance also had higher levels of abscisic acid than the cultivar with weak apical dominance.

In considering a mechanism for abscisic acid involvement in apical dominance, many investigators have attempted to implicate an interaction with auxin. TUCKER (1978) found that plants with high abscisic acid activity also had high levels of auxin activity. He concluded that the role of auxin is to maintain a high level of abscisic acid which inhibits the outgrowth of lateral buds. Auxin treatment of a plant following decapitation maintained the abscisic acid content at high concentrations in the stem tissue (KNOX and WAREING, 1984). An earlier theory implicating auxin control of abscisic acid levels suggested that abscisic acid is synthesized in lateral buds in response to the arrival of auxin from the apical bud (ARNEY and MITCHELL, 1969).

Evidence against the involvement of auxin has come from AMES, HILL, WALTON and DASHEK (1979) and TAL, IMBER, EREZ and EPSTEIN, (1979) who found no correlation between levels of abscisic acid and auxin activity.

An attractive theory proposed by TUCKER and MANSFIELD (1972, 1973) suggested that abscisic acid synthesized in the mature leaves may inhibit only the lateral buds they subtend by preventing the buds from responding to cytokinins in the bud or stem tissue. A downward gradient of free cis-trans abscisic acid was found to occur along the stem of Vicia faba, from the apical bud to the roots (EVERAT-BOURBOULOUX, 1987). This gradient correlates with the gradient of potential of lateral buds to extend following decapitation (HUSAIN and LINCK, 1966). Such a gradient is easily envisaged since mature leaves are considered

to be the primary site of abscisic acid synthesis, and translocation of this growth regulator occurs acropetally towards the apical bud (WAREING and SAUNDERS, 1971, HCAD, 1973).

The role of abscisic acid in apical dominance remains unresolved. Recently NISHITANI and HASEGAWA (1985) have suggested that auxin exerts its effects by increasing an inhibitor which is different from abscisic acid. A control of apical dominance, perhaps only under certain conditions (HILLMAN, 1986) may depend upon a balance between promotive factors and an inhibitory complex including abscisic acid.

1.6 Environmental factors affecting apical dominance

The morphology of a plant is a direct result of an interaction between the environment and genetic factors governing plant development and growth. Apical dominance, a developmental process determining the form of a plant is strongly influenced by environmental and physical factors. These factors may alter the growth regulators in the plant responsible for the regulation of apical dominance. A brief review of such factors acting on the plant is thus necessary to illustrate the complexity of a multiple control system likely to be operative in the regulation of plant form.

1.6.1 Light

Light intensity and quality, as well as the photoperiod experienced by the plant may influence the degree of apical dominance expressed. In general, high light intensities increase lateral bud growth, while lower light intensities result in a greater inhibition of buds (MITCHELL, 1953a, 1953b; GREGORY and VEALE,

1957; McINTYRE 1971a, 1971b; THIMANN et al., 1971; McINTYRE, 1973; FIELD and JACKSON, 1975b; ANDERSEN, 1976; ZIESLIN and MOR, 1981). The quality of the light may affect apical dominance and is related to light intensity. Red light promotes lateral bud growth, while far-red light, predominant under shading, will increase inhibition (KASPERBAUER, 1971; TUCKER and MANSFIELD, 1972, 1973; MORGAN and SMITH, 1976). Short-day photoperiods can reduce the degree of correlative inhibition, while increased inhibition can be experienced under long days (PHILLIPS, 1969a).

The mechanism by which light exerts an effect on apical dominance is unknown, but it may operate by altering the growth regulator and carbohydrate status of the plant, and/or the transport features of the plant.

PHILLIPS (1975) suggested that photoperiodic effects may be mediated through changes in hormone balance. In Pisum sativum, apical dominance is lost with increasing light intensity, possibly due to greater synthesis of auxin (ANDERSEN, 1976). Auxin distribution in the plant has been shown to change with changing light intensity. Low light decreased the auxin content of the Phaseolus vulgaris shoot apex, and increased the auxin levels at the base of the shoot (TILLBERG, 1972). TREWAVAS (1981), expanding on his still controvertial theory of growth regulator sensitivity, suggested that light reduces the level of auxin receptor levels in the plant. Etiolated tissues show a much greater sensitivity to auxin than green tissues (LEOPOLD and KRIEDEMANN, 1975) - possibly due to their having a greater number of cells than their green counterparts (BUTLER and LANE, 1959). However, no correlation has been noted between the effects of light on auxin levels in the plant (AUDUS, 1972).

The possibility that light affects apical dominance by altering the carbohydrate status of the plant underlines the importance of plant nitrogen in this phenomenon.

McINTYRE (1973) demonstrated that the effect of low light intensity is due to carbohydrate limitation for bud growth. A hypothesis of nutrient diversion, proposed by MORRIS and WINFIELD (1972) suggests that a light regime that increases vegetative growth will divert nutrients and growth regulators to the growing shoot apex.

Changes in transport features in the plant may be affected by light. Far-red and red light decreased the amount of diffusable and free auxin and coleoptile tips, while high light intensity increased auxin transport (LINO, 1982). In *Perilla fructescens* (L.) Britt., short-day treatments increased the translocation of cytokinins from the root to the shoot (BEEVER and WOOLHOUSE, 1973). This could be an effect on the sink strength of the shoot apex due to an enhanced unloading process (MOR, HALEVY and PORATH, 1980).

1.6.2 Water

Studies on the effects of water status on apical dominance have been largely neglected.

Early observations that the patterns of lateral bud outgrowth could be manipulated by changes in humidity (REMY, 1968) suggested that water relations in the plant could play a role in the controlling mechanism of apical dominance. *Pisum sativum* and *Phaseolus vulgaris* plants under water stress exhibited an increased degree of apical dominance which decreased after water stress was alleviated (McINTYRE 1971b, 1973). BLAKE and TSCHAPLINSKI (1986) suggested that decapitation of the shoot apex in woody plants leads to an increased availability of water by increasing the root : shoot ratio and reducing shoot competition for water. This increased availability of water would thus lead to bud release and accelerated growth.

The interacting effects of nitrogen and humidity on the water status of lateral buds may play a significant role in apical dominance. McINTYRE (1980) observed that under conditions of high humidity, inhibited lateral buds on Agropyron repens rhizomes were released from dominance - probably due to inhibition of the rhizome apex. McINTYRE (1987) has since determined that the inhibition of rhizome growth by high humidity results from a reduction in the supply of calcium to the rhizome apex. Transport of calcium is dependent on factors affecting the rate of transpiration (TIBBITTS, 1979). Thus, release of lateral buds occurs due to nitrogen now being transported to the lateral buds and not the rhizome apex.

1.6.3 Nutrients

The role of nutrient availability in apical dominance control has been reviewed in Section 1.4. Increases in organic and inorganic nutrient availability generally lead to a decrease in the degree of apical dominance, while plants under nutrient stress exhibit strong apical dominance. The effects of nitrogen availability may be influenced by the water status and light regime experienced by the plant (McINTYRE 1971a, 1973).

1.6.4 Gravity

The orientation of a shoot on a plant can influence the degree of lateral bud inhibition as well as the pattern of branching. Apical inhibition of lateral buds has been removed by placing a negatively geotropic shoot in a horizontal position (WAREING and NASR, 1958, 1961; SMITH and WAREING, 1964a, 1964b, 1966). These early investigators of gravistimulation suggested that reorientation of the shoot resulted in an inhibition of basipetal auxin transport from the shoot apex. SMITH and WAREING (1964b), noting that reorientation resulted in a decline in anterior branching and an increase in basal branching of lateral buds, suggested

that the change may be related to an increased availability of root derived cytokinins and nutrients. This hypothesis has received some support by PRAKASH, SHARMA, SHARMA and VIDHU (1985) and PRAKASH, KUMAR and SHARMA (1986).

The most convincing evidence for a role of gravistimulation in apical dominance has come from studies in Pharbitis nil, where shoot inversion induced the release of lateral buds from apical dominance (CLINE, 1983; CLINE and RILEY, 1984; CLINE and PRASAD, 1985; PRASAD and CLINE, 1985a, 1985b, 1986a, 1986b, 1987). Shoot inversion stimulates ethylene production which retards the elongation of the inverted shoot (PRASAD and CLINE, 1987). Similarly, physical restriction of the apical growth of a plant has been shown to induce ethylene production resulting in lateral bud outgrowth (HILLMAN and YEANG, 1979).

1.6.5 Other environmental factors

The effects of CO_2 (ANDERSEN, 1976) and temperature (BARNOLA, LAVARENNE and GERDRAUD, 1986; DE VRIES, DUBOIS and SMEETS, 1986) on apical dominance have received some attention but an understanding of these two factors remains limited.

1.7 A hypothesis of apical dominance control

The expression of apical dominance appears to rest with an interaction of environmental and physical factors. These factors can influence the degree of dominance expressed by limiting or modifying the growth regulators within the plant. Thus the control mechanism of lateral bud inhibition in the plant is mediated by a balance of growth regulators in the shoot system.

A working hypothesis of apical dominance control has been formulated from the following postulates interpreted from the literature:

1) The expression of plant shape or form resulting from the branching patterns of lateral buds is a direct result of gradients of nutrients, water and growth regulators in the plant. The controlled distribution of nutrients and growth regulators in the plant create a gradient of potential of lateral buds to extend.

2) Decapitation of the shoot apex changes the nutrient and growth regulator status of the plant. These changes could be viewed as the correlative signal in the plant.

3) Cytokinin applications to lateral buds on intact plants release the buds from apical dominance. Differential effects of different cytokinins suggest that the controlling mechanism may involve the controlled modification of cytokinin specificity at the bud site.

4) Events occurring in the plant following decapitation of the shoot apex and/or cytokinin application to lateral buds have indicated that the stem tissue adjacent to the lateral bud may be a site of activity related to the mechanism of action.

5) An interaction between a cytokinin and an inhibitor is likely in the control of apical dominance. If control resides in the modification of cytokinin specificity at the bud site, it is likely that this mechanism is mediated, directly or indirectly by the inhibitory influence from the shoot apex, possibly auxin.

The hypothesis thus formulated from the above postulates states:

Inhibited lateral buds on intact plants are not deficient in nutrients and growth regulators per se, but lack the biosynthetic capacity to utilize these essential resources. Decapitation of the shoot apex results in a redistribution of nutrients and growth regulators in the shoot system, resulting in a favourable auxin : cytokinin ratio at the lateral bud site. This ratio allows for the biosynthesis and utilization of specific cytokinins for a specific time period.

The study will thus be concerned with the role of cytokinins in the release of lateral buds of Pisum sativum L. The effect of applied cytokinins on lateral bud growth will be related to theoretical models of gradients of nutrients and inhibitory and promotive growth regulators in the plant. To demonstrate the polarity of these controlling factors in the plant, the patterns of lateral bud growth following removal of apical dominance will be correlated to the effects of cytokinin application. In this way, the study will encompass both aspects of apical dominance – the correlation between the shoot apex and a lateral bud, as well as the correlation between all lateral buds on a plant.

CHAPTER II

THE GROWTH AND DEVELOPMENT OF LATERAL BUDS

2.1 Introduction

The form of a plant is the result of the controlled growth or inhibition of modules along the developmental axis of the shoot. Each inhibited lateral bud on a plant represents a quiescent growth centre, the regulation of which, in terms of frequency, location and developmental potential, results from the correlation between the shoot apex and all lateral buds on the shoot. The performance of each meristem in relation to the other meristems is thus important in obtaining a holistic view of events occurring in the whole plant. CARR (1966) has stated that the metabolic interactions between parts of the plant offer considerable scope for the regulation of growth and development. With this in mind, the experiments in this chapter attempt to investigate the nature of these metabolic interactions. Of constant value will be references to theoretical models of growth regulator and resource distribution in the plant. In the words of MITCHELL (1962), 'we must not lose sight of the fact that transport processes in biology are integral with the activities of growth and survival'.

2.2 The response of lateral buds to decapitation: An introduction to the experimental system

2.2.1 Materials and methods

Plant material Seeds of <u>Pisum</u> <u>sativum</u> L. cv. Onward were pre-imbibed in distilled water for two hours and undamaged seeds not yet imbibed were selected for planting. This selection ensured uniform germination. The seeds were sown

in trays of vermiculite, eight to ten seeds to a tray saturated with distilled water in a greenhouse at $20^\circ C \pm 4^\circ C$. A high light intensity was provided by daylight. At the emergence of the first true leaf, the seedlings were watered daily with a full strength Hoagland's nutrient solution (HOAGLAND and SNYDER, 1933). Pre-trials in which intact and decapitated plants were watered with distilled water or a range of concentrations of nutrient solution indicated that the degree of apical dominance experienced by the lateral buds declined with increasing nutrient availability. Plants receiving only distilled water exhibited an experimentally more desirable complete apical dominance, but the growth of released lateral buds was insufficient as a result. It was decided to avoid imposing complete apical dominance artifically, but to apply a non-limiting supply of nutrients and select only uniform intact plants exhibiting complete apical dominance for experimentation. For all standard experiments performed, plants bearing four nodes with trifid bracts at the lower two nodes and true leaves at the upper two nodes were selected for experimentation (Figure 11). Where plants of a different age were required, sowing of seed was staggered so that treatment of a range of plant sizes could occur simultaneously. The lateral buds for all experiments were designated bud 1 to bud n from the basal to the anterior bud respectively. Using these plants cultivated under the standard conditions described, the following experiments were performed.

Decapitation of plants The shoot apex (the apical meristem and subtending young leaves) was excised 10 millimetres above bud 4, midway on the fifth internode of four-node plants. The length of each developing lateral bud was measured daily on replicates of 40 plants for 14 days. Plants not decapitated served as a control.
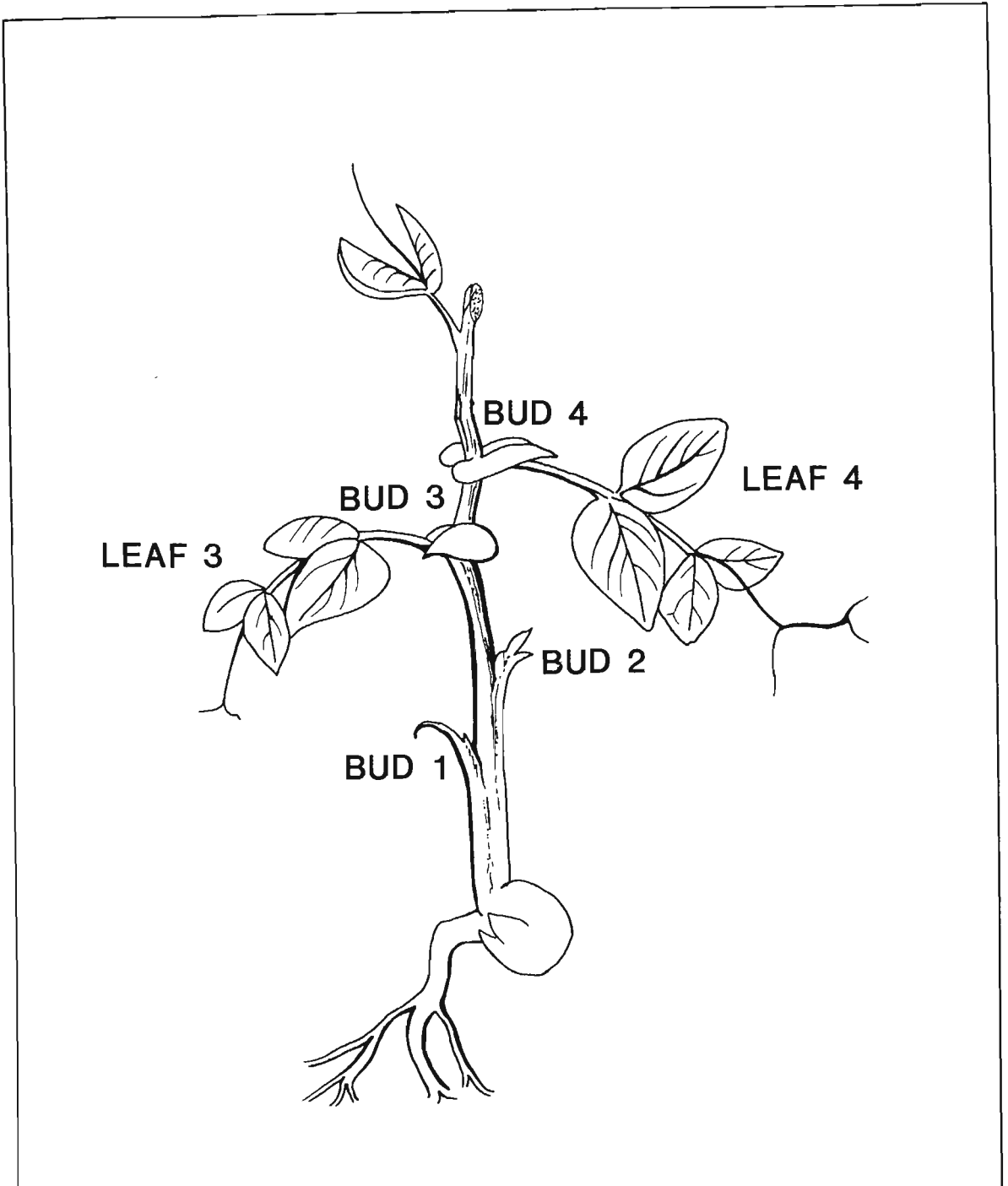
Figure 11 A <u>Pisum</u> <u>sativum</u> plant bearing four nodes with trifid bracts at the lower two nodes and true leaves at the upper two nodes. Lateral buds, nodes, internodes and leaves were numbered from the base to the shoot apex.

Serial decapitation of plants The shoot apex was excised as described from plants bearing four, six, eight or ten nodes. Replicates of 40 plants were used in each treatment. The length of each developing lateral bud was measured after 14 days. Plants not decapitated served as a control.

2.2.2 Results

The lateral buds of intact control plants in both experiments remained inhibited. In the decapitated four-node experiment, a gradient of lateral bud development was noted along the stem (Figure 12). Bud 2 grew most vigorously, followed by bud 3 and then by bud 4. Bud 1 failed to extend significantly. Extension of lateral buds on young decapitated plants of Pisum sativum could thus be described as basal.

In the second experiment, a definite changeover from basal to anterior lateral branching occurred with increasing age of the plant (Figure 13). Plants with six nodes showed a decline in bud 2 vigour, with an increase in growth of bud 1 and bud 3, but the orientation of branching was still basal. A visible changeover of branching first occurred on eight-node plants, where branching was both basal and anterior. The vigour of bud 2 declined even further. Following decapitation of the ten-node plants, lateral bud branching was entirely anterior in orientation. A compensatory effect was noted in all treatments, whereby the growth of additional lateral buds led to a decline in the previous vigour of the older lateral buds.
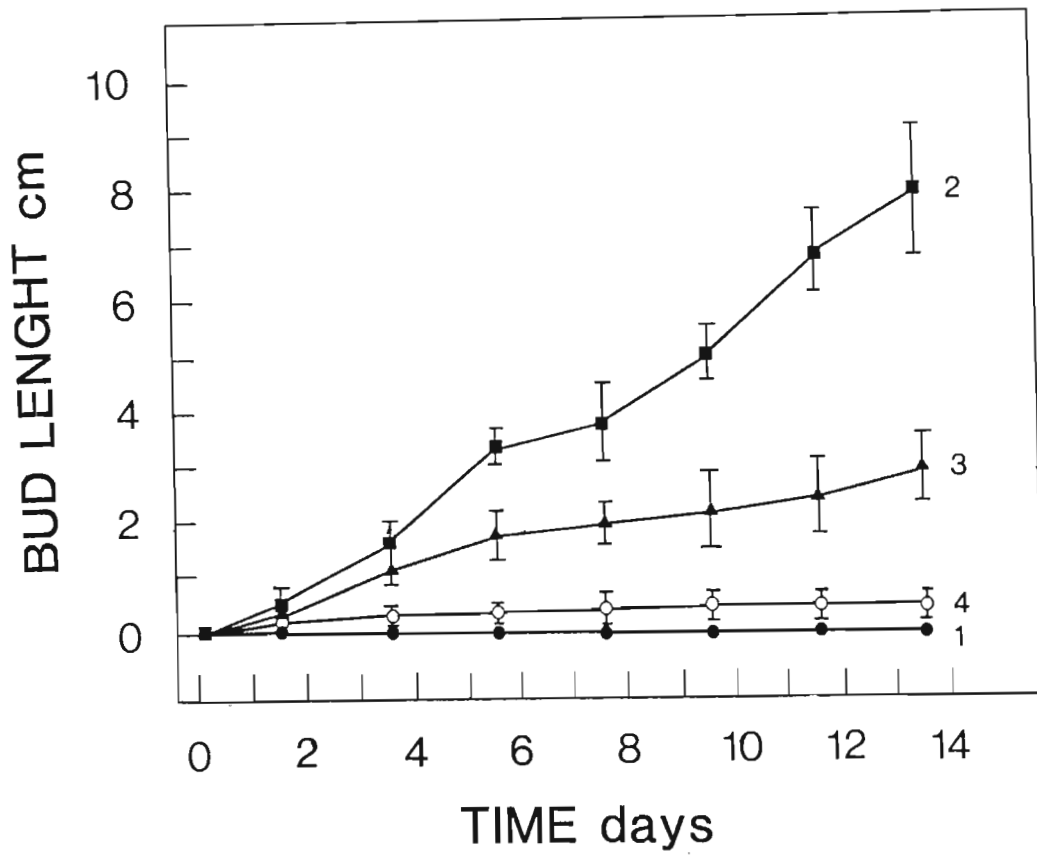
Figure 12 The extension of lateral bud 1, 2, 3 and 4 following decapitation of the shoot apex of four-node Pisum sativum plants after 14 days. Lateral buds on intact plants remained inhibited. Bars represent standard errors.
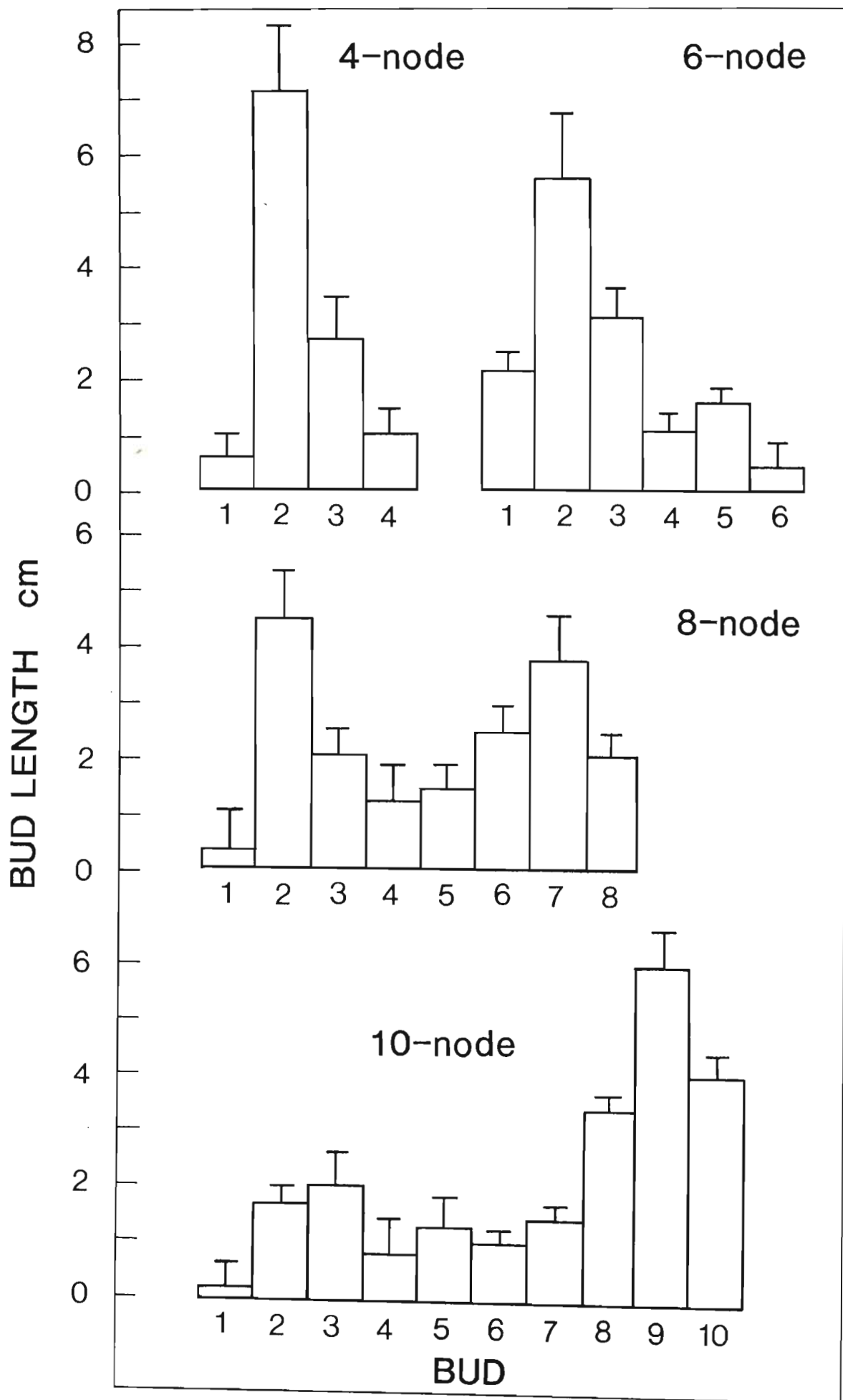
2.3 Correlative effects between lateral buds on the same plant following decapitation

2.3.1 Materials and methods

Four-node plants cultivated under the standard conditions described in Section 2.2.1 were selected for complete inhibition of all lateral buds. The shoot apex was decapitated and one or more of the inhibited lateral buds were excised from the leaf axils. In treatment (T)1 all buds (1-4) were excised leaving only the cotyledonary buds. In T 2-5, all buds but one were removed leaving bud 1, 2, 3 or 4 respectively. In T 6-11, two buds were removed, leaving two buds on the plant. This gave rise to six different permutations of bud combinations -T6 (buds 3 and 4), T7 (buds 2 and 4), T8 (buds 2 and 3), T9 (buds 1 and 4), T10 (buds 1 and 3) and T11 (Buds 1 and 2). In T12-15, only one bud was removed, leaving three buds on the plant. This gave rise to four permutations of bud combinations – T12 (buds 2, 3 and 4), T13 (buds 1, 3 and 4), T14 (buds 1, 2 and 4) and T15 (buds 1, 2 and 3). A further two treatments in which the apex was decapitated or left intact on plants with intact lateral buds served as controls. Secondary lateral buds emerging from mutilated nodes were removed daily. After 14 days, all lateral shoots were removed and their length was determined. Forty plants were included in each treatment.

2.3.2 Results

Control treatments Lateral buds on untreated intact plants remained inhibited. Decapitation of plants with intact lateral buds resulted in a distinct pattern of branching favouring a gradient of growth potential from bud 2 to bud 4 (Figures 15, 16, 17).

Treatment 1. Removal of four buds The removal of buds 1, 2, 3 and 4 on decapitated plants resulted in the emergence of three cotyledonary buds of various

lengths (Figure 14). The cotyledonary buds would only extend in the absence of all lateral buds and thus remained inhibited in all other treatments.

Treatments 2-5. Removal of three buds The removal of three buds left bud 1, 2, 3 or 4 to extend in the absence of competition from other lateral buds or the shoot apical bud. The results will thus reflect a bud's full potential to extend following decapitation. Little significant difference was noted in the lengths of buds 1, 2 and 3, while the length of bud 4 was only slightly shorter (Figure 15). The lengths of bud 1, 3 and 4 increased greatly over the corresponding buds in the control treatment (Figure 15). Bud 2 which showed the greatest potential to extend in the presence of competing buds, did not extend further when competition was removed.

Treatments 6-11. Removal of two buds The removal of two buds left two buds on the shoot to compete following decapitation. The results of these treatments (Figure 16) can be related to the growth potential of buds on control decapitated plants. Buds 2 and 3 appeared to be the most dominating buds on the shoot, and shared growth potential when competing in treatment 8. Buds 1 and 4 were the least dominant, and attained equal size when competing in the absence of buds 2 and 3 in treatment 9. The performance of two buds in isolation is affected by the proximity of another bud, the growth potential of that bud, as well as the basal or anterior orientation of the bud. Thus while bud 2 and 3 appeared to have equal growth potential status, bud 3 could not completely supress growth of bud 4 in treatment 6, as bud 2 did in treatment 7. This demonstrates the inherent greater growth potential of bud 2 when required to dominate a bud above it.

The opposite is true for buds below bud 3 and bud 2. Here, dominance of bud 1 by bud 2 in treatment 11 was less effective than dominance of bud 1 by bud 3 in treatment 10.
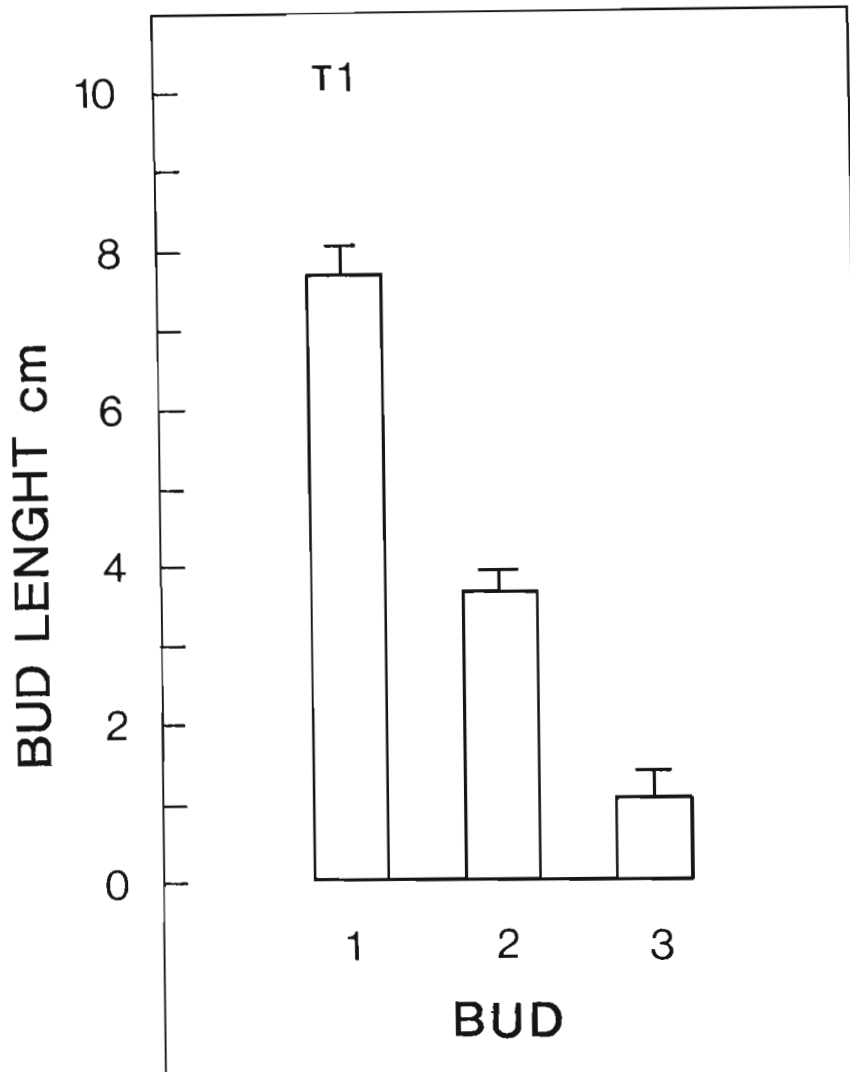
Figure 14 The extension of three cotyledonary buds on a decapitated four-node Pisum sativum plant 14 days after the excision of all lateral buds. T indicates treatment. Bars represent standard errors.
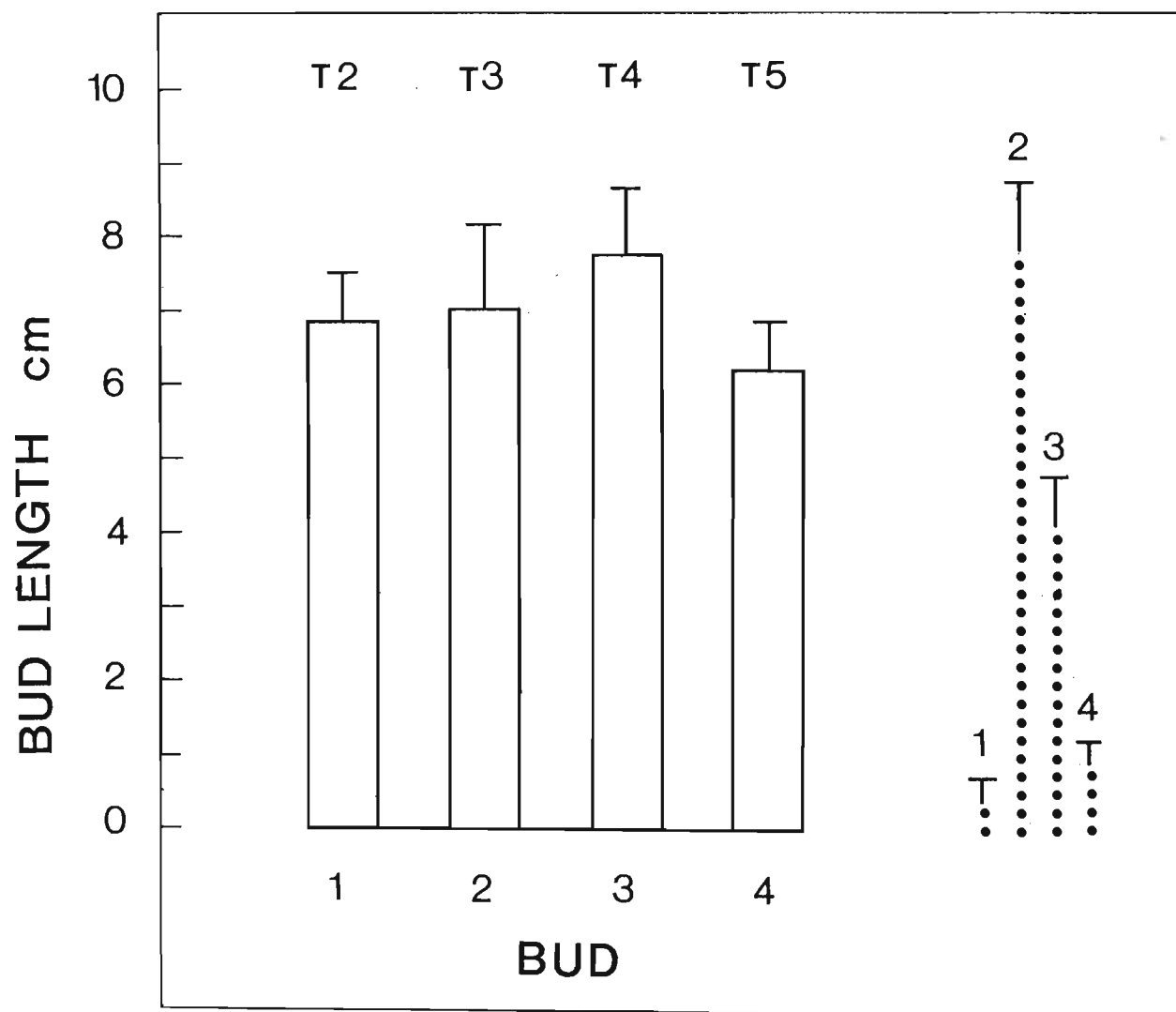
Figure 15   A comparison of the extension of lateral bud 1, 2, 3 and 4 on separate four-node Pisum sativum plants 14 days following excision of all but one lateral bud. The dotted lines represent the extension of all lateral buds on a decapitated control plant. T indicates treatment. Bars represent standard errors.

