ETHYLENE AND THE CONTROL OF AXILLARY BUD GROWTH IN *PHASEOLUS VULGARIS* L.

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Units of Measurement

The *Système International d'Unité* (SI metric units of measurement) has been adopted in the presentation of the results. An exception is made for the units of time, where it is felt that the concept of elapsed time is more readily grasped by the use of the units "hours" (h) and "minutes" (min) in references to periods exceeding 60 s.

Abbreviations

The following abbreviations were used in the text in addition to those for the SI units measurement:

ACC = 1-aminocyclopropane-1-carboxylic acid

AVG = aminoethoxyvinyl glycine

ca. = *circa*

cv. = cultivar

FID = flame ionization detector;
flame ionization detection

Fig. = Figure

GC = gas chromatograph;
gas chromatography

GC-MS = (combined) gas chromatography-mass spectrometry

h = hour(s)

IAA = indole-3-acetic acid

min = minute(s)

ppm = parts per million

SAM = *s*-adenosylmethionine

std. error = standard error

TIBA = tri-iodobenzoic acid

vpm = volumes per million

Contents

Summary

This thesis is concerned with investigations into the involvement of ethylene in the control of axillary bud growth in *Phaseolus vulgaris* L. Ancillary studies on the characteristics of axillary bud outgrowth and on the methodology of quantitative ethylene analyses were also carried out.

Growth of the leaves, internodes and axillary buds of intact young *Phaseolus* plants was governed by a fine degree of correlative control. The 1st trifoliate axillary bud, which was the bud studied in most of the experiments, showed slow but continuous growth at the time the plants were used for experiments (19 - 29 days old). Decapitation of the plant at the 3rd internode induced vigorous growth of this bud. The time lag between the decapitation stimulus and the detection of increased growth, determined by a photographic technique, was found to be 3-5 h. Much of the initial increase in the length of the bud internode may be attributed to cell expansion.

Vascular transport to axillary buds was investigated using decolorised basic fuchsin as a tracer. The results indicate that functional xylem continuity was already well established between the stem and the inhibited buds. The lack of vascular supply was not, therefore, the cause of growth inhibition. Phloem transport was not studied directly, but free lateral transport of radioactivity from labelled sucrose from the xylem to the more peripheral tissues was demonstrated.

The internal gaseous content of *Phaseolus* shoot tissue was sampled by vacuum extraction. The identities of ethylene and ethane in the gaseous extracts of apical shoots were established conclusively by a GC-MS selective ion detection method. Changes in the volume and composition of the gases recovered were observed when the evacuation conditions during extraction were altered. These changes were different in the primary leaves and in

the 2nd trifoliate leaves. Thus, estimates of internal ethylene and ethane concentrations in plant tissues were influenced by the morphology of the tissue and the evacuation conditions employed. The internal ethylene concentrations in the primary, 1st trifoliate and 2nd trifoliate leaves, considered together, were correlated to their respective rates of ethylene emanation. Only trace emanation of ethane was recorded.

Physical confinement of the developing apical shoot inhibited its growth and promoted the growth of axillary buds located basipetal to the treated portion of the shoot. These developments were thought to be brought about by ethylene since (a) the internal ethylene concentration and the rate of ethylene emanation were significantly increased in the treated tissue and (b) similar responses in shoot inhibition and axillary bud outgrowth were observed on plants with apical shoots treated with ethephon or ethylene. There is evidence to suggest that the promotion of axillary bud development by ethylene action on the apical shoot was associated with the availability of freely diffusible ethylene in the tissues of the treated shoot. Loss of apical dominance was not, apparently, directly dependent on either the internal ethylene concentration (i.e. concentration in the vacuum-extracted gases) or on the rate of ethylene emanation. Although treatment of the shoot with tri-iodobenzoic acid (TIBA) induced various morphological responses in the plant, including axillary bud outgrowth, which appeared similar to the responses to ethylene, the initial effect of TIBA is not thought to be ethylene-mediated.

Applications of ethephon or ethylene inhibitors - Ag^{+} or aminoethoxy-vinyl glycine (AVG) - directly to the axillary buds of the intact plant were ineffective in eliciting bud development. When applied in appropriate dosages to the axillary bud of the decapitated plant, ethephon or ethylene inhibitors inhibited the outgrowth of the released bud. The general conclusion that may be drawn from these observations is that ethylene is essential in maintaining normal axillary bud growth in the decapitated

plant, but the absence of bud development in the intact plant is not due to a lack of free ethylene.

Ethylene levels decreased in the stem upon decapitation of the shoot. However, it is not conclusive that a causal relationship exists between this decrease and the release of axillary buds from inhibition. The contention that auxin-induced ethylene is responsible for the suppression of axillary bud growth in the decapitated plant where the apical shoot is replaced by auxin is not borne out in this study.

Application of IAA directly to the axillary bud of intact plants gave rise to a transient increase in bud growth. The increment was annulled when a low concentration of AVG was supplied to the bud at the same time. This dosage of AVG did not affect bud outgrowth due to shoot decapitation or the normal slow growth rate in the bud of the intact plant. Thus, IAA-induced bud growth appears to be dissimilar to the growth arising from shoot decapitation.

Leaf and internode tissues subjected to wounding or to water-stress showed characteristic patterns of increased ethylene emanation. Wounding and water-stress generate different stimuli in the tissues although both forms of stress enhance ethylene production.

1. INTRODUCTION

In dicotyledonous plants, the primary meristems of the shoot are located at the shoot apex and at lateral buds on the stem. Under favourable environmental conditions, the apical bud develops actively; the development of lateral buds, on the other hand, is generally inhibited - either completely or incompletely according to the species - by the presence of the apex. In the event that the apical bud is lost or incapacitated by damage, the lateral buds are then released from inhibition and eventually a single lateral shoot assumes dominance.

Inhibition of lateral buds by the apex is one of several forms of correlative relationships which involve interactions between different organs of the plant in the regulation and co-ordination of the form and function of its tissues and organs. Such correlations additively modulate the physiological responses of the plant to environmental stimuli and define its morphological form in accordance with its genetic disposition. The correlative inhibition of lateral buds represents an excellent experimental model for the study of spatial organization of developmental activities in the plant, an aspect which has been emphasized in a recent review of the topic (Phillips, 1975).

Positional signalling at the intercellular level may involve chemical or biophysical processes. Very little is known about the rôle of biophysical processes in correlative phenomena, whereas there has been a considerable amount of research on the existence of chemical signals, particularly the plant growth substances (phytohormones). These compounds are thought to have an important rôle in the correlative inhibition of lateral buds. Among the major groups of plant hormones, ethylene is ubiquitous in the higher plants and is known to have wide-ranging and important effects on numerous aspects of plant physiology (Abeles, 1973).

Nevertheless, research on its involvement in the correlative inhibition of lateral buds has largely been neglected.

This introduction is divided into four parts, namely: a review of correlative inhibition of lateral buds; an appraisal of ethylene as a phytohormone; the selection of experimental plant material and the scheme and rationale of the research study.

1.1. CORRELATIVE INHIBITION OF LATERAL BUDS

Although primary growth of a shoot arises principally from the development of the apical meristem, quiescent lateral buds are, nevertheless, an asset to the plant in the maintenance of sustained active growth, or even in the survival of the plant itself. These lateral (often axillary) buds serve as ready replacements should the apex be lost or damaged. Lateral buds have a significant rôle in determining the shape of the plant and consequently can affect the ecology of natural habitats and areas modified by man. The inhibition of lateral bud growth is of considerable interest to horticulturalists and agriculturalists. Substantial expense is incurred in either the suppression or promotion of lateral bud growth in crop plants and ornamentals. In tobacco and tomato, for example, the outgrowth of incompletely inhibited buds is undesirable. In young rubber plants (*Hevea brasiliensis*), on the other hand, various methods have been devised to induce branching to attain a crown that is denser and hence more photosynthetically efficient.

In the literature, the terms "apical dominance" and "correlative inhibition" are often used interchangeably. Strictly speaking, apical dominance should also encompass the inhibition of the growth of one shoot by the presence of another dominant shoot and the apical effect on initiation and development of lateral organs such as branches, leaves, rhizomes and stolons, besides the correlative inhibition of lateral buds (Phillips, 1969a). Both terms are commonly accepted in current usage as being generally synonymous and reviews on the phenomenon of apical dominance appearing from time to time have dealt, in the main, with the correlative inhibition of lateral buds (Phillips, 1969a, 1975; Guern

and Usciati, 1971; Rubinstein and Nagao, 1976).

The expression of apical control is both diverse and complex. Despite investigations into the multiplicity of aspects of this phenomenon over the past century, numerous questions on the mechanisms governing the suppression of growth in lateral buds remain unresolved.

1.1.1. Characteristics of Bud Inhibition and Re-activation

Development of lateral buds proceeds to a pre-determined degree, after which, further development is curtailed. The extent of development in the bud that is attained before active development stops varies with the species. Fairly well formed lateral buds are characteristic of some species, while in others, the buds remain rudimentary in structure.

A distinctive ovoid group of non-dividing cells has been observed in the inhibited buds of *Tradescantia* (Naylor, 1958) and *Linum* (McIntyre and Larmour, 1974). In *Tradescantia* these cells were found to contain 2C DNA (Dwividi and Naylor, 1968). On shoot decapitation, the cells doubled in DNA content and underwent mitotic division. Many other observations have been made on the changes in chromosomal characters and nucleic acid turnover in the cells of active and inhibited buds in various species (Panigrahi and Audus, 1966; Dwividi and Naylor, 1968; Booker and Dwividi, 1973; Jablanović and Petrović, 1978).

It is quite possible, however, that such changes are indicative of the onset of active mitosis and represent the normal course of events associated with nuclear and cell division *following* the release of correlative inhibition. There is no firm evidence that these changes

are themselves closely concerned with the primary mechanism by which growth inhibition in the lateral bud is imposed or annulled.

Sorokin and Thimann (1964) suggested that maintenance of correlative inhibition in lateral buds of *Pisum* could be explained by the inadequacy of vascular supply to the inhibited buds. Wardlaw and Mortimer (1970) noted, however, that growth of the lateral buds in decapitated *Pisum* plants preceded any significant formation of new vascular elements. Marr and Blaser (1967), working with *Helianthus*, made a similar observation. In some other plants, substantial vascular continuity exists between inhibited lateral buds and the stem. Peterson and Fletcher (1973) demonstrated the presence of functional xylem and phloem connecting lateral buds of *Glycine* to the stem. In *Phaseolus vulgaris*, vascular elements from the stem to the primary leaf axillary bud (White, 1973) and to the first trifoliate leaf axillary bud (Mullins, 1970a) have been observed. The label from $^{14}CO_2$ supplied to the primary leaf of decapitated *Phaseolus vulgaris* plants (with or without IAA applied to the cut stem) has been recovered in the subtended axillary bud within 1 h from decapitation (Hall and Hillman, 1975), thus denoting translocation from the leaf to the bud before any substantial differentiation of additional vascular tissue. The lack of vascular supply to inhibited buds is therefore not a consistent feature in every instance.

Correlative inhibition of lateral buds is most effectively released by removal of the apical shoot. Considerable variation has been encountered in the time lag between decapitation and the onset of lateral bud growth. Such differences are perhaps to be expected since they reflect not only the variation between species, but also the experimental approach used in the different investigations. Studies on the rapid growth response in lateral buds have relied either on direct measurement of the bud or on the detection of an increase in mitotic division in the

bud. Using the former approach, the outgrowth of the lateral bud in *Pisum* was observed 6 to 10 h (Wardlaw and Mortimer, 1970) or 8 to 12 h (Nagao and Rubinstein, 1976) after decapitation of the shoot. By extrapolation of the data of Wardlaw and Mortimer (1970), onset of bud outgrowth could have taken place as early as 4 h following decapitation. Work by Hall and Hillman (1975) showed an even shorter time lag from decapitation occurring in *Phaseolus*. Using a photographic method, bud outgrowth was detected within 30 min of decapitation. Since there is obviously a lower limit to the *measurable* increment in growth, the time of the earliest detectable growth is likely to be influenced by the size and extent of development of the inhibited bud in different species. McIntyre (1977) proposed that the very rapid increase in bud length following decapitation of the apex was due primarily to cell extension resulting from an increase in water potential in the buds.

Mitotic division in the zone of quiescent cells at the tip of the lateral bud in *Tradescantia* did not occur until four days after decapitation (Booker and Dwividi, 1973). In *Glycine*, an increase in cell division occurred from about the 25th hour following decapitation of the shoot (Ali and Fletcher, 1970), or excision of the bud from the plant (Peterson and Fletcher, 1974). Cell division in the lateral bud is stimulated even earlier in *Pisum*, and increased mitoses were noted within 12 h of decapitation, shortly after an increase in lateral bud length was discerned (Nagao and Rubinstein, 1976).

1.1.2. Control of Bud Outgrowth

Notwithstanding the anatomical and physiological changes taking place in the re-activated bud, the correlative signal governing such changes appears to be derived from the shoot apex. In specific instances, other organs such as internodes (Phillips, 1971a; Peterson and Fletcher, 1975;

Zieslin and Halevy, 1976), cotyledons (Dostál, 1909; Sebánek, 1965; Sprent, 1968) and mature leaves (Goebel, 1880; Zieslin and Halevy, 1976) may influence lateral bud growth, but the apical portion of the shoot is the major and universal source of the correlative signal. White *et al.* (1975) found that the actively growing young leaves at the shoot apex of *Phaseolus*, rather than the terminal bud, were instrumental in suppressing lateral bud growth. This is consistent with the observations of Shein and Jackson (1971) in *Phaseolus* and Snow (1929), Cozens and Wilkinson (1966), Tucker and Mansfield (1973) and Aung and Byrne (1978) in other species. It is plausible that expanding apical leaves are principally responsible for correlative inhibition of lateral buds in many other plants where the rôle of immature leaves has not been examined critically. Even if the apical bud alone were removed, this would be tantamount to depriving the plant of young leaves once those present at the time of decapitation had matured. In some species, the spectral quality of light might influence maintenance of apical dominance. Treatments with far-red wavelengths at the end of the photoperiod have been successful in increasing lateral bud inhibition in *Xanthium* (Tucker and Mansfield, 1972, 1973) and *Lycopersicon* (Tucker, 1976). However, this treatment had no effect on intact *Phaseolus* (White and Mansfield, 1978).

Although removal of the apical region of the shoot is the simplest and most effective means of eliminating correlative inhibition, this can also be achieved by a variety of chemical, nutritional and environmental treatments, and by physical manipulation of the plant (see Phillips, 1975). The chemicals that have been used to promote bud growth are mainly chemical pruning agents (Cathey *et al.*, 1966; Cathey and Steffens, 1968), phytohormones and compounds which modify hormonal action. The effects of phytohormones on apical dominance are reviewed in section 1.1.2.2.,

while the effects of nutrients and water are outlined in section 1.1.2.1.

Bark ringing is a form of physical manipulation which has been effective in releasing apical dominance in some woody species (Phillips, 1975 ; Leong *et al.*, 1976). In herbaceous plants such as *Phaseolus*, ringing the stem has not been effective (Snow, 1925; White, 1973), perhaps because of the presence of the central pith of living cells. An alternative method in such plants is steam-girdling, which kills the treated tissue (White, 1973). A stem or shoot physically trained from its normal vertical habit to assume an arc or a loop exhibits the phenomenon of gravimorphism. This gravitational response reduces correlative inhibition in the highest upwardly directed bud; i.e. the bud at the top of the curvature of the stem or shoot (Wareing and Nasr, 1958, 1961; Smith and Wareing, 1964a, 1964b; Hillman, 1968; Leong *et al.*, 1976). Where the entire stem is secured horizontally, lateral buds closest to the roots were most strongly stimulated (Wareing and Nasr, 1961). In non-woody *Hevea* plants, lateral bud development can be initiated by physical restriction of apical growth (Mulder, 1941; Leong *et al.*, 1976). This technique involves covering the terminal shoot of the plant either with a paper bag or by folding and securing the uppermost whorl of leaves over the apex.

Yet another non-surgical method of releasing apical dominance is by the use of red light treatments. This approach has been successful in promoting lateral bud growth in *Nicotiana* (Kasperbauer, 1971) and *Xanthium* (Tucker and Mansfield, 1972, 1973).

1.1.2.1. Involvement of Nutrient and Water

The "nutritional theory" of correlative inhibition postulates that the apical meristem, which can be traced back to the seed embryo, functions as a strongly competitive metabolic sink. A substantial proportion of nutrients available to the plant is thought to be diverted to the growing apex, while the lateral buds, being deprived of sufficient nutrients, remain inhibited. Decapitation of a plant thus results in a re-distribution of nutrients and consequent compensation growth in the lateral buds. Arguing along these lines, a greater availability of nutrients in the intact plant, either by increased essential minerals supplied to the plant or through increased photosynthesis, might be expected to stimulate lateral bud development. Indeed, there is evidence in support of this hypothesis (see McIntyre, 1977). Applications of high levels of each of the three major mineral elements: nitrogen (McIntyre, 1971a, 1973; Yun and Naylor, 1973; McIntyre and Larmour, 1974), phosphorus (McIntyre, 1968; Thimann *et al.*, 1971) and potassium (Wakhloo, 1970) have been reported to be effective in stimulating lateral bud growth in various species. Plants grown under high light intensity, and thereby having a high photosynthetic turnover, also exhibit a greater outgrowth of lateral buds (McIntyre, 1971a, 1971b, 1973; Thimann *et al.*, 1971).