T6

T7

T8

T9

T10

BUD

T11
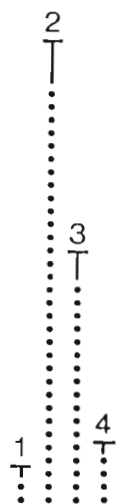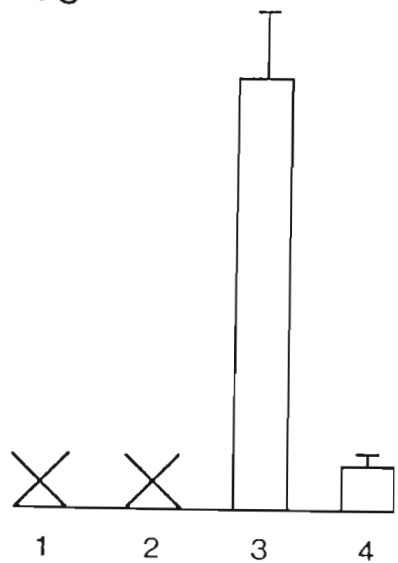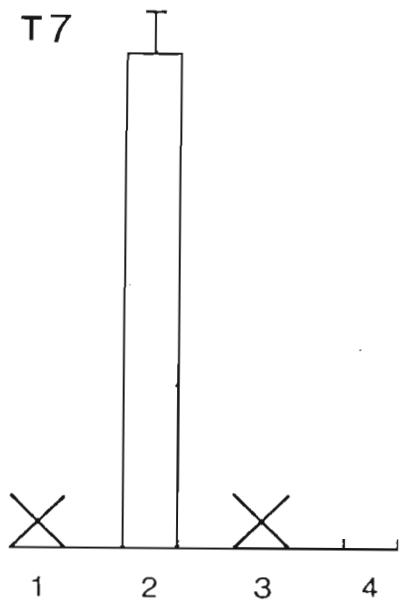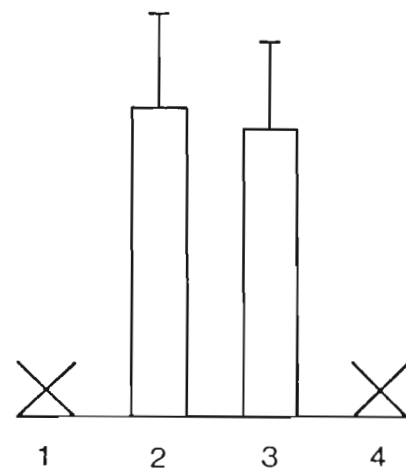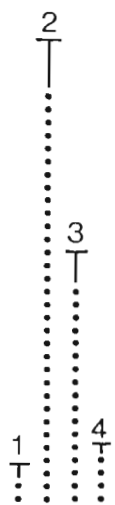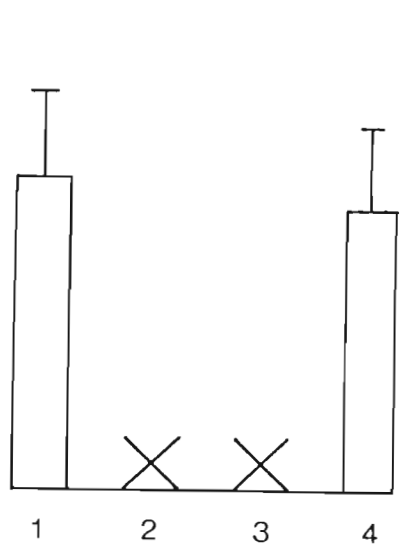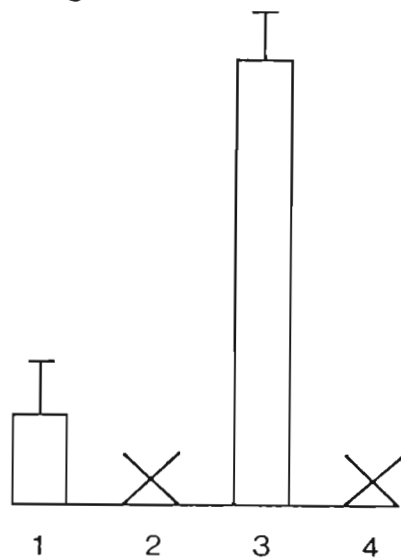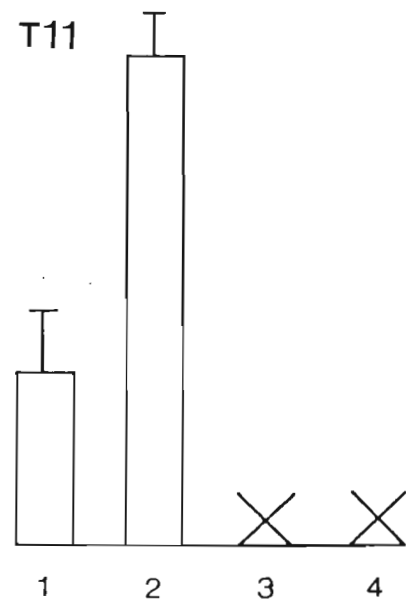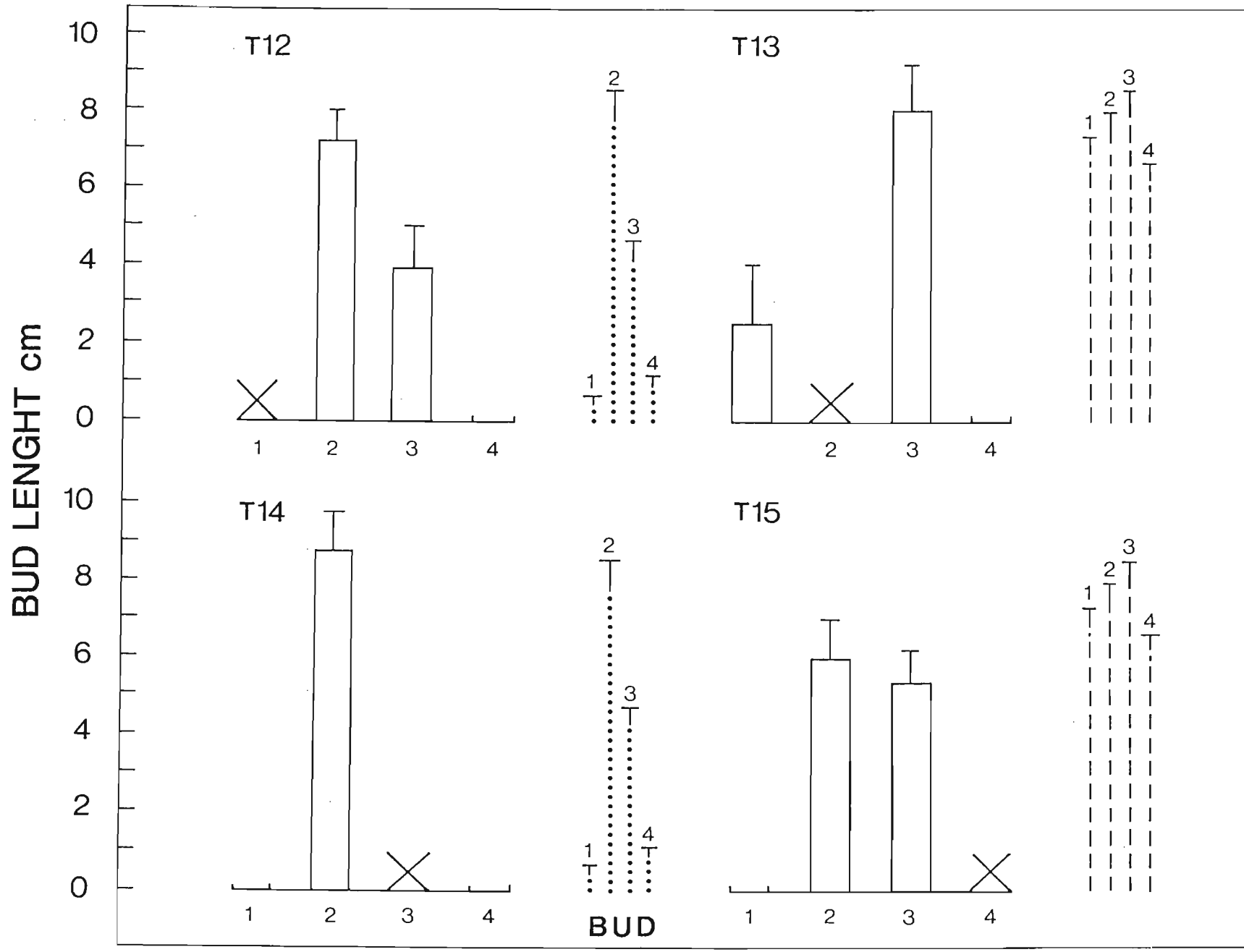
Treatments 12-15. Removal of one bud The removal of one bud left three buds on the shoot to compete following decapitation. The results of these treatments (figure 17) became complicated due to the introduction of a third (tertiary) competitive factor in the interaction between a secondary and a primary dominant bud.

The growth potential of a bud was now determined by the proximity of another bud able to weaken its influence over its primary competitor. Thus bud 3, which shared dominance with bud 2 in treatment 8 (Figure 16), lost its equal status when competing with both bud 2 and bud 4 in treatment 12 (Figure 17). While bud 4 itself did not extend, a weakening effect was exerted. The pattern of branching and length of shoot in this treatment was similar to those found in the control treatment. When bud 2 was removed, bud 3 again asserted dominance over bud 4 in treatment 13, but could not dominate bud 1. Bud 1 appeared to be strongly inhibited by the presence of bud 2 in treatments 14 and 15. On removal of bud 3, bud 2 asserted complete dominance over buds 1 and 4 in treatment 14, while the presence of bud 1 did not affect the competition between buds 2 and 3 when bud 4 was removed as in treatment 15.

2.4 Discussion

The pattern of lateral bud branching following decapitation of four-node Pisum sativum seedlings was found to be consistent with the view that apically synthesized auxin, moving basipetally from the shoot apex exerts an inhibitory influence on lateral bud growth. While the present results and those of BLAKE et al. (1983) showed that growth of lateral buds following decapitation was basally orientated, anterior growth has been demonstrated on decapitated Glycine max and Pisum

sativum (LEE, 1984) and Hygrophila (CUTTER and CHIU, 1975) seedlings. The lateral buds of different plants thus appear to respond differently to decapitation of the shoot apex. These differences are likely the result of differing gradients of promotive and inhibitory factors in the plant, as well as the developmental stage of the lateral buds.

The pattern of lateral bud growth, and thus the plant form, is a manifestation of growth regulator and possibly other gradients in the plant shoot. In the intact plant, auxin synthesized at the apical region of the shoot is transported basipetally towards the roots (GOLDSMITH, 1968, 1977). A gradient of auxin concentration thus exists in the plant, being highest at the shoot apex and declining down the stem as the auxin is immobilized or conjugated (SCOTT and BRIGGS, 1960; GOLDSMITH, 1968). The lowermost buds are therefore less under the inhibitory influence of the shoot apex, and considering their proximity to root supplied nutrients and growth regulators, have a high potential to develop following decapitation. This was found to be the case, with a basipetally increasing gradient of developmental potential being observed from bud 4 to bud 2. The anomalous performance of bud 1 will be discussed later. On decapitation the auxin gradients decline down the stem. In Pisum sativum, the level of extractable auxin at a node eight centimetres from the shoot apex started declining six hours after decapitation and was barely detectable after 12 hours (SCOTT and BRIGGS, 1960). It may be asked why basal growth of lateral buds occurs on a shoot when it is expected that the levels of auxin fall first at the apical region. This can be explained by the results of SCOTT and BRIGGS (1960), who distinguished between diffusable and extractable auxin in the plant. Thus while the concentration of diffusable auxin will fall first at the apical and then at the basal end of the stem, the more anterior lateral buds will not be able to assume dominance as expected, since in the absence of the normal auxin source, auxin moving down in the transport system will be gradually replaced by non-moving extractable auxin from the

stem tissue. This inhibitory effect of auxin is likely to be mediated by an antagonism with the gradient of root-derived cytokinins moving up in the plant. In shoots of Hygrophila, lateral buds at certain nodes along the shoot are more responsive than others to decapitation (CUTTER, 1975). She suggested that a substance derived from the shoot apex interacts with a cytokinin and reaches a threshhold value at certain positions along the stem. The lower buds on the plant probably achieve a more favourable ratio of auxin : cytokinin in or at the lateral bud site - a result of changes in the distribution of both these growth regulators following decapitation.

The regulation of the pattern of lateral bud growth is further complicated by the immediate activities occurring in the growing buds released from dominance. These buds now become a source of auxin (PHILLIPS, 1969a, HILLMAN et al., 1977) which may affect lateral buds basipetal to the auxin origin, as well as influencing the distribution of resources in the plant. In this way, the lower buds, because of their ability to grow faster and produce more auxin, dominate the upper buds which are still not self-sufficient in auxin, and thus deficient in promotive factors and/or nutrients. The position a lateral bud occupies on the gradient axis will thus determine how it will respond, and the response of all buds on the plant will in turn determine the form of the plant.

Additional examples of the manifestation of auxin polarity in the plant lend credibility to the above theory. THIMANN (1977) noted a differential uptake of radiolabelled auxin when applied to the serial segments of Pisum sativum internodes. Auxin uptake was greatest in the upper stem, low in the central region and again greater at the base. The distribution of radioactivity in intact Vicia faba plants following application of radiolabelled auxin to the shoot apex occurred as a gradient (EVERAT-BOURBOULOUX AND BONNEMAIN, 1980). Decapitation resulted in a marked lowering of the auxin in the nodes of bud 1 and bud 2 - the

buds which extend. Likewise, the distribution of auxin and abscisic acid in etiolated _Phaseolus vulgaris_ epicotyls was found to be maximal in the anterior regions and lowest in the basal regions (HOREMANS, VAN ONCKELEN and de GREEF, 1986). The parallel distribution of auxin and abscisic acid in the previous example is possibly correlated to the similarities in the physico-chemical properties and transport mechanisms for both growth regulators (SCHNEIDER and WIGHTMAN, 1978), and serves to underline the importance of growth regulator transport in the plant in relation to the control of apical dominance.

Of interest is that bud 1 did not extend significantly following decapitation of the shoot apex. Since it is the lowest bud, it is likely to attain a more favourable auxin : cytokinin ratio first. On the other hand, it is possible that auxin levels in this region may be suboptimal for events leading to bud release to occur. However, it is also possible that the inhibition of bud 1 is not totally under the control of the shoot apex, or not under the same controlling mechanism/s as the other lateral buds. Bud 1 occurs at the base of the shoot, and the vascular tissue in the transition zone from the shoot to the root may be unfavourable for the transmittance of the regulating signal. A further possibility exists that bud 1 may be developmentally retarded, and so may be under a localized inhibition from bud 2 above it. Whatever the mechanism, the behaviour of this bud serves as a cautionary warning that other factors, besides the distribution of growth regulators may be implicated in determining the pattern of lateral bud growth following decapitation.

The results of decapitation of four, six, eight and ten-node _Pisum sativum_ plants indicated that the pattern of lateral bud branching changes with the development of the plant. A gradual changeover from basal to anterior branching was noted as plants increased in age and size. Similar examples of this phenomenon have

been noted before. GREGORY and VEALE (1957) found that in Linum usitatissi-mum and Pisum sativum, growth of lateral buds was basal on younger decapitated plants but became anterior with increasing age of the plant. Performing serial decapitation of two, three and four-leafed Pisum sativum plants, HUSAIN and LINCK (1966) noted a changeover from basal to anterior branching. In addition, the stem apex of an intact plant seemed to lose its dominance with the increasing age of the plant. ZAMSKI, OSHRI and ZIESLIN (1985) also found that lateral buds at different positions along a Rosus shoot differed in their sprouting ability, with buds low down on the branch being more inhibited. Thus the developmental potential of lateral buds differs with the changing development of the plant. This phenomenon could be a useful investigative tool in studies on the control mechanism of apical dominance.

Examples related to this phenomenon have indicated that the response could be mediated, in part, by environmental or physical stimuli governing development in the whole plant. WAKHLOO (1970), applying high doses of potassium to the soil induced the extension of the lowermost lateral buds on intact Solanum sisym-brifolium plants. Similarly, when water stress to intact Pisum sativum plants was reduced, lateral buds grew out at the lowest (buds 1 and 2) nodes (McINTYRE, 1971b). These environmental influences on the orientation of branching are likely to be mediated by growth regulators in the plant. The polar movement of auxin has been shown to develop gradually in young seedlings (JACOBS, 1950; SMITH and JACOBS, 1968; GREENWOOD and GOLDSMITH, 1970); to decline in strength / polarity with age of the plant (McCREADY and JACOBS, 1963; LEOPOLD and DE LA FUENTE 1968; SMITH and JACOBS, 1968) and to show a graded change in properties along various developmental axes (JACOBS, 1950; LEOPOLD and LAM, 1962; JACOBS, 1978). A controlling role for the modulation of growth regulator flow thus seems plausible when considering the above evidence.

It would be expected that the anterior lateral buds on a plant, irrespective of plant age, would be inhibited by their proximity to the source of inhibitory auxin – the shoot apex. However, since this was not the case, it is likely that changes in the properties of growth regulator transport and possibly metabolism are responsible for this decline in inhibition. SNOW (1925) noted that the apical dominance of lateral buds of Pisum sativum increased with their distance from the shoot apex, and that the closer the lateral buds were to the shoot apex, the less was the inhibition. It is possible that unequal partitioning of nutrients and promotive growth regulators in older, larger plants results in the deprivation of the lateral buds. Alternatively, the auxin arriving at the lower buds may be suboptimal to initiate an interacting mechanism with another growth regulator. Applications of auxin to the decapitated stump of Pisum sativum plants reduced basal growth of lateral buds on young plants, but increased basal growth when applied to older plants (GREGORY and VEALE, 1957). The possibility that this auxin treatment to older plants may have acted by arresting the growth of the anterior lateral buds (and thus enhancing the growth of the basal buds) is unlikely considering the inability of a similar treatment to promote the growth of basal lateral buds on younger plants.

It would appear that lateral buds along a length of stem respond differently to factors which break apical dominance. PHILLIPS (1971a) showed that the interacting effects of gibberellin and auxin applied to lateral buds of Phaseolus multiflorus differed according to the stage of development of the buds themselves. In Hygrophila shoots, decapitation resulted in anterior branching while application of benzyladenine or kinetin to intact plants resulted in basal branching (CUTTER, 1975; CUTTER and CHIU, 1975). Likewise, LEAKEY and LONGMAN (1986) noted that the pattern of lateral shoot growth following decapitation of rooted Triplochiton schleroxylon cuttings varied in response to a range of environmental

conditions and growth regulator treatments. Therefore buds at certain nodes along a shoot, and thus presumably in a certain physiological state or stage of development, respond differently to factors which release lateral buds from inhibition.

The correlation between lateral buds on the same plant is likely to be an important factor controlling the patterns of lateral bud branching. Thus, while a lateral bud may have an innate developmental potential determined by its developmental and physiological state, its position on a length of stem in relation to other lateral buds will determine its behaviour before and subsequent to the release of apical dominance. This was demonstrated very clearly in the latter part of these growth experiments, where various combinations of lateral buds were left to compete with each other following removal of selected lateral buds on the shoot apex.

When all four lateral buds were excised, three cotyledonary buds grew out at various lengths, indicating a localized correlative effect at the cotyledonary node. These buds did not extend in the presence of another growing lateral bud above them. Considering that the lateral buds in the cotyledonary nodes of Pisum sativum were more likely to grow out after decapitation if the cotyledons were removed (SEBANEK, 1965, 1972; SPRENT, 1968), it is possible that a nutrient effect was operative.

Removal of all but one bud on the decapitated Pisum sativum shoot allowed each bud to grow to its full developmental potential without the influence of another bud. Buds 1, 2 and 3 all showed a similar developmental potential, with the growth of bud 4 only slightly less. Buds 1, 3 and 4 showed improved growth over their counterparts growing on the control plants, where no lateral buds were excised. These results raise the question as to what extent the developmental potential

of a bud is predetermined by the physiological state prior to decapitation, or by the differential growth rates developing later on. The results suggest that buds 1, 2 and 3, but to a lesser extent bud 4, are of a similar developmental state. However, due to their different positions of the polar axis of growth regulator distribution in the shoot, it is difficult to distinguish between the two effects. A comparison of events occurring immediately after decapitation in each bud may shed light on this aspect.

PHILLIPS (1975) has stated that all vegetative buds on a plant possess essentially equal developmental potential. This appeared to be the case in Pisum sativum. HUSAIN and LINCK (1966) showed that in this plant, the initial growth rates of all lateral buds on a plant were similar, and that differential growth rates developed later on. Contrary to these results, GREGCRY and VEALE (1957) showed that the behaviour of various lateral buds on isolated nodes of Pisum sativum closely resembled that of the lateral buds in situ. CUTTER and CHIU (1975) found that previously inhibited lateral buds of Hygrophila at the basal nodes were capable of growth when isolated. In addition, if the shoots were divided up into separate nodes, there was a delay in the outgrowth of the buds at the younger nodes. This appeared to be related to bud size. Thus it appears that if all the lateral buds are of a similar developmental size, the developmental potential to extend is determined firstly by the growth regulator status at the node or in the bud, and that differential growth rates which develop later on may be related to other factors. HUSAIN and LINCK (1966) noted that the developmental potential of the basal buds, and especially bud 2 on Pisum sativum plants was higher at the early stages of growth, while GOULD et al. (1987) showed that buds present at node 2 in intact seedlings have a measurable size advantage over other buds. Morphological and anatomical differences in buds could account for different developmental potentials of buds in certain plants. An inhibitory

gradient among lateral buds located at various positions along a Rosus shoot was demonstrated by ZIESLIN and HALEVY (1976). Several factors were involved in the maintenance of this inhibitory gradient, but it was shown that there are morphological and anatomical differences between lateral buds along the shoot (ZAMSKI et al., 1985).

Other factors involved in the maintenance of this inhibitory gradient are very likely to include the partitioning of resources between lateral buds on the plant, especially after growth has begun. This effect was manifest in the results of treatments where various combinations of two or three lateral buds were left to compete on the decapitated Pisum sativum shoot. A definite partitioning of growth potential was noted when two lateral buds were left intact on the stem. Their response was also influenced by their proximity to each other and their positions on the stem relative to the other bud. These effects were also noticeable when three lateral buds were left intact on the plant, but the interrelationships became very complex.

PHILLIPS (1968) ascribed control of apical dominance to the shoot apex as it is the first meristem to grow and command a nutrient supply. This would imply that a given organ enjoys its status at the expense of another organ. GOEBEL (1900) had earlier recognised that organs of a plant are not independent, but that there occur reciprocal influences among organs that may be under nutrient control. This was recognisable in the results of the 'growth competition' studies, where a form of compensatory growth was noted. In the event of the loss of an organ, some plant organs show regenerative growth which tends to make up (compensate) for the loss. Thus, removing all the lateral buds of intact Coleus blumei plants resulted in faster growth of the shoot apex (JACOBS and BULL-WINKEL, 1953). Likewise, by removal of the lateral bud on decapitated Phaseolus

vulgaris plants, a greater yield was achieved on bud 2 (BINNIE and CLIFFORD, 1980). It is tempting to explain the interrelationship between lateral buds as being determined by a competition for resources, but HARDWICK (1986) has preferred to describe the interaction in terms of cooperation. Thus while one lateral bud may appear to obtain resources at the expense of another, the interaction results in the successful survival of the whole plant. The growth of lateral buds following decapitation is thus likely to be related to the redistribution of nutrients and promotive growth regulators to those buds which, by virtue of their genome and/or their position on the plant, are best suited to develop following removal of the inhibitory signal.

The development of lateral buds following decapitation may be viewed as being under two possible controlling mechanisms mediated by auxin. Removal of the shoot apex leads to a significant increase in auxin levels in lateral buds (THOMAS, 1972; HILLMAN et al., 1977). These newly established sites of auxin synthesis could now redirect the flow of resources to these actively growing meristems. More rapid growth of lateral buds is an indication of greater endogenous auxin synthesis (SACHS and THIMANN, 1967), and thus greater cytokinin and nutrient mobilization. If nutrient supply is optimum or superoptimum, the lateral buds will probably obtain sufficient nutrients to develop, but will not do so until their cytokinin requirements are met. Thus, suboptimal nutrient availability has been shown to increase the level of lateral bud inhibition (GREGORY and VEALE, 1957; McINTYRE, 1973; LEAKEY and LONGMAN, 1986). Primary control by nutrient availability is thus likely to be operative only under conditions of nutrient deprivation. At optimal nutrient levels, auxin is likely to exert its effect via an interaction with cytokinin.

A hypothetical model of the interacting effects of lateral buds on the growth of each other will be proposed, based on the results of the experiments in this

chapter (Figure 18). When all lateral buds on a decapitated plant are left intact, the pattern of lateral branching occurring may be explained as follows. While all lateral buds show a similar potential to extend following decapitation, bud 4 and bud 3 are adjacent to an inhibitory stem concentration of auxin, and will not be able to extend until this auxin concentration has declined to an optimal level. Bud 2, the most dominant bud in all treatments assumes dominance and begins to produce auxin at its rapidly growing apex. This growing shoot will now mobilize root derived nutrients and growth regulators to the detriment of buds 3 and 4 acropetal to it. However, the auxin produced by the now dominant bud 2 will not move acropetally towards the buds above. Since these buds are not under the inhibitory action of auxin produced by bud 2, they will continue to develop at a reduced rate, competing for nutrients and thus influencing the buds below them. Their decline in size acropetally up the stem will be a result of their delayed release from dominance and their weaker sink strengths due to their reduced capacity to synthesize auxin. Buds below the dominant growing shoot will be influenced by the flow of the secondary source of auxin which could regulate cytokinin availability to these buds. Thus, some growth may be achieved, especially if nutrient conditions are optimal.

In this way, each lateral bud on a plant affects the degree of inhibition of all other buds by its ability to 1) mobilize nutrients, and 2) influence the action of cytokinins below it. Both processes are likely to be mediated by auxin - the extent of each being dependent on the nutrient status of the plant.

82.
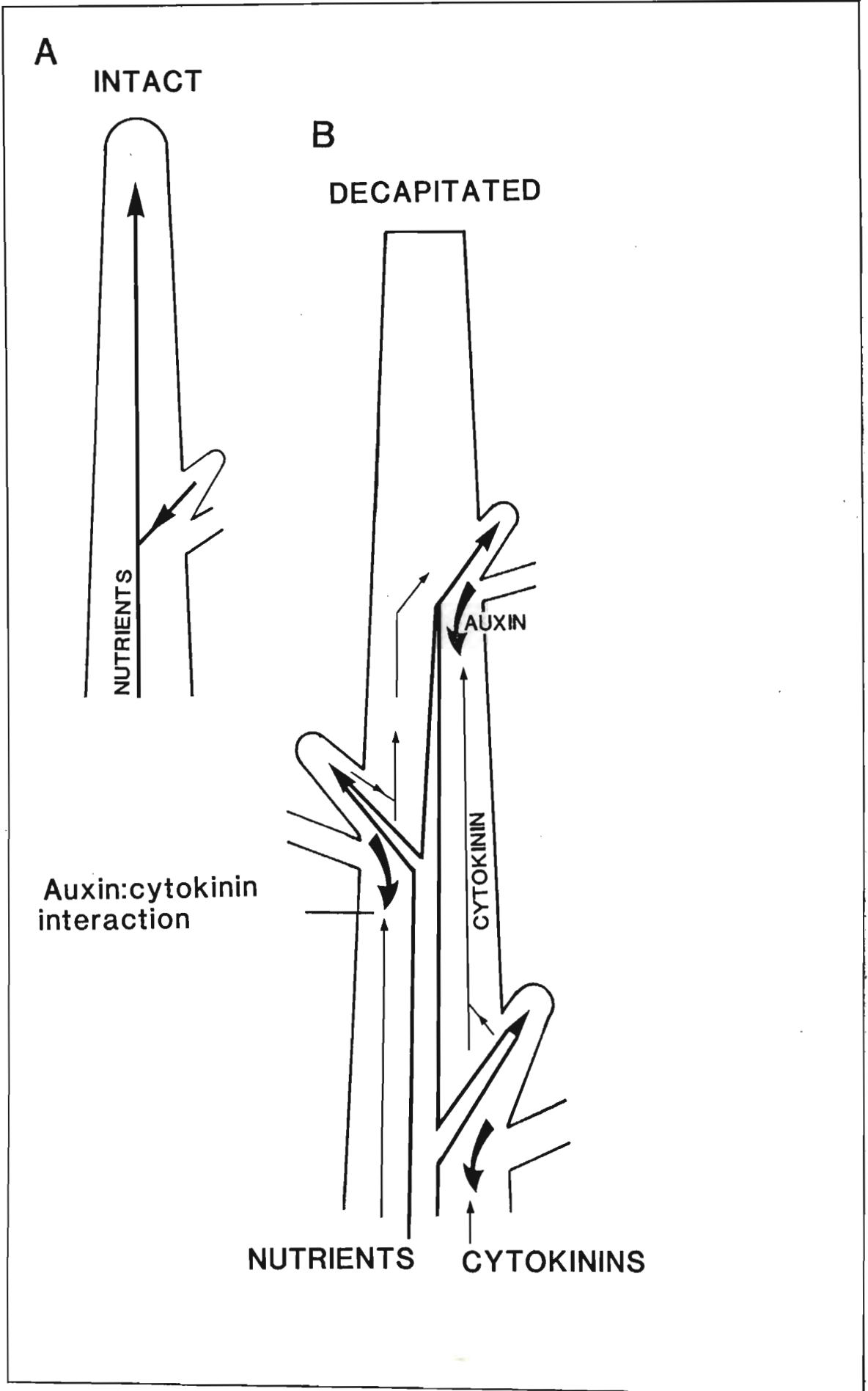
CHAPTER III

THE APPLICATION OF CYTOKININS TO LATERAL BUDS

3.1 Introduction

In Chapter 2, an attempt was made to formulate a working model of events occurring in the Pisum sativum plant which could influence the expression of apical dominance. This model was based, in part, on the responses of lateral buds to decapitation, and on theoretical concepts of growth regulator transport. Assuming an inductive role for cytokinins in lateral bud release and growth, it now became necessary to study, quantitatively, the biological response which results from the application of a cytokinin to a lateral bud.

However, in relation to growth regulator application studies, CARR (1966) stated that 'when hormones become available as pure compounds they lend themselves to the production of dramatic effects and there is a widespread and regrettable tendency to spray, paint or otherwise feed them indiscriminately to any and every kind of plant, plant organ, tissue or cell; this is scarcely science, but a form of exhibitionism'. This study will not be concerned with the 'indiscriminate' application of cytokinins to the lateral buds only, but will attempt to relate the changes induced to changes in sensitivity of the lateral buds to the applied cytokinins. In addition, the correlations between all lateral buds on a plant will be subject to an investigation by documenting their response to cytokinin application to one of four lateral buds on intact or decapitated plants.

3.2 An activity profile of the ability of cytokinins to release lateral buds from apical dominance

3.2.1 Materials and methods

Cytokinin application   A range of cytokinins were applied directly to the inhibited lateral buds of four-node intact Pisum sativum plants cultivated under the standard conditions described in Section 2.2.1. The cytokinins were dissolved in a mixture of 50 per cent ethanol and 0.8 per cent polyethylene glycol 2000 in an identical manner to that described by SACHS and THIMANN (1967). The ethanol was used as a surfactant to facilitate the entry of the cytokinin into the lateral buds, while the polyethylene glycol, due to its hygroscopic properties, maintained the aqueous solution in constant contact with the buds. A microlitre syringe was used to dispense 10 microlitre aliquots (each containing 3.3 micrograms of cytokinin) of solutions of adenine, adenosine, benzyladenine, dihydrozeatin, dihydrozeatin riboside, isopentenyladenine, isopentenyladenosine, kinetin, zeatin or zeatin riboside to bud 2 on intact plants. The complete nomenclature and structure of these compounds is presented in Figure 19. Since the inhibited lateral buds were very small, the treatment droplet was allowed to rest in the axil between the main shoot and the petiole or bract.

Ideally, dose-response curves for each cytokinin would be compared to determine their relative activities, but this would have to be done within a single experiment, and was logistically impossible. Therefore the activity comparisons were done at only one concentration (3.3 micrograms of cytokinin per lateral bud). This particular concentration has been used before (SACHS and THIMANN, 1967; PILLAY and RAILTON, 1983) and has proved to show that the regulatory potential is great at this range, and that different cytokinins being compared can show a greater or lesser activity quite clearly. Nevertheless, it was borne in mind that
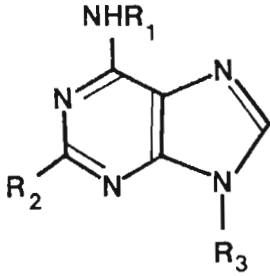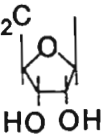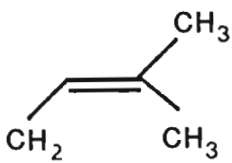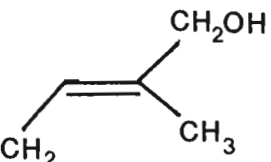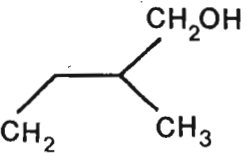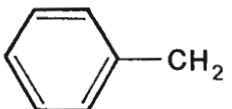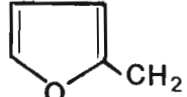
Figure 19   The structures, nomenclature and abbreviations of natural and synthetic cytokinins applied to the inhibited lateral buds of <u>Pisum</u> <u>sativum</u> plants. Riboside (R) refers to the β-D-ribofuranoside group.

the differential activity of exogenous cytokinins may be related to the concentration at which they are applied.

A control treatment was performed in which 10 microlitre aliquots of the ethanol and polyethylene glycol solution were applied to bud 2 on intact plants. A second control treatment was performed in which similar plants were decapitated as described in Section 2.2.1, and the ethanol and polyethanylene glycol solution then applied to bud 2 as before. This treatment served primarily as a control between experiments, but was also useful in comparing the degree of bud growth induced by a cytokinin with that induced by decapitation. The length of the lateral buds was measured daily for 14 days. Forty replicates were performed per treatment.

The soybean callus bioassay   The biological activities of the cytokinins used in the previous experiment were tested in the soybean (Glycine max cv. Acme) callus bioassay. This bioassay exhibits a linear relationship between response and concentration over a wide range of cytokinin concentrations (VAN STADEN and DAVEY, 1979). Callus was obtained from the cotyledons of soybeans according to the proceedure described by MILLER (1963, 1965). A nutrient medium (30 millilitres) adapted from MILLER (1963, 1965) (Table 2) and 3.3 micrograms of the cytokinin to be tested were added to 50 millilitre Erlenmeyer flasks containing 0.3 grams (one per cent) of agar. Replicates of 10 flasks per cytokinin were used. The flasks were stoppered with non-absorbent cotton wool bungs which were then covered with aluminium foil. The flasks were then autoclaved at a pressure of 1.05 bars for 20 minutes before being transferred to a sterile transfer chamber.

Three pieces of soybean stock callus, each weighing approximately 10 milligrams were placed on the medium in each flask. The flasks were then incubated in a growth room with a constant temperature (25°C $\pm$ 2°C) and continuous low light

Table 2: Basal medium for soybean callus bioassay (adapted from MILLER, 1963; 1965)

Stock solution	Chemical	gl^{-1} Stock solution	ml Stock solution l^{-1} medium
Stock 1	KH_2PO_4	3.0	100
	KNO_3	10.0	
	NH_4NO_3	10.0	
	$Ca(NO_3)_2.4H_2O$	5.0	
	$MgSO_4.7H_2O$	0.175	
	KCl	0.65	
	$MnSO_4 4H_2O$	0.14	
Stock 2	NaFeEDTA	1.32	10
	$ZnSO_4.7H_2O$	0.38	
	H_3BO_3	0.16	
	KI	0.08	
	$Cu(NO_3)_2.3H_2O$	0.035	
	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.01	
Stock 3	Myo-inositol	10.0	10
	Nicotinic acid	0.2	
	Pyridoxine HCl	0.08	
	Thiamine HCl	0.08	
Stock 4	NAA	0.02	10
Additional	Sucrose		30 gl^{-1} medium
	Agar		10 gl^{-1} medium

pH adjusted to 5.8 with NaOH

intensity (cool white fluorescent tubes). After 21 days, the three pieces of callus in each flask were weighed simultaneously.

3.2.2 Results

Cytokinin applications: structure – activity relationships   Control lateral buds, to which a solution of ethanol and polyethylene glycol was applied, remained inhibited in the presence of the shoot apex. In the decapitated control treatment, a gradient of lateral bud development was noted along the stem as described in Section 2.2.2 and indicated in Figure 12 (Figure 20). The ability of the different cytokinins to release bud 2 varied considerably. Benzyladenine exhibited the greatest activity, with zeatin riboside and zeatin being only slightly less active (Figure 20). Dihydrozeatin and isopentenyladenine exhibited a much lower, but intermediate activity, while isopentenyladenosine, kinetin and dihydrozeatin all had very low activity. Applications of adenine or adenosine to bud 2 were not successful in promoting growth of this bud. All untreated buds on the intact plants remained inhibited for the duration of the experiment.

The soybean callus bioassay   The ability of the different cytokinins to promote the growth of soybean callus in vitro appeared to parallel the activities of these same cytokinins in releasing lateral buds from apical dominance (Figure 20). Benzyladenine, zeatin riboside and zeatin all had similarly high activities in this bioassay, followed by dihydrozeatin with medium activity. Isopentenyladenine and kinetin had similar activities, being lower than the activity of dihydrozeatin but higher than the activities of isopentenyladenosine and dihydrozeatin riboside which exhibited low activity. Adenine and adenosine were inactive in this bioassay.

Kinetin appeared to be the only cytokinin that did not behave in a similar manner in both the promotion of lateral bud release on intact plants and the promotion
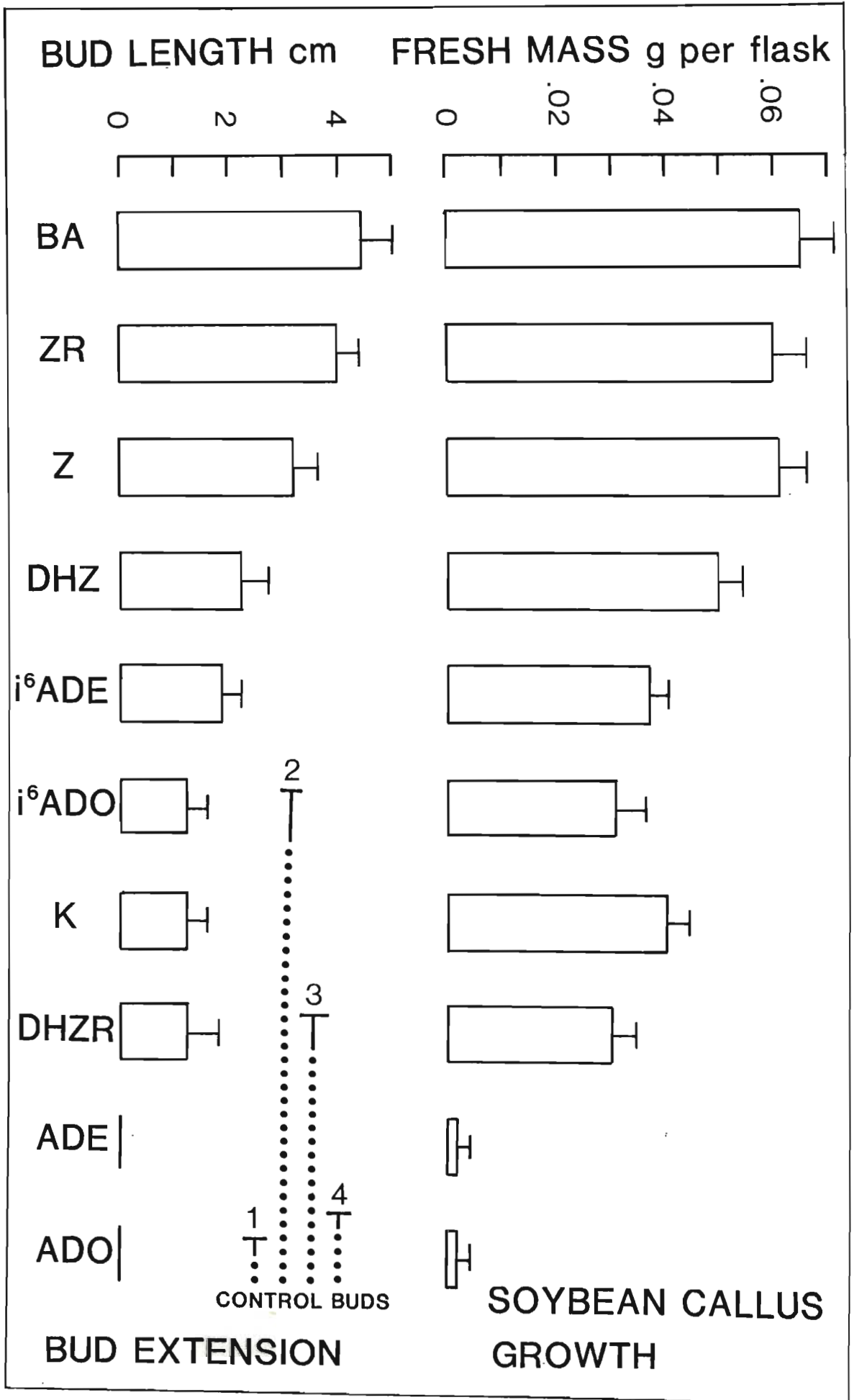
of cell division in the soybean callus bioassay. The increased activity of this cyto-kinin in the latter bioassay may be related to its synthetic nature and thus struc-tural stability.

While a detailed discussion of these results will be provided at the end of this chapter, it is pertinent that some comment be made to indicate the motivation for the following experiments. Of primary interest was the fact that the natural cytokinins isopentenyladenine and zeatin exhibited very different biological acti-vity in both systems tested, although they are very similar in structure (Figure 19). Zeatin appeared to be a potent antagonist of the inhibitory effect on bud 2, while isopentenyladenine showed only weak activity. PILLAY and RAILTON (1983) demonstrated that while a single application of zeatin to inhibited lateral buds of intact Pisum sativum could release these buds from inhibition, similar treatment with isopentenyladenine caused only limited outgrowth. These authors suggested that the inhibited lateral buds were unable to hydroxylate isopentenyladenine to zeatin. Hydroxylation of isopentenyladenine results in an increase in the cyto-kinin's activity (LEONARD, HECHT, SKOOG and SCHMITZ, 1969). Since these two cytokinins have been recovered from Pisum sativum plants (SYONO and TORREY, 1976; WIGHTMAN et al., 1980; DAVIES et al., 1986), it was decided to pursue an investigation of the role of isopentenyladenine and zeatin in the release of lateral buds from apical dominance.

3.3 Application of isopentenyladenine and zeatin to lateral buds

3.3.1 Materials and methods

Cytokinin application: growth effects   Four-node Pisum sativum plants cultivated under standard conditions described in Section 2.2.1 were used. Isopentenyladenine

or zeatin were applied directly to one of each of the four inhibited lateral buds as described in Section 3.2.1. Thus, only one lateral bud on a plant was treated with a cytokinin. In addition, equal replicates of plants treated in this manner were decapitated as described in Section 2.2.1. A single treatment thus comprised a batch of 40 plants, either decapitated or left intact, to which isopentenyladenine or zeatin was applied to bud 1, 2, 3 or 4 only. A control treatment was performed by decapitating similar plants not treated with the cytokinins. Replicates of 40 plants were used for each treatment. Since this experiment required approximately 700 plants, only the lengths of the treated buds were measured daily for 14 days.

<u>Cytokinin application: concentration effects</u>   The above experiment was repeated exactly as described, but 3.3, 6.6 or 9.9 micrograms of isopentenyladenine or zeatin were applied to one of each bud on replicates of 40 intact or decapitated plants. The scale of the experiment (2 000 plants) meant that daily bud lengths could no longer be measured, and the final lengths of both treated and untreated lateral buds were recorded after 14 days.