Criticisms against the nutritional theory have centred on the fact that no increase in carbohydrate "concentration" (expressed as quantity per unit weight of tissue) could be found in the stimulated lateral buds of decapitated *Pisum* (Wardlaw and Mortimer, 1970) and *Phaseolus* plants grown under high light intensity (McIntyre, 1973). The nitrogen, phosphorus and potassium concentrations in the released buds of decapitated *Phaseolus* plants were actually lower than those in IAA-inhibited buds (Phillips, 1968).

McIntyre (1977) also hypothesized that since active plant meristems have the competitive ability to obtain water at the expense of mature parts of the plant (Anderson and Kerr, 1943; Wilson, 1948), competition for water may play a critical rôle in apical dominance. He suggested that most plants in their natural environments suffer varying degrees of mild water stress. He pointed out that well-watered *Pisum*, *Phaseolus* and *Helianthus* plants grown in an environment of high air humidity showed outgrowth of lateral buds in synergism with high light intensity and mineral element supplementation.

Taken as a whole, the weakness of the nutrient-water stress theory lies in the fact that mineral nutrients, light and water are all fundamental essentials to growth and development of the whole plant. Augmenting these factors would naturally lead to accelerated growth in the plant as a whole, inclusive of the lateral buds. It would not be surprising for growth promotion to be especially prominent in the lateral buds in particular since these organs are already predisposed to further development. While evidence against the nutrient theory could be derived from the failure of direct applications of nutrients to inhibited buds to release correlative inhibition (Goodwin and Cansfield, 1967), the theory cannot be dismissed on the evidence of negative results alone. In advanced multicellular organisms, the assimilation of nutrients involves complex regulatory processes beyond mere diffusion of the nutrients into the tissue. As mentioned previously, there are other objections to the nutrient theory, and these concern the failure to detect increases in carbohydrate and inorganic ion concentrations in developing lateral buds of decapitated legumes (Phillips, 1968; Wardlaw and Mortimer, 1970). While such findings must question the appropriateness of the nutrient theory, they are by no means definitive arguments against the theory. The onset of lateral bud growth entails considerable morphological changes and physiological

re-organization at the cellular and biochemical level, complicating any direct comparison between growing and inhibited buds. In considering an acceptable premise by which such a comparison may be made, Phillips (1975) argued against the use of *total* bud nutrient content as the parameter for comparison since developing buds were physically larger than inhibited buds. He proposed that the consideration of nutrient *concentration* was more meaningful. This approach, however, is still not entirely satisfactory. In as much as high nutrient concentration implies availability of the nutrients, it can also be interpreted to denote an under-utilization of the nutrients and, hence, the accumulation of the surplus. An absence of any increase in nutrients in the developing bud might further be interpreted as reflecting a rapid turn-over of nutrients involved in the formation of new tissue. A low nutrient concentration, by this line of reasoning, only serves to underline the need for a sustained supply to the developing bud.

The validity of the nutrient theory seems, therefore, to be subject to the manner in which experimental findings are interpreted. If the theory is accepted provisionally, then the inhibitory effect on the lateral bud could either be effected through the nutrient gradient arising from the sink at the shoot apex, or, alternatively, a discrete correlative signal might be generated at the apex. The latter situation gives rise to a separate but related theory of correlative inhibition: the theory of hormone-directed transport.

Went (1936, 1939) first suggested a modification of the nutritive theory whereby growth factors are attracted towards the region of highest auxin concentration. Nutrient diversion by hormone-directed transport has been demonstrated experimentally by the accumulation of sucrose (Booth *et al.*, 1962, Bowen and Wareing, 1971), phosphorus (Booth *et al.*, 1962; Davies and Wareing, 1965; Hussein and Linck, 1966; Seth and Wareing, 1967) and nitrogen (Phillips, 1968) at the decapitated stem where auxin

had been applied. Hormone-directed transport of nutrients can be represented by two working models: a direct effect of the hormone, which on release from the apex affects the transport system; or the maintenance of a local sink activity at the apex and a physiological gradient for transport arising therefrom. Davies and Wareing (1965) showed that the former model was the more tenable by the use of the auxin transport inhibitor, tri-iodobenzoic acid (TIBA), which reduced acropetal movement of ^{32}P in *Pisum*. Sebánek (1967) has, however, shown that two concentrations of IAA, one that promoted lateral bud growth and another that inhibited bud growth, both induced accumulation of ^{32}P. He favoured the view that high concentrations of IAA inhibited bud growth not by nutrient diversion, but by a toxic effect on the buds. Besides auxins, other groups of phytohormones, the cytokinins (Turvey and Patrick, 1979) and gibberellins (Mulligan and Patrick, 1979) have since been shown also to have the property of attracting and concentrating nutrients. The mode of action of gibberellins and cytokinins appears to be different from that of auxins, and is more dependent on a localised sink effect.

1.1.2.2. Involvement of Phytohormones

Since Snow (1925) proposed the existence of a "diffusable inhibitor" from the shoot apex to explain the correlative inhibition of lateral buds, the involvement of phytohormones in this phenomenon has been widely researched. These substances seem particularly suited to the role of mediation in correlative phenomena. They may be transported from the site of synthesis to the site of action over short distances by diffusion and active transport. Movement over longer distances may involve passage in the vascular system. In auxins, where transport is best studied, all three modes of movement have been demonstrated in various experimental systems (see Goldsmith, 1977). Depending on the specific hormone being

studied, phytohormones are thought to act either as inhibitors or promoters of bud development. Investigations into the action of hormones may involve the analysis of the endogenous hormonal content in quiescent buds and buds released from inhibition. Alternatively, application of hormones is made to plants to examine their effect on correlative inhibition in lateral buds. Results from both experimental approaches need to be critically evaluated. In the first instance, very little is known about the fundamental mode of action of phytohormones. The intracellular sites of synthesis and action, distribution, biosynthesis, metabolism and interactions with other compounds and environmental stimuli remain unresolved. In many early investigations especially, misleading data could have arisen from imprecise methods of extraction and assay of endogenous plant hormones. In modern research, this problem has been alleviated to a great extent by the adoption of more definitive techniques of analysis (see Hillman, 1978). Another oft encountered difficulty in the analysis of endogenous phytohormones is in determining whether the changes observed are the cause or effect of the release of buds from correlative inhibition. In experiments involving the application of hormones, the problem lies in ensuring that sufficient applied hormone reaches the site of action in the plant tissue. Besides the physical barrier separating the site of application on the plant surface and the site of action of the hormone, the chemical breakdown of the compound might also result in it not achieving its intended effect. This consideration has often been employed as a justification for the use of very high concentrations of the applied hormone. In fact, such a solution could create problems of its own in that supra-normal concentrations of a hormone could radically modify various physiological processes in the plant in a pharmacological manner. Another method of circumverting the problem is through the use of synthetic hormones which are more stable than their

natural counterparts; for example, the substitution of naphthalene acetic acid for indoleacetic acid. Sometimes, synthetic hormones are used simply because they are more readily available: the cytokinin, kinetin, is one such example. While the use of these synthetic compounds to explain naturally occurring plant responses can be questioned, they do provide a broad understanding of the roles in correlative inhibition played by the groups of phytohormones to which they belong.

All five major types of phytohormones: auxins, abscisic acid, cytokinins, gibberellins and ethylene have been implicated in the control of correlative inhibition in plants. An outline of the experimental evidence for the involvement of the first four types of phytohormones is given below. The role of ethylene in correlative inhibition is described in section 1.2.2.

Experiments with pea seedlings carried out by Snow in 1925 showed that the inhibitory influence of the apical portion of the shoot could pass through a water-filled gap to sustain inhibition on a lateral bud of an adjoining decapitated seedling. Snow (1925) noted that steam girdling a zone of the stem of *Phaseolus* or *Faba* released apical dominance on the lateral buds beneath the girdle, whilst the shoot above the girdle maintained its growth. This finding pointed to the existence of a "diffusible inhibitor" originating from the apical bud which could not pass the girdled region of the stem. When Thimann and Skoog (1933, 1934) discovered that application of auxin or apex diffusate to decapitated *Faba vulgaris* maintained lateral bud inhibition, the identity of the "diffusible inhibitor" was ascribed to an auxin. Although to the present time, the replacement of the plant apex by auxin has been widely cited as evidence for an inhibitory role of auxin in apical dominance, it is unsatisfactory in several respects. White *et al.* (1975) determined

indoleacetic acid (IAA) levels in *Phaseolus* shoot tips to be 0.1 - 0.7 ng per shoot tip by mass spectrometry. However, the amount of replacement exogenous IAA required to maintain bud inhibition, as inferred from the results of White (1976), is of an order of a thousand times or more as much. Even allowing for a certain amount of loss through transport and degradation (Hall and Hillman 1975), there is some doubt as to whether this experimental approach simulates accurately the situation in the intact plant. The effect of some form of chemotoxicity by auxin applied in such large quantities must remain a possibility. Certainly, to show unequivocally the normal effect of a natural substance in a biological system, the substitution of the applied compound should be similar in quantity to what was removed from the plant to stop the process (Jacobs, 1959). Application of auxin to the cut end of the decapitated internode is often accompanied by massive tissue swelling in the treated region. The resultant sink effect in relation to the diversion of nutrients and other growth factors from the lateral bud must also be considered.

Nevertheless, there are various experimental observations in support of the "direct auxin theory" put forward by Thimann (1937) who proposed that while buds required auxin for their growth, they are inhibited by a concentration of auxin which would be optimal for elongation of the main stem. A correlation between the amount of auxin present in a bud of *Pisum* and the degree of its inhibition has been shown by Wickson and Thimann (1960), following treatment with ^{14}C-IAA at different concentrations and over different intervals. The auxin content in the stem of decapitated pea plants has been found to be reduced as compared with the intact plant (Scott and Briggs, 1960). Application of auxin transport inhibitors such as TIBA (Panigrahi and Audus, 1966; Little, 1970) or morphactins (White and Hillman, 1972; Chang, 1975) in a ring around an internode elicited a growth response of lateral buds basipetal to the point of application. These results must be accepted with caution, however, since

these compounds affect shoot growth markedly (White and Hillman, 1972).

Considerable criticism has been raised against the "direct auxin theory". Examination of auxin levels in inhibited and released buds even from early studies showed that auxin levels tend to rise rather than fall upon their release from inhibition (Overbeek, 1938). This observation has been confirmed more recently by Hillman *et al.* (1977) who analysed IAA extracted from *Phaseolus* buds by combined gas chromatography-mass spectrometry. Tucker and Mansfield (1973) detected lateral bud growth in *Xanthium* within 48 h, before any significant change in bud auxin content had occurred. Hall and Hillman (1975) found that while application of tritiated IAA to decapitated bean plants inhibited axillary bud growth, little or no radioactivity was transported into the bud during the 48 h following removal of the apex. This finding is again incompatible with the "direct theory". An "indirect theory" of auxin inhibition served to accommodate some of the objections raised against the "direct theory" by assuming that auxin from the apex acts to modify another factor(s) which in turn modulates bud growth. For example, Overbeek (1938) proposed that high auxin concentration in the stem could prevent auxin formation - and hence growth activity - in the lateral buds. Hormone-directed transport, mentioned in the previous section, is another example of the "indirect auxin theory".

The significance of vascular supply to inhibited buds has been mentioned earlier. The work of Gregory and Veale (1957), Sorokin and Thimann (1964) and Panigrahi and Audus (1966) correlated vascular connection development between lateral buds and the main stele with the release of apical dominance, and forms the basis of another mode of auxin-mediated bud inhibition. Auxin moving down the stem could be envisaged as inhibiting the development of adequate or fully functional vascular connections between lateral buds and the main vascular system of the stem, thereby depriving the buds of supply of nutrients.

Auxins are not the only phytohormone thought to inhibit lateral bud growth. An extracted inhibitor from *Faba*, now thought to be abscisic acid, was found to accumulate at higher concentrations in inhibited lateral buds than in the shoot apex (Kefford, 1955). In *Xanthium* (Tucker and Mansfield, 1972, 1973) there appears to be an interaction between putative abscisic acid and the spectral quality of light. Red light treatments promoted lateral bud growth, and this was accompanied by a marked fall in abscisic acid-like activity in the bud. The decrease was deemed to be apparent both in the concentration and the absolute amount of the inhibitor in the bud. However, there is as yet insufficient evidence to establish that the inhibition of buds by endogenous abscisic acid occurs widely in different species. In *Acer* and *Syringa*, for example, the absolute quantities of abscisic acid present in the outgrowing lateral buds following decapitation and defoliation of the shoot remain unchanged, even though there was a decrease in the concentration due to the increase in fresh weight of the bud (Dörffling, 1976). White and Mansfield (1977) found no significant fall in the level of endogenous abscisic acid-like substances in the axillary buds of *Phaseolus* following decapitation of the shoot.

The effects of exogenous application of abscisic acid on the outgrowth of buds in decapitated plants have also been investigated. Applied abscisic acid inhibited lateral bud growth in *Pisum*, but was not as effective as the intact apex (Arney and Mitchell, 1969). In *Phaseolus*, abscisic acid applied on its own to the decapitated stem slightly promoted axillary bud growth, but increased inhibition marginally when applied in combination with IAA and kinetin (Hillman, 1970). Phillips (1975) has criticized these results as the abscisic acid had been applied apically though the shoot apex is not the site of abscisic acid synthesis in the intact plant. This reasoning itself is open to question as young, unstressed leaves of *Phaseolus* contain relatively high levels of abscisic

acid (Hillman, unpublished). In any case, direct application of the inhibitor to lateral buds has been successful in suppressing active development of the buds in *Pisum* (Bellandi and Dörffling, 1974; White and Mansfield, 1977), *Lycopersicon* (Tucker, 1977) and also, when applied in high concentrations, in *Phaseolus* (White and Mansfield, 1977).

Phytohormones are also thought to have a stimulatory function in correlative inhibition and cytokinins may fulfil this rôle. Cytokinins applied directly to lateral buds have been shown to release correlative inhibition in *Pisum, Coleus, Sabiosa, Helianthus* (Sachs and Thimann, 1964), *Faba* (Panigrahi and Audus, 1966), *Glycine* (Ali and Fletcher, 1970, 1971), and *Lycopersicon* (Aung and Byrne, 1978). The stimulatory effects of cytokinins last only a few days, however, and do not normally result in a fully developed axillary shoot. Repeated applications of cytokinin give no additional effect (Sachs and Thimann, 1964; Ali and Fletcher, 1970, 1971), but addition of auxin following the initial response to cytokinin can prolong the duration of development (Sachs and Thimann, 1967). In *Pisum*, decapitation of the apex led to mobilization of radioactive labelled cytokinin from the roots to the lateral buds (Morris and Winfield, 1972). However, since this was noted 48 h following decapitation, the response could have resulted from, rather than been the cause of, lateral bud development. Cytokinin action appears to require supplementation with an essential factor in the stem because lateral buds completely excised from the stem respond very poorly to benzyladenine (Peterson and Fletcher, 1975). On the other hand, bud growth was dramatic when a 60 mm stem segment was excised along with the bud. The response to cytokinin follows a time lag of about 6 h, as determined by biochemical changes in the bud (Schaeffer and Sharpe, 1970; Usciati *et al.*, 1974) or by measurements of bud length (Nagao and Rubinstein, 1976).

Wooley and Wareing (1972a,b) proposed that lateral buds are deficient in cytokinins because acropetal transport of this hormone is inhibited by

auxin produced in the apex. They also found that acropetal movement of benzyladenine to lateral buds of decapitated *Solanum* was inhibited by indoleacetic acid and gibberellic acid applied to the cut end of the stump. For this hypothesis to be acceptable, it would have to be assumed that whereas outgrowth of inhibited lateral buds requires cytokinin, this is not required for sustaining growth of the apical shoot. Criticisms of the proposed requirement of cytokinin from the roots as a pre-condition for lateral bud development has arisen from the common observation of the development of buds borne on excised stem segments incubated in nutrient media. However, there is some evidence that shoots may be capable of synthesizing cytokinins in the absence of roots (Wang and Wareing, 1979).

Another class of growth stimulators, the gibberellins, have also been implicated in correlative inhibition. Promotion of lateral bud growth has been observed following the application of gibberellins to the bud (Catalano and Hill, 1969; Ali and Fletcher, 1970), or to the roots (Shien and Jackson, 1971; Jackson and Field, 1972). In several instances, the effect of gibberellins on quiescent buds was absent or only slight, but became evident only after an initial stimulatory treatment by a cytokinin. This was the case in soybean (Ali and Fletcher, 1971), pea (Sachs and Thimann, 1964) and tomato (Catalano and Hill, 1969). Such observations suggest that in these studies, gibberellins play no major rôle in the actual release of correlative inhibition, but that they act principally to enhance the growth of released buds.

Gibberellins applied in combination with auxins to the cut stem of decapitated plants gave conflicting results in different studies, and both the stimulation (Phillips, 1969b, 1971b; Hillman, 1970) and inhibition (Jacobs and Case, 1965; Scott *et al.*, 1967) of bud growth, as compared to the effect of auxin alone, have been observed. This discrepancy might be explained by differences in the age of the treated tissue and the location, relative to the bud, where the gibberellins were

applied. Whereas gibberellic acid applied close to the bud in decapitated *Phaseolus multifloras* promoted its growth, application made to the young internode some distance away from the bud reduced bud growth (Phillips 1971a). This might be due to an effect of compensation growth arising from gibberellin-induced extension of the internode.