3.3.2   Results

<u>Cytokinin application: growth effects</u>   The lateral buds of intact control plants remain inhibited. In the decapitated control treatment, a gradient of growth potential along the stem was noted to be similar to the identical treatment in Section 2.2.2, Figure 12 (Figure 21). Bud 2 grew most vigorously, followed by bud 3 and then bud 4. Bud 1 failed to extend.

Zeatin applied to the buds of intact plants promoted the extension of treated buds to a lesser or greater degree (Figure 22). Zeatin applied only to bud 4 on intact plants had little effect, but the response of buds to zeatin increased with distance from the apex. Of significance is that bud 1, which did not extend follow-

ing decapitation, extended the most following an application of zeatin to this bud. Zeatin applied to the various lateral buds on decapitated plants produced a similar gradient of extension, with bud 1 extending the most, and bud 4 the least (Figure 22). However, the combined zeatin application and decapitation caused a greater extension of all buds than did zeatin application to intact plants. In addition, extension of bud 1 and bud 4 on decapitated plants was greater when these buds were treated with zeatin.
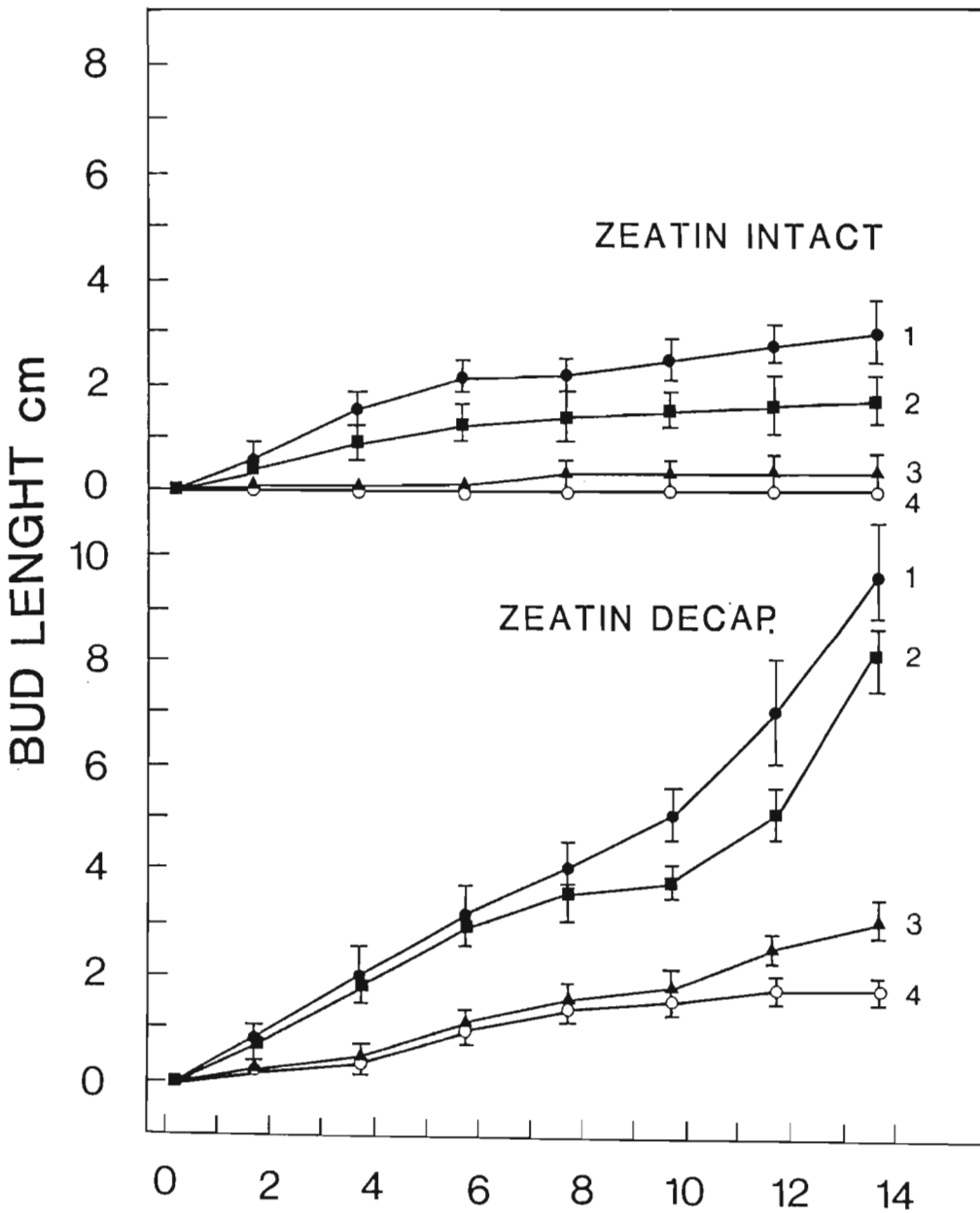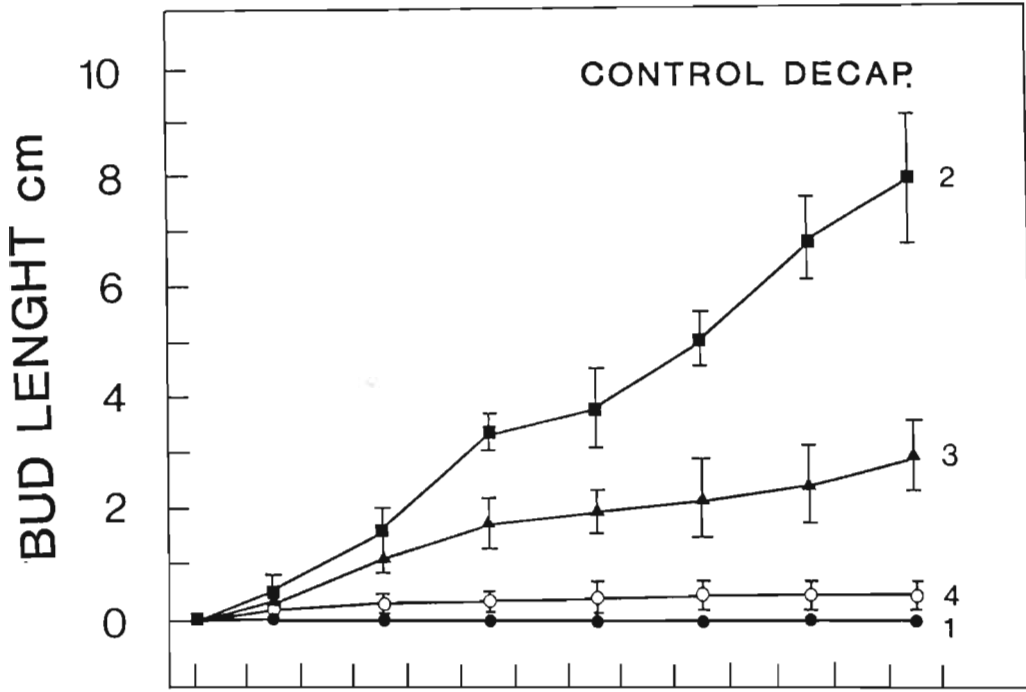
Applications of isopentenyladenine to inhibited buds of intact or decapitated plants caused the buds to respond in a dissimilar manner to those treated with zeatin. Isopentenyladenine applied to the buds of intact plants caused the extension of bud 2 and bud 3 but not bud 1 and bud 4 (Figure 23). Combining the isopentenyl-adenine application with decapitation allowed the treated buds to exert dominance first, and the overall growth of lateral buds 1, 3 and 4 was enhanced above the growth of buds on decapitated control plants (Figure 23). Bud 2 appeared to reach a maximum growth potential following decapitation, and further treatment with zeatin or isopentenyladenine did not enhance its growth.

Cytokinin application: concentration effects   Zeatin applied to the buds of intact plants at three concentrations suggested that certain buds are sensitive to cytokin-in concentration. Lateral buds 3 and 4 responded to higher concentrations of zeatin in a similar manner to buds in the previous experiment (Figure 24). Buds 1 and 2 responded better to lower concentrations of zeatin, suggesting that high concentrations of this cytokinin were inhibitory to these buds. On decapitated plants, growth of bud 1 increased with increasing concentration   of   zeatin. No difference was noted in the extension of buds 2 and 3 at different zeatin concentrations, but bud 4 appeared to respond better to lower concentrations of zeatin. Comparing the relative lengths of the untreated buds on the same plants
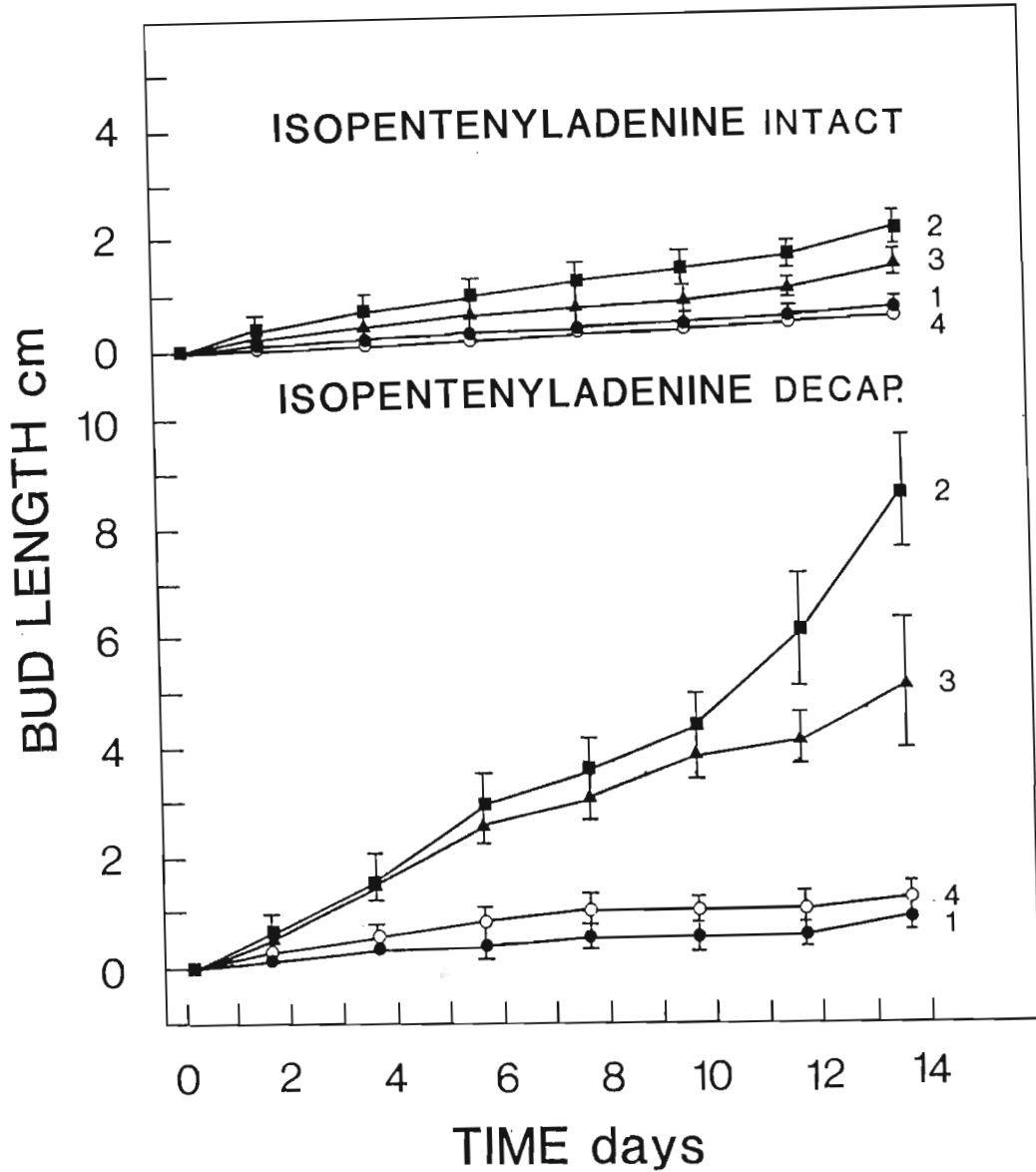
Figure 23   The extension of lateral bud 1, 2, 3 or 4 following a single application of isopentenyladenine to one bud only on intact or decapitated four-node Pisum sativum plants. Bud length was measured daily for 14 days. Buds treated with ethanol and polyethylene glycol on intact control plants remained inhibited. Bars represent standard errors.
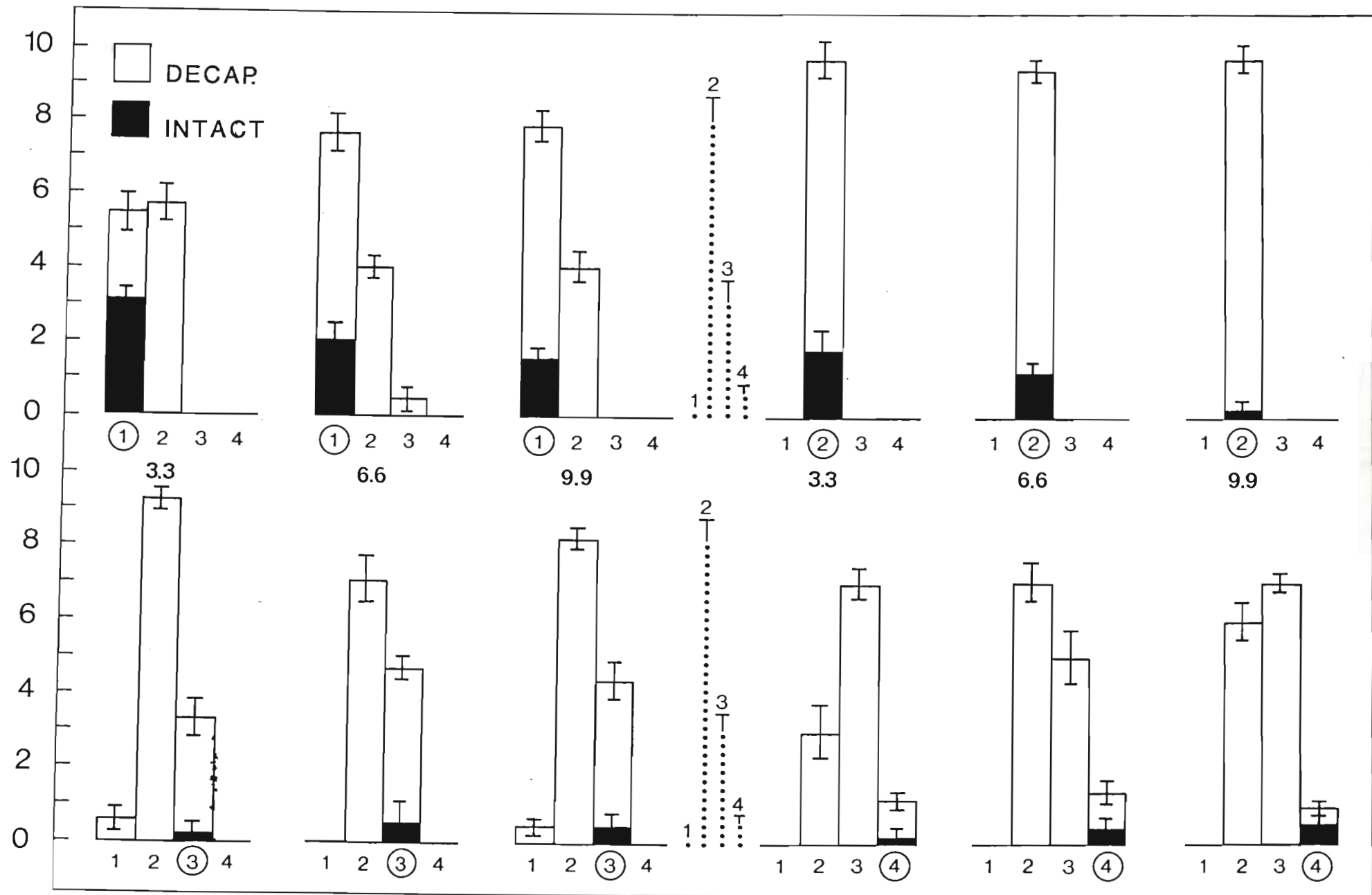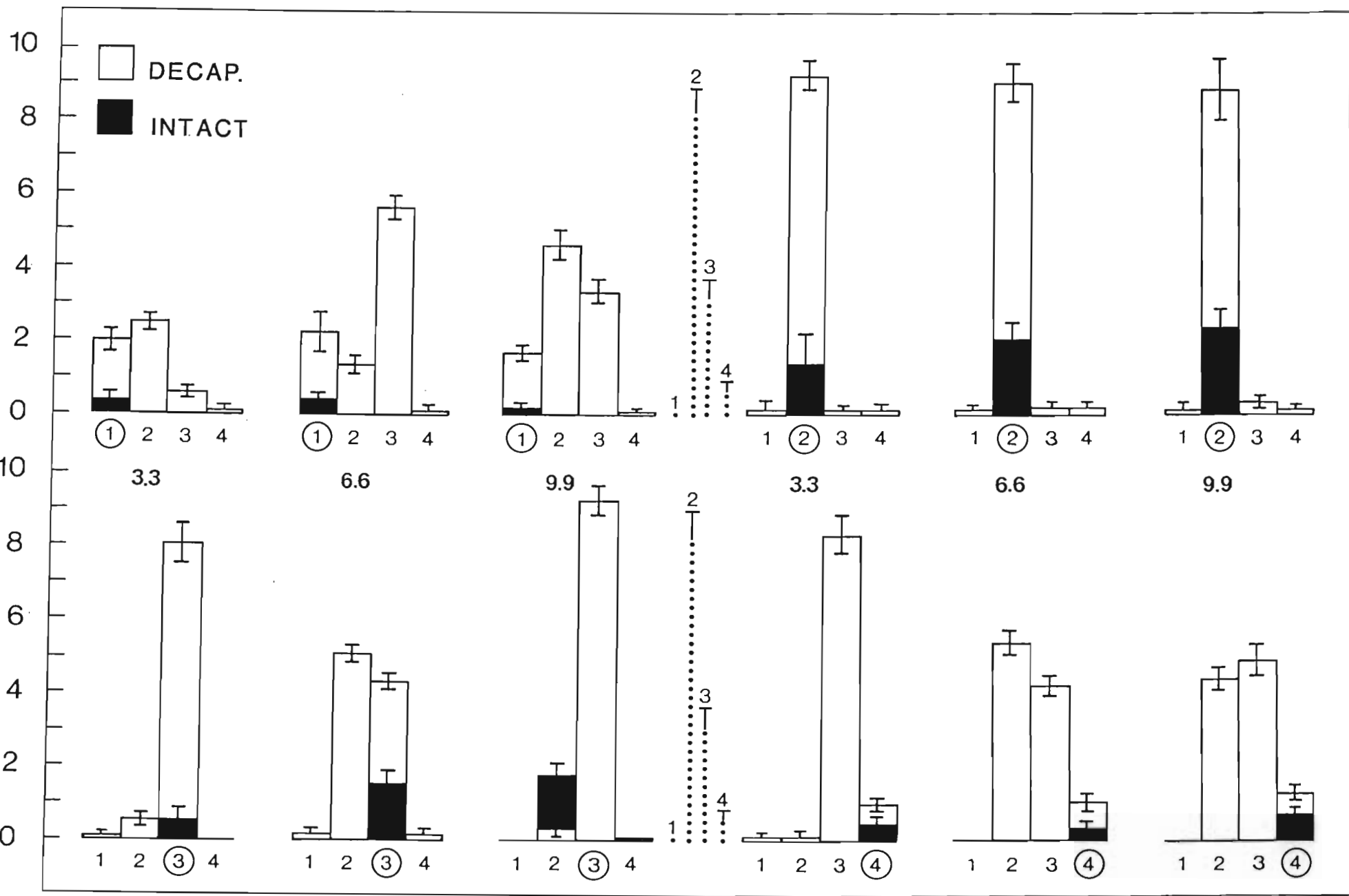
indicated that only bud 2 was able to exert complete dominance over the extension of other lateral buds. Bud 1 was found to exert a greater dominance over the extension of bud 2 with increasing zeatin concentration. Lateral buds 3 and 4 were less able to exert a dominating effect over the buds below them.

Applications of higher isopentenyladenine concentrations to bud 1 on intact plants failed to increase the ability of this bud to extend (Figure 25). Buds 2, 3 and 4 showed an increased ability to extend on the intact plant with increasing concentrations of isopentenyladenine applied. This effect was absent in the zeatin treatment (Figure 24). Bud 2 on the decapitated plant appeared to achieve maximum extension at all concentrations of isopentenyladenine applied and dominance over other buds on the same stem was complete (Figure 25). Extension of bud 3 was similar to that of bud 2 in this treatment, but where bud 2 had been able to assume partial dominance, extension of bud 3 was depressed. No difference in response of bud 4 to increasing isopentenyladenine concentrations was noted. However, the extension of buds below the treated bud indicated that they may be affected by the concentration of isopentenyladenine applied above them. When low concentrations of isopentenyladenine were applied to bud 4, only bud 3 asserted dominance, while at higher concentration both bud 3 and bud 2 were able to extend, in addition to the treated bud.

3.4 Discussion

The ability of the different cytokinins to release a lateral bud on Pisum sativum following a single application to the bud varied greatly, and appeared to parallel the same cytokinin biological activity recorded in the soybean callus bioassay. A similar, but not identical parallel profile of activity hierarchies was noted by SKOOG and ABDUL GHANI (1981) using the lateral buds of Pisum sativum and the tobacco callus bioassay. Since these two bioassays are both dependent on the stimulation of cell division and thus growth of the callus, it is very likely that

the mechanism of cytokinin release of lateral buds operates by a similar process. A comparison of these results and others of a variety of bioassays or biological systems reported in the literature is presented in Table 3. As can be seen, the relative activities of the cytokinins in the different bioassays vary considerably. These differences in activity hierarchies suggest (but do not prove) that this is due to the mechanism of action varying in the different biological systems used (LETHAM, 1967; GARRISON et al., 1984). The only consistent feature among the different bioassays reviewed is the low activities of isopentenyladenine and isopentenyladenosine in relation to the high activity of zeatin.

The differences in the cytokinin structure – activity relationships observed may be due to differences in cytokinin uptake (penetration into the tissue), their metabolism (stability) and possible differences in the structural requirements for cytokinin activity at the site/s of action in different plants (MOK et al., 1978; GARRISON et al., 1984). The performance of applied cytokinins in the experiments of this chapter will be discussed in relation to these factors.

Structure-activity relationships may reflect differences in the uptake of different cytokinins. HECHT, LEONARD, SCHMITZ and SKOOG (1970) found that the quantitative comparison between the activities of the cytokinin base isopentenyladenine and its riboside isopentenyladenosine was complicated by permeability differences. The penetration of the applied cytokinin into the plant tissue may also be dependent on the culture conditions used. Isopentenyladenine was very rapidly absorbed by tobacco callus cells via a liquid suspension medium, but not a solid agar medium (LALOUE et al., 1977). In addition, results of experiments employing different methods of cytokinin application could be inconsistent. Entry of cytokinins applied directly to the lateral buds is not likely to occur via the same route as xylem on root supplied cytokinins. Thus the observed biological response of a cytokinin may not be due only to its intrinsic activity.

Table 3   A comparison of cytokinin structure – activity hierarchies in various biological systems (1-6).*

Bioassay system	Decreasing biological activity of cyto-kinin tested ———→	Reference
Lateral bud growth[1]	BA ZR Z DHZ 1⁶Ade 1⁶Ado K DHZR iso-Z Z 1⁶Ade BA K BA=Z 1⁶Ade=K	SKOOG AND ABDUL GHANI (1981) PILLAY AND RAILTON (1983)
Soybean callus bioassay[2]	BA ZR Z DHZ 1⁶Ade 1⁶Ado K DHZ Z K	MILLER AND WITHAM (1964)
Tobacco callus bioassay[2]	Z 1⁶Ade K=1⁶Ado 1⁶Ade=K	SKOOG, HAMZI, SZWEYKOWSKA, LEONARD, CARRAWAY, FUJJI, HELGESON AND LOEPPKY (1967): LEONARD, HECHT, SKOOG AND SCHMITZ, (1968); LALOUE, TERRINE AND GUERN (1977). BEAUCHESNE AND GOUTAREL (1963).
Bean callus bioassay[2]	DHZ tZ cZ 1⁶Ade=1⁶Ado (cv.1) tZ DHZ cZ 1⁶Ade=1⁶Ado (cv.2)	MOK et al. (1978)
Soybean explant system[3]	DHZR BA DHZ ZR Z 1⁶Ade 1⁶Ado	GARRISON et al. (1984).
Leaf senescence bioassay[4]	BA K Z 1⁶Ade Z ZR DHZ DHZR	LETHAM (1967), BIDDINGTON AND THOMAS (1978) KUHNLE, FULLER, CORSE AND MACKEY (1977)
Papaw Shoot growth[5]	BA Z K 1⁶Ade	DREW AND SMITH (1986)
Spirodella growth[6]	Z=K 1⁶Ade 6.1 Z K 1⁶Ade 6.2	LETHAM (1967)

*   Results from this study have been included in bold for comparison. While the protocol for each system has usually been based on the key reference as indicated below, it is not implied that culture conditions, plant material and cytokinin concentration were standard throughout.

1. Lateral bud growth tests the relative activities of cytokinins in releasing lateral buds of Pisum sativum from apical dominance following a single application of cytokinin to the bud (SACHS and THIMANN, 1967).

2. The callus bioassays test the relative activities of cytokinins incorporated into the medium in promoting the cell division and growth activity of bean (Phaseolus sp.) (MOK, MOK and ARMSTRONG, 1978); tobacco (Nicotiana tabacum) (MURASHIGE and SKOOG, 1962, LINSMAIER and SKOOG, 1965) or soybean (Glycine max) (MILLER, 1963, 1965) callus in vitro.

3. The soybean explant system tests the relative activities of cytokinins incorporated into the medium of soybean (Glycine max) explants (leaf, pod and subtending stem segment) in retarding leaf yellowing, blade abscission and promoting pod development in vitro (GARRISON, BRINKER and NOODÉN, 1984).

4. The leaf senescence bioassay tests the relative activities of cytokinins in delaying the senescence of detached leaves of Nicotiana tabacum, Brassica pekenensis Rupr. or Raphanus sativus L. as determined by chlorophyll retention (OSBORNE and McCALLA, 1961).

5. Papaw shoot growth tests the relative activities of cytokinins in promoting shoot growth on pawpaw (Carica papaya L.) explants in vitro (DREW and SMITH, 1986).

6. Spirodella growth in darkness tests the relative activities of cytokinins in promoting the growth of Spirodella oligorrhiza in vitro as determined by an increase in fresh weight (6.1) and frond number (6.2) as done by HILLMAN (1957) with Lemna minor.

Abbreviations: BA, benzyladenine; DHZ, dihydrozeatin; DHZR, dihydrozeatin riboside; 1⁶Ade, isopentenyladenine; 1⁶Ado, isopentenyladenosine; K, kinetin; Z, zeatin; ZR, zeatin riboside.

However, the differences in the biological activity noted in the ten compounds tested for their ability to release inhibited lateral buds are likely to be, largely, a result of the intrinsic activities of the cytokinins due to their structure and to their subsequent metabolism in the plant following application. Natural cytokinins occur in different metabolic forms, the diversity of which may raise the following questions. Firstly, is the biological activity observed following a cytokinin application a result of the compound applied, or of a metabolite of that compound? Secondly, what is the significance of these metabolic transformations in terms of the regulation of apical dominance (or other growth processes) in the plant? GARRISON et al. (1984) has suggested that the differences between isopentenyladenine and zeatin effects may be due to their differential metabolism. This is highly significant in terms of the hypothesis of the thesis, and indicates a need for an introduction to cytokinin metabolism. This will be achieved by reviewing selected aspects of cytokinin metabolism as they become appropriate in this discussion and the discussion of the next chapter.

The structural requirements for a high order of cytokinin activity generally include an adenine molecule with the purine ring intact and with a N^6-substituent of moderate size (SKOOG and ARMSTRONG, 1970). Only N^6- adenine derivatives were found to be active in releasing the lateral buds of Pisum sativum from apical dominance. Many features of the N^6- side chain also markedly influence cytokinin biological activity, such as length, degree of saturation and configuration (SKOOG and LEONARD, 1968). Unsaturated side chains lead to higher activities than their corresponding saturated side chains (SKOOG and ARMSTRONG, 1970). Thus, the activities of zeatin (unsaturated) in releasing lateral buds was greater than than of dihydrozeatin with a saturated side chain, while isopentenyladenine (unsaturated) was similarly more active than isopentenyladenosine (saturated). Kinetin appeared to have the same lateral bud releasing activity as isopentenyladenine, but was ten-fold more active than isopentenyladenine in the

tobacco callus bioassay (ROGOZINSKA, HELGESON and SKOOG, 1964). Thus the presence of a double bond appears to confer stability on a cytokinin, with the loss thereof leading to a loss in activity (SKOOG and LEONARD, 1968).

The above interpretation assumes that the cytokinins applied were active in their original form, and were not metabolised by the plant. For example, it is not known to what extent the activity of the riboside isopentenyladenosine is derived from liberation of its free base isopentenyladenine. The conversion of cytokinin ribosides to the base is necessary for the expression of their stimulating effect on cell division (LALOUE and PETHE, 1982). Zeatin and zeatin riboside were clearly active in releasing lateral buds from apical dominance, but their putative precursors, isopentenyladenine and isopentenyladenosine, were relatively inactive. This was noted to be the trend in the other bioassays reviewed, and is considered to be the result of the allylic hydroxyl group in zeatin being a structural feature associated with high cytokinin activity (LETHAM, 1967).

Thus, the activity of a cytokinin may be related directly to its structure. However, applied cytokinins may also undergo four types of metabolic reactions:

  i)   interconversions of bases, nucleotides and nucleosides
 ii)   irreversible side chain modification
iii)   side chain cleavage leading to loss of biological activity
 iv)   conjugaion with sugars or amino acids leading to retention or loss of
       biological activity.

(HORGAN, 1986)

Therefore, some differences in the activity exhibited by applied cytokinins may be rationalised from a consideration of the relative stabilities of the metabolites formed (LETHAM and PALNI, 1983). In this way, the greater activity of dihydro-zeatin than zeatin in releasing lateral buds could be due to an inactivation by

side chain cleavage of zeatin but not the more stable dihydrozeatin (HENSON, 1978). In detached leaves of Phaseolus vulgaris, applied dihydrozeatin derivatives were more stable than the zeatin derivatives (PALMER, SCOTT and HORGAN, 1981a). Cytokinin stability may be conferred by the binding to a sugar, leading to the formation of a glucoside. Glucosides are important metabolites of both applied and endogenous cytokinins (LETHAM, TAO and PARKER, 1982), and may represent storage or protected forms (PARKER and LETHAM, 1973). In detached leaves of Phaseolus vulgaris, applied glucosides were more stable than their free bases (PALMER et al., 1981a). Likewise, 7- glucosylation, occurring significantly only with isopentenyladenine, protects the N^6- isopetenyl side chain from cleavage and confers stability (PARKER and LETHAM, 1973). In addition, a cytokinin-binding protein (CPD) which has a high affinity for isopentenyladenine and isopen-tenyladenosine has been isolated (POLYA and BOWMAN, 1979; KEIM, ERION and FOX, 1981). This CPD has a low affinity for zeatin (LETHAM and PALNI, 1983). A cytokinin thus bound to a CPD would probably be protected from enzymic degredation and could subsequently be released when the level of free cytokinin declined.

The activity of oxidase enzymes may be instrumental in regulating the nature and amounts of cytokinin occurring in a given organ. Cytokinins with saturated side chains (dihydrozeatin and dihydrozeatin riboside) and the synthetic cytokinins benzyladenine and kinetin are relatively resistant to attack by cytokinin oxidase which readily cleaves the unsaturated side chains of isopentenyladenine and zeatin and their ribonucleotides isopentenyladenosine and zeatin riboside (WITTY and HALL, 1974). In Phaseolus vulgaris the relative activities of a 'cytokinin oxidase' like degradation process (WHITTY and HALL, 1974) and a zeatin to dihydrozeatin interconversion mechanism in different parts of the plant effectively maintain a decreasing concentration gradient of zeatin derivatives and an increasing gradient of dihydrozeatin derivatives from the roots to the lamina (PALMER,

The biological activity of the cytokinin tested may thus be due, in part, to differences in metabolism as a result of enzyme activity. However, HORGAN (1986) has cautioned that the fate of externally applied cytokinins does not always mirror that of endogenous compounds, particularly in tissues which exhibit high rates of side chain cleavage.

The implications of cytokinin structure - activity relationships in the phenomenon of apical dominance should begin to be apparent following the above discussion. Since isopentenyladenine and zeatin both occur in the plant, yet differ greatly in their ability to release lateral buds when applied exogenously, it is possible that the modulation of the biological activity of these cytokinins via their metabolism may control the relative amounts of biologically active cytokinin arriving at the lateral bud site. Isopentenyladenine is the immediate precursor of zeatin in some systems (MIURA and MILLER, 1969; MIURA and HALL, 1973), and thus the correlative signal could be envisaged as acting on the promotion or inhibition of the enzyme system responsible for the hydroxylation reaction. A similar concept of metabolic control of cytokinin availability was proposed by LETHAM and PALNI (1983). They suggested that a large pool of cytokinin in a plant, bound to CPD and thus stabilized, could be in equilibrium with a pool of free cytokinins which would be subject to metabolism but maintained by synthesis. When the level of the free pool dropped, perhaps due to cessation of synthesis, free cytokinin would be released from the CPD.

A further investigation of the relative activities of isopentenyladenine and zeatin was thus undertaken on all lateral buds on both intact and decapitated plants.

The application of isopentenyladenine or zeatin to one of four lateral buds on intact or decapitated Pisum sativum plants yielded results suggesting that removal

of the shoot apex caused a change in response of the buds to these cytokinins. In addition, it appeared that the lateral buds responded differently to isopentenyladenine or zeatin application. Lateral buds nearer the intact shoot apex apparently had less ability to utilize the applied cytokinins than buds lower down on the stem. This gradient of potential to respond to exogenous cytokinins was likely a result of an interaction with the gradients of endogenous growth regulators in the plant, already discussed in Chapter 2. It is possible that this gradient of response was related to a difference in the metabolism of the applied cytokinins at each lateral bud. This concept was further investigated, the results of which will be presented in the following chapter. Of immediate concern is the question of how different lateral buds on the same plant can differ in their sensitivity (response capacity) to an applied cytokinin.

The hierarchy of lateral bud growth promotion by a cytokinin differed when isopentenyladenine or zeatin were applied to intact plants. Remembering that only one bud on a plant was treated at a time, the results are not directly comparable to the growth of lateral buds on decapitated control plants, but certain observations can be made. Zeatin was most effective in promoting lateral bud outgrowth when applied to bud 1, the lowest lateral bud, becoming less effective when applied to lateral buds nearer to the shoot apex. This effect was likely the result of zeatin or a zeatin derivative interacting with the endogenous levels of auxin at the lateral bud site. However, isopentenyladenine, when applied to lateral buds in a similar manner caused a different gradient of response. Here the hierarchy of lateral bud growth closely resembled that of buds released from apical dominance on decapitated control plants in that bud 2 was most sensitive to isopentenyladenine application, followed by bud 3, 4 and then by bud 1. This differential response of lateral buds on intact plants to isopentenyladenine or zeatin application suggests that the immediate response of the buds to these two cytokinins is different.

Bud 1, peculiar in its inability to extend even on the decapitated control plants, responded well to zeatin application. Does this imply a deficiency of zeatin at the lateral bud on the intact plant, or was this bud unable to fulfil its cytokinin requirement following decapitation? Isopentenyladenine application to this same bud on the intact plant was ineffective, indicating a possible inability of bud 1 to utilize or hydroxylate this cytokinin to zeatin. Similar analysis of the sensitivity of the other lateral buds to isopentenyladenine or zeatin suggests that certain buds may be in a better position to utilize both cytokinins to some degree, while other buds may respond to only one or the other. Since the greatest response was usually to zeatin and not isopentenyladenine, it may be postulated that inhibition of lateral buds on the intact plant is a result of a deficiency of zeatin at the bud site. A gradient of sensitivity may exist whereby only certain lateral buds are able to utilize exogenous isopentenyladenine, probably by metabolic modification of its structure and thus activity. Lateral buds most able to respond to this cytokinin were noted to be bud 2 and bud 3, which also appear to be least under the dominating influence of the shoot apex.

The growth of the lateral bud induced by cytokinin application on an intact plant was never as great as the growth of a corresponding bud on a decapitated control plant. This has been noted before (SCHAEFFER and SHARPE, 1969; SACHS and THIMANN, 1964, 1967; ALI and FLETCHER, 1970, 1971) and indicates that there is some residual type of apical inhibition which is not overcome by cytokinin application. This phenomenon was investigated further by the application of isopentenyladenine or zeatin to either bud 1, 2, 3 or bud 4 on decapitated plants. Again, a differential response was noted to cytokinin application in that the profile of extension following decapitation and isopentenyladenine application resembled that of the lateral buds on control decapitated plants, but zeatin similarly applied induced the greatest growth of bud 1, followed by bud 2, 3 and bud 4. Since only

one bud was treated on a plant, these treatments allowed for a distinction between the decapitation and the cytokinin effect. Thus, decapitation led to the growth of all lateral buds in a particular way. Addition of isopentenyladenine to one of these buds at the time of decapitation only enhanced the growth of bud 1, 3 or bud 4 over those on decapitated control plants. In other words, bud 2 on the decapitated plant was not as sensitive to applied isopentenyladenine as it was on the intact plant. Similarly, zeatin was only effective in promoting the greater growth of bud 1 or bud 4 on decapitated plants, indicating that bud 2 and bud 3 were no longer sensitive to exogenous zeatin once decapitation had occurred. A similar response was noted by NAGAO and RUBINSTEIN (1975), who found that benzyladenine, when applied to lateral buds on decapitated Pisum sativum plants, promoted greater bud growth than when applied to intact plants. Thus, removal of the shoot apex led to an increased sensitivity of only certain lateral buds to exogenous isopentenyladenine or zeatin. In this way bud 2 and bud 3 probably obtained optimal levels of zeatin following decapitation, and further applications of this cytokinin were not necessary for optimum growth. Lateral buds 1 and 4, which normally exhibit a high degree of inhibition, probably as a result of zeatin deprivation, responded well to exogenous applications of zeatin. Likewise, bud 2, insensitive to isopentenyladenine application on the decapitated plant can probably fulfil its zeatin requirements and so isopentenyladenine was not needed as a precursor for further zeatin. However, bud 1, 3 and bud 4, which could not utilize isopentenyladenine on the intact plant did so in the absence of the shoot apex. This indicates that the partitioning of isopentenyladenine in the intact plant may result in a gradient of this cytokinin along the shoot. On decapitation, a gradient of potential to extend would be directly related to the ability of the lateral bud to utilize this isopentenyladenine, probably by hydroxylating it to zeatin.

To summarise the discussion at this point, it may be stated that zeatin was biologically more active than isopentenyladenine in releasing inhibited lateral buds on

intact plants, yet neither of these two cytokinins promoted the growth of buds to a greater extent than those on decapitated plants. However, decapitation did result in an increasing ability of certain lateral buds to utilize isopentenyladenine, and may have been related to the metabolism of this cytokinin. At this stage, this proposal remains untested, but is likely to be dependent on the position a lateral bud occupies on the shoot.

To proceed, a comparison of the growth of treated and untreated lateral buds on the same plant revealed that cytokinins applied to one bud may exert their effect at other nodes. For example, if decapitation had initiated a strong metabolic sink strength at bud 2, cytokinins applied to bud 3 or bud 4 anterior to it, could probably have been translocated away from these buds. Only if the amount of cytokinin applied was sufficiently high would these lateral buds have been able to exert dominance over bud 2. Conversely, induction of bud extension using a cytokinin may have caused untreated lateral buds on the same plant to be inhibited. This was clearly illustrated in the responses of bud 2 and bud 3, and is considered to be the result of the induction of a strong sink at these buds when treated with a cytokinin. This effect was investigated further by applying a range of isopentenyladenine or zeatin concentrations to lateral buds of decapitated or intact plants, and recording the growth of treated as well as untreated lateral buds on the same plant.

The experiment introduced above was performed for two reasons. Firstly, it was expected that possible differences in the responses of lateral buds to cytokinin concentration would provide further explanation for the differential responses to isopentenyladenine and zeatin already noted. Secondly, if lateral buds supplied with cytokinin in this manner did respond differently, possible compensatory effects between the treated buds would provide further information on the correlation between all lateral buds on the plant. Indeed, since the hypothesis

of the thesis relied on the traditional but currently unfavourable premise that (some) developmental responses of plants are dependent on changes of growth regulator levels or concentrations, it was decided to investigate the relationship, if any, of lateral bud growth and cytokinin concentration.

A detailed analysis of these results in relation to the growth substance sensitivity / concentration debate (TREWAVAS, 1981, 1987; TREWAVAS and JONES, 1981; TREWAVAS and CLELAND, 1983; FIRN and DIGBY, 1984; FIRN, 1986; DAVIES, 1987; WEYERS, PATERSON and A'BROOK, 1987) is beyond the scope of this study, but certain terminology had best be defined before proceeding. The ensuing discussion will attempt to describe, quantitatively, the sensitivity of the lateral buds to endogenous cytokinins by referring to their responses to the exogenously applied isopentenyladenine and zeatin. In this case, 'sensitivity' will refer to the capability of an organ or plant to respond to a particular growth substance (TREWAVAS and CLELAND, 1983). Thus, a change in sensitivity will refer to the observation that the response to a given amount of applied cytokinin has changed (DAVIES, 1987). This of course will assume that uptake efficiency of the lateral buds for isopentenyladenine and zeatin is equal. In this way, the response of lateral buds to apical dominance will be viewed as being a result of sensitivity variations occurring in the plant during growth and development, provided that when sensitivity changed, there would be a response, to a change in concentration, of a different magnitude.

In general, it was found that high concentrations of zeatin were inhibitory to lateral bud growth on the intact plant, but high concentrations of isopentenyladenine were required to effect the release of lateral buds on similar plants. In addition, lateral buds closer to the apical bud required higher levels of both cytokinins to effect bud release. This indicates that the sensitivity of

a particular lateral bud differed with the application of isopentenyladenine or zeatin, and may have been related to:

i)   the requirement of that bud for a particular cytokinin,

ii)   the ability of the bud to utilize (metabolise) the applied cytokinin, or,

iii)   the threshold values of the cytokinin already at the lateral bud site.

The gradient of response observed along the shoot axis again implicated the gradient of auxin in the plant as a controlling factor in apical dominance.

Certain lateral buds (bud 1 and bud 2) were inhibited by high zeatin concentrations when on the intact plant. This suggests that zeatin levels in these buds were near optimal before cytokinin application, and thus high zeatin applications to these non-growing buds produced a toxicity effect. This was expected of bud 2, which has demonstrated an intrinsic advantage over the other lateral buds. However, bud 1, completely inhibited on decapitated control plants, would only respond to lower concentrations of zeatin. An increased sensitivity to isopentenyladenine by bud 2 on intact plants was noted with increasing isopentenyladenine concentration, indicating that this bud had a certain ability to utilize this cytokinin in the presence of the shoot apex. Similar application of isopentenyladenine to bud 1 led to the observation that this bud may lack an ability to utilize isopentenyladenine. Zeatin applied to bud 3 or bud 4 on intact plants induced an increase in growth with increasing cytokinin concentration. This increased sensitivity to zeatin may be due to a deficiency of zeatin as a result of unequal partitioning of root produced cytokinins in the whole plant, or a higher requirement for zeatin at these lateral buds resulting from the higher levels of inhibitory auxin at the lateral buds. In this way, higher isopentenyladenine concentrations to these same lateral buds on intact plants increased growth somewhat. These results of isopentenyladenine or zeatin application to lateral buds on intact plants indicate that the sensitivity of a lateral bud to an applied

cytokinin will depend on the position the lateral bud occupies on the plant, and is most likely related to a bud's ability to metabolise the applied cytokinin. This provides further evidence, if only circumstantial, for the controlling role of cytokinin specificity and metabolism in regulating the relative amounts of specific cytokinins available to the lateral buds and thus their ability to overcome apical dominance.

Decapitation of the plants led to a change in sensitivity of the lateral buds to applied cytokinin. An increase in the ability of lateral bud 1, 3 and bud 4 to respond to applied zeatin indicated that zeatin was no longer inhibitory at high concentrations. This was not unexpected, since the actively growing lateral buds released by decapitation could now utilize the applied cytokinin. However, bud 2 did not exhibit a change in sensitivity to either applied cytokinins following decapitation, since it was likely to fulfil its own cytokinin requirements as a result of its vigorous sink strength. Further indications of a change in sensitivity following decapitation were observed on bud 3, which increased markedly in sensitivity to increased isopentenyladenine application. Thus it is likely that this lateral bud could not achieve self sufficiency in cytokinin in the presence of other growing but untreated lateral buds, and so had to utilize the higher levels of applied isopentenyladenine, possibly by hydroxylating it to zeatin.

Such a change in response following decapitation was noted much earlier by SACHS and THIMANN (1967), who found a major difference in sensitivity to apical inhibition between growing and non growing lateral buds on Pisum sativum shoots. These authors concluded that breakage of such inhibition must entail a major sensitivity change. Support for this concept was provided by NAGAO and RUBIN-STEIN (1975), who demonstrated that removal of the shoot apex of Pisum sativum did not lead to an increase in sensitivity to applied benzyladenine, but that high concentrations of benzyladenine were no longer supraoptimal for growth when compared with the growth on intact plants.

The observed change in sensitivity of a lateral bud to exogenous cytokinin following decapitation also provides a satisfactory explanation of results in the literature long considered to be anomalous with the concept of a control of lateral bud growth by changes in the levels of growth regulators in the plant. TUCKER and MANSFIELD (1972, 1973) observed a decline in cytokinin levels in lateral buds of Xanthuim strumarium following decapitation, while the cytokinin levels of Pisum sativum did not, as apparently expected, increase after similar treatment (NAGAO and RUBINSTEIN, 1975). Likewise, lateral buds lower down on a Rosus stem, subject to inhibition, contained higher levels of cytokinin than upper, uninhibited buds (VAN STADEN, SPIEGELSTEIN, ZIESLIN and HALEVY, 1981). These results, together with the sensitivity changes observed in this study, clearly suggest that inhibited lateral buds are unable to utilize certain cytokinins, even at high concentrations, until the inhibitory signal is removed. Cytokinin levels in lateral buds do not increase following decapitation because they are being rapidly utilized for growth and development. If anything, they should, and do decline. Thus measurements of cytokinin concentrations provide little information about the concentration of physiologically active cytokinins at the lateral bud site, unless they can be related to the changes in the bud's sensitivity to them.

A final aspect of the concentration experiment to be discussed is the observation that increasing the concentration of a cytokinin to a lateral bud could induce an increase in the compensatory growth exhibited by other untreated lateral buds on the same plant. This compensatory affect was noted and discussed in Chapter 2. Increasing zeatin application to bud 1 on decapitated plants resulted in a decrease in the growth of the untreated bud 2 on the same plants. However, other correlative effects were also evident. Zeatin applied to bud 4 in increasing concentrations reduced the growth of untreated bud 3 while increasing, in a compensatory manner, the growth of bud 2. Thus a 'chain of events' could occur between two or more lateral buds on a shoot, whereby, depending on the response

of a given bud to an applied cytokinin, the other lateral buds would exhibit a secondary or tertiary effect as a result. This may occur, in part, as a result of a sink effect controlled by a partitioning of resources in the plant. Alternatively, the cytokinin may be exported from the lateral bud if applied in high enough concentrations, and exert an effect elsewhere.

To conclude the discussion of this chapter, it may be noted that a consistent feature of the cytokinin application experiments was the low biological activity of isopentenyladenine in releasing a lateral bud from inhibition in relation to the high activity of zeatin. This difference in the structure – activity relationship of these two cytokinins is likely the result of a particular structural requirement for cytokinin activity at the lateral bud site. Experiments with isopentenyladenine and zeatin indicated that lateral buds differ in their sensitivity to each of these two cytokinins, probably as a result of differences in their metabolic capacity. Decapitation of the shoot apex resulted in a change in sensitivity of the lateral buds to applied cytokinin. Lateral buds were more able to utilize the applied isopentenyladenine, and high zeatin concentrations were no longer supraoptimal for growth. This suggests that following removal of the inhibitory signal, certain lateral buds were able to hydroxylate the applied isopentenyladenine to zeatin. These responses occurred as a gradient along the lateral buds of the stem, and thus implicate apically synthesized auxin in a controlling role of apical dominance.

It became obvious that any further studies into the concept of a control by changes in cytokinin structure at the lateral bud site could only proceed via an investigation of the metabolism of the applied cytokinin, especially isopentenyladenine. It now became necessary to know the fate of the applied isopentenyladenine in relation to its distribution in the whole plant and its metabolism. For example, could the growth potential of a particular lateral bud be related to its ability

to hydroxylate applied isopentenyladenine to zeatin, and what would be the effect of removal of the shoot apex on this same process? These questions and others were investigated in the following chapter, using the radiolabelled cytokinin $[^3H]$-isopentenyladenine.

CHAPTER IV

CYTOKININ METABOLISM IN RELATION TO APICAL DOMINANCE

4.1 Introduction

The most fundamental control mechanisms of plant growth and development are likely to operate at the levels of enzymic regulation of metabolism (CHEN, 1981). Thus, the rate of cytokinin biosynthesis, transport, interconversion and degradation are all important in determining the physiological responses controlled by this growth regulator. Cytokinin metabolism studies have the potential to yield information concerning the functions and rates of turnover of cytokinins as well as information on cytokinin biosynthesis (HUTTON, 1982). From such studies, one can attempt to elucidate the molecular basis for the diverse growth regulatory activities of cytokinins (COWLEY, DUKE, LIEPA, MACLEOD and LETHAM, 1978). However, studies of cytokinin metabolism in the whole plant are of lesser physiological relevance if their physiological roles are not clear. Very few studies have been performed in which cytokinin metabolism has been closely related to plant development.

The metabolic studies in this chapter have been conducted in order to document the metabolism of isopentenyladenine in the Pisum sativum plant, and to ascertain whether or not this metabolism is related to the developmental process of apical dominance.

4.2 General Materials and Methods

A description of the general methods pertaining to the application, extraction and tentative identification of the $[^3H]$ isopentenyladenine or its metabolites will first be presented. The exact protocol of each metabolism experiment will be presented later in the relevant section.

Plant material and isolation of organs. Four-node or ten-node Pisum sativum plants, cultivated under standard conditions described in Section 2.2.1 were used for all experiments unless indicated otherwise. Where different organs were required for incubation studies, the intact four-node plants were divided into leaves, stems, roots and nodal explants bearing a stipule and an inhibited lateral bud. Leaves were derived from the upper (fourth) node of the plants; stems from internodal segments between the basal (first) and second node, and the second and third node. Nodal explants were taken from the second node. Root tip sections 20 millimeters in length were removed from the lateral roots. In the studies on stem metabolism, stem sections representing an entire internode were used, and were designated stem section 1 – 4 as illustrated in Figure 11 of Section 2.2.1.

Incubation of plant organs. Replicates of one gram of fresh tissue were incubated in 100 millilitres of distilled water containing 60×10^6 Bq of $[^3H]$ isopentenyladenine obtained from Amersham. The purity of the radiolabelled sample was previously determined and confirmed using thin layer chromatography (TLC), as described later in this section. The explants were incubated at 25°C on a shaker in the light, with the exception of root cultures which were maintained under identical conditions in the dark.

Harvesting of plant tissues. The tissues from whole plant in vivo studies and the organs from in vitro studies were removed after the required incubation time, washed in running distilled water and then frozen with liquid nitrogen.

Extraction of $[^3H]$ isopentenyladenine metabolites. To extract metabolites, the tissues were homogenised in cold 80 per cent ethanol and filtered through Whatman paper No. 1. The ethanol extract was taken to dryness in vacuo at 40°C, redissolved in three millilitres of 80 per cent ethanol and passed through a millipore filter (0.22 micrometers). The samples were taken to dryness in vacuo and redissolved in filtered distilled water. Samples of 100 microlitres were then analysed using TLC or high-performance liquid chromatography (HPLC).

Tentative identification of $[^3H]$ isopentenyladenine metabolites

i) **Thin layer chromatography:** Extracts were spot-loaded onto silica-gel-coated TLC plates ten millimetres from the base and developed in an ascending manner in two dimensions. Firstly, the plates were run in a solvent comprising n-Butanol : acetic acid : water (12 : 3 : 5) and secondly in a solvent comprising n-Butanol : ammonia : water (6:1:2) (upper phase). Once the solvent front had advanced approximately 150 millimetres from the origin, it was marked and the plates were dried in a stream of warm air. The plates were divided into ten R_f zones with each R_f zone being scraped off and placed in a 10 millilitre scintillation vial.

ii) **High-performance liquid chromatography** A Hypersil 5 ODS column (5 μm, C_{18} bonded, 250 x 5 mm i.d.) with a flow rate of one millilitre per minute, fitted to a Varian 5000 liquid chromatograph was used. Separation conditions were as follows. The aqueous buffer consisted of 0.2 Molar acetic acid adjusted to pH 3.5 with triethylamine. Samples were eluted with a linear gradient of methanol

(five to fifty per cent over 90 minutes) **in triethylamine** buffer. Absorbance was recorded with a Varian variable wavelength monitor at 265 nanometres, fitted with an 8 microlitre flow-through cell. One millilitre fractions were collected. Only 100 microlitres of each one millilitre (one minute) fraction were used for determination of radioactivity, with the remainder stored for subsequent analysis. Further separation of radioactive peaks co-eluting with isopentenyladenine and isopentenyladenosine was achieved using a Supelcosil LC 18 DB column (250 x 4.6 i.d.) with a flow rate of one millilitre per minute, in the manner described. This column yielded a superior separation of isopentenyladenine from isopentenyladenosine.

iii) **Determination of radioactivity:** Samples recovered from TLC plates were eluted with one millilitre of 100 per cent methanol before being treated as for the fractions recovered from HPLC. All fractions were counted in Ready Solv EP Scintillation fluid (Beckman) with a Beckman LS 3800 Scintillation counter. Individual radioactive peaks determined in this way were further investigated by chemical or enzyme treatment, and then re-chromatographed against authentic cytokinin standards using HPLC as described.

Chemical and enzyme treatments

i) **β-glucosidase treatment** To test for the presence of cytokinin glucosides, fractions collected after HPLC analysis were dried and redissolved in one millilitre of 0.03 Molar acetate buffer (pH 5.3). After adding 1.5 milligrams of β-glucosidase (Sigma – from almonds), the solution was incubated at 37°C for one hour. The reaction was terminated by adding two millilitres of 80 per cent ethanol and the solution was then filtered using a millipore filter (0.22 micrometres) before being taken to dryness in vacuo. The sample was then redissolved in 100 micro-litres of distilled water and analysed by HPLC. This procedure was adapted

from LEE, MOK, MOK, GRIFFIN and SHAW (1985). Since β-glucosidase will only hydrolyse the O-glucosides, compounds remaining after treatment may represent the 7- and 9- cytokinin glucosides resistant to this enzyme (HOAD, LOVEYS and SKENE, 1977; PALMER et al. 1981a) or other possible compounds.

ii) **Hydrochloric acid (HCl) treatment** was performed on metabolites suspected of having riboside- or glucoside substituted side chains. Side chain cleavage was achieved by hydrolysing the sample in boiling 1N HCl (two millilitres) for one hour before being dried *in vacuo*. The sample was then redissolved in 100 microlitres of distilled water and analysed by HPLC.

iii) **Potassium permanganate (KMnO$_4$) oxidation** was performed on metabolites suspected of having an unsaturated side chain. The fraction was dried and then redissolved in one millilitre of distilled water. A few drops of 0.01 per cent aqueous solution of KMnO$_4$ were added to the sample until the pink colour persisted for more than a few seconds (MILLER, 1965). An excess of 80 per cent ethanol was added and the resulting precipitate was removed with a millipore filter (0.22 micrometres). The sample was taken to dryness *in vacuo* before being redissolved in 100 microlitres of distilled water and analysed by HPLC. The double bond in the allyl group (isopentenyl side chain) of a cytokinin is broken and oxidised by such a treatment (MILLER, 1965).

iv) **Alkaline phosphatase treatment** was performed on metabolites suspected of being nucleotides. The dried sample was taken up in two millilitres of buffer (0.01 Molar magnesium chloride; 0.1 Molar tris-hydroxymethylaminoethane at pH 8.2) to which was added two milligrams of alkaline phosphatase (Sigma - from calf intestine) (MILLER, 1965). The sample was incubated at 30°C for 12 hours after which the reaction was terminated with an excess of 80 per cent ethanol.

The sample was then taken to dryness in vacuo before being redissolved in 100 microlitres of distilled water and analysed by HPLC. Alkaline phosphatase treatment causes the hydrolysis of nucleotide fractions with the subsequent detection of the base or its riboside (VAN STADEN and MENARY, 1976; VAN STADEN and DAVEY, 1977).

4.3 Application of $[^3H]$ isopentenyladenine to bud 2 on the whole plant

4.3.1 Materials and methods

Four-node Pisum sativum plants, cultivated under the standard conditions described were treated as follows. The $[^3H]$ isopentenyladenine was applied, as described in Section 3.2.1, to bud 2 on the intact plant or on plants decapitated simultaneously. A third treatment was performed in which the shoot apex of the plant was decapitated 24 hours prior to cytokinin application. Twenty replicates were used per treatment. At intervals of four, eight and twelve hours, the plants were harvested and separated into the roots, lateral bud explants from each of four nodes, leaf 3 and leaf 4 and the shoot apex (Figure 26). A lateral bud explant comprised the very small bud and its subtending nodal tissue. The fresh plant material was weighed into one gram samples which were immediately frozen in liquid nitrogen. Extraction of $[^3H]$ isopentenyladenine or its metabolites from these samples for total radioactivity analysis was performed as described in Section 4.2.

4.3.2 Results and discussion

Analysis of the distribution of total radioactivity recovered from the different plant organs provided an overview of the distribution of applied $[^3H]$ isopentenyl-adenine or its metabolites over a 12 hour period (Figure 27). On the intact
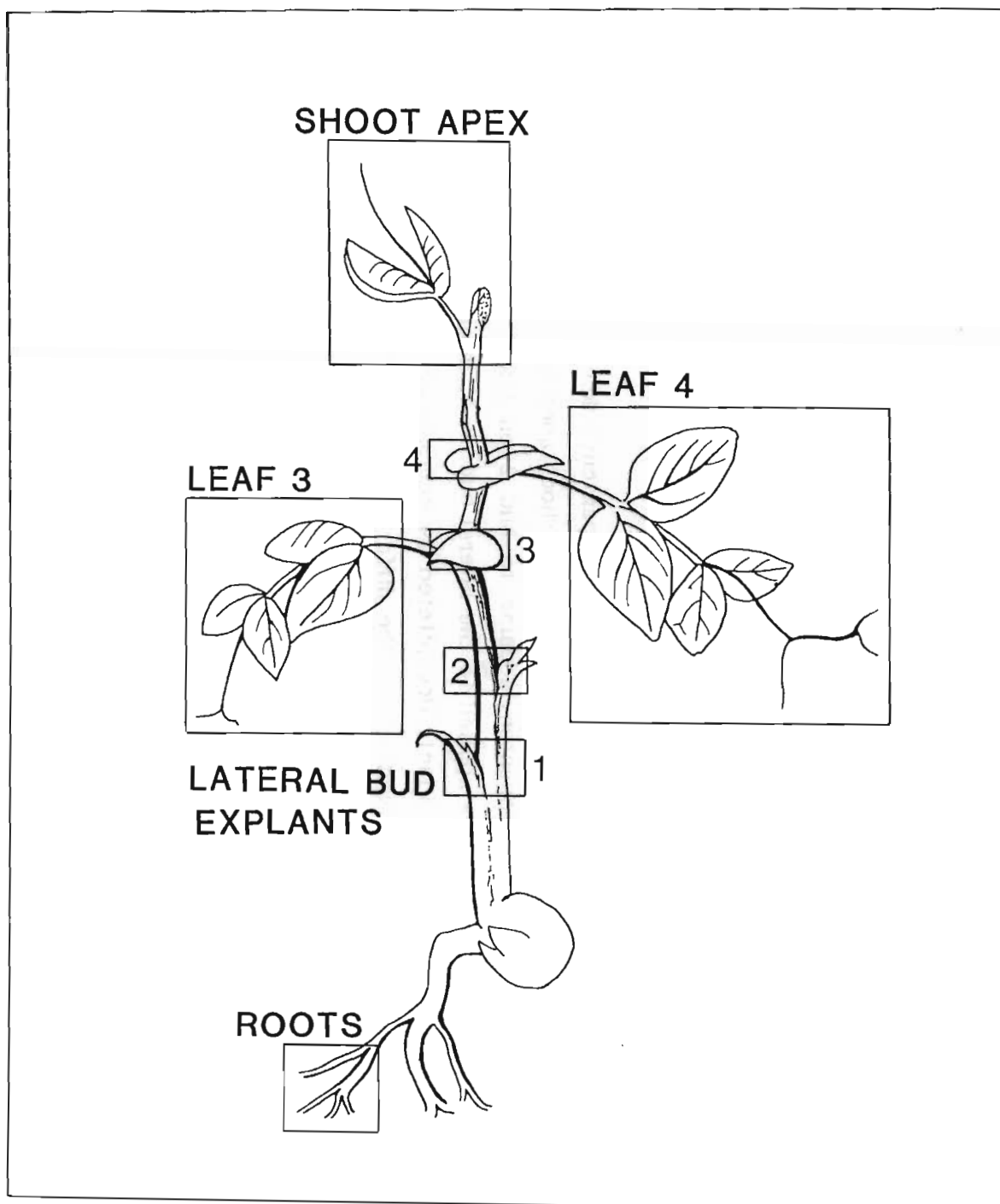
Figure 26 Explants excised following application of $[^3H]$ isopentenyladenine to lateral bud 2 on intact or decapitated Pisum sativum plants.
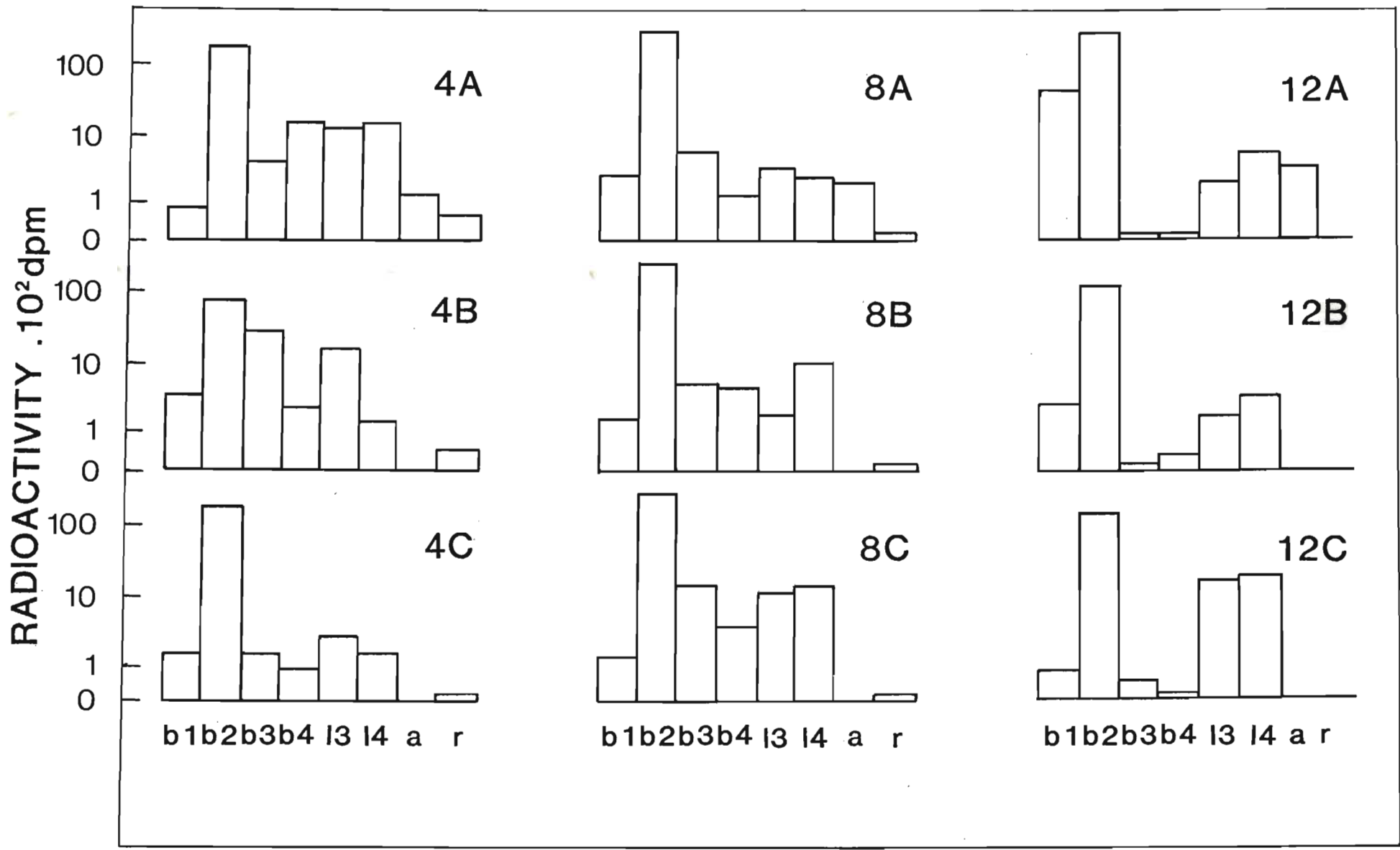
plant,the total radioactivity recovered was highest at bud 2, the site of application, but increased acropetally in the leaves and shoot apex with increasing time. This is in support of MORRIS and WINFIELD, 1972; CHANG and GOODIN, 1974 and PROCHAZKA et al., 1977, 1983, who demonstrated that cytokinins applied to intact plants move in an acropetal direction to regions of high meristematic activity. Thus the shoot apex was found to accumulate radioactivity with time. However, since cytokinins may be transported acropetally in the xylem and bi-directionally in the phloem (VAN STADEN and DAVEY, 1979), as well as more laterally between the xylem and the phloem (VAN STADEN and DAVEY, 1981; JAMESON et al., 1985), it was not surprising that radioactivity was recovered in all plant organs extracted.

The total radioactivity in the roots of intact plants declined to negligible amounts over 12 hours. It is likely that the metabolites associated with this radioactivity were incorporated into other compounds which were then exported to the shoot system. This may be correlated to the increase in radioactivity noted in the leaves and shoot apex with time. The lateral buds 3 and 4 (and their sub-tending nodal tissue), above the site of $[^3H]$ isopentenyladenine application, contained a high level of radioactivity after four hours which declined to negligible amounts after 12 hours. Since these lateral buds were not growing on the intact plant, it is unlikely that the labelled cytokinin was being utilized. It is possible that the high levels of radioactivity were associated with the acropetally moving labelled cytokinin in the phloem of the subtending stem tissue of these explants. However, since the levels of radioactivity remained constantly high at the site of application, it is unlikely that a 'preferential sink' effect at the shoot apex was solely responsible for the decline noted at bud 3 and bud 4. Concommitant with this decline was an increase in the radioactivity in leaves 3 and 4, the leaves associated with these lateral buds. It is therefore likely that part of this radio-

activity moved to the leaves. Application of $[^{14}C]$ zeatin to the xylem of Lupinus albus L. plants resulted in most of the radioactivity being recovered in the leaves (DAVEY and VAN STADEN, 1981). The major compounds recovered from the Lupinus albus leaves were found to be zeatin riboside and zeatin-0-glucoside, and reflect similar findings of HENSON (1978). It is possible that the increase in radioactivity at leaf 3 and leaf 4 was the result of the accumulation of radioactive metabolites and their conversion to glucosides. This glucosylation process may represent an attempt to inactivate excess cytokinins (VAN STADEN, 1976a) or prepare them for export from the leaves (DAVEY and VAN STADEN, 1981).

Of interest is that radioactivity associated with the $[^{3}H]$ isopentenyladenine applied to bud 2 moved basipetally to bud 1 and accumulated in high levels with time. This indicates an inability of this lateral bud to utilize this cytokinin, and in the absence of a subtending leaf, the radioactivity accumulated.

The change in the pattern of distribution of radioactivity occurring after the plants were decapitated was consistent with the view, expressed in Section 1.5.1.2, that a marked change in the translocation of cytokinins occurs towards lateral buds on plants released from apical dominance by decapitation (MORRIS and WINFIELD, 1972; PROCHAZKA et al., 1983). In plants treated with $[^{3}H]$ isopentenyladenine and decapitated simultaneously, a rise in the level of radioactivity recovered from lateral buds 3 and 4 was noted after four hours, but then declined rapidly until after 12 hours, negligible amounts remained. This effect could be the result of two processes occurring in the plant. Firstly, since the cytokinin was applied simultaneously with decapitation, the auxin gradient in the stem was still intact and the applied $[^{3}H]$ isopentenyladenine (or a metabolite thereof) was mobilized to the region of high auxin activity at the cut stump. The levels

of radioactivity at bud 3 and bud 4 were found to parallel the levels at leaf 3 and leaf 4 respectively. As the auxin levels declined down the stem with time, it is possible that less radioactivity associated with cytokinin metabolites was polarized in an acropetal direction. Secondly, since the lateral buds would have commenced active growth four to eight hours after decapitation, they may have utilized the radioactive isopentenyladenine. This effect was not apparent at bud 2 because it was saturated with supraoptimal levels of $[^3H]$ isopentenyladenine. Since the levels of radioactivity recovered from leaf 3 and leaf 4 appeared to parallel the radioactivity at their associated lateral buds, it is unlikely that a decline in the radioactivity of the latter would have been a result of glucosylation at the leaves. Such a process would have, in any case, resulted in an increase in radioactivity at the leaves. Cytokinin glucosides which accumulate during periods of slow growth appear to be hydrolysed to active cytokinins within the leaf during periods of active growth (HENDRY, VAN STADEN and ALLAN, 1982). This may have contributed to the decline in radioactivity in the leaves. The sequence of radioactivity distribution occurring after decapitation of the plant suggests that the optimal utilization of the $[^3H]$ isopentenyladenine began between four and eight hours after application, and this, together with the sequence of declining radioactivity in the leaves, indicates the beginning of a cytokinin requirement by the lateral buds.

Bud 1, below the point of cytokinin application accumulated a lower but consistent level of radioactivity than its counterpart on the intact plant. This may have been due to this bud being ineffective in mobilizing the $[^3H]$ isopentenyladenine in the presence of the other metabolic sinks (lateral buds 2, 3 and 4) or it may have rapidly exported the metabolites once they had been metabolised. It was suggested in the previous chapter that bud 1 appears to be able to utilize certain cytokinins if applied exogenously, but does not retain endogenous cytokinins for its own use.

Decapitation of the plants 24 hours prior to the $[^3H]$ isopentenyladenine applica-tion resulted in a similar distribution of radioactivity in the whole plant, but a reduction in radioactivity was noted in bud 3 and bud 4. This is likely the result of the inhibitory gradient of auxin having dispersed in the shoot, so that the localized application of $[^3H]$ isopentenyladenine to bud 2 would by now have resulted in the establishment of a strong sink at this bud. Thus bud 3 and 4, due to their reduced growth rates, would have been unable to mobilize the radiolabelled cytokinin. Unlike the previous treatment (simultaneous $[^3H]$ isopentenyladenine application and decapitation) which resulted in a parallel decline of radioactivity in both the lateral buds and the leaves, in this treatment the radioactivity in the leaves was found to increase. This supports the previous claim that the upper lateral buds were not growing, and therefore cytokinins glucosylated in the leaves were not needed for growth. In this way, the mainte-nance of the storage forms of cytokinin were not the cause of the decline in growth at lateral buds 3 and 4, but a result of their inhibition by the competitive effects of bud 2 below them.

In both decapitated treatments, the initial amount of radioactivity extracted from the roots was found to decline with time. It is likely that radioactivity initially transported to these roots was later required for growth in the form of a metabolite of $[^3H]$ isopentenyladenine.

The distribution of total radioactivity in the Pisum sativum plant following applica-tion of $[^3H]$ isopentenyladenine thus supports the view, expressed in the literature review and demonstrated in Chapters 2 and 3, that the distribution of cytokinin in relation to auxin gradients in the plant are important in maintaining the inhibi-tion of lateral buds. In addition, decapitation appears to result in the redistribution of cytokinins in the plant which can be related to the changes in the growth of the lateral buds.

Further identification of the radioactive metabolites was not attempted since it was felt that the possibility of invalid results would not warrant such an enormous undertaking ($\pm$ 720 HPLC analyses). VAN STADEN (1979) has stated that the occurrence of cytokinins in an organ can only be related to synthesis if the possibility of transport from other organs or tissues is completely eliminated.

It was therefore decided to investigate the metabolism of $[^3H]$ isopentenyladenine in isolated roots, leaves, stems and lateral bud explants of Pisum sativum. While cytokinin biosynthesis in isolated explants is not necessarily the same as in whole plants, CHEN, ERTL, LEISNER and CHANG (1985) are of the opinion that it is a valid measure of biosynthetic potential.

4.4 The metabolism of $[^3H]$ isopentenyladenine by isolated organs

4.4.1 Materials and methods

Explants of roots, stems, lateral buds and leaves were obtained from plants of Pisum sativum as described in Section 4.2. Replicates of one gram of fresh tissue were incubated in distilled water containing $[^3H]$ isopentenyladenine, harvested after three hours and six hours, extracted, and the metabolites tentatively identified by HPLC and TLC as described in section 4.2.

4.4.2 Results

Metabolism of $[^3H]$ isopentenyladenine by the roots Five major radioactive peaks were recovered from extracts of root tissue after three hours and six hours incubation (Figure 28). Four peaks were found to co-elute with adenine, adenosine, trans-zeatin riboside and isopentenyladenine / isopentenyladenosine. The amount

Figure 28 The distribution of [3H] isopentenyladenine derived radioactivity from root extracts (- - - - -) and the UV-trace of authentic cytokinin standards (————) after separation by HPLC using a Hypersil 5 ODS column. **A,** Total extract after 3 hours. **B,** Total extract after 6 hours. **C,** Peaks (49 - 53 minutes) for 3 and 6 hours after treatment with HCL. **D,** Peak (73 - 90 minutes) for 3 hours after further separation using a Supelcosil LC 18 DB column or TLC (vertical bars). **E,** Peak (75 - 90 minutes) for 6 hours after further separation using a Supel-

of radioactivity associated with these peaks remained constant for three hours and six hours. An unidentified peak designated a (56 - 60 minutes) was recovered after three hours. After six hours, this peak had increased and was designated b (56 - 60 minutes). Metabolites co-eluting with adenine for both time treatments were hydrolysed with acid and re-chromatographed on HPLC. Both subsequently co-eluted with adenine. Metabolites co-eluting with adenosine were similarly treated after which the radioactivity coincided with the elution position of adenine. Treatment of possible trans-zeatin riboside metabolites with acid resulted in the recovery of radioactive peaks co-eluting with trans-zeatin (Figure 28). Metabolites a and b failed to shift elution position following acid treatment. The radioactive peaks co-eluting with isopentenyladenine / isopentenyladenosine were re-chromatographed using a Supelcosil column which yielded separation of the base and riboside for both time treatments (Figure 28). Separation on TLC confirmed these results (Figure 28).

Metabolism of [^{3}H] isopentenyladenine by the stems Stem tissue showed the most extensive metabolic activity with six major radioactive peaks being recovered after three hours and nine peaks recovered after six hours (Figure 29). At three hours, radioactive peaks co-eluting with adenine, adenosine, dihydrozeatin and isopentenyladenine / isopentenyladenosine were recovered. Unidentified metabolites were designated c (55 - 59 minutes) and d (59 - 61 minutes). The metabolite co-eluting with adenine was hydrolysed with acid and re-chromatographed on HPLC, co-eluting with adenine again. The metabolite co-eluting with adenosine was similarly treated, after which the radioactivity coincided with the elution position of adenine. The metabolite co-eluting with dihydrozeatin was treated with potassium permanganate which did not cause it to shift, confirming that this peak was in all probability dihydrozeatin. Metabolite c when treated with acid shifted towards the elution positions of adenine and adenosine. Metabolite

Figure 29 The distribution of $[^3H]$ isopentenyladenine derived radioactivity from stem extracts (- - - - -) and the UV-trace of authentic cytokinin standards (————) after separation by HPLC using a Hypersil 5 ODS column. **A,** Total extract after 3 hours. **B,** Total extract after 6 hours. **C,** Peak c for 3 hours after treatment with HCL. **D,** Peak (34 - 39 minutes) for 6 hours after treatment with B-glucosidase. **E,** Peak f for 6 hours after treatment with HCL. **F,** Peak (73 - 90 minutes) for 3 hours after further separation using a Supelcosil LC 18 DB column or TLC (vertical bars). **G,** Peak (73 - 90 minutes) for six hours after

d failed to shift position after treatment with acid. The radioactive peak co-eluting with isopentenyladenine / isopentenyladenosine was treated as described for the root extract and separation of the base and riboside was achieved.

After six hours incubation, nine major radioactive peaks were recovered from extracts of stem tissue (Figure 29). Of these, four were similar in position to those found at the elution positions of adenine, adenosine, dihydrozeatin and isopentenyladenine / isopentenyladenosine after three hours, but had increased in radioactivity. Further, a radioactive peak co-eluting with the elution position of zeatin-0-glucoside, not found after three hours, was recovered. Unidentified metabolites were designated e (24 - 26 minutes), f (26 - 29 minutes), g (55 - 58 minutes) and h (59 - 63 minutes). Metabolites g and h, similar to metabolites c and d respectively, showed an increase in radioactivity from three hours to six hours. Radioactive peaks co-eluting with adenine and adenosine were treated with acid which caused them to respond in a similar manner to the corresponding peaks at three hours. Potassium permanganate treatment again failed to shift the radioactive peak co-eluting with dihydrozeatin (Figure 29). The radioactive peak co-eluting with zeatin-0-glucoside was treated with β-glucosidase which yielded a radioactive peak co-eluting with trans-zeatin when re-chromatographed (Figure 29). Treatment of metabolite e with acid failed to shift this peak, while similar treatment of metabolite f produced two radioactive peaks, one of which had shifted from the original position (Figure 29). Metabolites g and h, similar in elution position to metabolites c and d of the three hour treatment, responded to acid treatment in the same way. Metabolite g shifted towards the elution positions of adenine and adenosine, while metabolite h failed to shift position. The radioactive peak co-eluting with isopentenyladenine / isopentenyladenosine was treated as described for the root extract and separation of the base and riboside was achieved (Figure 29).

Metabolism of | ^{3}H | isopentenyladenine by the buds After three hours, three major radioactive peaks were recovered in extracts from bud explants. One of the peaks co-eluted with adenine, one with isopentenyladenine / isopentenyladenosine and the unidentified peak was designated as i (58 – 63 minutes). After six hours, four major radioactive peaks were recovered, three having elution positions identical to those of adenine, isopentenyladenine / isopentenyladenosine and metabolite i found after three hours (Figure 30). No increase in radioactivity was noted for these three peaks. The unidentified peak co-eluting with the position of metabolite i was designated j (58 – 63 minutes). The fourth metabolite was designated k (65 – 68 minutes). Metabolites co-eluting with adenine for both time intervals were treated with acid and re-chromatographed on HPLC. Both co-eluted with adenine. Metabolites i and j were treated with acid and β-glucosidase respectively which yielded radioactive peaks near the elution positions of isopentenyladenine and isopentenyladenosine (Figure 30). The metabolite k, not found at three hours was treated with acid but did not shift in position when re-chromatographed on HPLC. The radioactive peaks co-eluting with isopentenyladenine / isopentenyladenosine were treated as described for the root extract and separation of the base and riboside was achieved (Figure 30).