The involvement of the auxins, abscisic acid, cytokinins and gibberellins in apical dominance presents a complex picture indeed of their relative rôles. The understanding of the mechanisms governing correlative inhibition is further complicated by the interactions that can occur between various hormones (Hillman, 1970; Shien and Jackson, 1971), and the release of buds from inhibition might entail these hormones operating in a pre-determined sequence (Sachs and Thimann, 1967; Ali and Fletcher, 1971). A similar temporal element might, therefore, have to be incorporated into the experiment to obtain meaningful results.

1.2. ETHYLENE AS A PHYTOHORMONE

Ethylene - C_2H_4 - is a highly active regulator of plant growth and development. While much attention in early research was devoted to the rôle of ethylene in fruit ripening, the effects of the gas in numerous areas of both the vegetative and reproductive physiology of the plant has since been recognised. The involvement of ethylene has been implicated in such diverse plant processes as cell division and expansion, seed germination, shoot and root growth and development, stress responses, ageing and senescence. The sphere of influence of ethylene is further increased by its ability to interact with phytohormones such as the auxins, cytokinins and gibberellins, and to modify the action of these hormones in the regulation of plant development (see Abeles, 1973; Lieberman, 1979). Following its recognition as a natural plant product (Gane, 1934), ethylene has itself been gradually, and now widely, accepted as an important plant hormone. Certainly, many of its characteristics are consistent with its assignment as a phytohormone. It is readily recovered and identified from plant tissues, leaving no doubt that it occurs naturally in plants. Profound effects are elicited by trace quantities of endogenous ethylene, and many of these effects can be mimicked by equally small quantities of the applied compound. Finally, ethylene acts neither as a substrate nor a co-factor in reactions associated with its regulatory rôle (Lieberman, 1979). Perhaps the major consideration against it being defined as a hormone in the strict technical sense is the apparent absence of physiologically significant transport from its site of synthesis to its site of action (Zeroni *et al.*, 1971; Jerie *et al.*, 1978a). These two locations in terrestrial plants are generally co-incidental or in close proximity, although small amounts of ethylene may be channelled to distant parts of the plant when emanation is prevented, e.g. from the roots in

waterlogged soil (Jackson and Campbell, 1975). However, objections raised on the basis of the precise definition, in the view of Lieberman (1979), "only dwell on semantics". In so far as ethylene is regarded as a plant hormone, it is, nevertheless, not a pheromone in the sense analogous to the (often gaseous or volatile) animal hormones which are secreted into the environment. Pheromones released by animals serve to influence and stimulate, in specific ways, the behaviour or development of other individuals of the same species. While hormone-like substances, liberated into water or air, are known to play a rôle in sexual reproduction of certain fungi and algae, the ethylene produced by a plant affects its own physiological and developmental functions. There is no evidence that ethylene emanated from a plant influences, through genetic design, any physiological process in another plant.

The amount of ethylene required to elicit a response in plants is very small indeed. Work involving the ethylene-requiring tomato mutant, diageotropica, showed that abnormal development of the mutant plant could be rectified by the application of only 5×10^{-3} vpm ethylene (Zobel, 1973). Ethylene concentrations in excess of 0.1 vpm have been regarded as supra-optimal, and therefore inhibitory, in the regulation of certain aspects of plant growth and development, such as DNA synthesis (Apelbaum and Burg, 1972) and tissue differentiation (Zobel and Roberts, 1978). The range of ethylene concentrations within which the normal regulatory role of ethylene operates varies between different species. Konings and Jackson (1979) showed that whereas less than 1 vpm ethylene was required for the stimulation of root elongation in rice plants, the upper threshold value for tomato plants was only 0.02 vpm.

The basic substrate for the biosynthesis of ethylene is methionine, carbons 3 and 4 of the amino acid being converted to ethylene (Lieberman *et al.*, 1966; Baur *et al.*, 1971; Hanson and Kende, 1976). Adams and Yang (1979) proposed a model whereby ethylene is formed via the intermediate

s-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC). Lieberman (1977) put forward a different model of ethylene synthesis in which methionine is degraded to ethylene directly while attached to an enzyme complex.

The mode of ethylene action is a matter of some controversy. There are two general concepts, differing mainly on whether or not ethylene has to be metabolized to enable it to carry out its physiological functions. The first hypothesis involves no ethylene metabolism. It requires ethylene to form a transient complex with its receptor, from which it rapidly dissociates once the necessary changes to the receptor have been effected. Ethylene acts essentially as a switch in this instance. In the second hypothesis, ethylene metabolism gives rise to the formation of an ethylene-receptor complex which participates in further physiochemical reactions.

The view that ethylene metabolism does not occur derives support from the very low incorporation of exogenous ^{14}C-labelled ethylene into the treated plant tissue. The fact that many ethylene symptoms in plants disappear soon after removal of the applied ethylene also suggests the absence of viable ethylene-receptor complexes. On the other hand, ethylene metabolism has been positively demonstrated in pea seedlings (Beyer, 1975a,b) and carnation (Beyer, 1977) and morning glory flowers (Beyer and Sundin, 1978). The metabolic products are carbon dioxide, which is released, and mainly water-soluble metabolites which are incorporated into the tissue, although in very small amounts as mentioned above. Hall and Jerie (1978) have pointed out, however, that in many metabolism experiments, insufficient attention has been made towards the recovery of volatile ethylene metabolites. In *Faba* cotyledons, more than 95% of the ^{14}C-ethylene applied has been found to be metabolised within 25 h. Most of the radioactivity has been recovered as ethylene

oxide which is very volatile (b.p.285 K). However, the very high efficiency at which applied ethylene was oxidized raises the possibility that the ethylene oxide had been produced artifactually by a non-physiological reaction (Lieberman, 1979). The occurrence of ethylene metabolism in itself does not establish conclusively that the response to ethylene in plants is dependent on such metabolism. Some evidence of a causal relationship has been obtained by treatment of pea seedlings with high carbon dioxide concentrations, low oxygen concentrations and silver nitrate: these treatments inhibit the metabolism of ^{14}C-ethylene in pea seedlings while simultaneously reducing the response of the treated tissues to ethylene (Beyer, 1979). One potential weakness in this study lies in the fact that Ag^+ and carbon dioxide have been shown to increase endogenous ethylene production in tobacco leaf discs, possibly by a negative feedback regulatory mechanism (Aharoni and Lieberman, 1979a; Aharoni *et al.*, 1979). The reduction in ethylene metabolism might thus be a result of competition between the applied ^{14}C-ethylene and the increased amounts of endogenous ethylene and, therefore, be quite unrelated to the de-sensitization of the tissue to ethylene by Ag^+ and carbon dioxide. A further objection to a cause-effect relationship between the metabolism of ethylene and the physiological effects it induces is in regard to the saturation concentration of ethylene in either case. Whereas ethylene responses in etiolated peas saturate at around 10 vpm (Abeles *et al.*, 1972), the oxidation of ^{14}C-ethylene to ^{14}C-carbon dioxide and its incorporation into pea tissue do not saturate even at 140 vpm ethylene (Beyer, 1975b).

In phytohormone research, it is often desirable to inhibit the effect of the hormone to examine its specific rôles in the plant. In so far as ethylene is concerned, "anti-ethylene" agents are of two types; those that inhibit ethylene biosynthesis and those that inhibit ethylene action. The most important inhibitors of ethylene production are the enol ether

amino acid analogs, the L-2 amino 4-alkoxy-*trans*-3-butenoic acid molecules. Two such compounds that are commonly used are rhizobitoxine (L-2-amino-4-(2-amino-3-hydroxypropoxy)-*trans*-3-butenoic acid) and aminoethoxyvinyl glycine (L-2-amino-4-(2-aminoethoxy)-*trans*-3-butenoic acid). These compounds are highly potent, being effective at very low concentrations, specific in their action in inhibiting ethylene synthesis, and have little effect on the general metabolism of the plant. In reference to the biosynthesis of ethylene according to the model of Adams and Yang (1979) (see above), enol ether amino acid analogs are thought to inhibit the conversion of SAM to ACC. The ability of high carbon dioxide and low oxygen content in the air, and of Ag^+ ion in inhibiting the action of ethylene, has already been mentioned above. Ag^+ has the strongest effect, and is specific in its ability to desensitize a wide range of plant tissue against the action of ethylene (Beyer, 1976a,b; Veen and Geijin, 1978). Ag^+ ions block the incorporation of ^{14}C-ethylene into tissues but does not affect the oxidation of ^{14}C-ethylene to ^{14}C-carbon dioxide in the same tissue. In contrast, carbon dioxide inhibits the formation of ^{14}C-carbon dioxide but has no effect on tissue incorporation (Beyer, 1979). This suggests two sites at which ethylene may be metabolized. However, as has been mentioned above, the relationship between ethylene metabolism and action has yet to be convincingly demonstrated.

Since ethylene is a gas at normal temperatures and pressures, assessing the quantitative status of the hormone in plant tissue presents unique considerations and problems not encountered with the other groups of phytohormones. The ethylene status in a plant tissue is expressed either as its rate of emanation or as its concentration in the gaseous extract of the tissue. Determination of the rate of ethylene release most commonly involves enclosure of the tissue in an airtight container over a period of time, after which, the accumulated ethylene in the air

is sampled and analysed by gas chromatography. In such analyses, precautions must be observed in order that the rate of ethylene emanation is not vastly inflated through artifacts arising from stress ethylene induced by wounding (Hyodo, 1977; Jackson and Osborne, 1970; Saltveit and Dilley, 1978a) or water stress (Aharoni, 1978). In the assay of the internal ethylene content, the tissue is subjected to vacuum extraction below the surface of a solution in which ethylene is poorly soluble (e.g. a saturated salt solution). The gaseous extract collected is subsequently analysed by gas chromatography (Beyer and Morgan, 1970b). A detailed account of the analytical procedures for the assay of ethylene has been given by Ward *et al.* (1978).

1.2.1. Ethylene-Auxin Relationship

Although interactions between ethylene and other classes of phytohormones are known, the most significant interactions occur with the auxins. Since auxins have been widely implicated in the correlative inhibition of buds, a brief examination of the relationship between ethylene and auxin is given below.

Ethylene effects are in many instances a reversal of those of auxin. For example, auxin promotes elongation of shoot tissue cells while ethylene inhibits; auxin delays abscission while ethylene promotes it. If both growth regulators are applied at the same time, antagonistic effects are often observed: for instance, auxins can block the induction of abscission (Milbrath and Hartman, 1940) or the ripening of fruits (Vendrell, 1969) by ethylene. It is feasible, therefore, that ethylene may have a rôle in regulating auxin activity in the plant and/or *vice versa*.

The promotion of ethylene production by auxin is a well studied and widespread phenomenon. Several (but by no means all) of the effects of

auxins in the plant have been attributed to auxin induced ethylene (see Abeles, 1973 and Lieberman, 1979). The evidence available suggests that auxin induces ethylene formation *via* two systems (Steen and Chadwick, 1973). The first system is stimulated by low levels of auxin, requires no *de novo* synthesis of protein and occurs rapidly. (Franklin and Morgan (1978) reported the time lag between auxin treatment and ethylene stimulation in sorghum to be 15 min or less.) The second system responds to high auxin levels, requires protein synthesis and induces larger quantities of ethylene. Yu *et al.* (1979) investigated the stimulation of ethylene by auxin based on the model of Adams and Yang (1979) for ethylene synthesis, in which methionine is converted to ethylene *via* SAM and ACC. They proposed that the conversion of SAM to ACC was the rate-limiting step in the biosynthesis of ethylene, and that auxin stimulated ethylene production by inducing the synthesis of the enzyme involved in this reaction. Their results also suggested a single system, rather than two systems, by which auxin induces ethylene production. Conversion of ACC to ethylene was observed to proceed initially in the presence of cycloheximide, but sustained conversion was observed only when protein synthesis was allowed to take place.

While application of auxins can affect ethylene production, the converse is also true in that ethylene is also known to influence auxin transport and metabolism. Generally, ethylene reduces auxin levels (Valdovinos *et al.*, 1967; Burg *et al.*, 1971; Lieberman and Knegt, 1977) although cases where auxin content is raised after ethylene treatment are also known (Lyon, 1970). Valdovinos *et al.* (1967) claimed the conversion of tryptophan to auxin to be inhibited after treating the plants with ethylene. Burg *et al.* (1971) reported that the effect of ethylene varied according to the position of the plant studied. In the pea seedling, for example, the largest reduction of auxin occurred in the subapex, less

in the hook region, while an increase occurred in the apical region.

Another possible mechanism of the ethylene-induced reduction in auxin activity is the inhibition of transport of auxin from the site of production to the site of action. In plants pretreated with ethylene, a reduction in the polarised transport of auxin is commonly observed in isolated plant segments (e.g. Palmer and Hallsall, 1969; Beyer and Morgan, 1971). Susceptibility to ethylene-induced inhibition of auxin transport varies with species; cotton, for example, is highly susceptible, whereas sunflower shows little response (Morgan *et al.*, 1968). Burg and Burg (1967) have shown that it is the quantity rather than velocity of transport that is affected.

There are several possible mechanisms that can account for the observed disruption in auxin transport by ethylene. A reduction in auxin uptake into the plant tissue through the cut surface could be one possible cause. However, evidence against this has been produced in several studies (Burg and Burg, 1966; Beyer and Morgan, 1969, 1970a). Alternatively, there could be a gradual increase in auxin destruction in the course of transportation; or the auxin molecules could be increasingly immobilised by the action of ethylene. It is also possible that a breakdown occurs in the mechanisms involved in actively transporting auxin from cell to cell. Finally, ethylene might induce a breakdown of the structural integrity of pathways through which transport occurs. Beyer and Morgan (1970a) found an increase in IAA decarboxylation in ethylene-treated stem segments, but believed the IAA metabolites to be a result of, rather than the cause of ethylene mediated disruption of IAA transport. Osborne and Mullins (1969) noted that pretreatment of petiole segments with an auxin completely protected the auxin transport from both an uninduced and ethylene-induced fall in IAA transport. A decrease in protein synthesis accompanies this loss of transport capacity and treatments

with auxins which retain synthesis also retain transport.

Besides its effect on the longitudinal transport of auxin along the stem, ethylene has also been shown to modify geotropic-mediated lateral auxin transport in pea internodes and tomato petioles (Kang and Burg, 1974).

1.2.2. Involvement of Ethylene in Correlative Inhibition of Buds

Among the various groups of growth regulators, relatively little attention has been directed at ethylene as a factor governing correlative inhibition. Hall, Truchelut, Leinweber and Herrero (1957) reported the loss of apical dominance in cotton plants resulting from ethylene pollution of the air. *Petunia* plants (Burg, 1973) and potato sprouts (Catchpole and Hillman, 1976) supplied with ethylene showed development of inhibited lateral buds when the ethylene was subsequently withdrawn. Outgrowth of lateral buds also followed application of the ethylene-releasing agent, ethephon, to *Prosopsis fuliflora* and *Acacia farnesiana* (Morgan *et al.*, 1969) and to *Hevea brasiliensis* (Leong *et al.*, 1976).

That ethylene should influence correlative inhibition is perhaps not surprising in view of the known effects of auxins in this regard, and in view of the established ability of auxins in stimulating ethylene production (see previous section). In experiments where excised pea nodal sections were incubated in different concentrations of IAA solution, Burg and Burg (1968a) found that degree of inhibition of the bud outgrowth correlated well with the induction of ethylene formation by the various IAA concentrations. Treatment of the buds with ethylene alone produced a similar inhibitory effect. Burg and Burg (1968a) reported on this basis, that ethylene production could account for the inhibitory action of applied auxin. Inhibition of bud growth may, for example, act through the ability of ethylene to antagonize DNA synthesis and thereby inhibit cell division (Apelbaum and Burg, 1972; Apelbaum *et al.*, 1974).

When it was first observed that ethylene evolution in etiolated pea

plants was higher at the nodes than at the internodes, Burg and Burg (1968b) proposed that inhibition of the lateral buds, which were situated at the nodes, was due to ethylene. In a subsequent paper, however, it appears that the high ethylene emanation from the nodes could be traced to the scale leaves, and decapitated seedlings with the scale leaves removed showed no decrease in ethylene emanation from the nodes (Burg and Burg, 1968a). Another inconsistency in ascribing the rōle of bud growth inhibition to ethylene is that bud outgrowth induced by kinetin is not inhibited by ethylene even at concentrations as high as 1000 v.p.m. (Burg and Burg, 1968a).

Although the effect of applied ethylene on buds in experimental systems is generally one of inhibition, it must be remembered that ethylene is a natural plant hormone involved in growth and development. Thus, the release of *Malus hupehensis* buds from inhibition was accompanied by an increase in ethylene production. Treatment with the ethylene synthesis antagonist aminoethoxyvinyl glycine inhibited outgrowth of the bud (Zimmerman *et al.*, 1977)

1.3. EXPERIMENTAL PLANT MATERIAL

The dwarf French Bean, *Phaseolus vulgaris* c.v. Canadian Wonder has been used in this study. The advantages of using this plant are that it is easy to grow uniformly, it is small enough for a fairly large number to be accommodated in the experimental growth room, and it is fast growing, thereby allowing a rapid turnover of plants. The lateral (axillary) buds in *Phaseolus* are well formed and are large enough to enable measurements to be made to detect small increases in growth. A considerable amount of work has already been carried out with *Phaseolus* and interpretation of results obtained from this study can therefore be made in relation to previous work.

In previous studies on apical dominance in *Phaseolus* correlative inhibition of the axillary buds of the primary leaves have most commonly been investigated. In the present study, however, attention is placed on the third node axillary bud, i.e. the bud subtended by the first trifoliate leaf. The lateral buds at the axils of the primary leaves are paired, and may exert mutual effects on each other in the course of their growth (Hillman, 1970). On the other hand, there is only a single axillary bud at the insertion of the first trifoliate leaf (although supernumerary buds may develop at a later stage if the plant is decapitated).