Metabolism of [^{3}H] isopentenyladenine by the leaves Three major peaks were recovered from extracts of leaf tissue after three hours and six hours (Figure 31). These peaks co-eluted with adenine, dihydrozeatin riboside and isopentenyladenine / isopentenyladenosine. A slight decline in radioactivity associated with the adenine and dihydrozeatin riboside peaks was noted after six hours. Metabolites co-eluting with adenine for both treatments were treated with acid and re-chromatographed on HPLC. Both co-eluted with adenine. The metabolite co-eluting with dihydrozeatin riboside at six hours was treated with acid, after which the
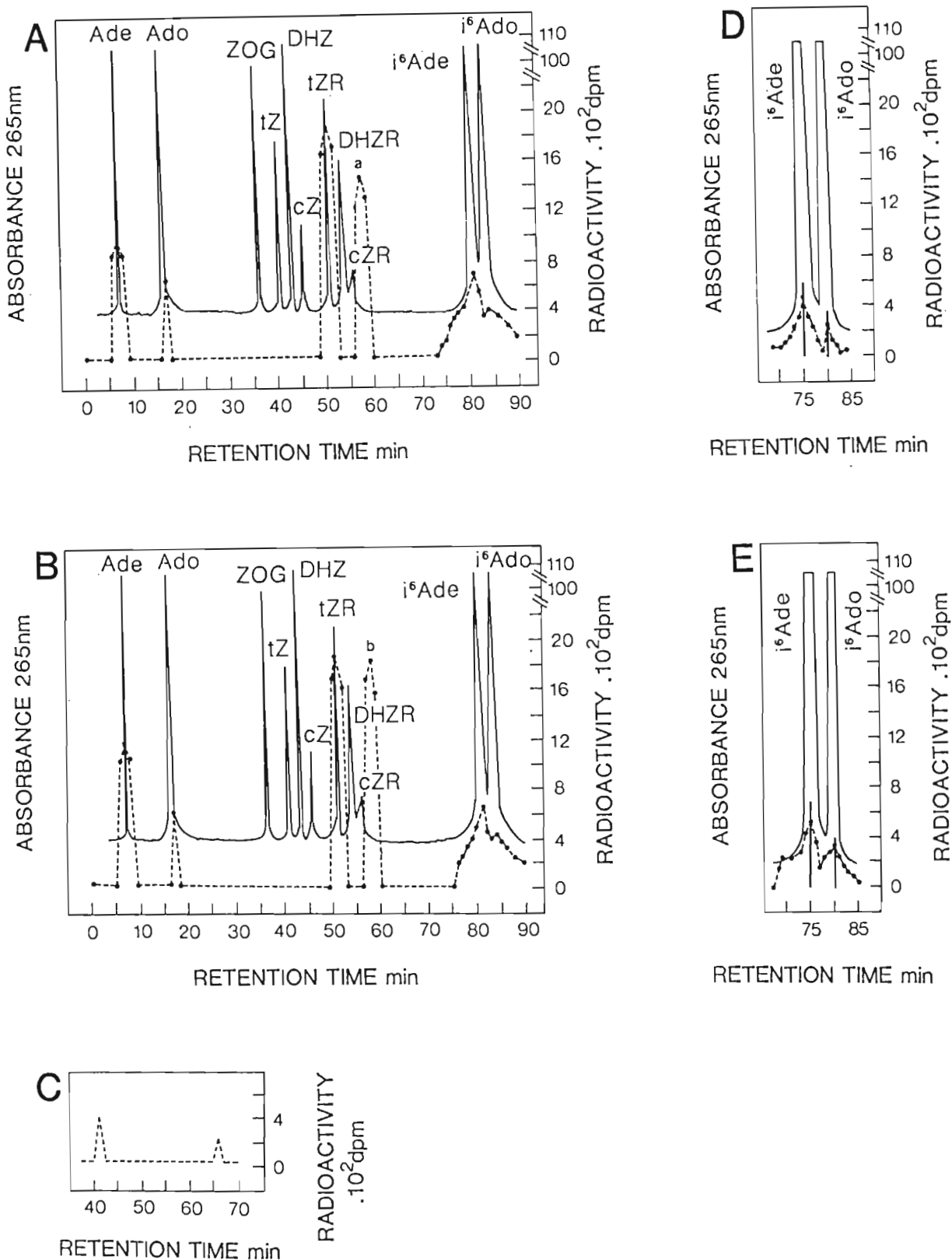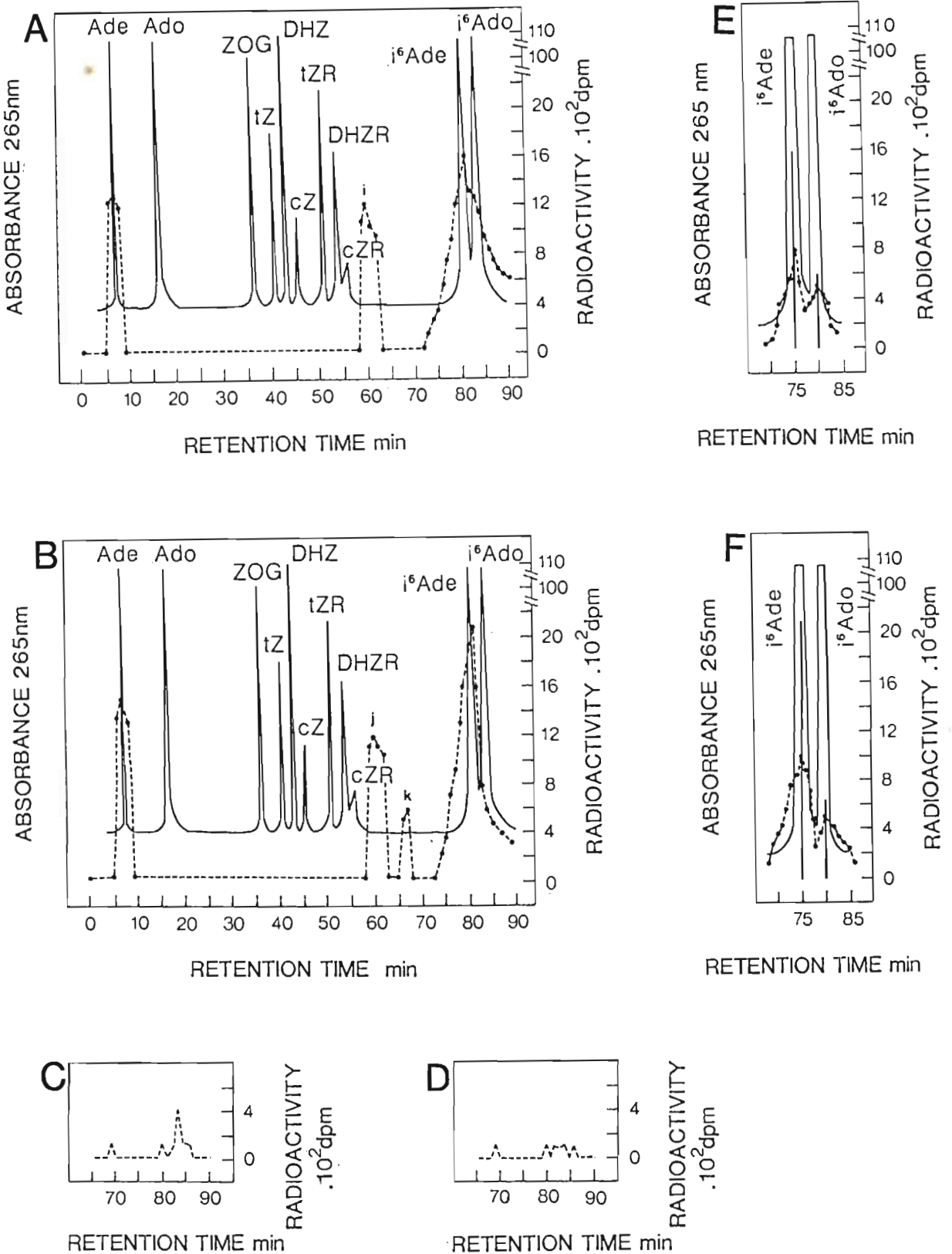
Figure 30 The distribution of [3H] isopentenyladenine derived radioactivity from bud explant extracts (- - - - -) and the UV-trace of authentic cytokinin standards (————) after separation by HPLC using a Hypersil 5 ODS column. **A,** Total extract after 3 hours. **B,** Total extract after 6 hours. **C,** Peak i for 3 hours after treatment with HCL. **D,** Peak j for 6 hours after treatment with B-glucosidase. **E,** Peak (72 - 90 minutes) for 3 hours after further separation using a Supelcosil LC 18 DB column or TLC (vertical bars). **F,** Peak (73 - 90 minutes) for 6 hours after further separation using a Supelcosil LC 18 DB column or TLC
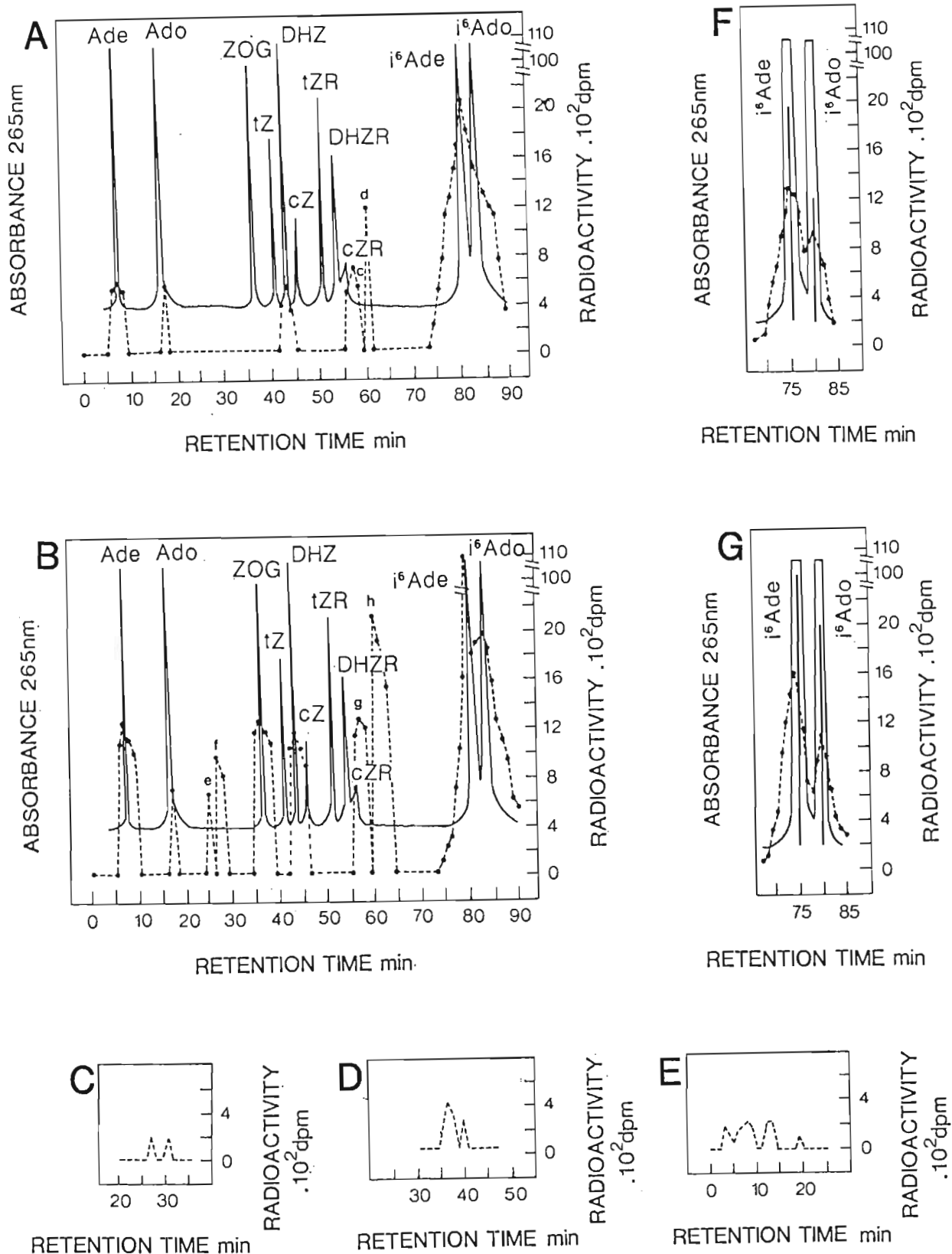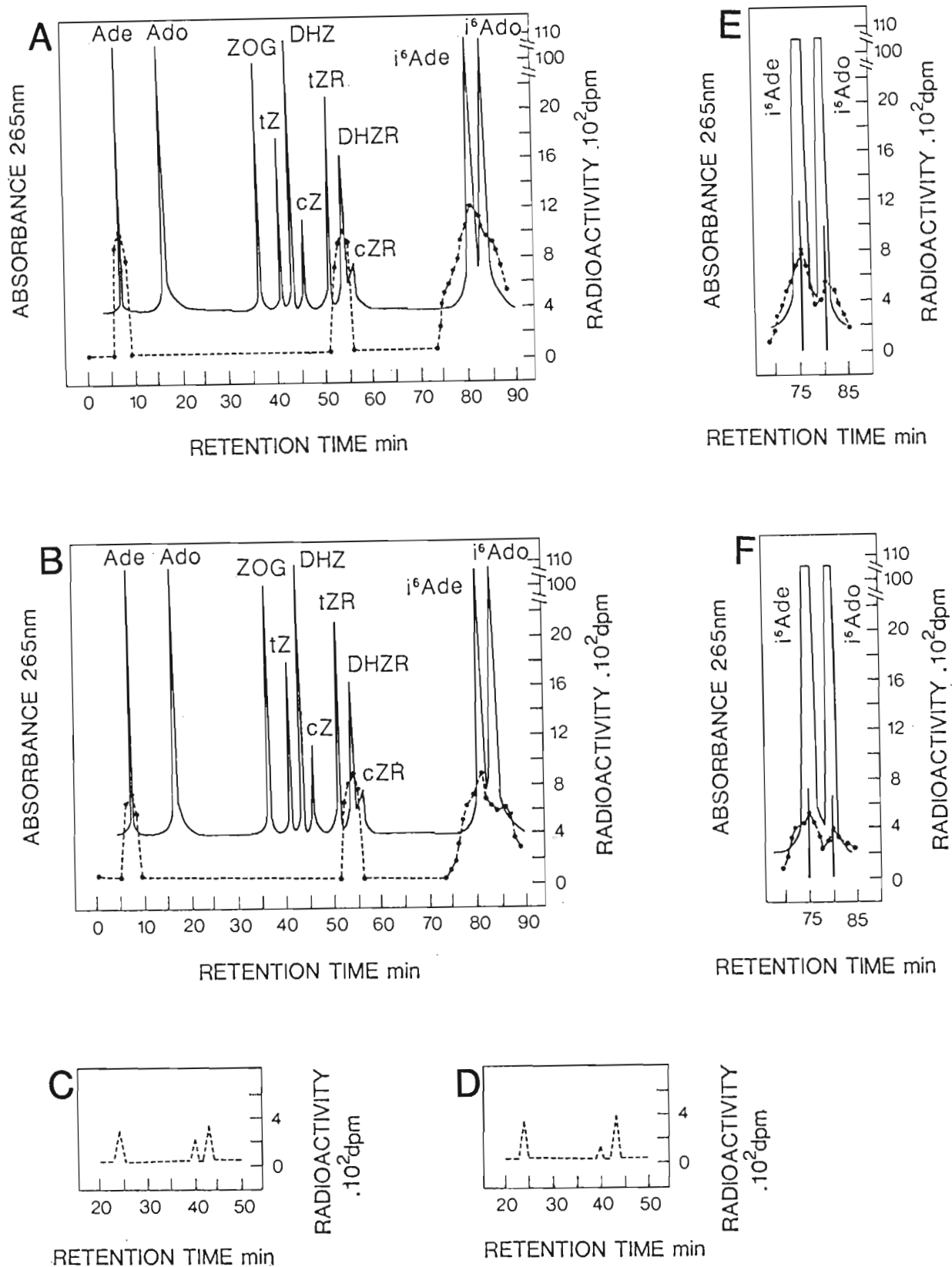
Figure 31 The distribution of [^{3}H] isopentenyladenine derived radioactivity from leaf extracts (- - - - -) and the UV-trace of authentic cytokinin standards (————) after separation by HPLC using a Hypersil 5 ODS column. **A,** Total extract after 3 hours. **B,** Total extract after 6 hours. **C,** Peak (51 - 56 minutes) for 3 hours after treatment with HPCL. **D,** Peak (51 56 minutes) for 6 hours after treatment with B-glucosidase. **E,** Peak (73 - 90 minutes) for 3 hours after further separation using a Supelcosil LC 18 DB column or TLC (vertical bars). **F,** Peak (74 - 90 minutes) for 6 hours after further separation using a Supelcosil

radioactivity coincided with the elution position of dihydrozeatin (Figure 31). The radioactive peaks co-eluting with isopentenyladenine / isopentenyladenosine were treated as described for the root extract and separation of the base and riboside was achieved (Figure 31).

Differential rates of uptake and metabolism by the organs The amounts of identifiable radioactive cytokinins recovered were quantified and have been expressed as the percentage of the total radioactivity supplied at the commencement of the experiment (Table 4).

Table 4 Distribution of tentatively identified radioactive cytokinins recovered in extracts of Pisum sativum organs after three and six hours following incubation with $[^3H]$ isopentenyladenine. Results are expressed as the percentage of total radioactivity supplied at the commencement of the experiment

Radioactivity co-eluting with:	ROOTS		STEMS		BUDS		LEAVES	
	3h	6h	3h	6h	3h	6h	3h	6h
Ade	1.0	1.2	0.6	1.3	1.3	1.5	1.1	0.7
Ado	0.6	0.65	0.6	0.75				
ZOG				1.3				
DHZ			0.5	1.2				
tZR	1.9	1.9						
DHZR							1.0	0.9
1^6Ade	0.7	0.7	2.9	11.0	1.6	2.3	1.2	0.7
1^6Ado	0.4	0.45	1.2	2.0	1.0	0.66	1.0	0.66
Total	4.6	4.9	5.8	17.5	3.9	4.5	4.3	3.0

Abbreviations: Ade, adenine; Ado, adenosine; ZOG, zeatin-0-glucoside; DHZ, dihydrozeatin; tZR, trans-zeatin riboside; DHZR, dihydrozeatin riboside; 1^6Ade, isopentenyladenine; 1^6Ado, isopentenyladenosine.

Uptake of $[^3H]$ isopentenyladenine was greatest in the stem tissue, with much lower levels of $[^3H]$ isopentenyladenine being recovered from root, bud and leaf explants. While levels of $[^3H]$ isopentenyladenine were found to increase

in the stem and bud explants with time, this compound remained consistantly low in the roots and was found to decline in the leaves. Recovery of radioactive isopentenyladenosine appeared to follow the same trends as for $[^3H]$ isopentenyladenine, but the percentage recovery was far lower. Of the radioactive zeatin metabolites recovered, both trans-zeatin riboside in the roots and dihydrozeatin riboside in the leaves remained constant, while dihydrozeatin in the stem was found to increase with time. The amounts of adenine recovered follow closely the pattern of recovery of $[^3H]$ isopentenyladenine, while the amounts of adenosine recovered can be correlated to the levels of isopentenyladenosine in both the root and stem tissues.

4.4.3 Discussion

Comparison of the uptake and metabolism of $[^3H]$ isopentenyladenine by different organs of Pisum sativum indicated that different types of cytokinin metabolites were associated with each organ. Cytokinin metabolism apparently changes quantitatively and qualitatively during different stages of development (PALNI, TAY and MACLEOD, 1987), in various organs or tissue systems (EINSET, 1985; DICKINSON, FORSYTH and VAN STADEN, 1986) and under different physiological conditions (CHEN and KRISTOPEIT, 1981). Cytokinin metabolism may also vary in different species (PALMER et al., 1981a; EINSET, 1986a; MOK and MOK, 1987). The very different metabolic patterns in the axes (SONDHEIMER and TZOU, 1971) and stems (PALMER et al., 1981b) of Phaseolus vulgaris point to an organ specific localization of cytokinin oxidase (McGAW, SCOTT and HORGAN, 1984). DICKINSON et al. (1986), noting a differential metabolism of $[^{14}C]$ adenine in cell free extracts or intact plants of Lycopersicon esculentum, suggested that the cytokinin biosynthetic processes may be strictly compartmentalized.

The findings that certain shoot organs of Pisum sativum have the capacity to metabolise applied [^{3}H] isopentenyladenine to zeatin derivatives supports recent evidence indicating that cytokinin biosynthesis may not be limited to the roots of plants. Rootless Nicotiana tabacum plants supplied with the cytokinin precursor adenine (EINSET and SKOOG, 1973; CHEN and ECKERT, 1976) were able to synthesize isopentenyladenine, isopentenyladenosine, zeatin and zeatin riboside (CHEN and PETSCHOW, 1978a). In addition, Actinidia tissue cultures (EINSET, 1984) and Daucus carota root cambium and Pisum sativum roots, stems and leaves (CHEN et al., 1985) have been found to synthesize cytokinins from applied [^{14}C] adenine or isopentenyladenine. Thus, cytokinin biosynthetic sites are also located in the shoot in addition to the presumed root sites. The implications of this in the control of apical dominance will be discussed later. Firstly, it will be necessary to present a brief overview of cytokinin biosynthesis before attempting an interpretation of the exact metabolic interactions found to occur in the plant organs studied.

The precise sites of cytokinin biosynthesis and metabolism of applied cytokinins in the shoot system remain to be elucidated. Cytokinins are believed to arise by biosynthesis de novo (CHEN, ECKERT and McCHESNEY, 1976; BURROWS, 1978) or as by-products released intact during turnover of the cytokinin-containing tRNA species (KLEMEN and KLAMBT, 1974; LEINWEBER and KLAMBT, 1974). It is possible that both pathways are employed. Adenine and adenosine have both been suggested as possible precursors of free cytokinins not related to the degradation of tRNA (CHEN and PETSCHOW, 1978a; NISHINARI and SYONO, 1980a, 1980b). NISHINARI and SYONO (1980a) proposed that the isopentenyl group (origin undetermined) is transferred directly to adenosine to form isopentenyladenosine. Isopentenyladenosine is then converted to zeatin via isopentenyladenine. A simplified pathway of cytokin biosynthesis is presented in Figure 32.
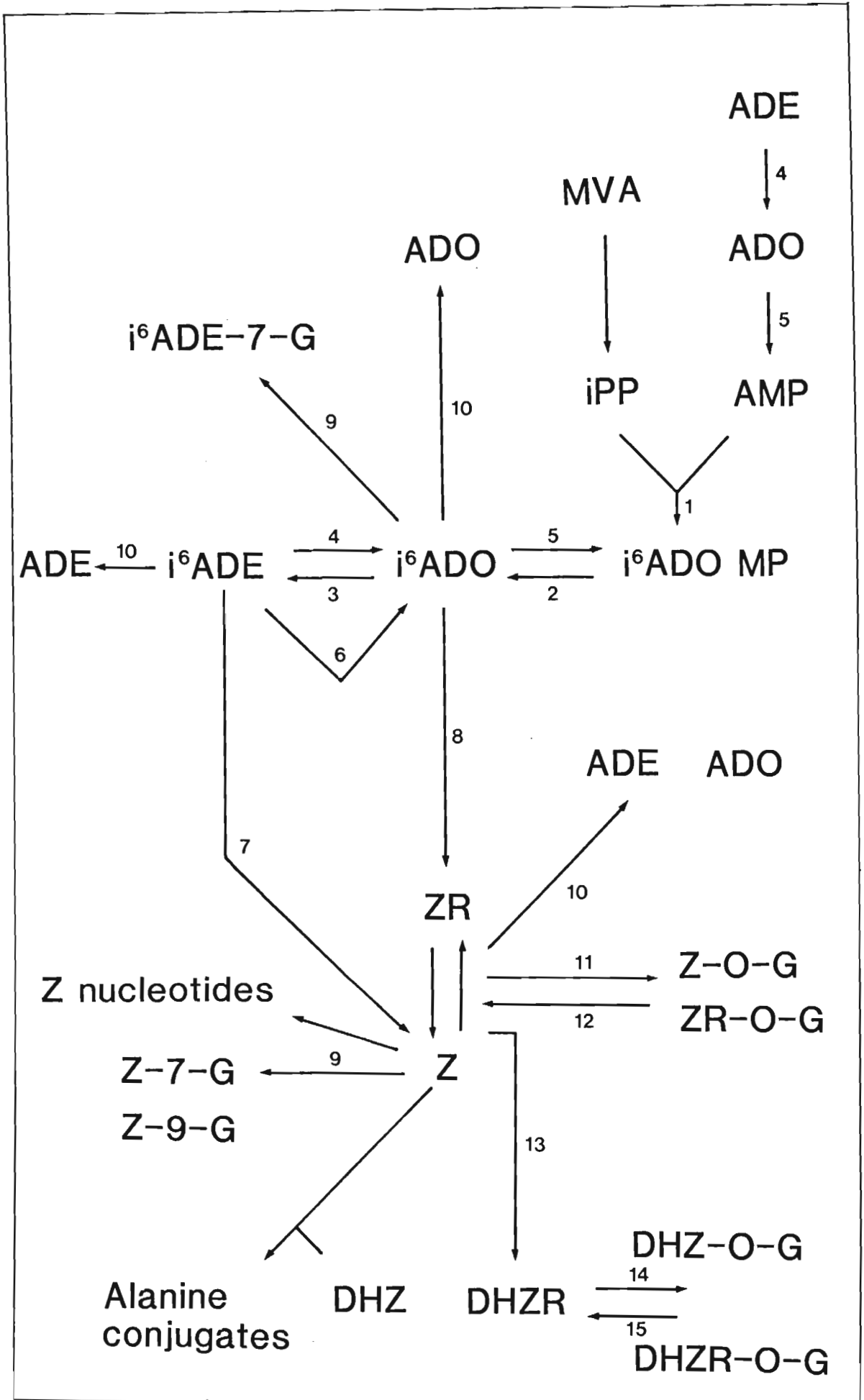
The functional significance of the cytokinin metabolites in the roots, stems, leaves and bud explants following incubation with $[^3H]$ isopentenyladenine will now be discussed. An attempt will be made to relate the results obtained to those of other studies on the metabolism of applied cytokinins and cytokinin levels in selected plant organs. For this purpose tables listing the distribution of metabolites following application of naturally occurring cytokinins (Table 5) and cytokinins in selected species and organs (Table 6) will be presented. However, there is a limited amount of data concerning cytokinin metabolism in Pisum sativum. Do exogenously applied cytokinins actually undergo the same metabolism as endogenous cytokinins? STUCHBURY, PALNl, HORGAN and WAREING (1979) proposed that the cytokinin metabolism observed should reflect the true capabilities of the tissues, since it is the distribution of enzymes which determines which cytokinins are formed. However, the demonstration that a metabolite derived from an exogenous source of cytokinin also occurs endogenously may not necessarily mean that this metabolism reflects the metabolism of the naturally occurring compound (HORGAN, PALNI, SCOTT and McGAW, 1981).

The recovery of radioactive peaks correlating with adenine and adenosine are likely the result of degradative metabolism of the applied cytokinin. Oxidative side chain cleavage yielding adenine, adenosine and adenine nucleotides is the predominant fate of exogenously applied isopentenyladenine, isopentenyladenosine, zeatin and zeatin riboside in many tissues (PARKER and LETHAM, 1974; SUMMONS, ENTSCH, LETHAM, GOLLNOW and MACLEOD, 1980; PALNI, 1980). In addition, significant amounts of isopentenyladenine were recovered in all organs extracted. CHEN and PETSCHOW (1978b) demonstrated the oxidative cleavage of isopentenyladenosine to isopentenyladenine by adenosine phosphorylase, while PACES, WERSTIUK and HALL (1971) showed the degradation of isopentenyladenosine to adenosine and then to adenine by enzyme action. The

Table 5 The metabolites formed in different plant tissues following application of naturally occurring cytokinins

tokinin	Tissue	Plant	Metabolites identified	References
Z	roots	Raphanus sativus	Z-7-G, ZR, ZMP, AMP	GORDON et al. (1974).
Z	roots	Zea mays	Ade, Ado, Z-7-G, AMP	PARKER and LETHAM (1974).
Z	leaves	Phaseolus vulgaris	DHZR, DHZMP	PALMER et al. (1981a)
or ZR	leaves	Populus spp.	Z-O-G, DHZ-O-G, DHZR-O-G, Ado	LETHAM, PARKER, DUKE, SUMMONS and MACLEOD (1977); DUKE, LETHAM, PARKER,MACLEOD and SUMMONS (1979).
Z	seeds	Malus	DHZ, DHZR, ZR, Z-9-G, Z-O-G, DHZ-O-G	ENTSCH, LETHAM, PARKER, SUMMONS and GOLLNOW (1980).
Z	embryos	Fraxinus	ZR, ZRMP, ZR-di and tri- phosphates	TZOU, GALSTON and SONDHEIMER (1973).
Z	crown gall tissue	Vinca rosea	AMP, Z-O-G, ZMP, Ade, Ado	PALNI (1980).
Z	derooted seedlings	Raphanus sativus	Z-7-G, ZRMP, ZR, Ade, Ado, AMP	PARKER, LETHAM, COWLEY and MACLEOD (1972); PARKER AND LETHAM (1973).
Z	derooted seedlings	Zea mays	Z-9-G, ZRMP, ZR, Ade, Ado, AMP, Z-9-G	PARKER, WILSON, LETHAM, COWLEY and MACLEOD (1973); PARKER and LETHAM (1974).
Z	derooted seedlings	Lupinus spp.	Z-O-G, ZRMP, DHZRMP, DHZ, ZR DHZR, Z-7-G, Z-9-G, DHZ-O-G ZR-O-G, DHZ-O-G, Ade, Ado	PARKER, LETHAM, WILSON, JENKINS, MACLEOD and SUMMONS (1975). PARKER, LETHAM, GOLLNOW, SUMMONS, DUKE and MACLEOD (1978). DUKE, MACLEOD, SUMMONS, LETHAM and PARKER (1978).
ZR	caryopses	Zea mays	DHZR-O-G, DHZ-O-G, Ado	SUMMONS et al. (1980).
O-G	detached leaves	Lupinus luteus	ZR-O-G, Z, DHZ, Ado	PARKER et al. (1978).
Ade	cell suspension	Nicotiana tabacum	i^6Ade-7-G, i^6AdoMP, i^6Ado-di and tri-phosphates	LALOUE, GAWER and TERRINE (1975); LALOUE et al. (1977).
Ade	crown gall tissue	Vinca rosea	AMP, ZMP, Ade, Ado, i^6AdoMP, Z, ZR	PALNI (1980).
Ado	cell suspension	Nicotiana tabacum	i^6AdoMP, i^6Ado-di and tri-phosphates	LALOUE, TERRINE AND GAWER (1974); LALOUE et al. (1977).

viations: Ade, adenine; Ado, adenosine; AMP, adenosine 5'-monophosphate; DHZ, dihydrozeatin, DHZR, dihydrozeatin riboside; DHZRMP, dihydrozeatin riboside nophosphate; DHZ-O-G, dihydrozeatin-0-glucoside; DHZR-O-G, dihydrozeatin riboside-0-glucoside; i^6Ade, isopentenyladenine; i^6Ade-7-G, isopentenyladenine-7- side; i^6Ado, isopentenyladenosine; i^6AdoMP, isopentenyladenosine 5'-monophosphate; Z, zeatin; Z-O-G, zeatin-0-glucoside; Z-7-G, zeatin-7-glucoside; Z-9-G, 9-glucoside; ZMP, zeatin 5'-monophosphate; ZR, zeatin riboside; ZR-O-G, zeatin riboside-0-glucoside; ZRMP, zeatin riboside 5'-monophosphate.

relative values of isopentenyladenine to adenine and isopentenyladenosine to adenosine recovered suggest that isopentenyladenosine is a likely intermediate in the conversion of isopentenyladenine to zeatin derivatives in these organs, since hydroxylation of isopentenyladenine and isopentenyladenosine is stereospecific (PALNI and HORGAN, 1983).

Table 6   Cytokinins extracted from Pisum sativum or related genera

Plant	Tissue	Cytokinins	Reference
Pisum sativum	roots	Z, Zr, 1^6Ade 1^6Ado	WIGHTMAN et al. (1980) FORSYTH and VAN STADEN (1981)
Pisum sativum	root nodules	Z, ZR, ZRMP 1^6Ade, 1^6Ado	SYONO and TORREY (1976) SYONO et al. (1976)
Phaseolus vulgaris	roots	Z-O-G	SCOTT and HORGAN (1984)
Lupinus sp.	stems	ZR, DHZR	PURSE, HORGAN, HORGAN and WAREING (1976); PARKER et al. (1978)
Phaseolus vulgaris	stems	ZR, DHZR, Z-O-G	PALMER et al. (1981b)
Phaseolus vulgaris	leaves	DHZ-O-G, DHZR	WANG, THOMPSON and HORGAN (1977) WANG and HORGAN (1978)
Lupinus sp	seed	ZR, DHZR, Z-O-G, ZR-O-G, DHZ-O-G, DHZR-O-G	SUMMONS, ENTSCH, PARKER and LETHAM (1979)

Abbreviations: DHZR, dihydrozeatin riboside; DHZ-O-G, dihydrozeatin-O-glucoside; DHZR-O-G, dihydrozeatin riboside-O-glucoside; 1^6Ade, isopentenyladenine; 1^6Ado, isopentenyladenosine; Z, zeatin; Z-O-G, zeatin-O-glucoside; ZR, zeatin riboside; ZR-O-G, zeatin riboside-O-glucoside; ZRMP, zeatin riboside 5'-monophosphate.

In the roots, trans-zeatin riboside appeared to be the major metabolite derived from $[^3H]$ isopentenyladenine. The ribosides and their 5'-monophosphates are among the most abundant naturally occurring cytokinins (McGAW et al., 1984). Zeatin and zeatin riboside appear to undergo similar metabolism when applied to plant tissue (VAN STADEN and DAVEY, 1977; SUMMONS et al., 1980; LETHAM and PALNI, 1983) and it is implied that these two cytokinins are interchangeable. Radiolabelled zeatin riboside has also been recovered in isolated roots of Raphanus sativus (GORDON et al., 1974), Zea mays (PARKER and LETHAM, 1974) and Lycopersicon esculentum (DICKINSON et al., 1986) following incubation with $[^{14}C]$ zeatin or $[^{14}C]$ adenine. Since root produced cytokinins move up to the shoot in the xylem sap, zeatin riboside may be considered to be the main translocational form of cytokinin in the xylem. The hydroxylation of exogenous isopentenyladenine to zeatin metabolites has also been reported for Actinidia roots (EINSET, 1984). WIGHTMAN et al. (1980) have extracted isopentenyladenine from Pisum sativum roots. Contrary to a previous report (SCOTT and HORGAN, 1984), zeatin-0-glucoside, considered to be a major endogenous cytokinin in Phaseolus vulgaris roots was not recovered in this study. Since glucosides are considered to represent storage or inactivation forms of cytokinin (VAN STADEN and DAVEY, 1979) it is likely that supraoptimal levels of trans-zeatin riboside had not yet occurred in the root tissue after six hours.

Recovery of relatively large amounts of trans-zeatin riboside in the roots suggested that this compound represents the form in which cytokinins are transported from the roots to the leaves and shoot apex. Indeed, the major cytokinins extracted from the stems of Lupinus plants and decapitated Phaseolus vulgaris plants have been found to be the ribosides and nucleotides of zeatin and dihydrozeatin (PURSE et al., 1976; PARKER et al., 1978; PALMER et al., 1981b). In the isolated stem segments of this study, dihydrozeatin was the major metabolite initially derived

from applied $[^3H]$ isopentenyladenine, with zeatin-0-glucoside being recovered later. In feeds of radiolabelled zeatin, the usual metabolites found in the stem are dihydrozeatin or its derivatives (PALMER, HORGAN and WAREING, 1981c; McGAW et al., 1984). Cytokinin glucosides have been extracted from the phloem of plants (HALL and BAKER, 1972; HENSON and WAREING, 1976; VAN STADEN and BROWN, 1977, 1978), so it appears that the recovery of radioactive zeatin-0-glucoside from isolated stem tissues resulted from the ability of this tissue to glucosylate excess zeatin derivatives with time. Minor quantities of zeatin-0-glucoside have been detected in stems of Phaseolus vulgaris (PALMER et al. 1981b), and the ability of stem tissues to glucosylate cytokinins could explain the cytokinin-like effects that some stems appear to have on in vitro bud growth and development (WOOLLEY and WAREING, 1972a; PETERSON and FLETCHER, 1975).

Apart from the identifiable cytokinin metabolites discussed, the stem tissues were found to accumulate six unidentified radioactive metabolites. Significance of these metabolite is not known, but it is likely that portions of these metabolites involve glucosylation of $[^3H]$ isopentenyladenine. In any event, the hydroxylation of applied $[^3H]$ isopentenyladenine to a zeatin derivative in the stem, together with the observed capacity for glucosylation indicates that the stem may play an active role in the biosynthesis of cytokinins transported in the shoot.

The major radioactive cytokinin metabolite recovered from leaf extracts was dihydrozeatin riboside. It has been demonstrated that the major endogenous cytokinins in Phaseolus vulgaris leaves are the riboside and 0-glucoside of dihydro-zeatin (WANG et al., 1977; WANG and HORGAN, 1978). In the intact plant, the dihydrozeatin in the stems could be a precursor for these ribosides and gluco-sides found in the leaves. However, since isolated Pisum sativum leaves have

been shown to synthesize isopentenyladenine from supplied $[^{14}C]$ adenine (CHEN et al., 1985), it would appear that cytokinins in leaf tissue need not represent translocated forms from the root. No glucosylated cytokinins were recovered from the leaf explants. It is possible that the explants were harvested before glucosylation was necessary, or that the leaves may have been too young in the seedling plant used. Zeatin and zeatin riboside have been found to be the predominant cytokinins in young expanding leaves (VAN STADEN, 1976a, 1976b; HENSON 1978, VAN STADEN and DAVEY, 1981), while cytokinin glucosides are the major form of cytokinin in mature and senescing leaves (ENGELBRECHT, 1972; DUKE et al., 1979; VONK and DAVELAAR, 1981). Following feeds of radiolabelled zeatin to the roots of Phaseolus vulgaris, the major radioactive cytokinins recovered in the leaf were dihydrozeatin, dihydrozeatin riboside and zeatin riboside-0-glucoside (PALMER et al., 1981c). In detached leaves of Phaseolus vulgaris supplied with dihydrozeatin, the major radioactive cytokinins recovered were dihydrozeatin and its glucosides (PALMER et al., 1981a).

The results of $[^{3}H]$ isopentenyladenine applications to the roots, stems and leaves of Pisum sativum are therefore consistent with the presence of endogenous cytokinins in these organs on whole plants. Thus the metabolism of applied $[^{3}H]$ isopentenyladenine by the isolated organs appeared to reflect the true capacities of these tissues.

Bud explants comprising a node bearing an inhibited lateral bud yielded few radioactive metabolites. The possibility exists that the inhibited lateral buds were unable to hydroxylate $[^{3}H]$ isopentenyladenine to zeatin, or that this tissue does not have a high cytokinin biosynthetic capacity per se. The significance of this in relation to lateral bud inhibition will be discussed later.