1.4. SCHEME AND RATIONALE OF RESEARCH

Growth patterns of the leaves, internodes and axillary buds of intact young bean plants are presented in a short introductory section.

The second section of this investigation is concerned with some fundamental characteristics of lateral bud growth. The timing of the onset of accelerated growth of the axillary bud following decapitation of the apical shoot is examined. The time lag between shoot decapitation and growth response in the bud gives a useful indication of the mechanisms governing the maintenance and release of correlative inhibition. McIntyre (1977) has proposed that the initial rapid bud outgrowth is due mainly to cell extension resulting from an increase in water potential in the bud. To verify the validity of this proposal, cell lengths in the bud of intact plants and in the bud of plants one and six days after decapitation are compared. The vascular supply and transport to axillary buds was investigated using decolonized basic fuchsin as a tracer. Although White (1973) has shown by anatomical studies that vascular connections exist between the primary leaf axillary buds and the stem, his results give no indication of the efficiency of transport of materials to these buds or if the vascular connections are functional at all. The present study considers these points and, in addition, extends the investigation on vascular transport to the axillary buds of the first and second trifoliate leaves. Models accounting for the characteristic features of acropetal and basipetal transport to the buds are proposed.

The third section of this study gives an appraisal of some aspects of ethylene methodology. In preliminary experiments, the three main hydrocarbon gases present in the gaseous extract of *Phaseolus* shoot tissue had been tentatively identified as methane (which is always present in the air), ethane and ethylene by gas chromatography-flame ionization detection, the retention times of the gases having been compared on a

Poropak R column and an activated alumina column. Combined gas chromatography-mass spectrometry of similar *Phaseolus* extracts was carried out to provide positive identification of ethylene and ethane. Vacuum extraction of ethylene and ethane from *Phaseolus* tissue is then considered in greater detail. Modifications on the basic method of Beyer and Morgan (1970b) which enable small volumes of gases (about 0.15 cm^3) to be recovered are described. Although the vacuum extraction method has been commonly used to assay the internal concentration of ethylene in plant tissue, the vacuum employed has varied in different studies, with 6.7 kPa (50 mm Hg) (Aharoni, 1978), 13.3 kPa (100 mm Hg) (Beyer and Morgan, 1970b), 40.0 kPa (300 mm Hg) (Kapuya and Hall, 1977) and 66.7 kPa (500 mm Hg) (Kapuya and Hall, 1977) having been used. The effect of varying the conditions of vacuum extraction on the concentration of ethylene in the extracted gas sample is not known. Another point of ambiguity is the relationship between the internal ethylene concentration in the tissue (as determined by vacuum extraction) and the rate of ethylene emanation by the same tissue. While there are reports of a correlation between these two measurements (Beyer and Morgan, 1970b; Aharoni and Lieberman, 1979b), this fact has been disputed (Zeroni *et al.*, 1977; Hall, 1977). Experiments were therefore set up to compare the ethylene and ethane concentration of *Phaseolus* leaf gaseous extracts recovered after applying a vacuum of 13.3 kPa for 2 min with extracts obtained using a stronger vacuum of 66.7 kPa for 3 min. Experiments were also carried out to determine whether a correlation exists between the internal ethylene concentration and the rate of ethylene emanation.

The next section examines the involvement of ethylene (and ethane) in the control of axillary bud growth. This section comprises two main parts: the first dealing with ethylene involvement in the apical shoot and the second part dealing with ethylene involvement in the axillary bud and adjacent tissue. The first part looks into the possible involvement of ethylene in the release of apical dominance by the physical confinement

of the developing shoot. The effect on correlative inhibition by the physical restriction of apical shoot growth and by treatment of the shoot with ethylene (supplied as the gas or as ethephon) are compared. The ethylene status of physically confined shoots, expressed as internal ethylene concentration and rate of ethylene release, is compared to that in the shoots of control plants. Apical shoots were also treated in ethylene-enhanced and ethylene-depleted atmospheres to observe the effects of these treatments on ethylene emanation, internal ethylene concentration and on the release of apical dominance. This experimental approach allows for a critical assessment of the relationship between the ethylene status of the apical shoot and the loss of correlative inhibition.

In the second part of the section, correlative inhibition is studied in relation to the rôle of ethylene in the bud and surrounding tissue (i.e. tissue in the region of the node). Ethephon was applied to the bud or cut stem of the decapitated plant to examine ethylene effect on bud outgrowth. Similar applications of ethylene antagonists - AVG, which inhibit ethylene synthesis and Ag^{+} ion, which inhibits ethylene action - were also carried out in separate experiments. The well-known ability of auxin both to inhibit lateral bud growth in a decapitated plant and to induce ethylene production raises the possibility of these two responses being related. Burg and Burg (1968 a,b) have shown that auxin-induced ethylene is responsible for an inhibition in the development of buds borne on nodal segments incubated in auxin solutions. It is plausible, therefore, that in the classic experiment demonstrating the substitution of the intact apex by IAA, the high concentration of auxin applied to the cut end of the decapitated stem is translocated basipetally to the bud where it induces ethylene production and the consequent inhibition in bud growth. To test this hypothesis, the internal ethylene concentration and the rate of ethylene release from the node bearing the bud, and from the internodes immediately above and below it, were assayed in decapitated plants with or

without IAA applied to the stump. Similar analyses also carried out on the intact plant facilitates a comparison of the ethylene status in nodal region of the intact and decapitated plant, and provides for a re-assessment of an earlier hypothesis of Burg and Burg (1968b) that high ethylene at the node maintains correlative inhibition in lateral buds.

The effect of IAA applied directly to the axillary bud has also been investigated in relation to the antagonism between auxin-promoted growth and growth inhibition resulting from auxin-induced ethylene. In some treatments, IAA was applied together with the ethylene antagonist AVG to observe the auxin response in the absence of an interaction with ethylene induced by the auxin.

The final section of the study is not directly related to correlative inhibition, but nevertheless has an important bearing to the ethylene analyses employed throughout the study. The characteristics of enhanced ethylene release resulting from tissue wounding or water stress in *Phaseolus* stem and leaves are described and compared. High ethylene emanation so induced can cause serious errors in the analyses of the basal rates of ethylene release, unless appropriate precautions are observed.

Although the rôle of ethylene in correlative inhibition is the major consideration in this study, the possible involvement of endogenous ethane has also been assessed alongside with ethylene in a large proportion of the experiments.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL

Seeds of the dwarf French bean, *Phaseolus vulgaris* L. cv. Canadian Wonder, were obtained from Charles Sharpe & Co. Ltd., Sleaford, Lincs., U.K. and stored at 283 K (10°C) in darkness. The seeds were sown singly into plastic pots, 8 cm diameter x 8.5 cm depth, containing a standard compost mixture based on loam. For some experiments requiring a large number of plants, seeds were sown in 37 x 24 x 9 cm polystyrene boxes, twenty-five seeds to a box.

Midway through the study, seedlings grown in the loam-compost were found to be severely infected by the soil pathogen *Thielaviopsis basicola* which caused drying and abscission of the primary leaves. Subsequent planting was therefore made in Levington compost (Fisons Garden Products, Harston, Cambridge, U.K.). Besides overcoming the problem of the infection, Levington compost also encouraged better emergence of the seedlings and subsequent growth of the plant. Table 1 compares seed emergence and plant vigour (as indicated by the size of the primary leaf and 2nd trifoliate leaf) between the loam-compost, a mixture of top- and sub-soil and Levington compost. The seeds sown were either unselected or selected to omit seeds with wrinkled testas or small lesions. The results show that seed selection had no significant bearing on the subsequent performance of the plant. Seedling emergence in the top- and sub-soil mixture was over 84% but this rather poor soil could not sustain vigorous plant growth. Levington compost was superior to the loam-compost in seedling emergence and subsequent plant vigour. Heat sterilizing the latter improved the rate of development of the plant (as denoted by the size of the 2nd trifoliate leaf), thus suggesting the presence of inhibitory micro-organisms in the soil. On the other hand, poor seedling emergence

TABLE 1. Effect of potting material, soil sterilization and seed selection on seedling emergence and plant development 22 days from sowing. Values in each column bearing the same letter are not significantly different ($p \leq 0.05$).

	Potting material	Seed Selection[1]	Seedling emergence[2] (%)	Length of primary leaf[3] (mm)	Length of central leaflet, 2nd trifoliate[3] (mm)
A	Loam-compost	No	63.1	84.75^{a}	22.22^{a}
B	Loam-compost, sterilized	No	56.3	91.14^{ab}	39.50^{c}
C	Loam-compost, sterilized	Yes	68.0	87.70^{a}	33.90^{bc}
D	Levington compost	No	96.1	101.40^{c}	38.90^{c}
E	Levington compost	Yes	94.2	96.30^{bc}	35.10^{c}
F	Top- and sub-soil mixture	No	84.5	84.22^{a}	26.50^{ab}
G	Top- and sub-soil mixture	Yes	96.1	88.85^{a}	24.56^{a}

[1]Seeds were selected to remove those with wrinkled testas or small lesions.

[2]Based on 103 pots.

[3]Based on the 10 most vigorous plants from a population of 53 per treatment.

in this soil (63%) appeared to be due to physical factors since soil sterilization had no effect.

Plants were grown in a glasshouse which was heated in winter. Supplementary illumination was provided daily for 16 h between September and April using Atlas Kolorlux 400W MBFR/U high-pressure mercury vapour lamps spaced 0.8 m apart and hung 1 m above the plants. The irradiance from these lamps was about 8 $W.m^{-2}$ at a height of 15 cm above the bench top, with the transmitted wavelengths mainly between 500 and 700 nm. The pots and boxes of plants were placed on moist gravel and watered twice daily.

Plants were normally used in experiments when they were 19-25 days old or 23-29 days old at which time, the 2nd trifoliate leaf or 3rd trifoliate leaf, respectively, were at the beginning of their grand phase of growth (Fig. 1). (Leaves, nodes and internodes are counted acropetally.) The plants were selected for uniformity on the basis of height and the stage of development of the 2nd trifoliate leaf. Plants with axillary buds showing precocious development were not selected. In a small proportion of plants, floral buds developed in place of the 4th trifoliate leaf at the apex. As spontaneous sprouting of axillary shoots accompanies the onset of flowering, these plants usually showed an early loss of apical dominance and were thus unsuitable for experiments on the correlative inhibition of buds. Such plants could normally be identified during selection by examination of the apical bud.

Most of the experiments on correlative inhibition were concerned with the growth of the axillary bud subtended by the first trifoliate leaf at the third node. The inhibited bud had a well-defined internode and an unopen trifoliate leaf enclosed (except for the leaf tips in some buds) by bracts.

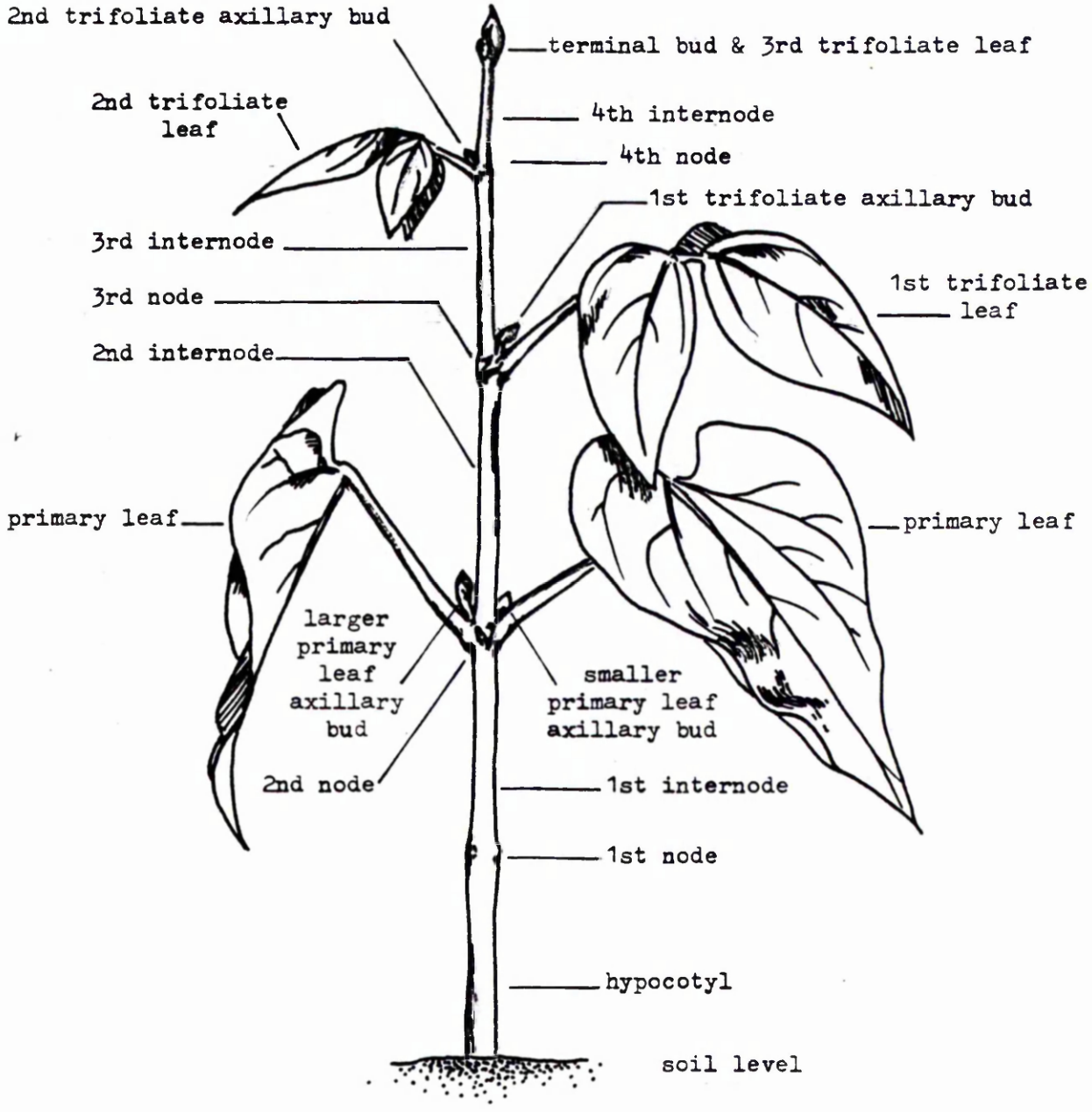


FIGURE 1. Phaseolus vulgaris L. cv. Canadian Wonder, approximately 22 days old.

2.2. ENVIRONMENTAL CONDITIONS FOR PLANT GROWTH EXPERIMENTS

Experiments involving plant growth were carried out in a 10.4 m^3 growth room. Plants in pots were placed on moist capillary matting on two side benches, each continuously illuminated by a bank of five 1.5 m 80W white fluorescent tubes affixed 0.75 m above the bench-top. The irradiance 20 cm above the bench was 11-13 $W \cdot m^{-2}$. A temperature of 298 ± 1.5 K (25 ± 1.5°C) was regulated by a thermostat and maintained by circulating warm or chilled air in the room. Humidity was not precisely controlled, but a steady relative humidity within the range of 65-80% could be maintained with small fluctuations occurring in phase with the cyclic variation in temperature (Fig. 2). The door of the growth room was left open for about 10 min daily for ventilation. Plants in the growth room were watered once a day and moisture in the capillary matting was replenished at the same time. The plants were transferred from the glasshouse into the growth room at least one day before the beginning of an experiment.

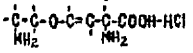
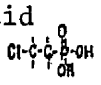
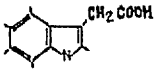
2.3. CHEMICALS AND GASES

Chemicals used in this study (other than the general laboratory chemicals) are listed in Table 2. Gases used are given in Table 3.

2.4. GROWTH MEASUREMENTS

Growth increments in leaves, internodes and buds over one day or longer were measured to the nearest 0.5 mm using a ruler and, where appropriate, a pair of dividers. Dry weight of plant tissue was determined after drying in an oven at 353 K (80°C) for two to three days. Leaf surface area was estimated by cutting out leaf shapes traced on to thick paper, and weighing.

TABLE 2. Chemicals

Compound	Source	Preparation for experimental use[1]
Basic fuchsin	British Drug House, Poole, U.K.	Prepared according to Talboys (1955): 100 cm^3 0.1% aqueous basic fuchsin was decolorized with 10 cm^3 10% potassium metabisulphite
U ^{14}C-sucrose	Radiochemical Centre, Amersham, U.K.	Aqueous solutions, 23 mol m^{-3} (74 x 10^2 Bq cm^{-3})
Aminoethoxyvinyl glycine hydrochloride (AVG) L-2-amino-4-(2-amino-ethoxy)-*trans*-3-butenoic acid-hydrochloride 	Dr. R. Maag Ltd., Dielsdorf, Switzerland	Aqueous solutions, various concentrations
Ethephon (4.8% formulation) 2-chloroethyl-phosphoric acid 	A.H. Marks & Co. Ltd., Wyke, U.K.	Aqueous dilutions to various concentrations
Mercuric perchlorate $Hg(ClO_4)_2$	Prepared in the laboratory	250 mol m^{-3} in 2 kmol m^{-3} perchloric acid. Prepared according to Young *et al.* (1952): 17.2 cm^3 of 70% perchloric acid was diluted to 25 cm^3. 5.4 g red mercuric oxide was dissolved in this solution by grinding in a mortar and made up to 100 cm^3 with water.
2,3,5-tri-iodobenzoic acid (TIBA) 	Koch-Light Laboratories Ltd., Bucks, U.K.	Dissolved in a minimal quantity of 70% ethanol and dispersed into molten hydrous lanolin.
Indole-3-acetic acid (IAA) 	Sigma Chemical Co., St. Louis, Mo., U.S.A.	Dissolved in a minimal quantity of 70% ethanol and dispersed into water or molten hydrous lanolin.

[1] Aqueous solutions applied to the plant surface incorporate 0.01% Triton X-100 as a wetting agent.

Control plants were treated with water or hydrous lanolin with the adjuvants (ethanol and Triton X-100) added and with only the test ingredient omitted.

TABLE 3. Gases

Gas	Source	Preparation for use
Ethylene (99.8%) C_2H_4	British Drug House, Poole, U.K.	Gas from the cylinder collected under water. Serial dilution[1] to appropriate concentration.
Ethylene (1 vpm; balance gas:air)	BOC Special Gases, London, U.K.	Gas from the cylinder collected under water. (For calibration)
Ethane (0.9%; balance gas: nitrogen) C_2H_6	Phase Separations Ltd., Queensferry, U.K.	Gas from the can collected under water. Serial dilution[1] to appropriate concentration.
Calibration gas mixture: 1100 vpm ethane 1095 vpm ethylene 1100 vpm methane 1090 vpm acetylene 5480 vpm CO_2 5300 vpm CO (Balance gas: nitrogen)	Phase Separations Ltd.	Gas from the can collected under water.
Artificial air	BOC Special Gases	No preparation required. (For flushing containers)

[1]Serial dilution involved the transfer of a sample of ethylene or ethane mixture from a donor container to a recipient container of known volume using an airtight hypodermic syringe. The containers were either test tubes or bottles fitted with rubber Subaseals or Clinbritic seals. Before the transfer was made, saturated sodium chloride solution (in which ethylene is only very slightly soluble) equal in volume to the donor sample was injected into the donor container to maintain the original pressure in the container when the sample was withdrawn. Similarly, an equal volume of air in the recipient container was removed before the transfer so that pressure was unchanged when the donor sample was subsequently injected into the container. The mixture in the recipient container was allowed to equilibrate before further dilution was carried out.

2.4.1. Rapid Axillary Bud Growth in the Decapitated Plants

A photographic method, basically similar to that described by Hall and Hillman (1975), was used to determine the time lag between shoot decapitation and onset of an increase in growth rate of the first trifoliate leaf axillary bud. Two plants were used in each experiment: one which was decapitated through the 3rd internode and the other serving as a control. The plants were secured to supporting stakes to ensure that no movement of the stem, and hence of the axillary buds, occurred during the course of the experiment. Besides carrying out the experiments in the growth room, the plants were further placed in a growth cabinet which had an open front and transparent top and side walls. This arrangement reduced the cyclic fluctuations in temperature and humidity in the micro-environment to which the plants were subjected (Fig. 2). Two single-lens reflex cameras were used: one each assigned to the control or decapitated plant. The cameras were fitted with extension bellows or extension rings and were focused on the internode of the first trifoliate leaf axillary bud (Fig. 3).

Nine experiments were carried out: five experiments using plants with the 2nd trifoliate leaf entering its grand phase of growth (16-25 days old) and four experiments involving physiologically older plants with the 3rd trifoliate beginning its grand phase of growth (22-27 days old). Short lengths (*ca.* 4 mm) of finely drawn glass were inserted into the proximal and distal ends of the internode of the axillary bud one day before the experiment to act as markers. Photographs of the axillary bud were taken at 20 min intervals for 2.7 h prior to decapitation of the shoot and at 30 min intervals for 10 h thereafter. Exposures of 1 s were made in available light. Measurements were made from the images of the buds magnified (x16) using a photographic enlarger. All measurements were made at least twice to check for consistency, and generally, measurements consistent to about 0.3 mm (in the enlarged image) were achieved.

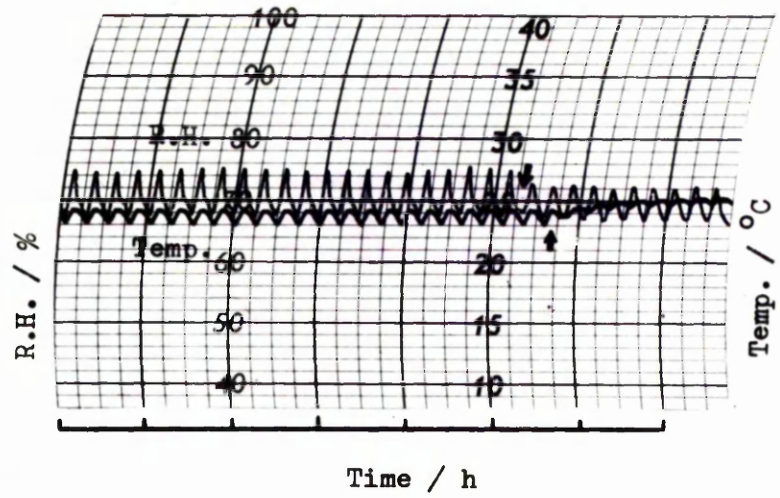


FIGURE 2. Cyclic fluctuations in temperature and relative humidity in the growth room and inside the growth cabinet (see Fig. 3 below) arising from the thermostat in the growth room switching on and off. The arrows denote the time the thermohydrograph was transferred into the growth cabinet.

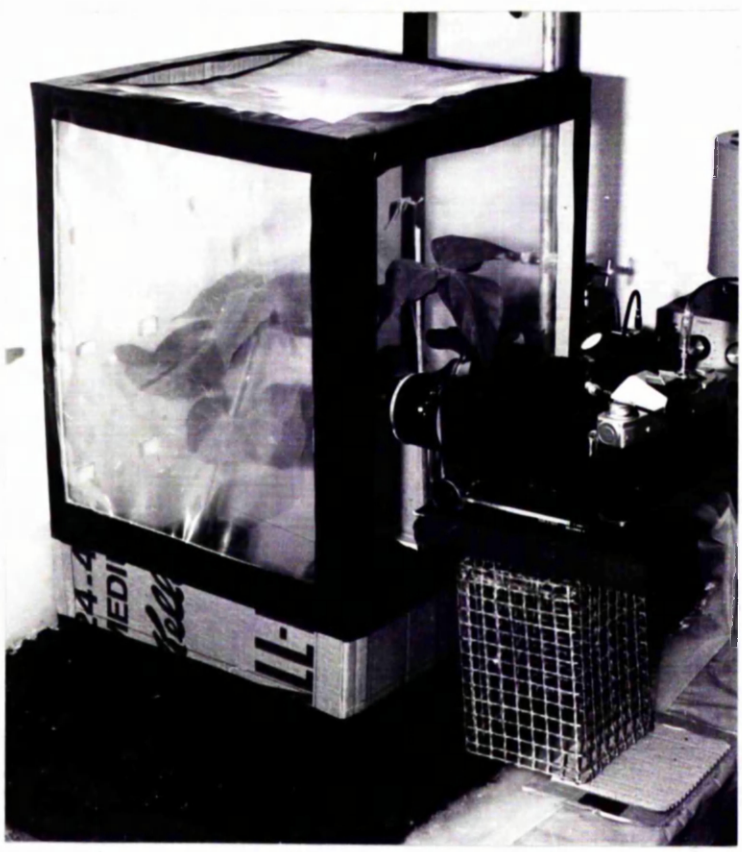


FIGURE 3. Experimental set-up for the measurement of rapid growth in the axillary bud following decapitation of the shoot.

2.4.2. Cell Extension in the Bud Internode

Plants were selected in batches of three, with the criteria for uniformity mentioned in section 2.1. more rigidly applied. In particular, the first trifoliate leaf axillary bud in the three plants were screened for similarity in the length of the bud internode, and in the general size and shape of the bud. Two plants were decapitated and the third left intact as a control. After 23-26 h, longitudinal hand sections were made of the internode of the first trifoliate leaf axillary bud from the control plant and from one of the decapitated plants. The sections were mounted in water and the length of the internode was measured under the microscope using a graduated eye-piece. The average length of the pith cells in the bud internode from the sub-apical portion of the internode to its mid-point was estimated by measuring files of cells under the microscope. Cells from this region were selected as they were generally uniform in shape and size. On the sixth day, the internode of the then developed axillary shoot in the second decapitated plant was measured with a ruler and the length of pith cells at the mid-point of the internode or at the proximal end of the internode, 3-6 mm from the axil, were measured under the microscope as before. Growth extension of the bud internode and pith cells was represented by the differences in these measurements between control and decapitated plants.

2.5. ASSESSMENT OF VASCULAR TRANSPORT

2.5.1. Transport of Basic Fuchsin in the Xylem

Decolorised basic fuchsin was prepared according to Talboys (1955), except that sodium metabisulphite was replaced by the potassium salt. Bean plants (150-200 mm tall) were cut under water at the middle of the first internode, or at the root 20 mm below soil level, with lateral roots present being removed. The cut ends were then immersed in vials containing decolorised basic fuchsin. Petioles of the primary leaf subtending the

larger axillary bud), and of the first and second trifoliate leaves, were cut two-thirds of the way up from the node and a well surrounding the cut end was formed from adhesive tape. Silicone grease was used to prevent leakage, and especially to fill in the petiolar groove. Decolorised basic fuchsin was applied to the well, and this was topped up from time to time as necessary. After intervals of 10, 40 and 80 min, batches of plants were examined for the uptake and transport of fuchsin. Transverse hand sections were made of the petioles of the primary leaves, first and second trifoliate leaves, the hypocotyl, the first to fourth stem internodes and the internodes of the lateral buds subtended by the primary leaf (the larger bud) and by the first and second trifoliate leaves. The sections were examined under the microscope and scored for the presence or absence of fuchsin which stained lignified vascular tissue red. At least six plants were used per treatment per time interval.

The cortical stem tissue of the stems in some plants was stripped away to examine the staining of the vessels lying beneath the cambium.

2.5.2. Lateral Transport of ^{14}C-Sucrose from the Xylem

Bean plants were cut through the middle of the first internode. The internode tissue peripheral to the cambium (consisting of the epidermis, cortex and the phloem and related tissue) was peeled away, leaving a 30-40 mm length of the first internode from the cut end to the 2nd node devoid of phloem elements (Fig. 4). The cut end was re-cut under water to obviate xylem embolisms and then immersed in 3.3 cm^3 of a solution of ^{14}C-sucrose contained in a vial. Half the number of plants so treated were removed after 40 min and the remaining after 75 min. A 25 mm section from the middle of the 2nd internode (which lay immediately above the treated internode) was excised and the tissue peripheral to the cambium (the "bark") was separated from the central core of xylem and pith tissue (the "wood"). The "bark" and "wood" tissues were placed separately into

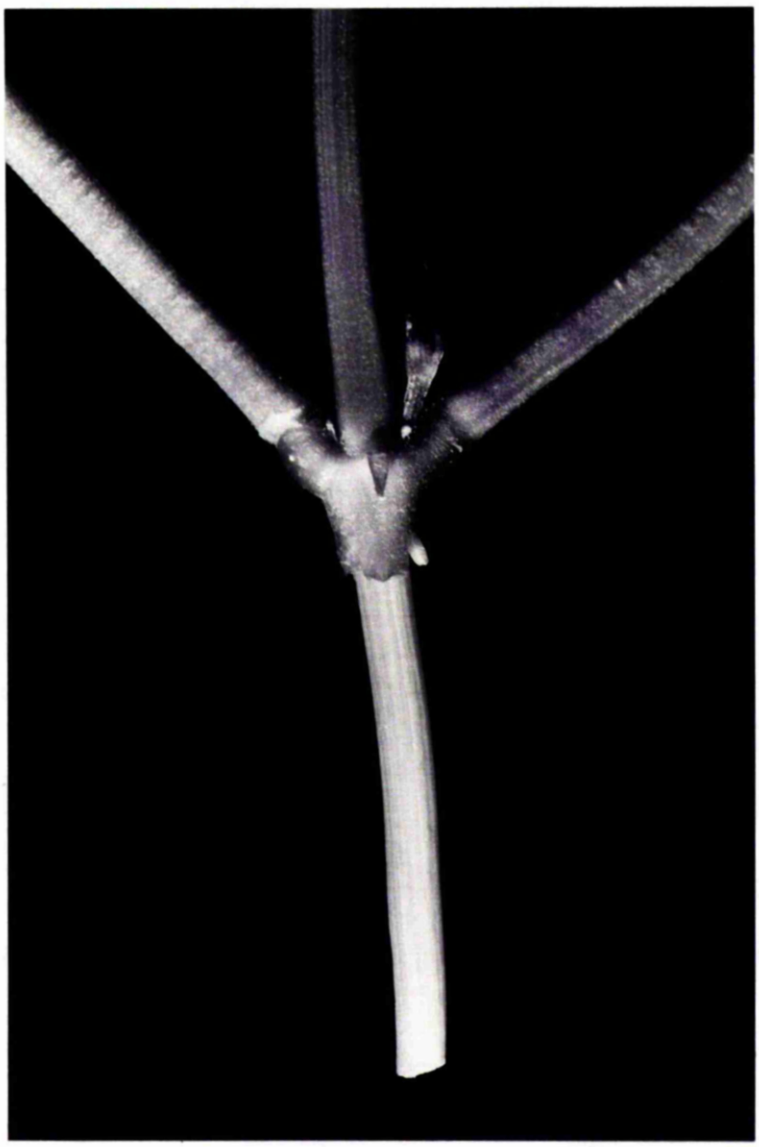


FIGURE 4. The "bark" (inclusive of the phloem) in the 2nd internode stripped away to facilitate uptake of ^{14}C-sucrose by the xylem alone through the cut end of the internode.

plastic scintillation vials each containing 10 cm^3 scintillant composed of 4 mg cm^{-3} 2,5-diphenyloxazole (P.P.O.) in toluene. The radioactivity in each tissue was estimated by counting in a Packard Tri-Carb liquid scintillation spectrometer (Model 3380) which used an external standard to calculate quenching. Background radioactivity was subtracted from the readings of the treated tissues. Quench correction was carried out using a calibration curve for the efficiency of the spectrometer at different quench levels. The calibration curve had been constructed from the quenching of a known amount of ^{14}C-hexadecane by the addition of varying quantities of a methanolic extract of chlorophyll.

2.6. ANALYSIS OF ENDOGENOUS ETHYLENE AND ETHANE

2.6.1. Sample Preparation

Rate of ethylene emanation was determined by enclosing the test tissue in a closed container fitted with a rubber Subaseal or Clinbritic seal (R. and J. Wood Ltd., Glasgow, U.K.). The size of the container depended on the amount of tissue to be analysed and vials, flasks, and small bell jars (lined with moist filter paper) ranging from 4.8 to 235 cm^3 were used. Ethylene released from the tissue was allowed to accumulate in the enclosed air for 20 min, thereafter the air was sampled for analysis by gas chromatography (GC). The needle of a "Gillette Scimitar" air-tight plastic syringe was inserted through the rubber seal and the plunger of the syringe was withdrawn and depressed a few times to flush out the "dead" space (*ca.* 0.05 cm^3) between the lower end of the plunger and the tip of the needle. An air sample (0.7 - 1 cm^3) was withdrawn and the needle was immediately pushed into a rubber stopper. The sample was normally injected into the gas chromatograph within 30 min. Preliminary tests showed that ethylene and ethane were retained in the syringe with negligible loss for up to 2.5 h. When air in very small containers (4.8 or 16 cm^3) was

sampled, withdrawal of the plunger of the syringe increased the effective volume of the container. An appropriate correction in the calculations was made in compensation. All ethylene emanation experiments were carried out in the growth room.