The metabolic reaction central to the hypothesis of the thesis, that of the hydroxylation of isopentenyladenine to zeatin or its derivatives was shown to occur in the roots, leaves and stems of Pisum sativum. It had been proposed earlier that the regulation of the amounts of specific cytokinins required at the lateral bud site in order to overcome apical inhibition could be controlled by this reaction. Thus a more thorough discussion of this reaction is necessary before discussing its significance in relation to growth processes in the plant. Hydroxylation of the isopentenyladenine side chain to give the corresponding trans-zeatin derivative was demonstrated in Actinidia callus (EINSET, 1984), Vinca rosea crown gall tissue (PALNI and HORGAN, 1983), and in microsomes from Brassica oleracea L. plants (CHEN and LEISNER, 1984). Other examples of hydroxylation of isopentenyladenine or related isopentenyl cytokinins have occurred in the fungus Rhizopogon roseolus (MIURA and MILLER, 1969; MIURA and HALL, 1973), immature Zea mays kernels (MIURA and HALL, 1973) and microsomes from Nicotiana tabacum callus cultures (CHEN, 1982). EINSET (1984, 1986a, 1986b) and EINSET and SILVERSTONE (1987) have pursued a study on the hydroxylation of isopentenyladenine by Actinidia plants which have yielded interesting and significant results, to be discussed in the next section of this chapter. The mechanism of this reaction is unclear, but preliminary characterization of the enzyme responsible for the hydroxylation reaction has been reported by CHEN (1982). CHEN (1982) and CHEN and LEISNER (1984) reported on microsomal activity from Nicotiana tabacum and Brassica oleracea cultures that will hydroxylate isopentenyladenine to zeatin. This prompted EINSET (1986a) to suggest the possibility that isopentenyladenine hydroxylation is mediated by a membrane-bound monooxygenase. However, in extracts of many higher plants, isopentenyladenine and isopentenyladenosine have not been detected in the free form (VAN STADEN and DAVEY, 1979; PALMER et al., 1981b; SCOTT, BROWNING and EAGLES, 1980). In addition, reports have been made of experiments involving isopentenyladenine or isopen-

tenyladenosine application to plants and tissues in which zeatin and zeatin riboside levels were unaffected or not detected (STUCHBURY et al., 1979; MOK, MOK, DIXON, ARMSTRONG and SHAW, 1982). Since it is now known that isopentenyl-adenine occurs in plants as a free cytokinin, if only in small amounts, it seems probable that the isopentenyladenine or an intermediate are so rapidly hydroxylated that their steady state levels are extremely low (LETHAM and PALNI, 1983; McGAW et al., 1984).

Since the stem, but not the lateral buds appeared to be a major site of cytokinin metabolism, it was decided to investigate further the metabolism of $[^3H]$ isopen-tenyladenine by stem sections along the whole shoot. If, as suggested earlier, the inhibition of lateral buds is influenced by the distribution of hydroxylase activity of cytokinins in the plant, mediating the distribution and activity in the shoot, this would be manifest by a differential cytokinin metabolism occurring in different stem sections along the length of the shoot.

4.5 The metabolism of $[^3H]$ isopentenyladenine by stem segments

4.5.1 Materials and methods

Stem explants from the internodes of Pisum sativum plants were obtained as described in Section 4.2. Fourteen-day-old (four node) plants and 21-day-old (ten node) plants were used. Stem explants from the internodes of four-node plants were designated internodes 1 - 5 from the base to the shoot apex respective-ly, and were treated separately. Stem explants from the internodes of the ten-node plants were designated internodes 1 - 10 from the base to the shoot apex and treated separately. Replicates of one gram of each of these stem internodes were incubated in distilled water containing $[^3H]$ isopentenyladenine, harvested

after six hours, extracted, and the metabolites tentatively identified by HPLC as described in Section 4.2. The availability of a wider range of cytokinin standards allowed for a more thorough identification of cytokinin metabolites than previously possible in this study.

4.5.2 Results

Metabolism of $[^3H]$ isopentenyladenine by the stem   Ten major radioactive peaks were recovered from the stem extracts of four-node and ten-node plants after six hours incubation (Figure 33, Table 7, Table 8). Nine peaks were found to co-elute with adenine, adenosine, dihydrozeatin riboside 5'-monophosphate / dihydrozeatin-9-glucoside / zeatin-0-glucoside, dihydrozeatin, dihydrozeatin riboside, isopentenyladenosine 5'-monophosphate, isopentenyladenine-9-glucoside, isopentenyladenine and isopentenyladenosine. An unidentified peak (58 - 59 minutes) was designated a. Chemical treatment of the peaks co-eluting with adenine, adenosine and isopentenyladenosine confirmed the presence of these metabolites as previously reported in this chapter. Acid treatment of the peak co-eluting with the dihydrozeatin riboside 5'-monophosphate / dihydrozeatin-9-glucoside / zeatin-0-glucoside complex caused the radioactivity to shift to the elution position of dihydrozeatin, indicating the removal of the ribose monophosphate. Alternative treatment of this peak with alkaline phosphatase resulted in a shift of radioactivity to the elution position of dihydrozeatin riboside, indicating the removal of the monophosphate. Treatment of this same peak with β-glucosidase resulted in a partial shift of radioactivity to the elution position of trans-zeatin, suggesting that this peak represented both dihydrozeatin riboside 5'-monophosphate and zeatin-0-glucoside. The presence of dihydrozeatin-9-glucoside was not confirmed. The metabolite co-eluting with dihydrozeatin was treated with potassium permanganate which did not cause it to shift, confirming
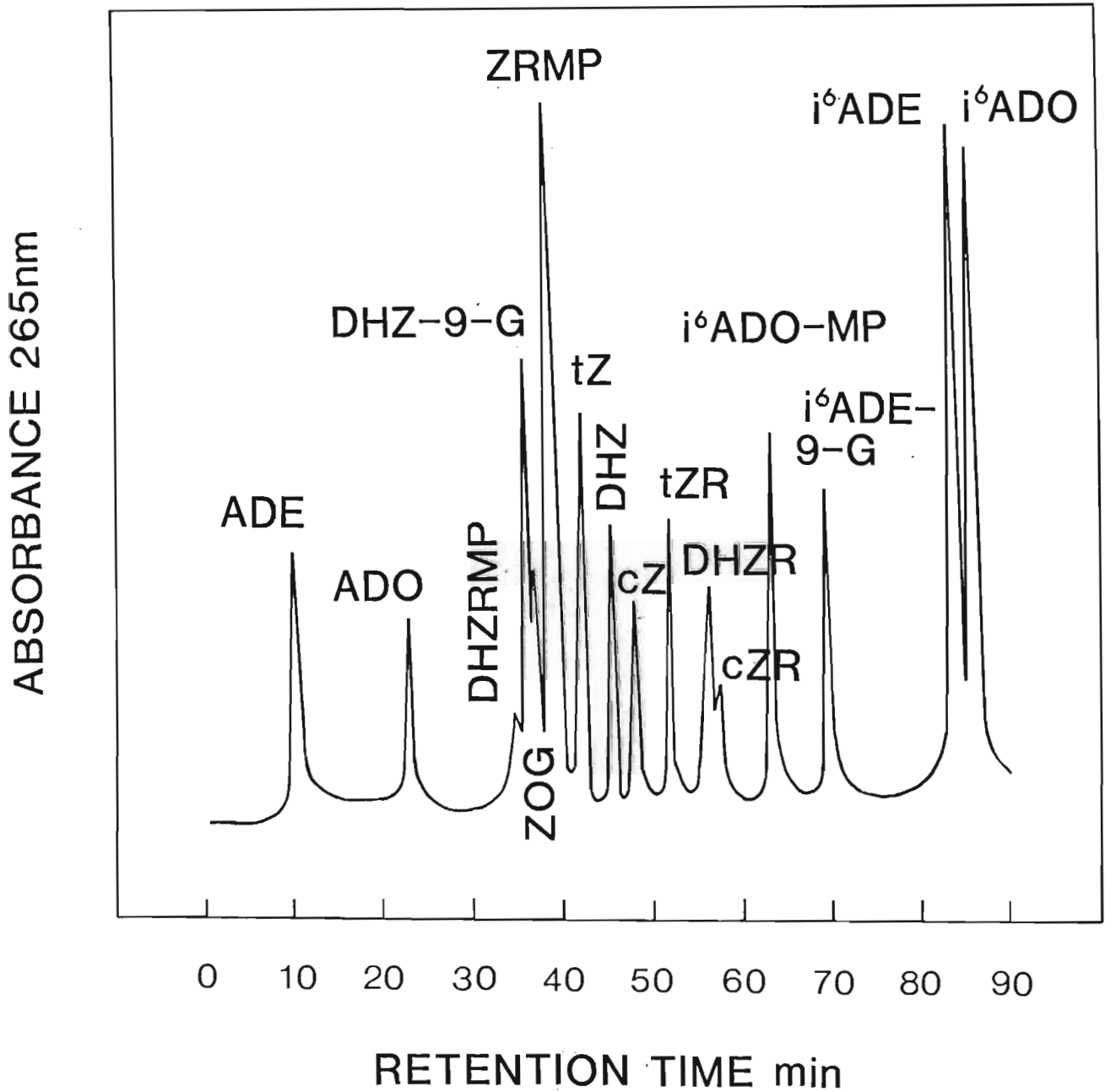
Figure 33   The UV-trace of authentic cytokinin standards after separation by HPLC using a Hypersil 5 ODS column. The UV-trace was used in the tentative identification of radioactive metabolites recovered in stem sections of Pisum sativum incubated with [^{3}H] isopentenyladenine.

Table 7 Distribution of tentatively identified radioactive cytokinins recovered in extracts of <u>Pisum</u> <u>sativum</u> stem sections (1-5) incubated with $[^3H]$ isopentenyladenine

Internodal stem sections were excised from 14 day old plants. Results are express-ed as the percentage of the relative recovery of each radioactive metabolite in each internode.

Stem inter-node No.	Ade	Ado	DHZ RMP/ ZOG	DHZ	DHZR	a	1^6Ado MP	1^6Ade 9-G	1^6Ade	1^6Ado
5	16.0	22.0	15.8	11,8	20,0	22.8	16.4	10.3	14.3	16.0
4	10.0	16.5	12.4	10.4	13.5	13.1	16.4	10.3	12.2	16.9
3	11.0	11.0	1.0	18.6	17.6	11.2	16.7	18.4	10.5	8.7
2	23.0	23.1	24.8	27.8	19.4	24.0	20.7	26.3	28.0	20.4
1	40.0	27.5	37.1	31.3	29.4	28.8	29.8	136.0	35.0	37.9

Table 8 Distribution of tentatively identified radioactive cytokinins recovered in extracts of <u>Pisum</u> <u>sativum</u> stem (1-10) sections incubated with $[^3H]$ isopentenyladenine

Internodal stem sections were excised from 21 day old plants. Results are express-ed as the percentage of the relative recovery of each radioactive metabolite in each internode.

Stem inter-node No.	Ade	Ado	DHZ RMP/ ZOG	DHZ	DHZR	a	1^6Ado MP	1^6Ade 9-G	1^6Ade	1^6Ado
10	15.6	13.5	16.8	11.8	17.8	12.4	17.0	16.5	14.7	14.5
9	13.5	10.8	13.4	10.7	11.2	9.5	12.0	12.6	9.8	9.0
8	7.9	10.2	8.4	8.0	6.7	7.6	10.0	8.6	7.8	7.8
7	5.0	8.6	6.7	6.4	5.0	5.7	8.0	7.3	6.8	6.0
6	7.8	7.5	5.8	5.3	5.9	6.6	7.0	6.6	6.8	6.0
5	9.2	7.5	5.0	6.9	7.6	8.5	8.0	8.6	7.8	7.2
4	9.9	7.5	5.8	7.5	7.6	8.5	9.0	8.6	8.8	8.4
3	12.0	13.5	10.9	12.3	10.2	11.4	10.0	9.3	10.7	11.5
2	10.6	10.8	13.4	16.1	13.5	15.2	10.0	10.6	13.7	16.3
1	8.5	9.7	13.4	14.5	12.7	14.3	9.0	11.2	12.7	13.3

Abbreviations for Tables 7 and 8: Ade, adenine; Ado, adenosine; DHZRMP/ZOG, dihydrozeatin riboside 5'-monophosphate/zeatin-0-glucoside; DHZ, dihydrozeatin; DHZR, dihydrozeatin riboside; 1^6Ado MP, isopentenyladenosine 5'-monophosphate; 1^6Ade-9-G, isopentenyladenine-9-glucoside; 1^6Ade, isopentenyladenine; 1^6Ado, isopentenyladenosine.

that this peak was in all probability dihydrozeatin. Acid treatment of the metabolite co-eluting with dihydrozeatin riboside resulted in a shift of radioactivity to dihydrozeatin, indicating the removal of the ribose. Metabolite a was treated with both acid and alkaline phosphatase but could not be tentatively identified as it retained its original elution pattern. Following treatment with alkaline phosphatase, the peak co-eluting with isopentenyladenosine 5'-monophosphate shifted to the elution position of isopentenyladenosine. Alkaline phosphatase treatment of the peak co-eluting with isopentenyladenine-9-glucoside caused a partial shift of the radioactivity to isopentenyladenine.

A comparison of the radioactive peaks recovered from the five different stem internodes of four-node plants revealed a gradient of metabolic activity (Table 7).The greatest metabolic activity occurred at the base of the stem, declining towards the shoot apex. Dihydrozeatin, the major metabolite recovered from all internodes was three-fold greater at the base than at the shoot tip. Extracts from internodes of ten-node plants revealed a different gradient of metabolic activity (Table 8). While some activity was still associated with internodes 1, 2 and 3 at the base of the stem, a zone of equal or higher activity was found to occur at internodes 8, 9 and 10 near the shoot apex. A zone of low activity was associated with internodes 5, 6 and 7 from the middle of the shoot.

4.5.3   Discussion

The results of this experiment confirmed the results of the previous experiment in this chapter, which indicated that the stem is a major site of cytokinin metabolism in the plant. Ten metabolites of $[^3H]$ isopentenyladenine were recovered from stem extracts, including dihydrozeatin and dihydrozeatin riboside as major metabolites.

Of particular interest was that the capacity to metabolise the supplied $[^3H]$ isopentenyladenine occurred as a gradient in the stem and appeared to be related to the age of the plant. In four-node plants, the cytokinin metabolic activity appeared to be greatest near the base of the shoot, while in ten-node plants, this same activity had begun to shift to the anterior end of the shoot. This pheno-menon can be related to the results of the experiment in Chapter 2, in which the shoot apex was removed from plants bearing four, six, eight or ten nodes. In this study, a basally orientated branching pattern in younger four-node plants changed to being anterior on older plants. This surely implies that the branching pattern of Pisum sativum plants is influenced by the distribution of hydroxylase activity in the plant mediating the distribution and activity of cytokinins in the shoot. Further evidence that the growth potential of lateral buds is related to their location on the stem and the age of the plant has already been reviewed in Chapter 1 (GREGORY and VEALE, 1957; HUSAIN and LINCK, 1966; CUTTER and CHIU, 1975).

Evidence in support of a distribution of cytokinin hydroxylase activity in the stem has been provided by EINSET (1984; 1986b) and EINSET and SILVERSTONE (1987), using Actinidia stem sections or whole plants. In these studies, the distribu-tion of isopentenyladenine hydroxylase activities were determined by measuring the accumulation of zeatin in explants incubated in a medium containing unlabelled isopentenyladenine. In isolated stem segments, hydroxylase activity was very low in the shoot tip, highest in the region corresponding to rapid leaf growth and very low in the mature stem (EINSET, 1984). The results of this whole shoot study correlate well with those of the serial incubation study of internodes 1 - 10 in this chapter. Application of isopentenyladenine to Actinidia plants indicated that the isopentenyladenine hydroxylation activity is not evenly distributed in the plant (EINSET, 1986b; EINSET and SILVERSTONE, 1987). In mature plants,

the hydroxylation activity was anterior, while in young seedlings this activity was basal.

The correlation between the gradients of cytokinin metabolic activity in the stem and the gradients of growth potential of lateral buds suggest a control of apical dominance in which root derived cytokinins or cytokinin precursors, moving acropetally in the stem to the growing apex are metabolised to cytokinins that the lateral buds can utilize. It is unlikely that lateral buds can fulfil their own cytokinin requirements, and the cytokinins from the stem tissue are likely to be very important. Lateral bud explants supplied with $[^3H]$ isopentenyladenine in this study did not hydroxylate the applied cytokinin to zeatin or a zeatin metabolite. Likewise, in experiments with isolated lateral buds in culture, little growth occurred, suggesting that a factor associated with the stem was necessary (BALLARD and WILDMAN, 1964; PETERSON and FLETCHER, 1975). In addition, PETERSON and FLETCHER (1975) found that the longer the internode below a lateral bud on an isolated stem segment, the greater the growth of that bud. PALMER et al. (1981b) have stated that the stem may have the capacity to abstract from and modify and contribute to cytokinins that are transported in the shoot, and that the stems' significance appears to be greater than simply a channel for root produced material.

However, while the distribution of cytokinins in the stem can be rationalised by the distribution or activity of enzymes in the shoot system, it is yet unclear how this distribution or activity is controlled. It is generally agreed that a primary component of the inhibitory correlative signal is the synthesis of auxin in the region of the shoot apex and its transport down the stem. As already reviewed in Chapter 1, the predominantly basipetal movement of auxin results in a gradient of auxin concentration in the stem tissues, with a decline in auxin towards the base of the shoot (SCOTT and BRIGGS, 1960; GOLDSMITH, 1968). This distribution

of auxin in the stem can be correlated to the gradient of cytokinin metabolic activity observed in the experiment of this chapter, as well as the growth studies of Chapter 2 and the cytokinin application studies of Chapter 3. It thus seemed obvious to investigate the possibility that the cytokinin metabolism observed in the stem could be controlled by auxin in the stem tissue. This was achieved in the following experiment in which isolated stem segments were incubated in distilled water and [^{3}H] isopentenyladenine including a range of auxin concentrations.

4.6 The effect of auxin on the metabolism of [^{3}H] isopentenyladenine in stems

4.6.1 Materials and methods

Replicates of stem internode 3 excised from four-node Pisum sativum plants as described in Section 4.2 were rinsed in running distilled water for 90 minutes. The purpose of this rinse was to allow the diffusion out of the stem of any endogenous auxins. The stem internodes (replicates of one gram) were then transferred to beakers containing 100 millilitre solutions of indole-3-acetic acid in distilled water (pH 6.0) at a range of concentrations. A distilled water treatment constituted a control. After 90 minutes pre-incubation on a rotary shaker in the dark, during which time the segments were allowed to equilibrate in their different auxin concentrations, 60×10^6 Bq of [^{3}H] isopentenyladenine was added to each beaker of indole-3-acetic acid solution or control distilled water and incubated for a further six hours on a rotary shaker in the dark. The stem explants were then harvested, extracted and the metabolites tentatively identified by HPLC as described in Section 4.2.

4.6.2   Results

Ten major radioactive metabolites were recovered from all stem extracts and their tentative identification was the same as for the previous two experiments in Section 4.4 and Section 4.5.

Indole-3-acetic acid appeared to affect the metabolism of $[^3H]$ isopentenyladenine supplied to the stem explants. A similar dose – response curve was noted for each of the metabolites recovered from the stem extracts (Figure 34). Optimum metabolism of $[^3H]$ isopentenyladenine occurred in a 10^{-6} Molar solution of indole-3-acetic acid. A decline in metabolic activity occurred both with a decrease and an increase in indole-3-acetic acid concentration from this optimum. Indole-3-acetic acid at a concentration of 10^{-4} Molar appeared to be inhibitory to cytokinin metabolism, while an absence of endogenous or exogenous auxin in the washed control segments resulted in an inability of the stem tissue to metabolise the applied $[^3H]$ isopentenyladenine. Comparison of the dose response curves for isopentenyladenine and the major metabolites dihydrozeatin and dihydrozeatin riboside indicate that the uptake of $[^3H]$ isopentenyladenine by all explants was similar, with the pronounced dose – response curve being the result of cytokinin metabolism.

4.6.3   Discussion

The results of the previous experiment suggest that cytokinin metabolism in the plant may be controlled by auxin. In the absence of exogenous indole-3-acetic acid or other endogenous auxins, the isolated stem tissue was incapable of metabolizing the applied $[^3H]$ isopentenyladenine. Cytokinins have been found to be inactive in inducing cell division in prewashed Helianthus tuberosus tuber tissue (ADAMSON, 1962; SETTERFIELD, 1963; KAMISAKA and MASUDA, 1970;
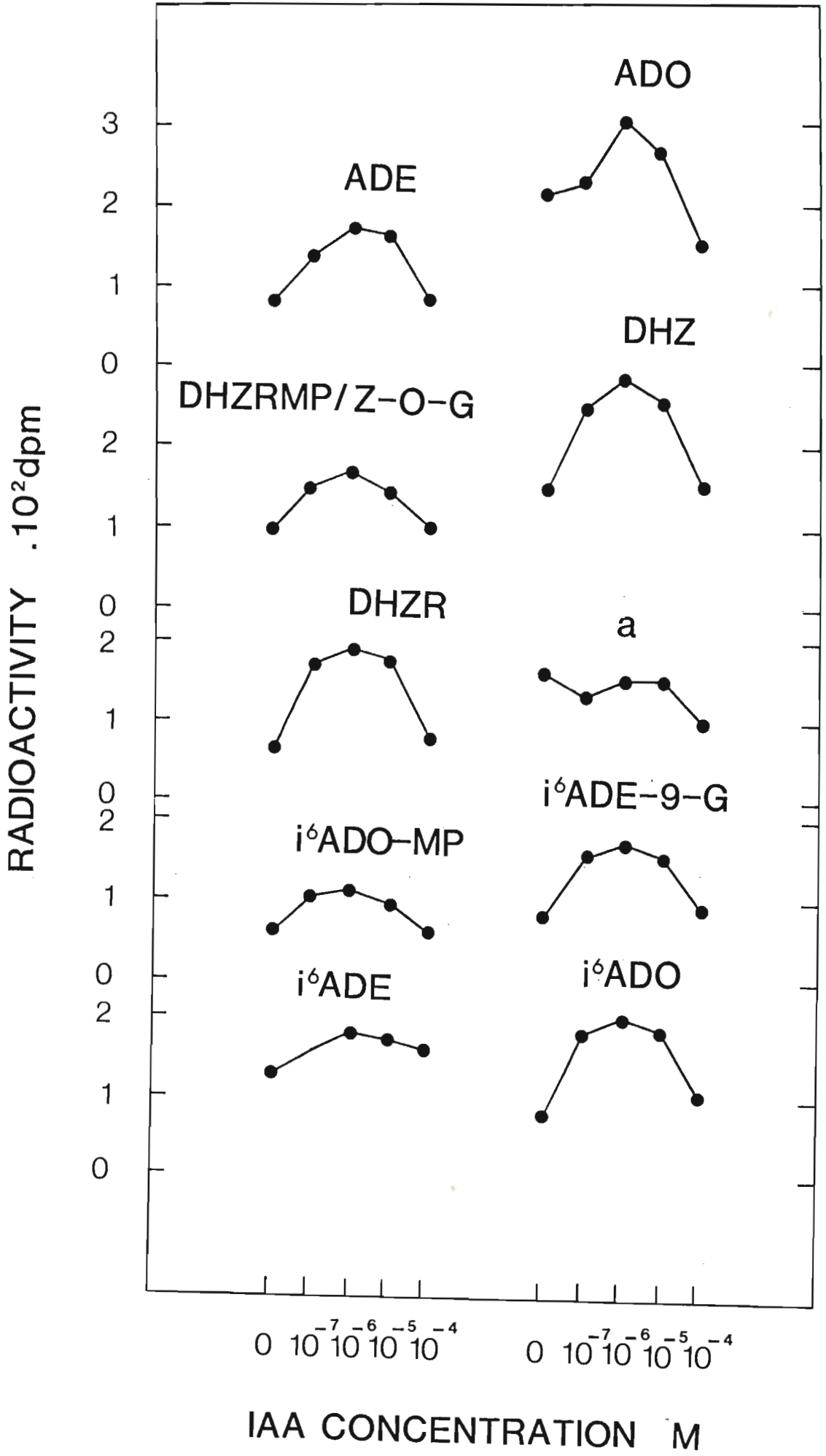
MINOCHA, 1979). Thus plant cell division and morphogenesis are very sensitive to, and probably controlled by, relative levels of auxin and cytokinin in the plant (PALMER, LETHAM and GUNNING, 1984). Throughout this study, the control of apical dominance has been envisaged as being a result of the relative levels of these two growth regulators in the plant, which are known to occur as a gradient in the shoot. Thus it is not surprising that high indole-3-acetic acid concentrations $(10^{-4}$ Molar) were inhibitory to cytokinin metabolism in this study.

The concept of an auxin - cytokinin interaction in the control of apical dominance was first noted by WICKSON and THIMANN (1958) who documented the antagonism between these two growth regulators in the growth of lateral buds of Pisum sativum. Their experiments on bud inhibition with applied auxin in solution required indole-3-acetic acid concentrations in the region of 10^{-4} Molar, the concentration which was found to inhibit cytokinin metabolism in this study. Other early circumstantial evidence supporting a controlling role of auxin in cytokinin metabolism and action was provided by KURASHI (1959), who demonstrated the enhancement by indole-3-acetic acid of kinetin induced growth of Raphanus sativus leaf discs, and the demonstration that kinetin in combination with indole-3-acetic acid increased the stimulating effect of the latter on the growth of Avena sativa coleoptiles (DENIZCI, 1966; WRIGHT, 1968; HEMBERG and LARSSON, 1972).

Studies on the in vitro growth of Nicotiana tabacum callus cultures have also shown that the presence of kinetin in the medium greatly influences the response of the tissue to auxin (MURASHIGE and SKOOG, 1962). Recently, BHATTACHARYYA and BASU (1985) noted that oxidation of endogenous indole-3-acetic acid was essential for kinetin induced growth of Triticum vulgare L. coleoptiles. These examples all illustrate that the growth processes mediated

by cytokinins are dependent on an optimum ratio of cytokinin : auxin occurring at the site of action.

Recent evidence now suggests that the interaction between these two growth regulators probably resides at the level of cytokinin metabolism. Indole-3-acetic acid has been reported to modify cytokinin metabolism in a number of plant tissues. In Solanum andigena stem cuttings, the formation of unidentified metabolites of benzyladenine were induced with auxin (WOOLLEY and WAREING, 1972a), while in Helianthus tuberosus tuber tissue, a more rapid metabolism of zeatin nucleotide resulted from auxin application (PALMER, et al, 1984). HANSEN, MEINS and MILANI (1985) and HANSEN, MEINS and AEBI (1987) observed an inhibition of endogenous cytokinin accumulation by Nicotiana tabacum crown gall tissues or normal tissues when cultivated on an auxin containing medium, likely the result of increased cytokinin metabolism. More recently, PALNI, BURCH and HORGAN (1988) demonstrated that the stability of $[^3H]$ zeatin riboside supplied to Nicotiana tabacum pith explants was inversely related to the naphthalene-3-acetic acid in the incubation medium. At higher auxin concentrations, a greater breakdown of $[^3H]$ zeatin riboside occurred. NELSON (1988) also noted that the benzyladenine : indole-3-acetic acid ratio affected the metabolism of benzyladenine supplied to Nicotiana tabacum callus tissue. The benzyladenine was more stable at lower auxin ratios, and more ribosylation of benzyladenine occurred at higher auxin ratios.

How does auxin affect the metabolism of endogenous or applied cytokinins? PARKER, ENTSCH and LETHAM (1986) have stated that one technique for the regulation of the action of plant growth substances is to influence the synthesis or inactivation of the active compounds at the level of enzyme function. Treatment of Salix babylonica L. bark explants with naphthalene-3-acetic acid resulted

in a decrease in the levels of extractable cytokinins (CHOVEAUX and VAN STADEN, 1981), prompting these authors to suggest that auxin action was not related to cytokinin biosynthesis per se as believed by WITHAM (1968) and INOUE, MAEDA, YOSHIDA and ORITANI (1979), but was the result of auxin action on cytokinin oxidase-type enzymes which stimulated cytokinin metabolism. Indole-3-acetic acid has been found to inhibit competitively an enzyme involved in cytokinin conjugation, indicating that cytokinins and auxins interact in a complex manner to mutually control their metabolism (PARKER et al., 1986). PALNI et al. (1988) have demonstrated an in vitro stimulation of cytokinin oxidase by auxin on a partially purified enzyme preparation which paralleled their in vivo observations that the stability of $[^3H]$ zeatin riboside supplied to Nicotiana tabacum explants was inversely related to auxin concentration.

The results of this study, as well as the examples discussed above again emphasize that auxins and cytokinins interact in a manner to control their metabolism and levels. Exogenously applied cytokinins have, in turn, been reported to elevate auxin levels and production in tissue, to suppress conjugation of auxins to amino acids and to affect the formation of enzymes which oxidise auxin (SCHNEIDER and WIGHTMAN, 1978; MONTAGUE, ENNS, SIEGEL and JAWORSKI, 1981; PARKER et al., 1986). Thus, while the results of the study on indole-3-acetic acid effects on $[^3H]$ isopentenyladenine metabolism in Pisum sativum stems cannot be considered conclusive, the very recent research impetus experienced by this aspect to cytokinin metabolism support the view that shoot produced auxin, moving down in the stem, may mediate the levels of cytokinins available to the lateral buds by affecting the enzymes responsible for the conversion of isopentenyladenine to zeatin metabolites. The hydroxylation of $[^3H]$ isopentenyladenine to zeatin derivatives have been demonstrated in the stem, roots and leaves of Pisum sativum, and this reaction in the stem has been correlated to the pattern of lateral bud

branching on the intact and decapitated plant, as well as the sensitivity of lateral buds to different cytokinin applications. It is believed that these growth processes are controlled by differential auxin gradients in the plant.

CONCLUSION

The response of lateral buds of <u>Pisum</u> <u>sativum</u> plants to decapitation or cytokinin application was consistent with the view that these lateral meristems are inter-dependent on the growth of each other. Correlative influences between lateral buds were found to coordinate and regulate the patterns of bud branching and thus plant form. These correlative influences or signals, ultimately controlled by environmental factors, were shown to be the result of gradients of growth regulators and nutrients in the whole plant. In particular, the patterns of cytokinin and auxin distribution and their modulation of flow through the plant were found to be important in the regulation of apical dominance. These endogenous growth regulator patterns in the plant were shown to be the result of their biosynthesis, translocation and metabolism. Together with the nutrient status of the plant, the distribution of growth regulators determined the growth potential of an inhibited lateral bud. This growth potential was manifest in the response of the lateral buds to direct applications of cytokinin. Of significance was that lateral buds responded differently to different cytokinins applied to them on the intact or decapitated plant. Thus it was shown, as hypothesized at the commencement of this study, that inhibited lateral buds on intact or decapitated <u>Pisum</u> <u>sativum</u> plants are not deficient in cytokinins and/or nutrients <u>per se</u>, but lack the capacity to utilize specific types of cytokinin for growth and development. Decapitation of the shoot apex results in a redistribution of growth regulators and nutrients in the plant, creating a more favourable auxin : cytokinin ratio at the lateral bud site. This ratio allows for the modification of cytokinins, previously inactive, to forms that the lateral buds can utilize.

The initial studies relating to lateral bud development and cytokinin application concerned an investigation of the correlations existing between different lateral buds, with particular reference to theoretical models of growth regulator and

nutrient distribution in the plant. The pattern of lateral bud branching following decapitation of plants supported the view that apically synthesized auxin, moving basipetally from the shoot apex exerts an inhibitory influence on lateral bud growth. Decapitation of plants of different sizes indicated that the pattern of lateral bud branching changes with the development of the plant. The position a lateral bud occupied on the shoot, and thus the regulating gradient determined how it would respond following decapitation. In addition, parallel responses between lateral buds released by decapitation or cytokinin application indicated that these two methods of bud release are influenced by the same gradient of growth regulators in the plant. Later, it was shown that the cytokinin metabolic activity in the stem occurred as a gradient in the plant, and could be related to plant age. These similar responses to experiments relating to different aspects of correlative control indicate a central controlling role for gradients of growth regulators in the shoot.

The control of lateral bud inhibition and their release and subsequent development are thus brought about by quantitative changes in the amounts and correlative influences between auxins, cytokinins and nutrients. However, what determines the relative supplies of these factors, essential for lateral bud growth? If auxins and cytokinins play a role in the control of apical dominance, mechanisms must exist for the control of their levels in plants. The differences in the biological activity of the natural cytokinins isopentenyladenine and zeatin in releasing lateral buds from apical dominance led to the suggestion that the correlative signal may operate via a control of the relative levels of these two cytokinins (or others) in the plant. The application of isopentenyladenine or zeatin to one of four lateral buds on intact or decapitated plants yielded results suggesting that removal of a shoot apex causes a change in response of lateral buds to exogenous cytokinin. Lateral buds nearer the intact shoot apex showed less ability

to utilize the applied cytokinins than buds lower on the shoot, while decapitation resulted in an increased ability of certain lateral buds to utilize isopentenyladenine.

The results of the cytokinin application studies led to the proposal that a factor, probably related to the inhibitory signal from the shoot apex controlled the relative amounts and forms of cytokinin at the lateral bud site. A correlative control signal was envisaged as acting on the promotion or inhibition of the enzyme system responsible for the hydroxylation of isopentenyladenine to zeatin. Since the correlative signal appeared to arrive at the site of primary events very soon after apical dominance was removed, but was inactive in isolated buds, it was concluded that this modulation of cytokinin activity occurred in the stem.

This was found to be the case following cytokinin metabolism studies which revealed that stems, roots and leaves, but not lateral buds of Pisum sativum possess the capacity to metabolise $[^3H]$ isopentenyladenine to zeatin metabolites. In addition, it was demonstrated that this metabolic capacity occurs as a gradient in the stem which can be related to the patterns of lateral bud release following decapitation or cytokinin application, as well as the age of the plant. It was concluded that stem tissue has the capacity to abstract from and modify and contribute to cytokinins that are translocated in the shoot. Auxin from the shoot apex appeared a likely candidate for the control of this cytokinin metabolic activity, and it was indeed shown, in the final experiment of this study, that auxin concentration had a direct effect on $[^3H]$ isopentenyladenine metabolism in stem sections in vitro. In this way, a control mechanism of apical dominance in Pisum sativum can be envisaged as operating at the level of the enzymic regulation of cytokinin metabolism, mediated by auxin.

This proposed mechanism is in keeping with the concept of a phytohormone, or plant growth regulator, since it does not include a localized site of synthesis and direct transport to a target tissue, as found with animal hormones. The cytokinins have been shown to occur in a wide range of plant tissues, yet if transported from these tissues, do not necessarily arrive at their site of action in the same molecular form. Thus, in this case, cytokinin mobility provides an opportunity for cytokinin modification as it is transported through tissues responsive to environmental or other stimuli governing the regulation of lateral bud inhibition. Whether or not such a mechanism is utilized by the plant as a primary control of apical dominance will depend on the existence of conditions under which the limited availability of specific cytokinins and possibly nutrients inhibit lateral bud growth. The final expression of apical dominance will thus represent the nett effect of interactions between the morphological environment experienced by the lateral bud and growth regulator systems at the level of synthesis, translocation, metabolism or ultimate growth regulator action.

REFERENCES

ABELES, F.B. 1966. Auxin stimulation of ethylene evolution. Plant Physiology 41: 585-588.

ABELES, F.B. 1985. Ethylene and plant development : an introduction. In: Ethylene and Plant Development. Roberts, J.A. and Tucker, G.A. (eds). Butterworths, London. pp. 1-8.

ABELES,F.B. and RUBINSTEIN, B. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiology 39: 963-969.

ADAMSON, D. 1962. Expansion and division in auxin-treated plant cells. Canadian Journal of Botany 40: 719-744.

ADDICOTT, F.T. and LYON, J.R. 1969. Physiology of abscisic acid and related substances. Annual Review of Plant Physiology 20: 139-164.

ALI, A. and FLETCHER, R.A. 1970. Hormonal regulation of apical dominance in soybeans. Canadian Journal of Botany 48: 1989-1994.

ALI, A. and FLETCHER, R.A. 1971. Hormonal interaction in controlling apical dominance in soybeans. Canadian Journal of Botany 49: 1727-1731.

AMES, I.H., HILL, C.A., WALTON, D.C. and DASHEK, W.V. 1979. Hormonal regulation of genetic tumor induction: cytokinin and abscisic acid. Plant and Cell Physiology 20: 1055-1061.

ANDERSEN, A.S. 1976. Regulation of apical dominance by ethephon, irradiance and CO_2. Physiologia Plantarum 37: 303-308.

ANDREAE, W.A. and VAN YSSELSTEIN, M.W.H. 1956. Studies on 3-indoleacetic acid metabolism. III. The uptake of 3-indoleacetic acid by pea epicotyls and its conversion to 3-indoleacetylaspartic acid. Plant Physiology 31: 235-240.

APELBAUM, A. and BURG, S.P. 1972a. Effect of ethylene on cell division and deoxyribonucleic acid synthesis in Pisum sativum. Plant Physiology 50: 117-124.

APELBAUM, A. and BURG, S.P. 1972b. Effects of ethylene and 2,4 dichloro-phenoxyacetic acid on cellular expansion in Pisum sativum. Plant Physiology 50: 125-131.

APELBAUM, A., SFAKIOTAKIS, E. and DILLEY, D.R. 1974. Reduction in extrac-table deoxyribonucleic acid polymerase activity in Pisum sativum seedlings by ethylene. Plant Physiology 54: 125-128.

ARNEY, S.E. and MITCHELL, D.L. 1969. The effect of abscisic acid on stem elongation and correlative inhibition. New Phytologist 68: 1001-1015.

ASPINALL, D. 1961. The control of tillering in the barley plant: I. The pattern of tillering and its relation to nutrient supply. Australian Journal of Biological Science 14: 493-505.

AUDUS, L.J. 1972. Plant Growth Substances. Leonard Hill, London.

AUNG, L.H. and BYRNE, J.M. 1978. Hormones and young leaves control de-velopment of cotyledonary buds in tomato seedlings. Plant Physiology 62: 276-279.

AUNG, L.H. 1984. Antimetabolites and hormones on the release and growth of Lycopersicon esculentum Mill. cotyledonary buds. Phyton (Horn) 44: 51-62.

BALLARD, L.A.T. and WILDMAN, S.G. 1964. Induction of mitosis in excised and attached dormant buds of sunflower (Helianthus annuus). Australian Journal of Biological Science 17: 36-43.

BARNOLA, P., LAVARENNE, S. and GERDRAUD, M. 1986. Apical bud dormancy of ash (Fraxinus excelsior): an evolution of the nucleotide triphosphate pool and the range of temperatures acting on bud opening during dormancy. Annales des Sciences Forestiers 43: 339-350.

BEAUCHESNE, G. and GOUTAREL, R. 1963. Activite de certaines purines substituees sur le developpement des cultures de tissue de moëlle de Tabarc en presence d'acide indolylacetique. Physiologia Plantarum 16: 630-635.

BEEVER, J.E. and WOOLHOUSE, H.W. 1973. Increased cytokinin from root system of Perilla fructescens and flower and fruit development. Nature 246: 31-32.

BELLANDI, D.M. and DORFFLING, K. 1974. Effect of abscisic acid and other plant hormones on growth of apical and lateral buds of seedlings. Physiologia Plantarum 32: 322-327.

BHATTACHARYYA, R.N. and BASU, P.S. 1985. Dependence of kinetin induced elongation of wheat coleoptiles on IAA metabolism. Biochemie und Physiologie der Pflanzen: 180: 269-276.

BIDDINGTON, N.L. and THOMAS, T.H. 1978. Influence of different cytokinins on the transpiration and senescence of excised oat leaves. Physiologia Plantarum 42: 369-374.

BINNIE, R.C. and CLIFFORD, P.E. 1980. Effects of some defoliation and decapitation treatments on the productivity of French beans. Annals of Botany 46: 811-813.