The level of internal ethylene in tissues was determined by vacuum extraction under a saturated solution of ammonium sulphate. The method used was basically that of Beyer and Morgan (1970b), except that a stronger vacuum of 66.7 kPa (500 mm Hg) was applied for 3 min for apical shoots and 4 min for stem sections. The apparatus (Fig. 5) consisted of a 16.5 cm diameter desiccator containing a saturated solution of ammonium sulphate. A bottle with its bottom removed and sealed at its mouth with a rubber Clinbritic seal was filled completely with the salt solution and stood in the desiccator. The plant tissue to be analysed was excised and immediately immersed into a solution of 0.02% Triton X-100 which served as a wetting agent. The tissue was gently agitated to remove all air bubbles adhering to the tissue surface. It was then transfered into the salt solution where it was again checked for air bubbles; the bubbles, if present, being dislodged. The plant tissue was subsequently shifted into the bottle; the cover of the desiccator moved into place and the vacuum pump started. As the pressure inside the desiccator was reduced, internal gases inside the plant tissue appeared as bubbles on the tissue surface. With mature tissue (e.g. fully expanded primary leaves), the bubbles detached from the tissue surface and collected at the neck of the bottle beneath the Clinbritic seal. When young tissues (e.g. apical shoots) were extracted, the air bubbles normally remained adhered to the tissue surface partly because the bubbles were smaller than those from mature tissue, and partly because the surface of young *Phaseolus* tissue was generally pubescent. After the vacuum had been applied, the tissue (both mature and young) was gently agitated with the aid of a length of flexible, braided wire to dislodge bubbles on the tissue surface and these collected beneath the

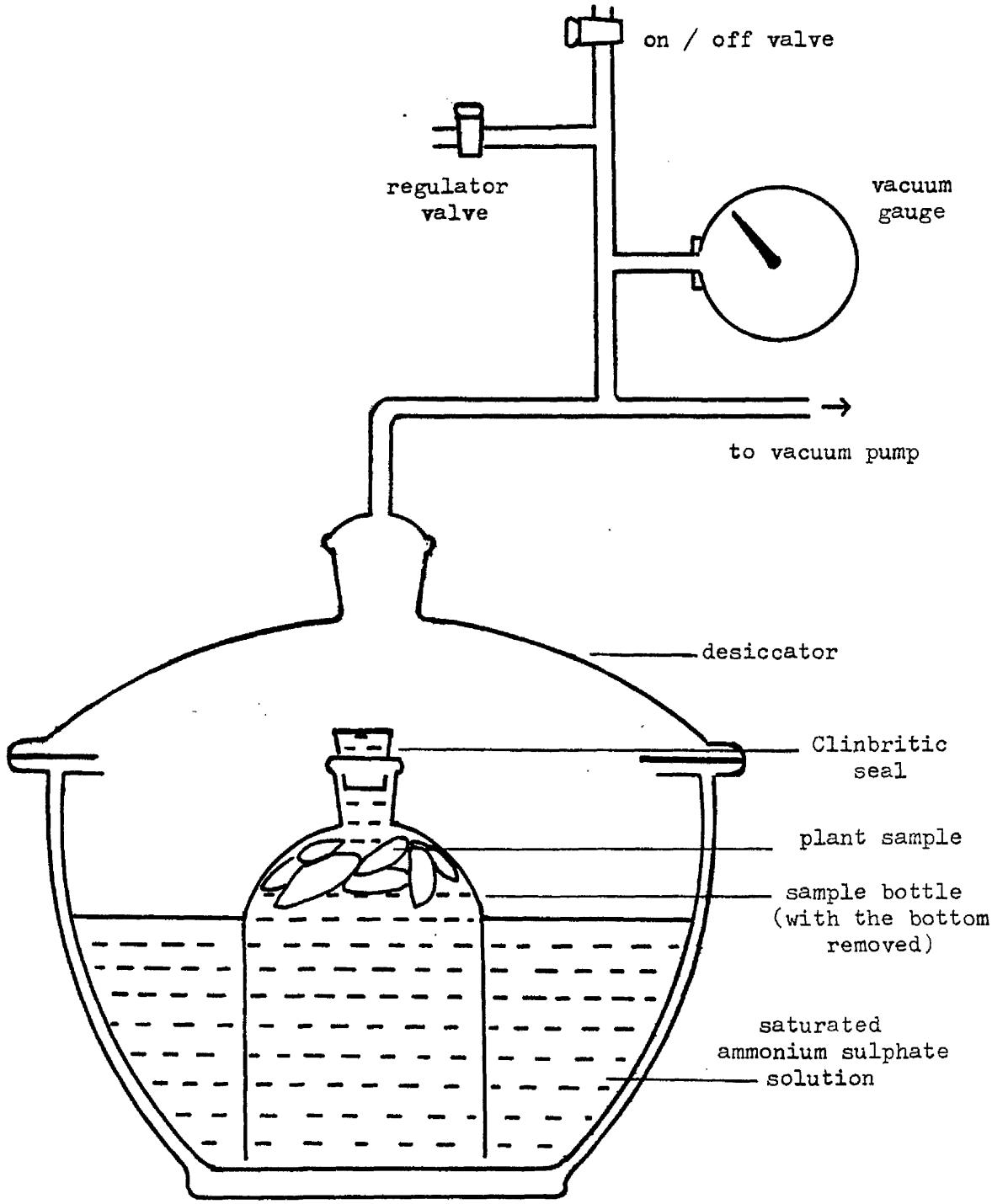


FIGURE 5. Apparatus used to extract internal gases from plant tissues.

Clinbritic seal. A sample was then withdrawn through the seal using an airtight syringe and analysed by GC. Extractions were carried out within 20 min from the time the test tissue was excised from the plant. The volumes of the gases extracted depended on the type and amount of tissue, and ranged from about 0.15 cm^3 to more than 1 cm^3. However, most extractions involved small tissue samples where additional precautions and experimental manipulations were required. This is dealt with in detail in section 3.3.2.

2.6.2. Gas Chromatography

Quantitative analysis of ethylene and ethane by GC was carried out on a Pye 104 gas chromatograph equipped with a flame ionization detector (GC-FID). Separation of ethylene and ethane was carried out mainly on an activated alumina column, but a Poropak R column was also used in some analyses. Experimental details for GC-FID and also for GC separation on a silica column carried out in relation to combined gas chromatography-mass spectrometry (GC-MS; see following section) are given in Table 4.

TABLE 4. Gas chromatography

Adsorbant	Activated alumina (100-120 mesh)	Poropak R[1]	SilicAR, CC-4 special[2]
Column	910 x 4 mm, glass	910 x 4 mm, stainless steel	910 x 4 mm, glass
Carrier Gas	nitrogen at 560 mm^3 s^{-1}	nitrogen at 560 mm^3 s^{-1}	helium at 333 mm^3 s^{-1}
Oven temperature	383 K (110°C)	318 K (45°C)	353 K (80°C)
Detector	FID	FID	MS
Typical retention time of ethylene	52 s	2.4 min	5.0 min
Typical retention time of ethane	44 s	3.2 min	3.0 min

[1]Phase Separation Ltd., Queensferry, U.K.
[2]Mallinckrodt Chemical Works, St. Louis, Mo., U.S.A.

The hydrogen supply to the FID was set at 700 mm^3 s^{-1} while the air supply was adjusted to 5.5 cm^3 s^{-1}. Gas lines to the FID were coupled to molecular sieve traps to remove water vapour and light hydrocarbon impurities in the gases. Typical ethylene and ethane peaks observed after separation in an activated alumina column or a Poropak R column are shown in Fig. 6. Peak heights of both gases were found to vary linearly with the amount of the gas injected and the straight line regression between volume of the gas and the peak height elicited passed very close to the origin (Figs. 7 and 8). Thus, it was possible to calculate the concentration of ethylene and ethane in a gas sample by proportional peak height compared to a standard; calculation of peak areas was unnecessary. An ethylene standard (1 vpm ethylene) was used for calibration of the FID response each time the GC was operated. An ethane standard was not used, but ethane peaks were calibrated against the ethylene standard. The ratios of the ethylene peak : ethane peak as elicited by equal amounts of each gas were 0.62 and 1.18 for the activated alumina column and Poropak R column respectively. These ratios were largely unaffected by slight changes in the carrier gas speed, oven temperature or hydrogen supply to the FID, the variations being less than 5% (Tables 5 and 6).

TABLE 5. Effect of small changes in experimental conditions of the gas chromatograph on the ratio of the peak heights of ethylene and ethane. (Activated alumina column) Injections (0.5 cm^3) of a mixture of 1 vpm ethylene and 1 vpm ethane were made. Peak heights are the means of duplicated readings.

Experimental conditions	Peak height of ethylene (mm)	Peak height of ethane (mm)	$\frac{\text{ethylene}}{\text{ethane}}$ ratio
Standard conditions (see Table 4)	28.0	45.5	0.62
Decreased carrier flow by 55 mm^3 s^{-1}	24.5	38.8	0.63
Increased temperature by 10 K	29.0	44.5	0.65
Increased hydrogen pressure at the cylinder by 562 kg m^{-2}	25.8	40.3	0.64

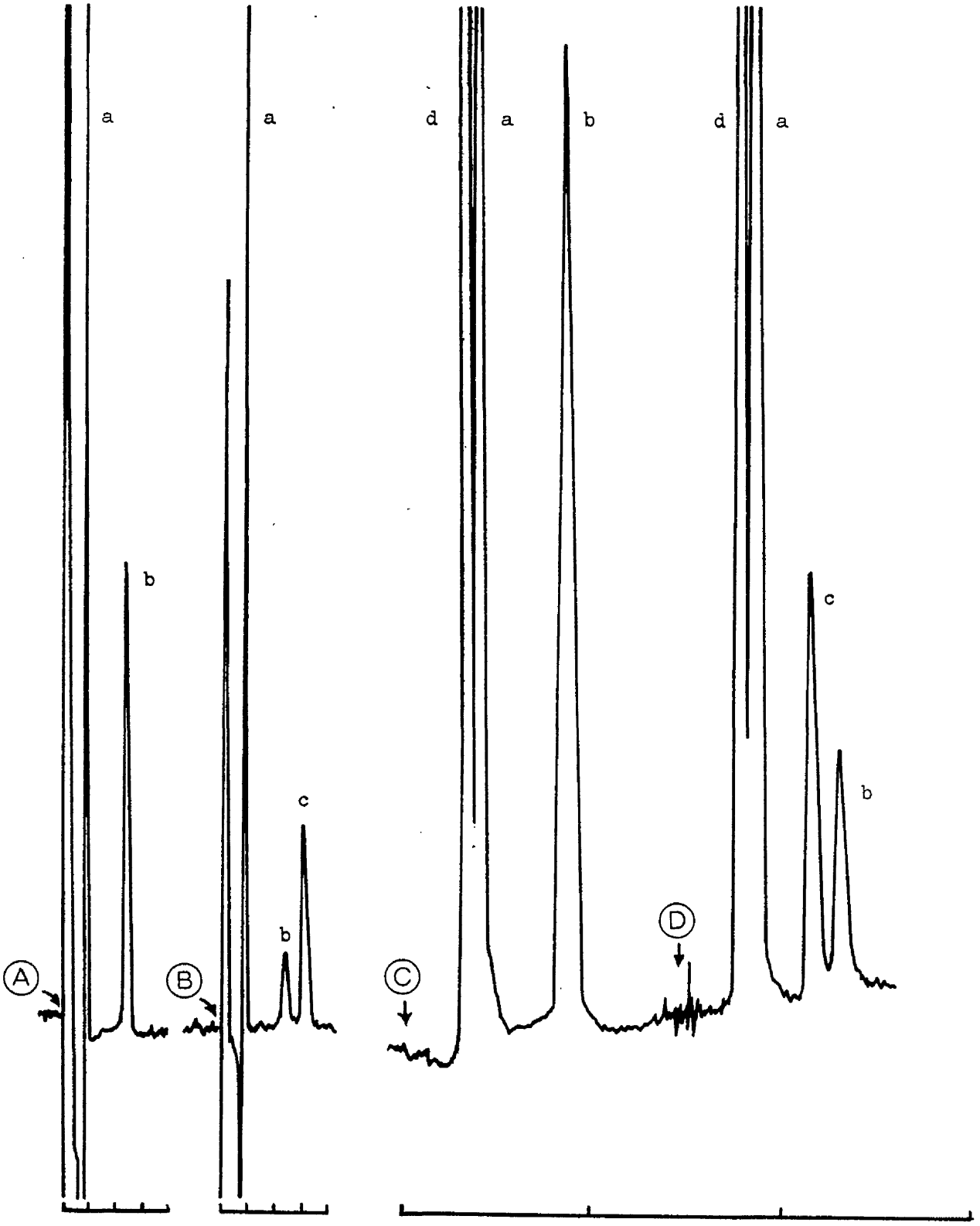
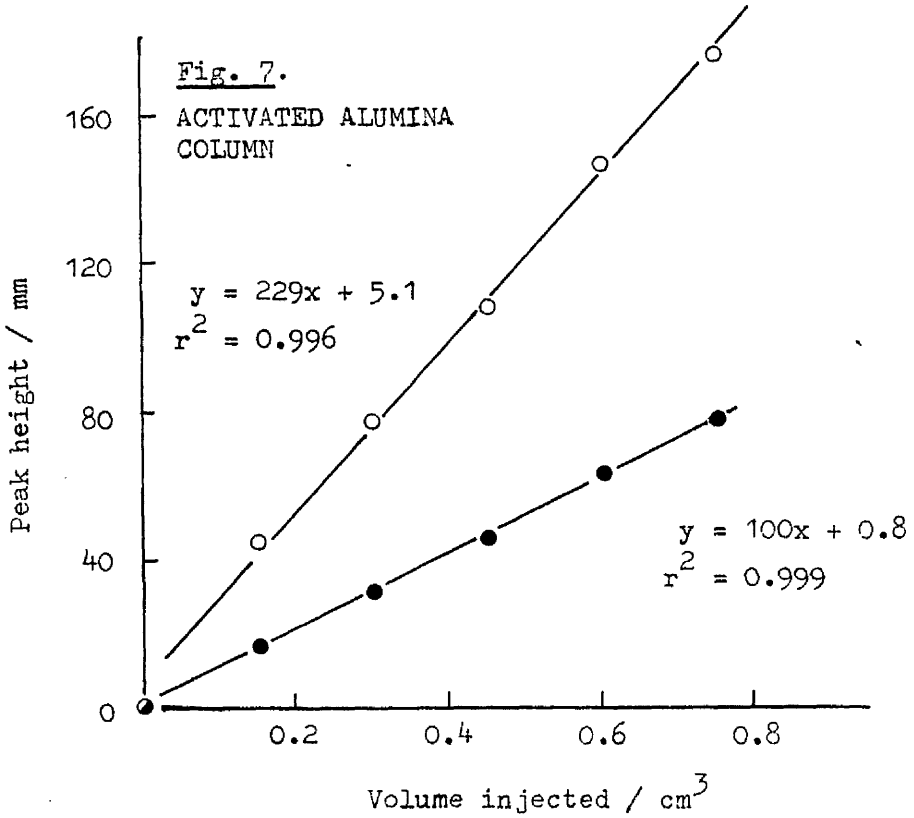
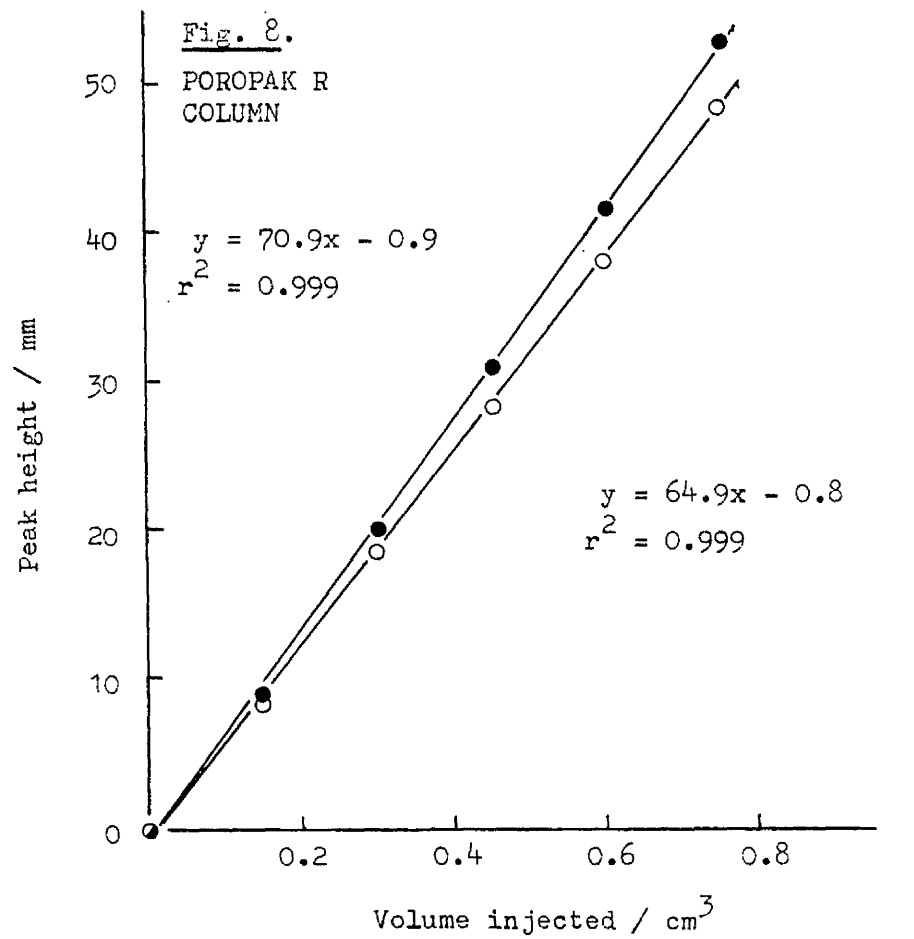


FIGURE 6. Gas chromatography-flame ionization detection. FID response to (A) 2 cm^3 1 vpm ethylene standard: Poropak R column; (B) 0.5 cm^3 gaseous extract from Phaseolus apical shoots: Poropak R column; (C) 0.25 cm^3 1 vpm ethylene standard: activated alumina column; (D) 0.24 cm^3 gaseous extract from Phaseolus apical shoots: activated alumina column. Key to peaks: a = methane; b = ethylene; c = ethane; d = oxygen. Arrows denote sample injections. 1 division on the x-axis = 1 min.





FIGURES 7 & 8. Relationship between the amount of a mixture of 1 vpm ethylene and 1 vpm ethane injected into the GC and the FID response expressed as the peak height on the recorder trace.

● = ethylene; O = ethane

TABLE 6. Effect of small changes in experimental conditions of the gas chromatograph on the ratio of the peak heights of ethylene and ethane (Poropak R column). Injections (0.5 cm^3) of a mixture of 1 vpm ethylene and 1 vpm ethane were made. Peak heights are the means of duplicated readings.

Experimental conditions	Peak height of ethylene (mm)	Peak height of ethane (mm)	$\frac{\text{ethylene}}{\text{ethane}}$ ratio
Standard conditions (see Table 4)	35.8	30.3	1.18
Decreased carrier flow by 55 mm^3 s^{-1}	28.3	24.8	1.14
Increased temperature by 5 K	36.8	31.5	1.17
Increased hydrogen pressure (at the cylinder) by 422 kg m^{-2}	32.8	28.5	1.15

With the GC adjusted to maximum sensitivity, 0.005 vpm ethylene and 0.003 vpm ethane could be detected in a 1 cm^3 sample (peak height = 2 x baseline) using the activated alumina column. The lower limit of detectability of ethylene and ethane using the Poropak R column was only of an order of 0.04 vpm. This was partly because the column was used in the early part of the study before the experimental conditions required for optimum GC sensitivity had been fully determined.

2.6.3. Combined Gas Chromatography-Mass Spectrometry

Gaseous samples from *Phaseolus* were taken from the portion of the shoot apical to the third node by vacuum extraction as described in section 2.6.1. Material from batches of 20 to 35 non-stressed plants (*ca.* 34 g) was used for each extraction. The gas samples (2 cm^3) were separated in an on-line Pye 104 gas chromatograph coupled to an AEI MS30 mass spectrometer *via* a single-stage glass jet separator. Experimental

conditions for the chromatographic separation have been given in section 2.6.2. After injection, the air peak was pumped off for 20 s and the gas chromatographic effluent connected to the jet separator by closing the dump valve. The sample line and ion source temperatures were maintained at 493 K (220°C) and 523 K (250°C) respectively. An ionization voltage of 24 eV was employed.

Single and multiple ion monitoring were carried out with an AEI Multipeak Selectro WF-055 monitor. At an instrument resolution of 3000, the triplet at m/e 28 due to $CO^{+\cdot}$ (27.9949), $N_2^{+\cdot}$ (28.0061) and $C_2H_4^{+\cdot}$ (28.0313) ions was resolved (Fig. 22). Using a single peak scanner, the collector signal at m/e 28 was displayed on an oscilloscope, and the magnet current adjusted to focus on the molecular ion of the ethylene (Channel 1). The molecular ion of ethane ($M^{+\cdot}$ = 30.0469) was then tuned on Channel 2 of the multiple peak monitor by appropriate selection of mass ratio (1.0723 found, 1.0719 theoretical). Scanning of the peaks was then stopped and multiple ion monitoring commenced.

2.7. TREATMENTS IN RELATION TO AXILLARY BUD GROWTH

2.7.1. Apical Shoot Treatments

Physical confinement of the shoot above the 3rd node was carried out using tubes of glass (14 mm diameter x 120 mm), plastic mesh (18mm x 124mm) or paper (12mm x 110mm). The paper tubes were closed at the upper ends and were perforated for ventilation. Plastic mesh tubes were open at either end, while the ends of the glass tubes were either left open or were sealed by a Clinbritic seal at the upper end and a split cork at the lower end. The split cork was bored in the centre to allow passage of the stem, the opening being lined with cotton wool impregnated with silicone vacuum grease. The glass and plastic tubes were held in place by clamps while the paper tubes were light enough not to require support (see Figs. 37-39).

Growth of the shoot in an ethylene-enhanced atmosphere, but without physical restriction of growth, was carried out using large glass vessels about 125 mm diameter x 100 mm and having an internal volume of approximately 1300 cm^3. Tubular extensions from the top and bottom of the vessels were sealed with Clinbritic seals and split corks as above (Fig. 45a). The shoots were enclosed above the 3rd node. Ethylene was injected through the Clinbritic seal to attain a concentration of 0.5 vpm inside the vessel. In control vessels where ethylene was not supplied, endogenously produced ethylene from the shoot was removed by ventilating 10 min daily with the aid of an aquarium pump. Alternatively, ethylene was absorbed by mercuric perchlorate solution contained in a shallow dish in the vessel. In vessels injected with ethylene, the ethylene absorbant was replaced with water or 2 kmol m^{-3} perchloric acid (the solvent for mercuric perchlorate).

Aqueous solutions of test chemicals (ethephon, silver nitrate, AVG) were applied with a paint brush to the terminal bud and/or abaxial surface of the 2nd trifoliate leaf. Single applications were made except when silver nitrate and AVG were applied, in which case the apical shoots (2nd trifoliate and terminal bud) were treated at the beginning of the experiment and after two days. The volume of test solution applied varied with the size of the leaves.

2.7.2. Stem Treatments

In the intact plant, tri-iodobenzoic acid dispersed in hydrous lanolin was applied as a ring around the 3rd internode just below the 4th node. A 1 cm^3 hypodermic syringe (without the needle attached) was used to dispense 30 mm^3 of the lanolin dispersion. The dispersion was wiped away two days later.

In experiments involving the removal of the apex, the shoot was decapitated at the 3rd internode. The cut surface of stem was treated with aqueous solutions of test chemicals (silver nitrate, AVG, ethephon)

by applying 0.1 cm^3 of the solution to a small wad of cotton wool wrapped around the cut end of the stem. The wad was then sealed with adhesive tape to inhibit evaporation (Fig. 9). IAA dispersed in hydrous lanolin was applied to the decapitated plant by dispensing 20 mm^3 of the dispersion on to the cut stem surface using a hypodermic syringe (without the needle attached).

2.7.3. Treatments to the Axillary Bud and Adjacent Tissue

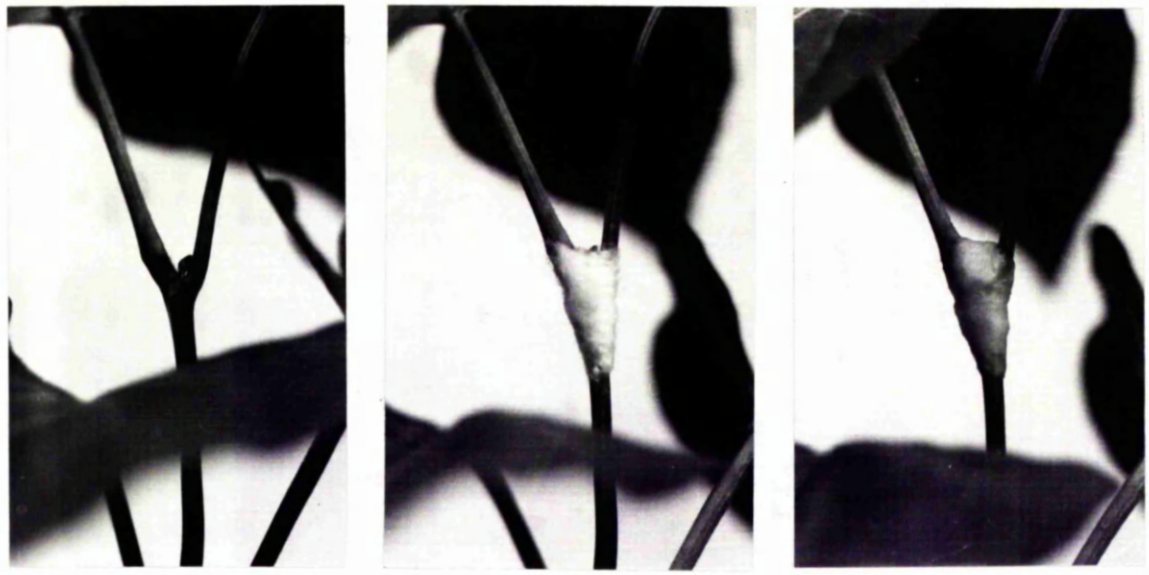
Ethephon was applied liberally (*ca.* 30 mm^3) to the third node (from where the first trifoliate arises) and its axillary bud using a paint brush. Other chemicals in aqueous solutions (silver nitrate, AVG, IAA) were applied by dispensing 0.3 cm^3 of the test solution to cotton wool wrapped around the node and axillary bud. In some experiments, the nodal region (but not the bud itself) was first abraded lightly with moist carborundum powder to assist penetration (Hocking, 1973). Adhesive tape was used to inhibit evaporation (Fig. 9). The cotton wool was removed after one day to measure bud growth and so as not to obstruct its growth.

Total chlorophyll content of the bud was carried out spectrophotometrically following extraction in hot methanol (Šestak, 1971).

2.8. INDUCTION AND ANALYSIS OF STRESS ETHYLENE

2.8.1. Ethylene induced by wounding

Shoots of *Phaseolus* were treated in ethylene-enhanced and ethylene-depleted atmospheres in glass vessels as detailed in section 2.7.1. Intact plants acted as controls. The plants were used for experiment after 4 days of treatment, by which time, the 2nd trifoliate leaf and 4th internode were at the end of their grand phase of growth. The glass vessels were removed from the plants and 1.5 h ventilation was allowed before the leaves and internodes were harvested. Three plants were used per treatment.



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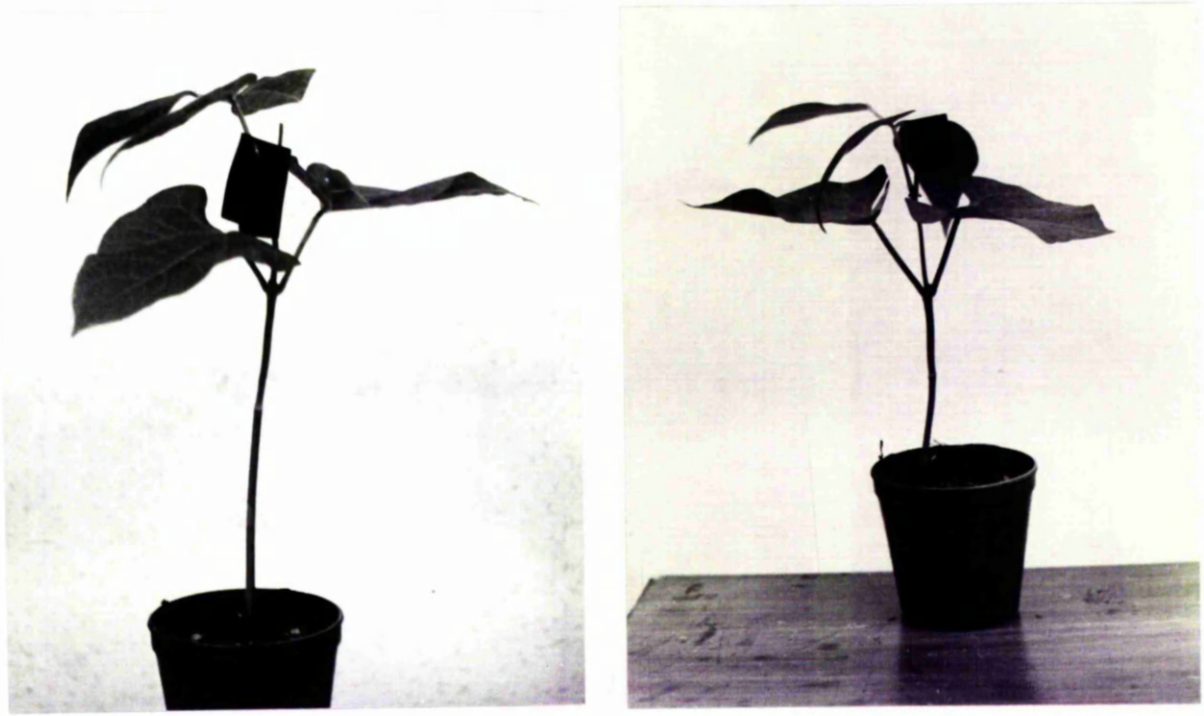


d e

FIGURE 9. Application of aqueous solutions of test chemicals to (a - d) the 3rd node and axillary bud and to (e) the cut end of the stem in the decapitated plant.

(a) The 3rd node and 1st trifoliate axillary bud.
(b) Cotton wool is wrapped around the 3rd node.
(c) A wad of cotton wool is placed straddling the axillary bud.
(d) After adding 0.3 cm^3 of the test solution to the cotton wool, it is sealed with adhesive tape to inhibit evaporation.
(e) Test chemical applied to the stump of the decapitated stem. The test solution (0.1 cm^3) is added to cotton wool wrapped around the cut end of the stem. Adhesive tape is then used to inhibit evaporation.

Internode sections, 5 mm long, were cut from the entire length of the 4th internode. No further wounding of the internode sections was necessary as the wound reaction was readily initiated by the process of sectioning alone. Leaf discs, 4.7 mm diameter, were punched out of all three leaflets of the second trifoliate leaf using a cork borer. Again, no further wounding of the leaf discs was necessary.

Twenty internode sections or thirty leaf discs were placed quickly into 4.8 cm^3 cylindrical glass vials and sealed with Subaseals. High humidity in the vials was maintained by a strip of moist filter paper. The vials were floated horizontally in a water bath (298 ± 0.5 K; 25 ± 0.5°C) such that two-thirds of the vial remained submerged below the surface of the water. The water bath was set beneath a bank of fluorescent lamps which, in combination with available daylight, gave an irradiance of about 10 Wm^{-2} at the water level. Air samples (0.7 cm^3) were withdrawn from the vials at 10 min intervals and analysed by gas chromatography. After each sampling, the vials were flushed with humidified ethylene-free artificial air for 40 s. No ethylene was detected in air samples extracted immediately after flushing, indicating that the procedure was effective. The incubation period between flushing and the next sampling was 9 min. The small quantities of ethylene produced precluded shorter sampling intervals. In plotting the time course for the wound reaction, the mid-point of the time taken to cut the sections and discs was taken as the time of wounding. The time ascribed to a calculated rate of ethylene release was the mid-point of the incubation period concerned.

In another series of experiments, 15 4th internode sections were floated in 2 cm^3 of water or 0.7 kmol m^{-3} mannitol in a 16 cm^3 flask for 30 min. Thereafter, the water or mannitol solution was decanted away, and the flasks sealed. After 90 min, the amount of ethylene that had accumulated in the flask was estimated by GC.

2.8.2. Ethylene induced by water-stress

Plants bearing an expanding 3rd trifoliate leaf were used, and they were therefore of the same physiological age as the plants in the previous section when the wounding treatments were administered. The central leaflet of the 2nd trifoliate leaf was excised at the proximal end of the rachilla, which was then re-cut under water and then immersed into a vial of water. The 4th internode was excised and the basal end re-cut under water and immersed in water contained in a test tube. Five leaflets and internodes were used in each experiment. The excised organs were transferred into a 22 cm diameter desiccator where high humidity was maintained by a layer of water and moist filter paper around the wall of the desiccator. After one day, the leaflets and internodes were removed from the desiccator, blotted dry briefly and then subjected to a warm airstream from a hair-drier held 60 cm away. The air temperature around the organs rose to 303 - 305 K (30 - 32°C) and the internodes and leaflets were treated for 3 min and 1.5 min respectively. The internodes were then transferred into a 6.2 cm^3 test tube and leaflets into a 213 cm^3 bell jar standing on a sheet of glass. Both containers were fitted with Subaseals for air sampling which was carried out at 10 min intervals. The incubation period was 9 min for internodes, with the air flushed for 40 s after each sampling using an aquarium pump. The leaflets were incubated over periods of 8 min with the air flushed for 100 s. The experiment was carried out in the growth room. Timing of ethylene release was as for the ethylene wound reaction described in the previous section.

2.9. BIOMETRY

2.9.1. Experimental Design

Even after selection for uniformity, a small amount of variability in the plants still remained. In the growth room, the plants were grouped

according to their apparent vigour and assigned to various treatments in the experiment such that plants from each group were equally distributed among the treatments. The plants were then arranged in blocks on the benches, with each block containing plants of similar apparent vigour and represented by a plant from each treatment (randomized block design).

The experiments were carried out on at least three occasions except where indicated otherwise. When essentially the same results were obtained on separate occasions, the results from only one experiment are presented.

2.9.2. Computation

A Hewlett Packard HP67 programmable calculator was used in processing most of the quantitative data. Prepared programme cards were used in curve-fitting, analyses of variance, the t-tests and in calculations of means and standard errors.

The t-test or a one-way analysis of variance was used to compare the mean values of two experimental treatments. Although the plants were arranged in blocks in the growth room, there was generally no consistent difference between the blocks. It was thus felt unnecessary to perform a two-way analysis to extract the block effects. When data from more than one replicate experiment were combined to obtain a larger number of observations, replicates which showed comparable treatment effects were selected. Thus, extraction of between-replicate variance was similarly not carried out since this would confer only a marginal advantage. When more than two treatments were compared, Duncan's Multiple Range Test (Duncan, 1955) was applied.

The analysis of variance and the t-test generally require normality in the distribution of data and homogeneity of the standard deviations of the series being compared. Since the data available were insufficient to test-fit for normality, this was assumed unless, by inspection, the data were obviously not normal (see below).

Homogeneity of standard deviations was tested and confirmed in representative sets of data. Studies have indicated that for cases showing only minor departures from the assumption of normal distribution and equal variances, the analysis of variance and the t-test still give adequate results (Adler and Roessler, 1968; Aitchison, T., pers. comm.).

In the measurements of small growth increments in the axillary bud over different time intervals (section 2.4.1.), the growth rate data were apparently non-parametric. In this instance, the Wilcoxon matched-pairs signed-rank test (Wilcoxon, 1945) was used to compare the growth rates.

In the presentation of the results of statistical tests, the usual notation was adopted to indicate the significance of the differences between two series:

N.S. = Not significant ($p > 0.05$); * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.

When more than two series were compared, a superscript letter was ascribed to the mean value of each series, the values bearing the same letter being not significant at the level of confidence indicated.

3. RESULTS AND DISCUSSION

3.1. GENERAL CHARACTERISTICS OF SHOOT GROWTH

Preliminary growth measurements were made of the shoot of *Phaseolus vulgaris* to obtain a basic understanding of the patterns of primary growth in the intact and decapitated plant. This work extends the study by White (1973).