BIONI, S. and CANCIANI, L. 1984. Uptake and translocation of benzyladenine by elm shoots cultured in vitro. Canadian Journal of Botany 62: 2385-2390.

BLACK, M.K. and OSBORNE, D.J. 1965. Polarity of transport of benzyladenine, adenine and indole-3-acetic acid in petiole segments of Phaseolus vulgaris. Plant Physiology 40: 676-680.

BLAKE, T.J., REID, D.M. and ROOD, S.B. 1983. Ethylene, indoleacetic acid and apical dominance in peas: a reappraisal. Physiologia Plantarum 59: 481-487.

BLAKE, T.J. and TSCHAPLINSKI, T.J. 1986. Role of water relations and photosynthesis in the release of buds from apical dominance and the early reinvigoration of decapitated poplars. Physiologia Plantarum 68: 287-293.

BOOKER, C.E. and DWIVEDI, R.S. 1973. Ultrastructure of meristematic cells of dominant and released buds of Tradescantia paludosa. Experimental Cell Research 82: 255-261.

BOOTH, A., MOORBY, J., DAVIES, C.R., JONES, H. and WAREING, P.R. 1962. Effects of indolyl-3-acetic acid on the movement of nutrients within plants. Nature 194: 204-205.

BOPP, M. 1980. The hormonal regulation of morphogenesis in mosses. In: Plant Growth Substances 1979. Skoog, F. (ed.). Springer-Verlag, Berlin. pp. 351-361.

BOWEN, M.R. and WAREING, P.F. 1971. Further investigations into hormone-directed transport in stems. Planta 99: 120-132.

BRAUN, J.W. and KENDER, W.J. 1985. Correlative bud inhibition and growth habit of the strawberry as influenced by application of gibberellic acid, cytokinin and chilling during short daylength. Journal of the American Society for Horticultural Science 110: 28-34.

BRENNER, M.L., WOLLEY, D.J., SJUT, V. and SALERNO, D. 1987. Analysis of apical dominance in relation to IAA transport. HortScience 22: 833-835.

BRIAN, P.W., HEMMING, H.G. and RADLEY, M. 1955. A physiological comparison of gibberellic acid with some auxins. Physiologia Plantarum 8: 899-912.

BRIAN, P.W., HEMMING, H.G. and LOWE, D. 1959. The effect of gibberellic acid on shoot growth of Cupid sweet peas. Physiologia Plantarum 12: 15-29.

BRUINSMA, J. 1980. The endogenous hormonal pattern and its interference by exogenous plant growth regulators. In: Physiological Aspects of Crop Productivity. Proceedings of the 15th Colloq. Int. Potash Institute, Bern. pp. 117-124.

BURG, S.P. 1973. Ethylene and plant growth. Proceedings of the National Academy of Sciences U.S.A. 70: 591-597.

BURG, S.P. and BURG, E.A. 1967. Ethylene action independent of protein synthesis. Plant Physiology 42: 531.

BURG, S.P. and BURG, E.A. 1968. Ethylene formation in pea seedlings, its relation to the inhibition of bud growth caused by indole-3-acetic acid. Plant Physiology 43: 1069-1074.

BURROWS, W.J. 1978. Incorporation of ^{3}H-adenine into free cytokinins by cytokinin - autonomous tobacco callus tissue. Biochemical and Biophysical Research Communications 84: 743-748.

BURROWS, W.J. and CARR, D.J. 1969. Effects of flooding the root system of sunflower plants on the cytokinin content in the xylem sap. Physiologia Plantarum 22: 1105-1112.

BUTLER, R.D. and LANE, G.R. 1959. The study of apical development in relation to etiolation. Journal of the Linnean Society of London Botany 56: 170-176.

CARPENTER, W.J. and RODRIGUEZ, R.C. 1971. The effect of plant growth regulating chemicals on rose shoot development from basal and axillary buds. Journal of the American Society for Horticultural Science 96: 389-391.

CARR, D.J. 1966. Metabolic and hormonal regulation of growth and development. In: Trends in Plant Morphogenesis. Cutter, E.G. (ed.). Longmans, London. pp. 253-283.

CARR, D.J. and BURROWS, W.J. 1966. Evidence of the presence in xylem sap of substances with kinetin like activity. Life Sciences 5: 2061-2077.

CATALANO, M. and HILL, T.A. 1969. Interaction between gibberellic acid and kinetin in overcoming apical dominance, natural and induced by IAA, in tomato (Lycopersicon esculentum Mill. cv. potentate). Nature 222: 985-986.

CATCHPOLE, A.H. and HILLMAN, J.R. 1976. The involvement of ethylene in the coiled sprout disorder of potato. Annals of Applied Biology 83: 413-423.

CHAMPAGNAT, P. and DALZON, M. 1958. Inhibition par carence sur les plantules de dicotyledones. Revue de Cytologie et de Biologie Vegetales 19: 337-343.

CHANG, W.C. and GOODIN, J.R. 1974. The role of root systems in lateral bud growth of pea (Pisum sativum L., var. Alaska). Botanical Bulletin of Academia Sinicia (Taipei) 15: 112-122.

CHEN, C-M. 1981. Biosynthesis and enzymic regulation of the interconversion of cytokinin. In: Metabolism and Molecular Activities of Cytokinins. Guern, J. and Péaud-Lenoël, C. (eds). Springer-Verlag, Heidelberg. pp. 34-43.

CHEN, C-M. 1982. Cytokinin biosynthesis in cell-free systems. In: Plant Growth Substances 1982. Wareing, P.F. (ed.). Academic Press, London. pp. 155-163.

CHEN, C-M. and ECKERT, R.L. 1976. Evidence for the biosynthesis of transfer RNA-free cytokinin. FEBS letters 64: 429-434.

CHEN, C-M., ECKERT, R.L. and McCHESNEY, J.D. 1976. Evidence for the biosynthesis of transfer-RNA-free cytokinin. FEBS letters 64: 429-434.

CHEN, C-M., ERTL, J.R., LEISNER, S.M. and CHANG, C.C. 1985. Localization of cytokinin biosynthetic sites in pea plants and carrot roots. Plant Physiology 78: 510-513.

CHEN, C-M. and KRISTOPEIT, S.M. 1981. Deribosylation of cytokinin ribonucleoside by adenosine nucleosidase from wheat germ cells. Plant Physiology 68: 1020-1023.

CHEN, C-M. and LEISNER, S.M. 1984. Modification of cytokinins by cauliflower microsomal enzymes. Plant Physiology 75: 442-446.

CHEN, C-M. and PETSCHOW, B. 1978a. Cytokinin biosynthesis in cultured rootless tobacco plants. Plant Physiology 62: 861-865.

CHEN, C-M. and PETSCHOW, B. 1978b. Metabolism of cytokinin. Ribosylation of cytokinin bases by adenosine phosphorylase from wheat germ. Plant Physiology 62: 871-874.

CHOVEAUX, N.A. and VAN STADEN, J. 1981. The effect of 1-naphthaleneacetic acid on the endogenous cytokinin content of aseptically cultured bark segments of Salix babylonica. Plant and Cell Physiology 22: 1207-1214.

CLINE, M.G. 1983. Apical dominance in Pharbitis nil: effects induced by inverting the apex of the main shoot. Annals of Botany 52: 217-227.

CLINE, M.G. and RILEY, L. 1984. The presentation time for shoot inversion release of apical dominance in Pharbitis nil. Annals of Botany 53: 897-900.

CLINE, M.G. and PRASAD, T.K. 1985. Effects of simulated gravity nullification on shoot-inversion release of apical dominance in Pharbitis nil. Physiologist 28: 97-98.

COWLEY, D.E., DUKE, C.C., LIEPA, A.J., MACLEOD, J.K. AND LETHAM, D.S. 1978. The structure and synthesis of cytokinin metabolites I. The 7- and 9-β-D-glucopuranosides and pyranosides of zeatin and 6-benzylamino-purine. Australian Journal of Chemistry 31: 1095-1111.

CUTTER, E.G. 1972a. In: The Dynamics of Meristem Cell Populations. Miller, M.M. and Kuehnert, C.C. (eds). Plenum, New York. pp. 51-53. Cited by: Phillips, I.D.J., 1975. Annual Review of Plant Physiology 26: 341-367.

CUTTER, E.G. 1972b. Regulation of branching in decussate species with unequal lateral buds. Annals of Botany 36: 207-220.

CUTTER, E.G. 1975. A morphogeneticist's view of correlative inhibition in the shoot. Advances in Experimental Medicine and Biology 18: 51-73.

CUTTER, E.G. and CHIU, H. 1972. Grafting experiments on correlative effects between lateral buds. Annals of Botany 36: 221-228.

CUTTER, E.G. and CHIU, H. 1975. Differential response of buds along the shoot to factors involved in apical dominance. Journal of Experimental Botany 26: 828-839.

DARWIN, C. 1881. The Power of Movement in Plants. D. Appleton, New York.

DAVEY, J.E. and VAN STADEN, J. 1981. Cytokin activity in Lupinus albus V. Translocation and metabolism of [8 - 14 C] zeatin applied to the xylem of fruiting plants. Physiologia Plantarum 51: 45-48.

DAVIES, P.J. 1987. The plant hormone concept: Transport, concentration and sensitivity. In: Plant Hormones and their Role in Plant Growth and Development. Martinus Nijhoff, Dordrecht. pp. 12-23.

DAVIES, P.J., HORGAN, R., HEALD, J.K. and McGAW, B.A. 1986. Endogenous cytokinins in vegetative shoots of peas. Plant Growth Regulation 4: 311-323.

DAVIES, C.R. and WAREING, P.F. 1965. Auxin-directed transport of radio-phosphorus in stems. Planta 65: 139-156.

DENIZCI, R. 1966. Uber den einfluss von kinetin und indolylessigsaure auf des austreiben der knospen von Pisum sativum. Planta 68: 141-156.

DE VRIES, D.P., DUBOIS, L.A.M. and SMEETS, L. 1986. The effect of temperature on axillary bud-break of hybrid tea-rose seedlings. Scientia Horticulturae 28: 281-287.

DICKINSON, J.R., FORSYTH, C. AND VAN STADEN, J. 1986. The role of adenine in the synthesis of cytokinins in tomato plants and in cell-free root extracts. Plant Growth Regulation 4: 325-334.

DORFFLING, K. 1964. Uber das wuchsstoff-hemmstaffsystem van Acer pseudo-platanus L. II. Die bedeutung van "Inhibitor β" fur die korrelative knospen-hemmung und fur die regulation der kambiumtatigkeit. Planta 60: 413-433.

DORFFLING, K. 1965. Berichte de Deutschen Botanischen Gesellschaft 78: 122-128. Cited by: Phillips, I.D.J. 1975 Annual Review of Plant Physiology 26: 341-367.

DORFFLING, K. 1966. Weitere untersuchungen uber korrelative knospenhem-ming. Planta 70: 257-274.

DORFFLING,K. 1976. Correlative bud inhibition and abscisic acid in Acer pseudo-platanus and Syringa vulgaris. Physiologia Plantarum 38: 319-322.

DORSTAL, R. 1909. Die korrelationsbeziehung zwischen dem blatt und seiner axillarknospe. Berichte de Deutschen Botanischen Gesellschaft 27: 547-554.

DORSTAL, R. 1926. Uber die wachstumsregulierende wirkung des laubblattes. Acta Societatis Scientiarum Naturalium Moravo-Silesiacae 3: 83-209.

DREW, R.A. and SMITH, N.G. 1986. Growth of apical and lateral buds of papaw (Carica papaya, L.) as affected by nutritional and hormonal factors. Journal of Horticultural Science 61: 535-543.

DUKE, C.C., LETHAM, D.S., PARKER, C.W., MACLEOD, J.K. and SUMMONS, R.E. 1979. The complex of O-glucosylzeatin derivatives formed in Populus species. Phytochemistry 18: 819-824.

DUKE, C.C., MACLEOD, J.K., SUMMONS, R.E., LETHAM, D.S. and PARKER, C.W. 1978. Lupinic acid and O-β-D glucopyranosylzeatin from Lupinus angustifolium. Australian Journal of Chemistry 31: 1291-1301.

DWIVEDI, R.S. and NAYLOR, J.M. 1968. Influence of apical dominance on the nuclear proteins in cells of the lateral bud meristem in Tradescantia paludosa. Canadian Journal of Botany 46: 289-298.

EINSET, J.W. 1984. Conversion of N6 - isopentenyladenine to zeatin by Actinidia tissues. Biochemical and Biophysical Research Communications 124: 470-474.

EINSET, J.W. 1985. Cytokinin metabolism in autonomous and crown gall tissue cultures. Biologia Plantarum 27: 276-280.

EINSET, J.W. 1986a. Zeatin biosynthesis from N^6-(Δ^2-isopentenyl) adenine in Actinidia and other woody plants. Proceedings of the National Academy of Sciences U.S.A. 83: 972-975.

EINSET, J.W. 1986b. Biosynthesis of zeatin from N^6-(Δ^2isopentenyl) adenine in Actinidia: Sites and seasonal changes in activity. Proceedings of the National Academy of Sciences U.S.A. 83: 7751-7754.

EINSET, J.W. and SILVERSTONE, A. 1987. Hydroxylation of N^6-(Δ^2-isopentenyl) adenine to zeatin. Relative activities of organ systems from Actinidia arguta. Plant Physiology 84: 208-209.

EINSET, J.W. and SKOOG, F. 1973. Biosynthesis of cytokinins in cytokinin - autotrophic tobacco callus. Proceedings of the National Academy of Sciences U.S.A. 70: 658-660.

ELFVING, D.C. 1985. Comparison of cytokinin and apical-dominance-inhibiting growth regulators for lateral-branch induction in nursery and orchard apple trees. Journal of Horticultural Science 60: 447-454.

ENGELBRECHT, L. 1972. Cytokinins in leaf cuttings of Phaseolus vulgaris L. during their development. Biochemie und Physiologie der Pflanzen 163: 335-343.

ENTSCH, B., LETHAM, D.S., PARKER, C.W., SUMMONS, R.E. and GOLLNOW, B.I. 1980. Metabolites of cytokinins. In: Plant Growth Substances 1979. Skoog, F. (ed.). Springer-Verlag, New York. pp. 109-118.

ERNEST, L.C. and VALDOVINOS, J.G. 1971. Regulation of auxin levels in Coleus blumei by ethylene. Plant Physiology 48: 402-406.

ERRERA, L. 1904. Conflits de preseance et excitations inhibitoires ches les vegetaux. Bulletin de la Societe Royale de Botanique de Belgique 42: 27.

EVERAT-BOURBOULOUX, A. 1987. Distribution of free and bound forms of cis-trans and trans-trans abscisic acid in broad-bean plants in relation to apical dominance. Physiologia Plantarum 70: 648-652.

EVERAT-BOURBOULOUX, A. and BONNEMAIN, J.L. 1980. Distribution of labelled auxin and derivatives in stem tissues of intact and decapitated broad-bean plants in relation to apical dominance. Physiologia Plantarum 50: 145-152.

EVERAT-BOURBOULOUX, A. and CHARNAY, D. 1982. Endogenous abscisic acid levels in stems and axillary buds of intact or decapitated broad-bean plants (Vicia faba L.). Physiologia Plantarum 54: 440-445.

FELDMAN, L.J. 1975. Cytoknin and quiescent centre activity in roots of Zea. In: The Development and Function of Roots. Third Cabot Symposium. Torrey, J.G. and Clarkson, D.T. (eds). Academic Press, London. pp. 55-72.

FELDMAN, L.J. 1979. Cytokinin biosynthesis in roots of corn. Planta 145: 315-322.

FIELD, R.J. and JACKSON, D.I. 1975a. A hormone balance theory of apical dominance. In: Mechanisms of Regulation of Plant Growth. Bieleski, R.L. (ed.). The Royal Society of New Zealand, Bulletin 12.

FIELD, R.J. and JACKSON, D.I. 1975b. Light effects on apical dominance. Annals of Botany 39: 369-374.

FISHER, J.B. 1971. Control of bud inhibition in Cyperus. Planta 97: 257-268.

FLETCHER, G.M. and DALE, J.E. 1974. Growth of tiller buds in barley: Effects of shade treatment and mineral nutrition. Annals of Botany 38: 63-76.

FIRN, R.D. 1986. Growth substance sensitivity: The need for clearer ideas, precise terms and purposeful experiments. Physiologia Plantarum 67: 267-272.

FIRN, R. and DIGBY, J. 1984. A third dimension in the control of plant development. Trends in Biological Sciences 9: 49.

FORSYTH, C. and VAN STADEN, J. 1981. The effects of root decapitation on lateral root formation and cytokinin production in Pisum sativum. Physiologia Plantarum 51: 375-379.

GALSTON, A.W. and DALBERG, L.Y. 1954. The adaptive formation and physiological significance of indoleacetic acid oxidase. American Journal of Botany 41: 373-380.

GARRISON, F.R., BRINKER, A.M. AND NOODÉN, L.D. 1984. Relative activities of xylem-supplied cytokinins in retarding soybean leaf senescence and sustaining pod development. Plant and Cell Physiology 25: 213-224.

GOEBEL, K. 1900. Organography of Plants Especially of the Archegoniate and Spermaphyta. Part 1. General Organography. Clarendon Press, Oxford.

GOLDSMITH, M.H.M. 1968. The transport of auxin. Annual Review of Plant Physiology 19: 347-360.

GOLDSMITH, M.H.M. 1977. The foliar transport of auxin. Annual Review of Plant Physiology 28: 439-478.

GOLDSMITH, M.H.M. and THIMANN, K.V. 1962. Some characteristics of movement of indoleacetic acid in coleoptiles of Avena. I. Uptake, destruction, immobilization and distribution of IAA during basipetal translocation. Plant Physiology 37: 492-505.

GOODWIN, P.B. and CANSFIELD, P.E. 1967. The control of branch growth on potato tubers. III. The basis of correlative inhibition. Journal of Experimental Botany 18: 297-307.

GORDON, S.A. 1946. Auxin-protein complexes of the wheat grain. American Journal of Botany 33: 160-169.

GORDON, M.E., LETHAM, D.S. and PARKER, C.W. 1974. The metabolism and translocation of zeatin in intact radish seedlings. Annals of Botany 38: 809-825.

GOULD, K.S., CUTTER, E.G., YOUNG, J.P.W. and CHARLTON, W.A. 1987. Positional differences in size, morphology, and in vitro performance of pea axillary buds. Canadian Journal of Botany 65: 406-411.

GREENWOOD, M.S. and GOLDSMITH, M.H.M. 1970. Polar transport and accumulation of indole-3-acetic acid during root regeneration by Pinus lambertiana embryos. Planta 95: 297-313.

GREGORY, F.G. and VEALE,J.A. 1957. A reassessment of the problems of apical dominance. Symposia of the Society for Experimental Biology 11: 1-20.

GUERN, J. and SADORGE, P. 1967. Polarite du transport de la 6-benzylamino-purine dans les jeunes plants etiolas de Cicer arietinum. Comptes Rendus de L'Academie des Sciences Serie III Sciences de la Vie 264: 2106-2109.

GUERN, J. and USCIATI, 1972. The present status of the problem of apical dominance. In: Hormonal Regulation in Plant Growth and Development. Kaldeway, H. and Vardar, Y. (eds). Verlag Chemie, Weinheim. pp. 383-400.

HALL, S.M. and BAKER, D.A. 1972. The chemical composition of Ricinus phloem exudate. Planta 106: 131-140.

HALL, S.M. and HILLMAN, J.R. 1975. Correlative inhibition of lateral bud growth in Phaseolus vulgaris L. Timing of bud growth following decapitation. Planta 123: 137-143.

HALL, W.C., TRUCHELUT, G.B., LEINWEBER, C.L. and HERRERO, F.A. 1957. Ethylene production by the cotton plant and its effects under experimental and field conditions. Physiologia Plantarum 10: 306-317.

HANSEN, C.E., MEINS, F. and AEBI, R. 1987. Hormonal regulation of zeatin-riboside accumulation by cultured tobacco cells. Planta 172: 520-525.

HANSEN, C.E., MEINS, F. and MILANI, A. 1985. Clonal and physiological variation in the cytokinin content of tobacco cell lines differing in cytokinin require-ment and capacity for neoplastic growth. Differentiation 29: 1-6.

HARDWICK, R.C. 1986. Physiological consequences of modular growth in plants. Philosophical Transactions of the Royal Society of London B Biological Sciences 133: 161-173.

HARRISON, M.A. and KAUFMAN, P.B. 1984. The role of hormone transport and metabolism in apical dominance in oats. Botanical Gazette 145: 293-297.

HARTUNG, W. and FUNGER, C. 1981. Abscisic acid and apical dominance in Phaseolus coccineus L. The role of tissue age. Annals of Botany 47: 371-375.

HARTUNG, W. and STEIGERWALD, F. 1977. Absciscic acid and apical dominance in Phaseolus coccineus L. Planta 134: 295-299.

HECHT, S.M., LEONARD, N.J., SCHMITZ, R.Y. and SKOOG, F. 1970. Cytokinins: synthesis and growth promoting activity of 2-substituted compounds in the N^6-isopentenyladenine and zeatin series. Phytochemistry 9: 1173-1180.

HEMBERG, T. and LARSSON, U. 1972. Interaction of kinetin and indole acetic acid in the Avena straight growth test. Physiologia Plantarum 26: 104-107.

HENDRY, N.S., VAN STADEN, J. and ALLAN, P. 1982. Cytokinins in Citrus. I. Fluctuations in the leaves during seasonal and developmental changes. Scientia Horticulturae 16: 9-16.

HENSON, I.E. 1978. Cytokinins and their metabolism in leaves of Alnus glutinosa L. Gaertn. Zeitschrift für Pflanzenphysiologie 86: 363-369.

HENSON, I.E. and WAREING, P.F. 1976. Cytokinins in Xanthium strumarium L. : Distribution in the plant and production in the root system. Journal of Experimental Botany 27: 1268-1278.

HEPLER, P.K. and WAYNE, R.O. 1985. Calcium and plant development. Annual Review of Plant Physiology 36: 397-439.

HEWETT, E.W. and WAREING, P.F. 1973. Cytokinins in Populus x robusta: Changes during chilling and bud burst. Physiologia Plantarum 28: 393-399.

HILLMAN, J.R. 1970. The hormonal regulation of bud outgrowth in Phaseolus vulgaris L. Planta 90: 222-229.

HILLMAN, J.R. 1984. Apical dominance. In: Advanced Plant Physiology. Wilkins, M.B. (ed.). Pitman, London. pp. 127-148.

HILLMAN, J.R. 1986. Apical dominance and correlations by hormones. In: Plant Growth Substances 1985. Bopp, M. (ed.). Springer-Verlag, Berlin. pp. 341-349.

HILLMAN, J.R., MATH, V.B. and MEDLOW, G.C. 1977. Apical dominance and the levels of indole acetic acid in Phaseolus lateral buds. Planta 134: 191-193.

HILLMAN, J.R. and YEANG, H.Y. 1979. Correlative inhibition of lateral bud growth in Phaseolus vulgaris L. Ethylene and the physical restriction of apical growth. Journal of Experimental Botany 30: 1075-1083.

HILLMAN, J.R., YEANG, H.Y. and FAIRHURST, V.J. 1985. Ethylene, lateral bud growth and indole-3-acetic acid transport. In: Ethylene and Plant Development. Roberts, J.A. and Tucker, G.A. (Eds). Butterworths, London. pp. 213-227.

HILLMAN, W.S. 1957. Nonphotosynthetic light requirement in Lemna minor and its partial satisfaction by kinetin. Science 126: 165-166.

HOAD, G.V. 1973. Effect of moisture stress on abscisic acid levels in Ricinus communis L. with particular reference to phloem exudate. Planta 113: 367-372.

HOAD, G.V., LOVEYS, B.R. and SKENE, K.G.M. 1977. The effect of fruit removal on cytokinins and gibberellin-like substances in grape leaves. Planta 136: 25-30.

HOAGLAND, D.R. and SNYDER, W.C. 1933. Nutrition of strawberry plants under controlled conditions. Proceedings of the American Society for Horticultural Science 30: 288-294.

HOREMANS, S., VAN ONCKELEN, H.A. and de GREEF, J.A. 1986. Longitudinal gradients of indole-3-acetic acid and abscisic acid in the hypocotyl of etiolated bean seedlings. Journal of Experimental Botany 37: 1525-1532.

HORGAN, R. 1986. Cytokinin biosynthesis and metabolism. In: Plant Growth Substances 1985. Bopp, M. (ed.). Springer-Verlag, Heidelberg. pp. 92-98.

HORGAN, R., PALNI, L.M.S., SCOTT, I. and McGAW, B. 1981. Cytokinin biosynthesis and metabolism in Vinca rosea crown gall tissue. In: Metabolism and Molecular Activities of Cytokinins. Guern, J. and Péaud-Lenoël, C. (eds). Springer-Verlag, Heidelberg. pp. 56-65.

HUGON, E. 1958. Interaction entre saccharose, thiamine et auxine dans les correlations entre bourgeons cotyledonaires ches Cicer arietinium L. Comptes Rendus de L'Academie des Sciences 247: 339-341.

HUSAIN, S.M. and LINCK, A.J. 1966. Relationship of apical dominance to the nutrient accumulation pattern in Pisum sativum var. Alaska. Physiologia Plantarum 19: 992-1010.

HUTTON, M.J. 1982. Transport and Metabolism of 8 (^{14}C) t-Zeatin. Ph.D. Thesis, University of Natal, Pietermaritzburg, South Africa.

HUTTON, M.J. and VAN STADEN, J. 1984. Transport and metabolism of labelled zeatin applied to the stems of Phaseolus vulgaris at different stages of development. Zeitschrift für Pflanzenphysiologie 114: 331-339.

INOUE, M., MAEDA, E., YOSHIDA, R. and ORITANI, I. 1979. On the occurrence of a high content of cytokinins in rice callus tissue. Plant and Cell Physiology 20: 917-924.

JACOBS, W.P. 1950. Auxin transport in the hypocotyl of Phaseolus vulgaris L. American Journal of Botany 37: 248-254.

JACOBS, W.P. 1970. The movement of plant hormones: auxins, gibberellins, and cytokinins. In: Plant Growth Substances, 1970. Carr, D.J. (Ed.). Springer-Verlag, Heidelberg. pp. 701-709.

JACOBS, W.P. 1978. Regulation of development by the differential polarity of various hormones as well as by the effects of one hormone on the polarity of another. In: Regulation of Developmental Processes in Plants. Schütte, H.R. and Gross, D. (eds). VEB Gustaf Fischer Verlag, Jena. pp. 361-380.

JACOBS, W.P. and BULLWINKEL, B. 1953. Compensatory growth in coleus shoots. American Journal of Botany 40: 385-391.

JACOBS, W.P. and CASE, D.B. 1965. Auxin transport, gibberellin and apical dominance. Science 148: 1729-1731.

JACOBS, W.P., DANIELSON, J., HURST, V. and ADAMS, P. 1959. What substance normally controls a given biological process? II. The relation of auxin to apical dominance. Developmental Biology 1: 534-554.

JAMESON, P.E., ZHANG, R. and LETHAM, D.S. 1985. Metabolism and translocation of ^{3}H-zeatin riboside in blue lupin plants. In: Abstracts of the 12th International Conference on Plant Growth Substances, Heidelberg. pp. 20.

JONES, R.L. and PHILLIPS, I.D.J. 1966. Organs of gibberellin synthesis in light grown sunflower plants. Plant Physiology 41: 1381-1386.

KALDEWAY, H. 1965. Wuchstofftransport, temperatur und pflanzenalter. Berichte de Deutschen Botanischen Gesellschaft 78: 128-143.

KAMISAKA, S. and MASUDA, Y. 1970. Auxin induced growth of Helianthus tuber tissue. V. Role of DNA synthesis in hormonal control of cell growth. Physiologia Plantarum 23: 343-350.

KASPERBAUER, M.J. 1971. Spectral distribution of light in a tobacco canopy and effects of end-of-day light quality on growth and development. Plant Physiology 47: 775-778.

KATO, J. 1958. Studies on the physiological effect of gibberellin II. On the interaction of gibberellin with auxin and growth inhibitors. Physiologia Plantarum 11: 10-15.

KATSUMI, M. and IKEO, Y. 1970. Stimulation of lateral bud growth of pea by (-)-Kauren-19-oic acid. Plant and Cell Physiology 47: 775-778.

KEIM, P., ERION, J. and FOX, J.E. 1981. The current status of cytokinin-binding moieties. In: Metabolism and Molecular Activities of Cytokinins. Guern, J. and Péaud-Lenoël, C. (eds). Springer-Verlag, Berlin. pp. 179-190.

KENDE, H. 1964. Preservation of chlorophyll in leaf sections by substances obtained from root exudate. Science 145: 1066-1067.

KENDE, H. 1965. Kinetin-like factors in the root exudate of sunflowers. Proceedings of the National Academy of Sciences U.S.A. 53: 1302-1307.

KENDE, H. and SITTON, D. 1967. The physiological significance of kinetin and gibberellin-like root hormones. Annals of the New York Academy of Sciences 144: 235-243.

KLEMEN, F. AND KLÄMBT, D. 1974. Half-life of sRNA from primary roots of Zea mays. A contribution to the cytokinin production. Physiologia Plantarum 31: 186-188.

KNOX, J.P. and WAREING, P.F. 1984. Apical dominance in Phaseolus vulgaris L.: The possible roles of abscisic acid and indole-3-acetic acid. Journal of Experimental Botany 35: 239-244.

KODA, Y. and OKAZAWA, Y. 1980. Cytokinin production by Asparagus shoot apex cultured in vitro. Physiologia Plantarum 49: 193-197.

KRAMER, D., DESBIEZ, M.O., GARREC, J.P., THELLIER, M., FOURCY, A. and BOSSY, J.P. 1980. The possible role of potassium in the activation of axillary buds of Bidens pilosus L. after decapitation of the apex. Journal of Experimental Botany 31: 771-776.

KRELLE, E. and LIBBERT, E. 1968. Inhibition of the polar auxin transport by a morphactin. Planta 80: 317-320.

KUHNLE, J.A., FULLER, G., CORSE, J. and MACKEY, B.E. 1977. Antisenescent activity of natural cytokinins. Physiologia Plantarum 41: 14-21.

KURASHI, S. 1959. Effect of kinetin analogs on leaf growth. Scientific Papers of the College of General Education, University of Tokyo (Biological Part) 9: 67-104.

LALOUE, M., GAWER, M. and TERRINE, C. 1975. Modalites de l'utilisation de cytokines exogenes par les cellules de tabac culturées en milieu liquide agite. Physiologia Vegetale 13: 781-796.

LALOUE, M. and PETHE, C. 1982. Dynamics of cytokinin metabolism in tobacco cells. In: Plant Growth Substances 1982. Wareing, P.F. (ed.). Academic Press, London. pp. 185-195.

LALOUE, M., TERRINE, C. and GAWER, M. 1974. Cytokinins: formation of the nucleoside-5'-triphosphate in tobacco and Acer cells. FEBS Letters 46: 45-50.

LALOUE, M., TERRINE, C. and GUERN, J. 1977. Cytokinins: metabolism and biological activity of N^6-(Δ^2-isopentenyl) adenosine in tobacco cells and callus. Plant Physiology 59: 478-483.

LANG, A. 1970. Gibberellins: Structure and metabolism. Annual Review of Plant Physiology 21: 537-570.

LEAKEY, R.R.B. and LONGMAN, K.A. 1986. Physiological, environmental and genetic variation in apical dominance as determined by decapitation in Triplochiton schleroxylon. Tree Physiology 1: 193-207.

LEE, T.T. 1984. Release of lateral buds from apical dominance by glyphosate in soybean and pea seedlings. Journal of Plant Growth Regulation 1: 37-48.

LEE, P.K., KESSLER, B. and THIMANN, K.V. 1974. The effect of hadacidin on bud development and its implications for apical dominance. Physiologia Plantarum 31: 11-14.

LEE, Y-H., MOK, M.C., MOK, D.W.S., GRIFFIN, D.A. and SHAW, G. 1985. Cytokinin metabolism in Phaseolus embryos. Genetic difference and the occurrence of novel zeatin metabolites. Plant Physiology 77: 635-641.

LEINEWEBER, M. and KLÄMBT, D. 1974. Half life of sRNA and its consequence for the formation of cytokinins in Lactobacillus acidophilus ATCC 4963 grown in the logarithmic and stationary phases. Physiologia Plantarum 30: 327-330.

LEOPOLD, A.C. and DE LA FUENTE, R.K. 1968. A view of polar auxin transport. In: The Transport of Plant Hormones. Vardar, Y. (ed.). North-Holland, Amsterdam. pp. 24-47.

LEOPOLD, A.C. and GUERNSEY, F.S. 1953. Auxin polarity in the Coleus plant. Botanical Gazette 115: 147-154.

LEOPOLD, A.C. and KRIEDEMANN, 1975. Plant Growth and Development. McGraw-Hill, New York.

LEOPOLD, A.C. and LAM, S.L. 1962. The auxin transport gradient. Physiologia Plantarum 15: 631-638.

LEONARD, N.J., HECHT, S.M., SKOOG, F. and SCHMITZ, R.Y. 1968. Cytokinins: Synthesis of 6-(3-methyl-3-butanylamino)-9-β-D-ribofuranosylpurine (3iPA) and the effect of side-chain unsaturation on the biological activity of isopentenylaminopurines and their ribosides. Proceedings of the National Academy of Sciences U.S.A. 59: 15-21.

LEONARD, N.J., HECHT, S.M., SKOOG, F. and SCHMITZ, R.Y. 1969. Cytokinins: synthesis, mass spectra and biological activity of compounds related to zeatin. Proceedings of the National Academy of Sciences U.S.A. 63: 175-182.

LETHAM, D.S. 1967. Regulators of cell division in plant tissues V. A comparison of the activities of zeatin and other cytokinins in five bioassays. Planta 74: 228-242.

LETHAM, D.S. and PALNI, L.M.S. 1983. The biosynthesis and metabolism of cytokinins. Annual Review of Plant Physiology 34: 163-197.

LETHAM, D.S., PARKER, C.W., DUKE, C.C., SUMMONS, R.E. and MACLEOD, J.K. 1977. O-Glucosylation and related compounds - a new group of cytokinin metabolites. Annals of Botany 41: 261-263.

LETHAM, D.S., TAO, G.Q. and PARKER, C.W. 1982. An overview of cytokinin metabolism. In: Plant Growth Substances 1982. Wareing, P.F. (ed.). Academic Press, London. pp. 143-153.

LIBBERT, E. 1964. Kontrolliert auxin die apikaldominanz? Physiologia Plantarum 17: 371-378.

LINO, M. 1982. Action of red light on indole-3-acetic acid status and growth in coleoptiles of etiolated maize seedlings. Planta 156: 21-22.

LINSMAIER, E.M. and SKOOG, F. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiologia Plantarum 18: 100-127.

LITTLE, C.H.A. 1970. Apical dominance in long shoots of white pine (Pinus strobus). Canadian Journal of Botany 48: 239-253.

LOEB, J. 1917. The chemical basis of axial polarity in regeneration. Science 46: 547-551.

LOEB, J. 1918. Chemical basis of correlation. I. Production of equal masses of shoots by equal masses of sister leaves in Bryophyllum calycinum. Botanical Gazette 65: 150-174.

LOEB, J. 1924. Regeneration from a Physiochemical Viewpoint. McGraw Hill, New York.

LOEFFLER, J.E. and VAN OVERBEEK, J. 1964. Kinin activity in coconut milk. In: Regulateurs Naturels de la Croissance Vegetale. Nitsch, J.P. (ed.). Centre National de la Recherche Scientifique, Paris. pp. 77-82.

MARTH, P.C., AUDIA, W.C. and MITCHELL, J.W. 1956. Effects of gibberellic acid on growth and development of plants of various genera and species. Botanical Gazette 118: 106-111.

MARTIN, G.C. 1987. Apical dominance. HortScience 22: 824-833.

McCREADY, C.C. 1966. Translocation of growth regulators. Annual Review of Plant Physiology 17: 283-294.

McCREADY, C.C. and JACOBS, W.P. 1963. Movement of growth regulators in plants. IV. Relationships between age, growth and polar transport in petioles of Phaseolus vulgaris. New Phytologist 62: 360-366.

McGAW, B.A., SCOTT, I.M. and HORGAN, R. 1984. Cytokinin biosynthesis and metabolism. In: The Biosynthesis and Metabolism of Plant Hormones. Crozier, A. and Hillman, J.R. (eds). Cambridge University Press, Cambridge. pp. 105-133.

McINTYRE, G.I. 1964. Mechanisms of apical dominance in plants. Nature 203: 1190-1191.

McINTYRE, G.l. 1968. Nutritional control of the correlative inhibition between lateral shoots in the flax seedling (Linum usitatissimum). Canadian Journal of Botany 46: 147-155.

McINTYRE, G.I. 1971a. Apical dominance in the rhizome of Agropyron repens. Some factors affecting the degree of dominance in isolated rhizomes. Canadian Journal of Botany 49: 99-109.

McINTYRE, G.I. 1971b. Water stress and apical dominance in Pisum sativum. Nature 230: 87-88.

McINTYRE, G.I. 1972. Studies on bud development in the rhizome of Agropyron repens. II. The effect of nitrogen supply. Canadian Journal of Botany 50: 393-340.

McINTYRE, G.I. 1973. Environmental control of apical dominance in Phaseolus vulgaris. Canadian Journal of Botany 51: 293-299.

McINTYRE, G.I. 1975. The correlative inhibition of bud and shoot growth in flax (Linum usitatissimum). Some factors affecting the pattern and degree of inhibition. Canadian Journal of Botany 53: 390-402.

McINTYRE, G.I. 1980. Apical dominance in the rhizome of Agropyron repens: The influence of humidity and light on the regenerative growth of isolated rhizomes. Canadian Journal of Botany 59: 549-555.

McINTYRE, G.I. 1987. Studies on the growth and development of <u>Agropyron</u> <u>repens</u>: Interacting effects of humidity, calcium and nitrogen on growth of the rhizome apex and lateral buds. <u>Canadian Journal of Botany</u> 65: 1427-1432.

McKEE, N.D. 1968. Correlative inhibition between axillary shoots of decapitated seedlings of <u>Pisum sativum</u>. Hons. B.Sc. thesis. Queens University, Belfast. Cited by Cutter, E.G. 1972. <u>Annals of Botany</u> 36: 207-220.

MILLER, C.O. 1963. Kinetin and kinetin-like compounds. In: <u>Modern Methods of Plant Analysis 6</u>. Peach, K. and Tracey, M.V. (eds). Springer-Verlag, Berlin. pp. 194-202.

MILLER, C.O. 1965. Evidence for the natural occurrence of zeatin and derivatives: Compounds from maize which promote cell division. <u>Proceedings of the National Academy of Sciences U.S.A.</u> 54: 1052-1058.

MILLER, C.O. and WITHAM, F.H. 1964. A kinetin-like factor from maize and other sources. In: <u>Regulateurs Naturels de la Croissance Végétale</u>. Nitsch, J.P. (ed.). C.N.R.S., Paris. pp. 1-6.

MINOCHA, S.C. 1979. The role of auxin and abscisic acid in the induction of cell division in Jerusalem artichoke tuber tissue cultured <u>in vitro</u>. <u>Zeitschrift für Pflanzenphysiologie</u> 92: 431-441.

MITCHELL, K.J., 1953a. Influence of light and temperature on the growth of ryegrass (<u>Lolium</u> spp.): 1. Pattern of vegetative development. <u>Physiologia Plantarum</u> 6: 21-46.

MITCHELL, K.J., 1953b. Influence of light and temperature on the growth of ryegrass (<u>Lolium</u> spp.): II. The control of lateral bud development. <u>Physiologia Plantarum</u> 6: 425-443.

MITCHELL, P. 1962. 'Metabolism, transport and morphogenesis: Which drives which?' Journal of General Microbiology 29: 25-37.

MIURA, G.A. and HALL, R. 1973. trans-Ribosylzeatin: Its biosynthesis in Zea mays endosperm and the mycorrhizal fungus, Rhizopogon roseolus. Plant Physiology 51: 563-569.

MIURA, G.A. and MILLER, C.O. 1969. 6-(γ,γ-Dimethylallylamino)purine as a precursor of zeatin. Plant Physiology 44: 372-376.

MOK, D.W.S. and MOK, M.C. 1987. Metabolism of ^{14}C-zeatin in Phaseolus embryos. Plant Physiology 84: 596-599.

MOK, M.C., MOK, D.W.S. and ARMSTRONG, D.J. 1978. Differential cytokinin structure-activity relationships in Phaseolus. Plant Physiology 61: 72-75.

MOK, M.C., MOK, D.W.S., DIXON, S.C., ARMSTRONG, D.J. and SHAW, G. 1982. Cytokinin structure – activity relationships and the metabolism of N^6-(Δ^2-isopentenyl) adenosine-[8-^{14}C] in Phaseolus callus tissues. Plant Physiology 70: 173-178.

MONTAGUE, M.J., ENNS, R.K., SIEGEL, N.R. and JAWORSKI, E.G. 1981. Inhibition of 2,4-dichlorophenoxyacetic acid conjugation to amino acids by treatment of cultured soybean cells with cytokinins Plant Physiology 67: 701-704.

MOR, Y., HALEVY, A.H. and PORATH, D. 1980. Characterization of the light reaction in promoting the mobilizing ability of rose shoot tips. Plant Physiology 66: 996-1000.

MORGAN, P.W. and GAUSMAN, H.W. 1966. Effects of ethylene on auxin transport. Plant Physiology 41: 45-52.

MORGAN, D.C. and SMITH, H. 1976. Linear relationship between phytochrome photoequilibrium and growth in plants under simulated natural radiation. Nature 262: 210-212.

MORELAND, C.F. 1934. Factors affecting the development of the cotyledonary buds of the common bean, Phaseolus vulgaris. Memoirs of the Cornell University Agricultural Experimental Station 167.

MORRIS, D.A. 1977. Transport of exogenous auxin in two-branched dwarf pea seedlings (Pisum sativum L.). Planta 136: 91-96.

MORRIS, D.A. 1981. Distribution and metabolism of root-applied cytokinins in Pisum sativum. Physiologia Plantarum 52: 251-256.

MORRIS, D.A. 1982. Hormonal regulation of sink invertase activity: Implications for the control of assimilate partitioning. In: Plant Growth Substances. Wareing, P.F. (ed.). Academic Press, London. pp. 659-669.

MORRIS, D.A., BRIANT, R.E. and THOMPSON, P.G. 1969. The transport and metabolism of ^{14}C-labelled indoleacetic acid in intact pea seedlings. Planta 89: 178-197.

MORRIS, D.A. and WINFIELD, P.J. 1972. Kinetin transport to axillary buds of dwarf pea (Pisum sativum L.). Journal of Experimental Botany 23: 346-355.

MOTHES, K., ENGELBRECHT, L. and KULAJEWA, O. 1959. Uber de werkung des kinetins auf stickstoffverteiling und eiweissynthese in isolierten blattern. Flora 147: 445-464.

MURASHIGE, T. and SKOOG, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497.

NAGAO, M.A. and RUBINSTEIN, B. 1975. Relationship of cytokinin to lateral bud growth at early stages after decapitation. Botanical Gazette 136: 366-371.

NAGAO, M.A. and RUBINSTEIN, B. 1976. Early events associated with lateral bud growth of Pisum sativum L. Botanical Gazette 137: 39-44.

NAKAMURA, E. 1964. Effect of decapitation and IAA on the distribution of radioactive phosphorus in the stem of Pisum sativum. Plant and Cell Physiology 5: 521-524.

NAYLOR, J.M. 1958. Control of nuclear processes by auxin in axillary buds of Tradescantia paludosa. Canadian Journal of Botany 36: 221-232.

NELSON, R.E. 1988. The relationship between IAA concentration and organogenesis, and benzyladenine metabolism in tobacco callus cultured in vitro. B.Sc. Hon. Thesis, University of Natal, Pietermaritzburg, South Africa.

NIEDERGANG-KAMEIN, E. and LEOPOLD, A.C. 1957. Inhibitors of polar auxin transport. Physiologia Plantarum 10: 29-38.

NISHINARI, N. and SYONO, K. 1980a. Cell-free biosynthesis of cytokinins in cultured tobacco cells. Zeitschrift für Pflanzenphysiologie 99: 383-392.

NISHINARI, N. and SYONO, K. 1980b. Biosynthesis of cytokinins by tobacco cell cultures. Plant and Cell Physiology 21: 1143-1150.

NISHITANI, K. and HASEGAWA, K. 1985. Mode of action of IAA in apical dominance in peas. In: Abstracts of the 12th International Conference on Plant Growth Substances, Heidelberg. pp. 126.

NITSCH, J.P. and NITSCH, C. 1965. Presence d'une phytokinine dan le cambium. Bulletin de la Societe Botanique de France 112: 1-10.

NONHEBEL, H.M., HILLMAN, J.R., CROZIER, A. and WILKINS, M.B. 1985. Metabolism of $[^{14}C]$ indole-3-acetic acid by coleoptiles of Zea mays L. Journal of Experimental Botany 36: 99-109.

NOUGAREDE, A., LANDRE, P. and REMBUR, J. 1983. Activites ATPasiques du noeud cotyledonaire et du bourgeon cotyledonaire du pois inhibe, reactivé ou soumis a la fusicoccine. Canadian Journal of Botany 61: 119-134.

NOUGAREDE, A., RONDET, P., LANDRE, P. and REMBUR, J. 1987. Effect of abscisic acid treatment on cell division, DNA content and coteledonary bud elongation in decapitated pea plants. Canadian Journal of Botany 65: 907-915.

OSBORNE, D.A. and BLACK, M.K. 1964. Polar transport of a kinin, benzyladenine. Nature 201: 97.

OSBORNE, D.J. and McCALLA, D.R. 1961. Rapid bioassay for kinetin and kinins using senescing leaf tissue. Plant Physiology 36: 219-221.

PACES, V., WERSTIUK, E. and HALL, R.H. 1971. Conversion of N^6-(Δ^2-isopentenyl) adenosine to adenosine by enzyme activity in tobacco tissue. Plant Physiology 48: 775-778.

PALMER, J.H. and PHILLIPS, I.D.J. 1963. The effect of the terminal bud, indoleacetic acid and nitrogen supply on the growth and orientation of the petiole of Helianthus annuus. Physiologia Plantarum 16: 572-584.

PALMER, M.V., SCOTT, I.M. and HORGAN, R. 1981a. Cytokinin metabolism in Phaseolus vulgaris L. II. Comparative metabolism of exogenous cytokinins by detached leaves. Plant Science Letters 22: 187-195.

PALMER, M.V., HORGAN, R. and WAREING, P.F. 1981b. Cytokinin metabolism in Phaseolus vulgaris L. III: Identification of endogenous cytokinins and metabolism of [$8-^{14}C$] dihydrozeatin in stems of decapitated plants. Planta 153: 297-302.

PALMER, M.V., HORGAN, R. and WAREING, P.F. 1981c. Variation in cytokinin levels in leaves of decapitated plants in relation to bud outgrowth. Journal of Experimental Botany 32: 1231-1241.

PALMER, M.V., LETHAM, D.S. and GUNNING, B.E.S. 1984. Cytokinin metabolism in nondividing and auxin-induced dividing explants of Helianthus tuberosus L. tuber tissue. Journal of Plant Growth Regulation 2: 289-298.

PALNI, L.M.S. 1980. Biosynthesis and metabolism of cytokinins. Ph.D. thesis. University of Wales, United Kingdom.

PALNI, L.M.S., BURCH, L. and HORGAN, R. 1988. The effect of auxin concentration on cytokinin stability and metabolism. Planta 174: 231-234.

PALNI, L.M.S. and HORGAN, R. 1983. Cytokinin biosynthesis in crown-gall tissue of Vinca rosea: metabolism of isopentenyladenine. Phytochemistry 22: 1597-1601.

PALNI, L.M.S., TAY, S.A.B. and MACLEOD, J.K. 1987. Determination by gas chromatography - mass spectrometry of $[^{15}N_5]$ adenine incorporation into endogenous cytokinins and the effect of tissue age on cytokinin biosynthesis in Datura innoxia crown gall tissue. Plant Physiology 84: 1158-1165.

PANIGRAHI, B.M. and AUDUS, L. 1966. Apical dominance in Vicia faba. Annals of Botany 30: 457-473.

PARKER, C.W., ENTSCH, B. and LETHAM, D.S. 1986. Inhibitors of two enzymes which metabolise cytokinins. Phytochemistry 25: 303-310.

PARKER, C.W. and LETHAM, D.S. 1973. Metabolism of zeatin by radish cotyledons and hypocotyls. Planta 114: 199-218.

PARKER, C.W., and LETHAM, D.S. 1974. Metabolism of zeatin in Zea mays seedlings. Planta 115: 337-344.

PARKER, C.W., LETHAM, D.S., COWLEY, D.E. and MACLEOD, J.K. 1972. Raphanin, an unusual purine derivative and a metabolite of zeatin. Biochemical and Biophysical Research Communications 49: 460-466.

PARKER, C.W., LETHAM, D.S., GOLLNOW, B.I., SUMMONS, R.E., DUKE, C.C. and MACLEOD, J.K. 1978. Metabolism of zeatin by lupin seedlings. Planta 142: 239-251.

PARKER, C.W., LETHAM, D.S., WILSON, M.M., JENKINS, I.D., MACLEOD, J.K. and SUMMONS, R.E. 1975. The identity of two new cytokinin metabolites. Annals of Botany 39: 375-376.

PARKER, C.W., WILSON, M.M., LETHAM, D.S., COWLEY, D.E., MACLEOD, J.K. 1973. The glucosylation of cytokinins. Biochemical and Biophysical Research Communications 55: 1370-1376.

PATRICK, J.W. 1982. Hormonal control of assimilate transport. In: Plant Growth Substances. Wareing, P.F. (ed.). Academic Press, London. pp. 669-678.

PATRICK, J.W. and STEAINS, K.H. 1987. Auxin promoted transport of metabolites in stems of Phaseolus vulgaris L. Auxin dose response curves and effects of inhibitors of polar auxin transport. Journal of Experimental Botany 38: 203-210.

PETERSON, C.A. and FLETCHER, R.A. 1975. Lateral bud growth on excised stem segments: effect of the stem. Canadian Journal of Botany 53: 243-248.

PHILLIPS, I.D.J. 1968. Nitrogen, phosphorus and potassium distribution in relation to apical dominance in dwarf bean (Phaseolus vulgaris, cv. Canadian Wonder). Journal of Experimental Botany 19: 617-627.

PHILLIPS, I.D.J. 1969a. Apical dominance. In: The physiology of Growth and Development. Wilkins, M.B. (ed.). McGraw Hill, New York. pp. 165-202.

PHILLIPS,I.D.J. 1969b. Auxin-gibberellin interaction in apical dominance: experiments with tall and dwarf varieties of pea and bean. Planta 86: 315-323.

PHILLIPS, I.D.J. 1971a. Effect of relative hormone concentration on auxin-gibberellin interaction in correlative inhibition of axillary buds. Planta 96: 27-34.

PHILLIPS, I.D.J. 1971b. Factors influencing the distribution of growth between stem and axillary buds in decapitated bean plants. Journal of Experimental Botany 22: 465-471.

PHILLIPS, I.D.J. 1975. Apical dominance. Annual Review of Plant Physiology 26: 341-367.

PILET, P.E. 1967. Variations of the auxin transport in the stems. Action of several effectors. Wissenschaftliche Zeitschrift der Universitaet Rostock Mathematisch - Naturwissenschaftliche Reihe 16: 479-486.

PILET, P.E. 1968. In vivo and in vitro auxin and kinin transport. In: Biochemistry and Physiology of Plant Growth Substances. Wightman, F. and Setterfield, G. (eds). Runge, Ottawa. pp. 993-1004.

PILLAY, I. and RAILTON, I.D. 1983. Complete release of axillary buds from apical dominance in intact, light grown seedlings of Pisum sativum L. following a single application of cytokinin. Plant Physiology 71: 972-974.

POLYA, G.M. and BOWMAN, J.A. 1979. Ligand specificity of a high affinity cytokinin - binding protein. Plant Physiology 64: 387-392.

PRAKASH, G., KUMAR, N. and SHARMA, P.K. 1986. Experiments on yield of Vaccaria pyramidata Medik. controlled by apex, lateral organs, stem orientation, irradiations and growth substances. Acta Botanica Indica 14: 30-42.

PRAKASH, G., SHARMA, P.K., SHARMA, R.K. and VIDHU. 1985. Correlative control of apex and lateral organs in Vaccaria pyramidata in response to certain physical and chemical factors. Acta Botanica Indica 13: 246-256.

PRASAD, T.K. and CLINE, M.G. 1985a. Shoot inversion-induced ethylene in Pharbitis nil induces the release of apical dominance by restricting shoot elongation. Plant Science 38: 163-172.

PRASAD, T.K. and CLINE, M.G. 1985b. Gravistimulus direction, ethylene production and shoot elongation in the release of apical dominance in Pharbitis nil. Journal of Experimental Botany 36: 1969-1975.

PRASAD, T.K. and CLINE, M.G. 1986a. The control of apical dominance: Localization of the growth region of the Pharbitis nil shoot. Journal of Plant Physiology 125: 185-190.

PRASAD, T.K. and CLINE, M.G. 1986b. Kinetics of shoot inversion-induced ethylene production in Pharbitis nil. Botanical Gazette 147: 437-442.

PRASAD, T.K. and CLINE, M.G. 1987. Gibberellin-enhanced elongation of inverted Pharbitis nil shoot prevents the release of apical dominance. Plant Science 49: 175-179.

PROCHAZKA, S. and JACOBS, W.P. 1984. Transport of benzyladenine and gibberellin A_1 from roots in relation to the dominance between the axillary buds of pea (Pisum sativum L.) cotyledons. Plant Physiology 76: 224-227.

PROCHAZKA, S., NAVRATILOVA, D. and BLAZKOVA, J. 1983. Transport of benzyl-8-^{14}C-adenine from roots into shoots of pea (Pisum sativum L.) seedlings with regard to outgrowth of lateral buds. Beitraege zur Biologie der Pflanzen 58: 421-426.

PROCHAZKA, S., SCHRAUDOLF, H. and SONKA, J. 1977. Transport of ^{14}C-kinetin in intact and decapitated pea seedlings (Pisum sativum L.). Zeitschrift für Pflanzenphysiologie 81: 308-313.

PURSE, J.G., HORGAN, R., HORGAN, J.M. and WAREING, P.F. 1976. Cytokinins of sycamore spring sap. Planta 132: 1-18.

REED, H.S. and HALMA, F.F. 1919. On the existence of a growth inhibiting substance in the Chinese lemon. University of California Publications in Agricultural Sciences 4: 99-112.

REMY, M. 1968. Effects d'une modification du mouvement de L'eau dans des epicotyles de Pois sur la Croissance des bourgeons axillaines et leurs correlations. Comptes Rendus de L'Academie des Sciences Paris 266: 676-679.

ROGOZINSKA, J.A., HELGESON, J.P. and SKOOG, F. 1964. Tests for kinetin-like growth promoting activities of triacanthine and its isomer, 6-(γ,γ-dimethylallylamino)-purine. Physiologia Plantarum 17: 165-176.

ROWNTREE, R.A. and MORRIS, D.A. 1979. Accumulation of ^{14}C from exogenous labelled auxin in lateral root primordia of intact pea seedlings (Pisum sativum L.). Planta 144: 463-466.

RUBINSTEIN, B. and NAGAO, M.A. 1976. Lateral bud outgrowth and its control by the apex. The Botanical Review 42: 83-113.

SACHS, J. 1874. Uber das wachsthum der haupt-und nebenwurzeln. II. Arbeitin aus dem Botanischen Institut des Würzburg 1: 584-634.

SACHS, J. 1880. Stoff und form der pflanzenorgane.I. Arbeitin aus dem Botanischen Institut des Würzburg 2: 452-488.

SACHS, J. 1882. Stoff und form der pflanzenorgane. II. Arbeitin aus dem Botanischen Institut des Würzburg 2: 689-718.

SACHS, T. 1966. Senescence of inhibited shoots of peas and apical dominance. Annals of Botany 30: 447-456.

SACHS, T. and THIMANN, K.V. 1964. Release of lateral buds from apical dominance. Nature 201: 939-940.

SACHS, T. and THIMANN, K.V. 1967. The role of auxins and cytokinins in the release of buds from dominance. American Journal of Botany 54: 136-144.

SATTELMACHER, B. and MARSCHNER, H. 1978. Nitrogen nutrition and cytokinin activity on Solanum tuberosum. Physiologia Plantarum 42: 185-189.

SCHAEFFER, G.W. and SHARPE, F.T. 1969. Release of axillary bud inhibition with benzyladamine in tobacco. Botanical Gazette 130: 107-110.

SCHAEFFER, G.W. and SHARPE, F.T. 1970. Kinetics of benzyladenine stimulation. Annals of Botany 34: 707-719.

SCHAEFFER, G.W. and SHARPE, F.T. 1971. Cytokinin function: Increase in [methyl-^{14}C] metabolism to phosphatidylcholine and a decrease in oxidation to carbon dioxide -^{14}C. Physiologia Plantarum 25: 456-460.

SCHAEFFER, G.W. and SHARPE, F.T. 1974. Cytokinin function: Kinetics of [Methyl - ^{14}C] metabolism in dormant and BA-activated axillary buds. Proceedings of the Royal Society of New Zealand Bulletin 12. Cited by: Rubinstein, B. and Nagao, M.A. 1976. The Botanical Review 42: 83-113.

SCHNEIDER, G. 1970. Morphactins: Physiology and performance. Annual Review of Plant Physiology 21: 499-536.

SCHNEIDER, E.A. and WIGHTMAN, F. 1974. Metabolism of auxin in higher plants. Annual Review of Plant Physiology 25: 487-513.

SCHNEIDER, E.A. and WIGHTMAN, F. 1978. Auxins. In: Phytohormones and Related Compounds - A Comprehensive Treatise. Vol. I. Letham, D.S., Goodwin, P.B. and Higgins, T.J.V. (eds). Elsevier/North Holland, Amsterdam. pp. 29-105.

SCHNEPF, E. 1986. Cellular polarity. Annual Review of Plant Physiology 37: 23-47.

SCOTT, T.K. and BRIGGS, W.R. 1960. Auxin relationships in the Alaska pea (Pisum sativum). American Journal of Botany 47: 492-499.

SCOTT, T.K. and BRIGGS, W.R. 1962. Recovery of native and applied auxin from the light-grown Alaska pea seedling. American Journal of Botany 49: 1056-1063.

SCOTT, I.M., BROWNING, G. and EAGLES, J. 1980. Ribosylzeatin and zeatin in tobacco crown gall tissue. Planta 149: 267-273.

SCOTT, T.K., CASE, D.B. and JACOBS, W.P. 1967. Auxin-gibberellin interaction in apical dominance. Plant Physiology 42: 1329-1333.

SCOTT, I.M. and HORGAN, R. 1984. Root cytokinins of Phaseolus vulgaris L. Plant Science Letters 34: 81-87.

SCOTT, T.K. and PRITCHARD, J.B. 1968. The control of apical dominance in the Alaska pea. In: The Transport of Plant Hormones. Vardar, Y. (ed.). North Holland, Amsterdam. pp. 308-319.

SEBANEK, J. 1965. The interaction of endogenous gibberellins in correlation with cotyledons and axillary buds in the pea (Pisum sativum L.). Biologia Plantarum 7: 194-198.

SEBANEK, J. 1972. The effect of exogenous gibberellin and auxin on the dominance between axillary buds of pea (Pisum sativum L.) cotyledons. Biologia Plantarum 14: 337-342.

SEBANEK, J. 1973. Die stimulations - und korrelationswirkung der abszissäure auf Pisum sativum und Scrophularia nodosa. Acta Universitatis Agricultura (Brno). 9: 15-23.

SEMENIUK, P. and GRIESBACH, R.J. 1985. Bud applications of BA induces branching of a nonbranching poinsettia. HortScience 20: 120-121.

SETH, A.K., DAVIES, C.R. and WAREING, P.F. 1966. Auxin effects on the mobility of kinetin in the plant. Science 151: 587-588.

SETH, A.K. and WAREING, P.F. 1964. Interaction between auxins, gibberellins and kinins in hormone directed transport. Life Sciences 3: 1483-1486.

SETTERFIELD, G. 1963. Growth regulation in excised slices of Jerusalem artichoke tissue. Symposia of the Society for Experimental Biology 17: 98-126.

SHEIN, T. and JACKSON, D.I. 1971. Hormone interaction in apical dominance in Phaseolus vulgaris L. Annals of Botany 35: 555-564.

SHEIN, T. and JACKSON, D.I. 1972. Interaction between hormones, light, and nutrition on extension of lateral buds in Phaseolus vulgaris L. Annals of Botany 36: 791-800.

SHIGEURA, H.T. and GORDON, C.N. 1962. The mechanism of action of hadacidin. Journal of Biological Chemistry 237: 1937-1940.

SHORT, K.C. and TORREY, J.G. 1972. Cytokinins in seedling roots of pea. Plant Physiology 49: 155-160.

SIEGEL, S.M. and GALSTON, A.W. 1953. Experimental coupling of indoleacetic acid to pea root protein in vivo and in vitro. Proceedings of the National Academy of Sciences U.S.A. 39: 1111-1118.

SKOOG, F. and ABDUL GHANI, A.K.B. 1981. Relative activities of cytokinins and antagonists in releasing lateral buds of Pisum from apical dominance compared with their relative activities in the regulation of growth of tobacco callus. In: Metabolism and Molecular Activities of Cytokinins. Guern, J. and Péaud-Lenoël, C. (eds). Springer-Verlag, Berlin. pp. 140-150.

SKOOG, F. and ARMSTRONG, D.J. 1970. Cytokinins. Annual Review of Plant Physiology 21: 359-384.

SKOOG, F. HAMZI, Q., SZWEYKOWSKA, A.M., LEONARD, N.J., CARRAWAY, K.L., FUJJI, T., HELGESON, P. and LOEPPKY, R.N. 1967. Cytokinins: structure / activity relationships. Phytochemistry 6: 1169-1192.

SKOOG, F. and LEONARD, N.J. 1968. Sources and structure: activity relationships of cytokinins. In: Biochemistry and Physiology of Plant Growth Substances. Wightman, F. and Setterfield, G. (eds). Runge Press, Ottawa. pp. 1-18.

SKOOG, F. and MILLER, C.O. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symposia of the Society for Experimental Biology 11: 118-131.

SKOOG, F. and TSUI, C. 1948. Chemical control of growth and bud formation in tobacco stem segments and callus cultured in vitro. American Journal of Botany 35: 782-787.

SMITH, C.W. and JACOBS, W.P. 1968. The movement of IAA-^{14}C in the hypocotyl of Phaseolus vulgaris. In: The Transport of Plant Hormones. Vardar, Y. (ed.). North-Holland, Amsterdam. pp. 48-59.

SMITH, H. and WAREING, P.F. 1964a. Gravimorphism in trees. 2. The effect of gravity on bud break in Osier willow. Annals of Botany 28: 283-295.

SMITH, H. and WAREING, P.F. 1964b. Gravimorphism in trees. 3. The possible implication of a root factor in the growth and dominance relationships of the shoots. Annals of Botany 28: 297-309.

SMITH, H. and WAREING, P.F. 1966. Apical dominance and the effect of gravity on nutrient distribution. Planta 70: 87-94.

SNOW, R. 1925. The correlative inhibition of the growth of axillary buds. <u>Annals of Botany</u> 39: 841-859.

SNOW, R. 1929. The transmission of inhibition through dead stretches of stem. <u>Annals of Botany</u> 43: 261-267.

SNOW, R. 1931. Experiments on growth and inhibition. II. New phenomena of inhibition. <u>Proceedings of the Royal Society of London B Biological Sciences</u> 108: 305-316.

SNOW, R. 1932. Experiments on growth and inhibition. III. Inhibition and growth formation. <u>Proceedings of the Royal Society of London B Biological Sciences</u> 111: 86-104.

SNOW, R. 1937. On the nature of correlative inhibition. <u>New Phytologist</u> 36: 283-300.

SONDHEIMER, E. and TZOU, D.S. 1971. The metabolism of hormones during seed germination and dormancy II. The metabolism of 8^{-14}C-zeatin in bean axes. <u>Plant Physiology</u> 47: 516-520.

SPONSEL, V.M. 1985. Gibberellins in <u>Pisum</u> <u>sativum</u> – their nature, distribution and involvement in growth and development of the plant. <u>Plant Physiology</u> 65: 533-538.

SPRENT, J.K. 1968. Effects of benzyladenine on cotyledon metabolism and growth of peas. <u>Planta</u> 81: 80-87.

STUCHBURY, T., PALNI, L.M.S., HORGAN, R. and WAREING, P.F. 1979. The biosynthesis of cytokinins in crown-gall tissue of <u>Vinca</u> <u>rosea</u>. <u>Planta</u> 147: 97-102.

SUMMONS, R.E., ENTSCH, B., LETHAM, D.S., GOLLNOW, B.I. and MACLEOD, J.K. 1980. Regulators of cell division in plant tissues. XXVIII. Metabolites of zeatin in sweet-corn kernels: Purifications and identifications using high-performance liquid chromatography and chemical-ionization mass spectrometry. Planta 147: 422-434.

SUMMONS, R.E., ENTSCH, B., PARKER, C.W. and LETHAM, D.S. 1979. Quantitation of the cytokinin glucoside complex of lupin pods by stable isotope dilution. FEBS Letters 107: 21-25.

SUSSEX, I.M. 1955. Morphogenesis in Solanum tuberosum L.: Apical structure and developmental pattern of the juvenile shoot. Phytomorphology 5: 253-273.

SYONO, K., NEWCOMB, W. and TORREY, J.G. 1976. Cytokinin production in relation to the development of pea root nodules. Canadian Journal of Botany 54: 2155-2162.

SYONO, K. and TORREY, J.G. 1976. Identification of cytokinins of root nodules of the garden pea Pisum sativum. Plant Physiology 57: 602-606.

TAL, M., IMBER, D., EREZ, A. and EPSTEIN, E. 1979. Abnormal stomatal behaviour and hormonal balance in Flacca, a wilty mutant of tomato. Plant Physiology 63: 1044-1048.

TAMAS, I.A. 1987. Hormonal regulation of apical dominance. In: Plant Hormones and their Role in Plant Growth and Development. Davies, P.J. (ed.). Martinus Nijhoff, Dordrecht. pp. 393-410.

TAMAS, I.A., OZBUN, J.L., WALLACE, D.H., POWELL, L.E. and ENGELS, C.J. 1979. Effects of fruits on dormancy and abscisic acid concentrations in the axillary buds of Phaseolus vulgaris L. Plant Physiology 64: 615-619.

TANIMOTO, S. and HARADA, H. 1984. Rates of auxin and cytokinin in organo-genesis in Torenia stem segments cultured in vitro. Journal of Plant Physi-ology 115: 11-18.

THIMANN, K.V. 1977. Hormone Action in the Whole Life of Plants. University of Massachusetts Press, Amherst. pp. 289-326.

THIMANN, K.V. and LALORAYA, M.M. 1960. Changes in nitrogen in pea stem sections under the action of kinetin. Physiologia Plantarum 13: 165-178.

THIMANN, K.V., SACHS, T. and MATHUR, K.N. 1971. The mechanism of apical dominance in Coleus. Physiologia Plantarum 24: 68-72.

THIMANN, K.V. and SKOOG, F. 1933. Studies on the growth hormone of plants. III. The inhibiting action of the growth substance on bud development. Pro-ceedings of the National Academy of Sciences U.S.A. 19: 714-716.

THIMANN, K.V. and SKOOG, F. 1934. On the inhibition of bud development and other functions of growth substance in Vicia faba. Proceedings of the Royal Society of London B Biological Sciences 114: 317-339.

THOMAS, T.H. 1972. The distribution of hormones in relation to apical dominance in Brussels sprouts (Brassica oleracea var. gemmifera L.) plants. Journal of Experimental Botany 23: 294-301.

THOMSON, K.S. and LEOPOLD, A.C. 1974. In-vitro binding of morphactins and 1-N-naphthylphthalmic acid in corn coleoptiles and their effects on auxin transport. Planta 115: 259-270.

TIBBITTS, T.W. 1979. Humidity in plants. Bioscience 29: 358-363.

TILLBERG, E. 1972. Some effects of light on the auxin content and inhibitors in bean seedlings (Phaseolus vulgaris L.). MSc Thesis, University of Stockholm, Stockholm, Sweden.

TREWAVAS, A.J. 1981. How do plant growth substances work? Plant Cell and Environment 4: 203-228.

TREWAVAS, A.J. 1987. Sensitivity and sensory adaptation in growth substance responses. In: Hormone Action in Plant Development. Hoad, G.V., Lenton, J.R., Jackson, M.B. and Atkin, R.K. (eds). Butterworths, London. pp. 19-38.

TREWAVAS, A.J. and CLELAND, R.E. 1983. Is plant development regulated by changes in the concentration of growth substances or by changes in the sensitivity to growth substances? Trends in Biochemical Sciences 8: 354-357.

TREWAVAS, A.J. and JONES, A.M. 1981. Consequences of hormone binding studies for plant growth substance research. Physiologia Plantarum 12: 5-8.

TUCKER, D.J. 1977a. Hormonal regulation of lateral bud outgrowth in the tomato. Plant Science Letters 8: 105-111.

TUCKER, D.J. 1977b. Apical dominance in the 'Rogue' tomato. Annals of Botany 41: 181-190.

TUCKER, D.J. 1977c. The effects of far-red light on lateral bud outgrowth in decapitated tomato plants and the associated changes in the levels of auxin and abscisic acid. Plant Science Letters 8: 339-344.

TUCKER, D.J. 1978. Apical dominance in the tomato: The possible roles of auxin and abscisic acid. Plant Science Letters 12: 273-278.

TUCKER, D.J. and MANSFIELD, T.A. 1972. Effects of light quality on apical dominance in Xanthium strumarium and the associated changes in endogenous levels of abscisic acid and cytokinins. Planta 102: 140-151.

TUCKER, D.J. and MANSFIELD, T.A. 1973. Apical dominance in Xanthium strumarium. A discussion in relation to current hypotheses of correlative inhibition. Journal of Experimental Botany 24: 731-740.

TZOU, D., GALSTON, E.C. and SONDHEIMER, E. 1973. The effects and metabolism of zeatin in dormant and nondormant ash embryos. Plant Physiology 51: 894-897.

USCIATI, M., CODACCIONI, M. and GUERN, J. 1972. Early cytological and biochemical events induced by a 6-benzylaminopurine application on inhibited axillary buds of Cicer arietinum plants. Journal of Experimental Botany 23: 1009-1020.

USCIATI, M., CODACCIONI, M., RAUTUREAU, C., MAZLIAK, P. and GUERN, J. 1974. Lipogenesis modification induced by application of 6-benzylamino-purine to inhibited axillary buds of Cicer arietinum L. plants. Plant Science Letters 2: 295-301.

VAN STADEN, J. 1976a. Occurrence of a cytokinin glucoside in the leaves and in honeydew of Salix babylonica. Physiologia Plantarum 36: 225-228.

VAN STADEN, J. 1976b. Seasonal changes in the cytokinin content of Ginko biloba leaves. Physiologia Plantarum 38: 1-5.

VAN STADEN, J. 1979. Changes in the endogenous cytokinin levels of excised buds of Salix babylonica L., cultured aseptically. Botanical Gazette 140: 138-141.

VAN STADEN, J. and BROWN, N.A.C. 1977. The effect of ringing on cytokinin distribution in Salix babylonica. Physiologia Plantarum 39: 266-270.

VAN STADEN, J. and BROWN, N.A.C. 1978. Changes in the endogenous cytokinins of bark and buds of Salix babylonica as a result of stem girdling. Physiologia Plantarum 43: 148-153.

VAN STADEN, J. and DAVEY, J.E. 1977. The metabolism of zeatin and zeatin riboside by soya bean callus. Annals of Botany 41: 1041-1048.

VAN STADEN, J. and DAVEY, J.E. 1979. The synthesis, transport and metabolism of endogenous cytokinins. Plant Cell and Environment 2: 93-106.

VAN STADEN, J. and DAVEY, J.E. 1981. Cytokinin activity in Lupinus albus VI. Translocation and metabolism of $[8-^{14}C]$ zeatin applied to the leaves and fruits of fruiting plants. Physiologia Plantarum 51: 49-52.

VAN STADEN, J. and MENARY, R.C. 1976. Identification of cytokinins in the xylem sap of tomato. Zeitschrift für Pflanzenphysiologie 78: 262-265.

VAN STADEN, J., SPIEGELSTEIN, H., ZIESLIN, N. and HALEVY, A.H. 1981. Endogenous cytokinins and lateral bud growth in roses. Botanical Gazette 142: 177-182.

VARDAR, Y. and KALDEWAY, H. 1972. Auxin transport and apical dominance in Pisum sativum. In: Hormonal Regulation in Plant Growth and Development. Kaldeway,H. and VARDAR, Y. (eds). Verlag Chemie, Weinheim. pp. 401-411.

VEEN, A. 1969. Auxin transport, auxin metabolism and ageing. Acta Botanica Neerlandicia 18: 447-454.

VONK, C.R. and DAVELAAR, E. 1981. $8-^{14}C$-zeatin metabolites and their transport from leaf to phloem exudate of Yucca. Physiologia Plantarum 52: 101-107.

WAKHLOO, J.L. 1965. Evidence for indole-3-acetic acid and tryptophan in the shoot of Solanum nigrum and the effects of potassium nutrition on their levels. Planta 65: 301-314.

WAKHLOO, J.L. 1970. Role of mineral nutrients and growth regulators in the apical dominance in Solanum sisymbrifolium. Planta 91: 190-194.

WANG, T.L. and HORGAN, R. 1978. Dihydrozeatin riboside, a minor cytokinin from the leaves of Phaseolus vulgaris L. Planta 140: 151-153.

WANG, T.L., THOMPSON, A.G. and HORGAN, R. 1977. A cytokinin glucoside from the leaves of Phaseolus vulgaris L. Planta 135: 285-288.

WANG, T.L. and WAREING, P.F. 1979. Cytokinins and apical dominance in Solanum andigena: Lateral shoot growth and endogenous cytokinin levels in the absence of roots. New Phytologist 82: 19-28.

WARDLAW, I.F. and MORTIMER, D.C. 1970. Carbohydrate movement in pea plants in relation to axillary bud growth and vascular development. Canadian Journal of Botany 48: 229-237.

WAREING, P.F. and NASR, T.A.A. 1958. Effects of gravity on growth, apical dominance and flowering in fruit trees. Nature 182: 379-381.

WAREING, P.F. and NASR, T.A.A. 1961. Gravimorphism in trees. I. Effects of gravity on growth and apical dominance in fruit trees. Annals of Botany 25: 321-340.

WAREING, P.F. and PHILLIPS, I.D.J. 1981. Growth correlation. In: Growth and Differentiation in Plants. Pergamon Press, England. pp. 132-138.

WAREING, P.F. and SAUNDERS, P.F. 1971. Hormones and Dormancy. Annual Review of Plant Physiology 22: 261-288.

WENT, F.W. 1936. Allgemeine betrachtungen über das auxin-problem. Biologisches Zentralblatt 56: 449-463.

WENT, F.W. 1939. Some experiments on bud growth. American Journal of Botany 26: 109-117.

WENT, F.W. and BONNER, D.M. 1943. Growth factors controlling tomato stem growth in darkness. Archives of Biochemistry and Biophysics 1: 439-452.

WEYERS, J.D.B., PATERSON, N.W. and A'BROOK, R. 1987. Towards a quantitative definition of plant hormone sensitivity. Plant Cell and Environment 10: 1-10.

WHITE, J.C. and MANSFIELD, T.A. 1977. Correlative inhibition of lateral bud growth in Pisum sativum and Phaseolus vulgaris L.: Studies on the role of abscisic acid. Annals of Botany 41: 1163-1170.

WHITE, J.C., MEDLOW, G.C., HILLMAN, J.R. and WILKINS, M.B. 1975. Correlative inhibition of lateral bud growth in Phaseolus vulgaris L. Isolation of indoleacetic acid from the inhibitory region. Journal of Experimental Botany 26: 419-424.

WICKSON, M. and THIMANN, K.V. 1958. The antagonism of auxin and kinetin in apical dominance. Physiologia Plantarum 11: 62-75.

WICKSON, M. and THIMANN, K.V. 1960. The antagonism of auxin and kinetin in apical dominance. II. The transport of IAA in pea stems in relation to apical dominance. Plant Physiology 13: 539-554.

WIGHTMAN, F., SCHNEIDER, E. and THIMANN, K.V. 1980. Hormonal factors controlling the initiation and development of lateral roots. II. Effects of exogenous growth factors on lateral root formation. Physiologia Plantarum 49: 304-314.

WILDMAN, S.G. and GORDON, S.A. 1942. The release of auxin from isolated leaf proteins of spinach by enzymes. Proceedings of the National Academy of Sciences U.S.A. 28: 217-228.

WITHAM, F.H. 1968. Effect of 2,4-dichloro-phenoxyacetic acid on cytokinin requirement of soybean cotyledon and tobacco stem callus tissues. Plant Physiology 43: 1455-1457.

WITT, J. 1964. Uber den einflub von stickstoffmangel auf den wuchsstoffhaushalt von Helianthus annuus L. Portugaliae Acta Biologia 8: 196-248.

WHITTY, C.D. and HALL, R.H. 1974. A cytokinin oxidase in Zea mays. Canadian Journal of Biochemistry 52: 789-799.

WOOLLEY, D.J. and WAREING, P.F. 1972a. The interaction between growth promoters in apical dominance. I. Hormonal interaction, movement and metabolism of a cytokinin in rootless cuttings. New Phytologist 71: 781-793.

WOOLLEY, D.J. and WAREING, P.F. 1972b. The role of roots, cytokinins and apical dominance in the control of lateral shoot form in Solanum andigena. Planta 105: 33-42.

WRIGHT, S.T.C. 1968. Multiple and sequential roles of plant growth regulators. In: Biochemistry and Physiology of Plant Growth Substances. Wightman, F. and Setterfield, G. (eds). Runge Press, Ottawa. pp. 521-542.

YEANG, H.Y. and HILLMAN, J.R. 1981a. Internodal extension of the first trifoliate leaf axillary bud of Phaseolus vulgaris L. following shoot decapitation. Annals of Botany 48: 25-32.

YEANG, H.Y. and HILLMAN, J.R. 1981b. Control of lateral bud growth in Phaseolus vulgaris L. by ethylene in the apical shoot. Journal of Experimental Biology 32: 395-404.

YEANG, H.Y. and HILLMAN, J.R. 1982. Lateral bud growth in Phaseolus vulgaris L. and the levels of ethylene in the bud and adjacent tissue. Journal of Experimental Botany 33: 111-117.

ZAJACZKOWSKI, S. and WODZICKI, T.J. 1978. On the question of stem polarity with respect to auxin transport. Physiologia Plantarum 44: 122-126.

ZAMSKI, E., OSHRI, S. and ZIESLIN, N. 1985. Comparative morphology and anatomy of axillary buds along a rose shoot. Botanical Gazette 146: 208-212.

ZIESLIN, N. and HALEVY, 1976. Components of axillary bud inhibition in rose plants. I. The effect of different plant parts (correlative inhibition). Botanical Gazette 137: 291-296.

ZIESLIN, N. and KHAYAT, E. 1983. Involvement of cytokinin, ABA and endogenous inhibitors in sprouting of basal buds in rose plants. Plant Growth Regulation 1: 279-288.

ZIESLIN, N. and MOR, Y. 1981. Plant management of greenhouse roses. Formation of renewal canes. Scientia Horticulturae (Amsterdam) 15: 67-75.