Plants were transferred from the greenhouse into the growth room twelve days after sowing. The growth of internodes, leaves and axillary buds thereafter is shown in Fig. 10. A fine degree of correlative control was evident in the sequential development of leaves and internodes. The growth pattern in these organs conformed to the sigmoid curve typical of biological systems: an initial phase of slow growth followed by the rapid grand phase of growth, before the growth rate decreased and finally stopped. A leaf or internode entered its grand phase of growth just as the preceding leaf or internode was completing this growth phase. Thus, at any given time, only one leaf or internode was undergoing rapid extension growth. Development of a trifoliate leaf began at about the same time as that of the internode immediately below the node of its insertion. The duration of growth in the internode was, however, shorter than in the leaves.

There was very little growth in the internode of the larger axillary bud of the primary leaves over most of the period of observation. In the 1st and 2nd trifoliate leaf axillary buds, on the other hand, a slow but continuous growth (about 0.2 mm a day) of the bud internode was noted throughout the period of observation. However, the rate and duration of bud growth can vary between batches of plants.

When the plant was decapitated at the 3rd internode, vigorous growth of the 1st trifoliate axillary bud and a less pronounced growth of the larger primary leaf axillary bud were observed (Fig. 11). Development of the smaller primary leaf axillary bud and cotyledonary buds were usually

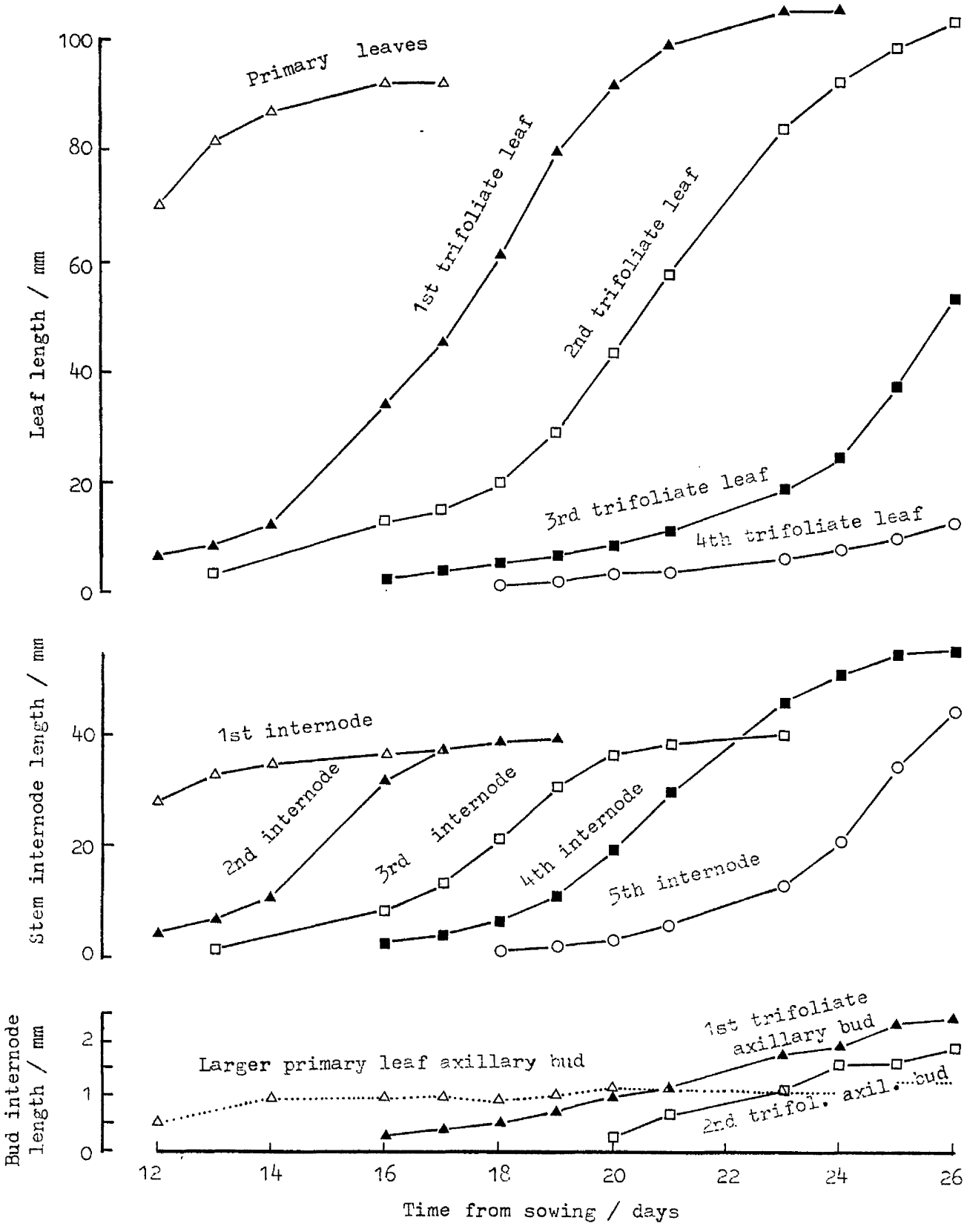


FIGURE 10. Growth patterns of leaves, stem internodes and axillary buds in the intact plant. Growth in trifoliate leaves was determined by measuring the lengths of the central leaflets. Axillary bud growth was determined by measuring the 1st internode lengths of the buds. Values are means of twelve plants.

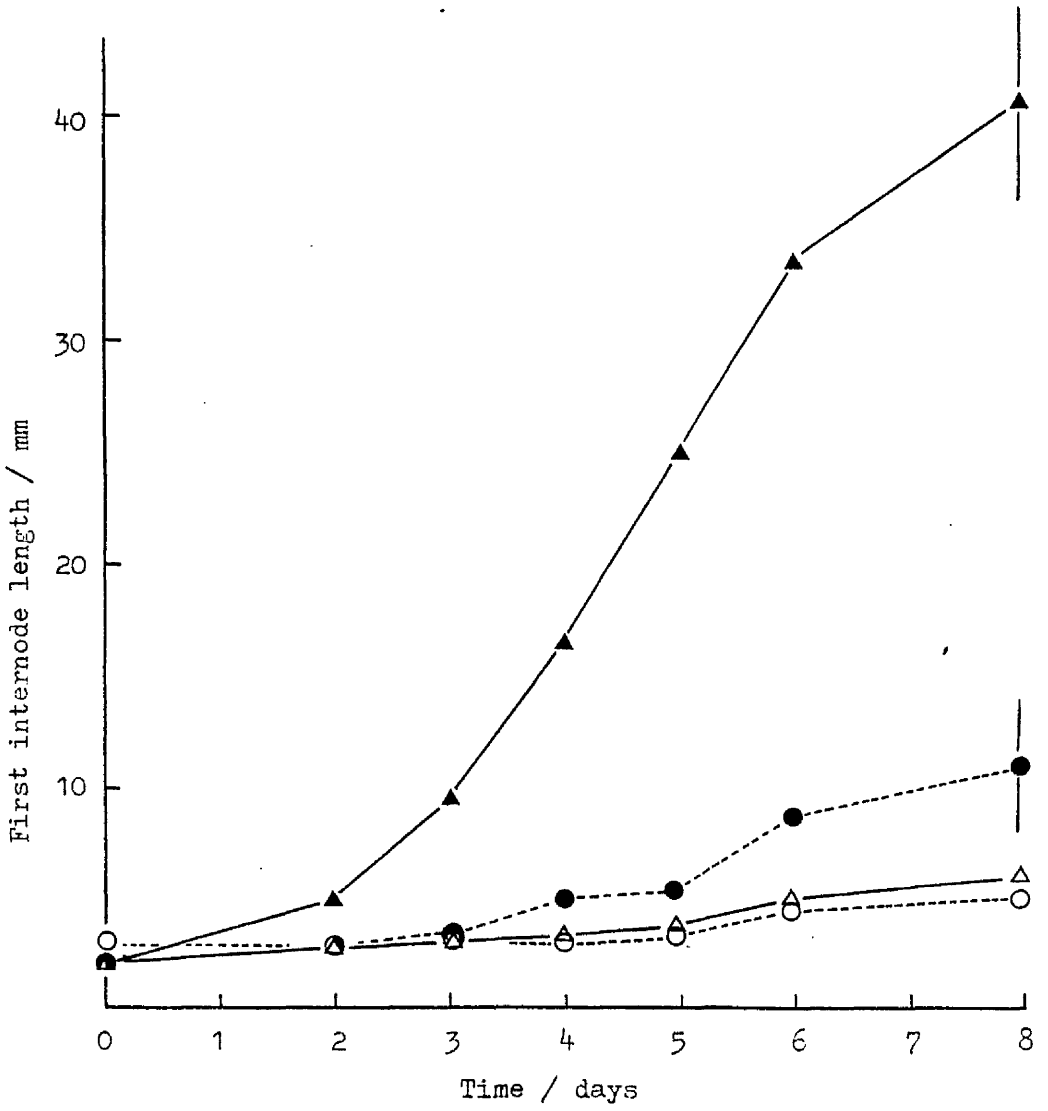


FIGURE 11. Growth of the 1st trifoliate axillary bud and the larger primary leaf axillary bud in intact and decapitated plants. Values are the means of eleven plants per treatment. The vertical bars represent ± std. errors.

—△ = 1st trifoliate axillary bud, intact plant.
—▲ = 1st trifoliate axillary bud, decapitated plant.
---○ = Primary leaf axillary bud, intact plant.
---● = Primary leaf axillary bud, decapitated plant.

insignificant although some growth in the former could occur over an extended period. In decapitated plants showing vigorous growth, a limited development of supernumerary buds at the 3rd node was sometimes seen. Development of the 1st trifoliate axillary bud was determinate. The bud developed into an axillary shoot comprising of a single internode bearing a trifoliate leaf and floral buds at its distal node (Fig. 12). Occasionally, a second internode might develop. The internode, trifoliate leaf and floral structures were all present in their rudimentary forms in the inhibited bud.

Although the dry weight of the axillary bud or shoot gives an indication of the growth attained, this measurement is not always convenient as it involves destructive sampling. In most of the experiments described in this thesis, growth of the axillary bud or shoot is quantified by measurements of the length of the bud or shoot internode. The sigmoidal growth curve of the internode was generally similar to that of the stem internodes. A comparison of the internode length with the dry weight of the entire 1st trifoliate axillary bud or shoot showed a positive correlation, indicating that the internode length is a reasonable representation of the growth of the entire bud or shoot (Fig. 13). The bud internode length and the dry weight of the entire bud generally conform to the relationship: Dry weight of entire bud = 0.423 x (bud internode length)$^{1.35}$, (r^2 = 0.948), where weight and length were in g and mm respectively.

Growth of the trifoliate leaf was monitored by measurement of the length of the central leaflet. While the total surface area of the three leaflets of the trifoliate is arguably a more appropriate parameter of leaf lamina growth, measurements of leaf areas are time consuming. A comparison between the length of the central leaflet and the total surface area of the three leaflets showed a positive correlation generally conforming to the relationship:

FIGURE 12. Development of axillary shoots following decapitation of the plant. The 1st trifoliate axillary bud develops vigorously after decapitation. Propensity to growth in the primary leaf axillary buds vary between batches of plants. The larger primary leaf axillary bud may show relatively active development or only slight development (plant on the left and right respectively). Normally, growth of the 1st trifoliate axillary bud is less vigorous where the primary leaf axillary bud also develops actively. This is not apparent in the above plants as they are from different batches, the plant on the left having been decapitated earlier.

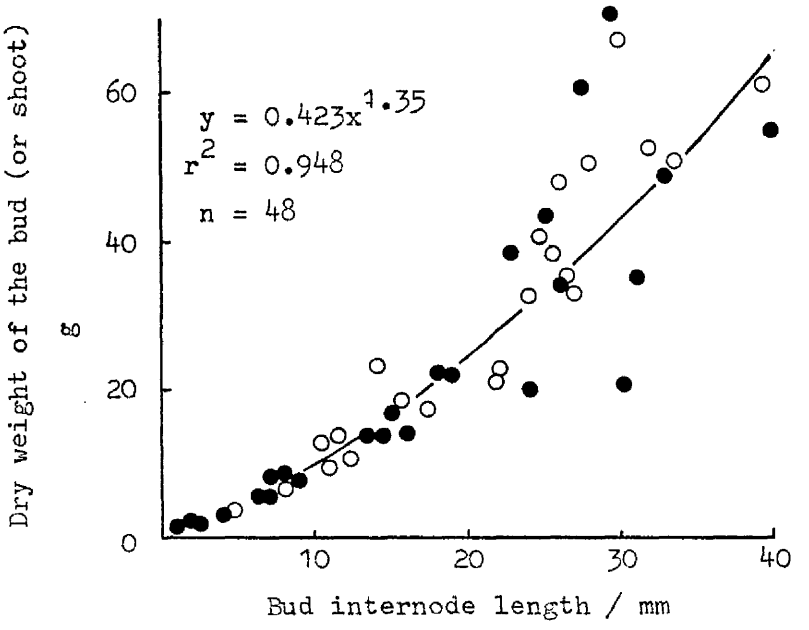


FIGURE 13. Relationship between the length of the bud internode and the dry weight of the entire axillary bud (or shoot). Results from two experiments are indicated by different symbols.

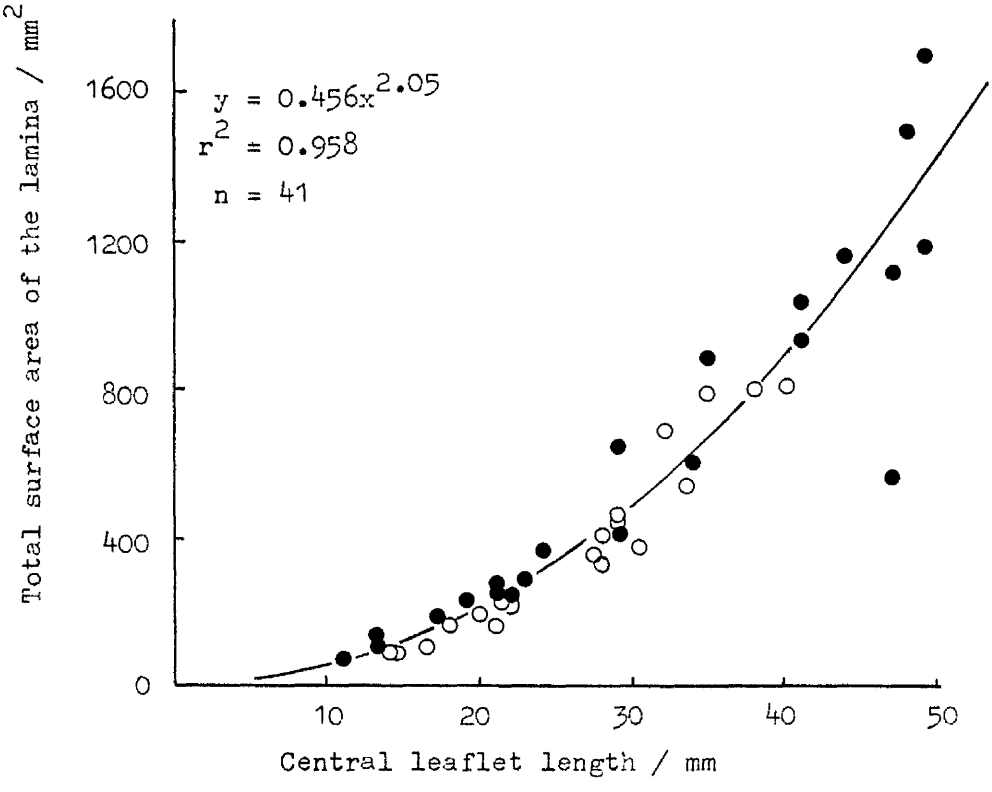


FIGURE 14. Relationship between the length of the central leaflet and the total surface area of the trifoliate leaf lamina. Results from two experiments are indcated by different symbols.

Total surface area = 0.456 x (length of the central leaflet)$^{2.05}$, (r^2 = 0.958), where surface area and length were in mm^2 and mm respectively (Fig. 14). The measurements in Fig. 14 are for the trifoliate leaf of the 1st trifoliate axillary shoot. Since the leaf is similar in shape and proportion to the trifoliate leaves of the main shoot, the above relationship should also apply to the latter.

Considerable differences exist between batches of plants in relation to the general vigour of the plant, the size of the different organs and their relative proportions to one another and the vigour of released axillary buds. Although the measurements were made in the growth room, seed germination and the initial growth of the plant took place in the glasshouse where environmental conditions could not always be rigidly controlled. McIntyre (1973) has shown that outgrowth of axillary buds in *Phaseolus* was enhanced by favourable conditions of light and humidity. The effect of temperature and light on internode length and axillary bud growth and the seasonal effect on the outgrowth vigour of the axillary buds of *Phaseolus* have been described by White and Mansfield (1975, 1978). In relation to the seasonal effect, growth of the plants in winter was influenced by the location of the pots in the glasshouse. The temperature and light intensity gradients between the centre and sides of the glasshouse were quite significant, and batches of plants grown at the centre of the glasshouse were larger.

In plants which were decapitated or otherwise treated to remove correlative inhibition, growth of the 1st trifoliate axillary buds was influenced by the extent of development in the primary leaf axillary buds. In batches of plants where the latter was not pronounced, the development of the 1st trifoliate axillary buds was more vigorous than in batches where the primary leaf axillary buds showed active growth (data not presented).

3.2. CHARACTERISTICS OF AXILLARY BUD OUTGROWTH

3.2.1. Rapid Bud Growth following Decapitation

The growth response in the axillary bud of the 1st trifoliate leaf was studied in relation to two main aspects. The time lag between the shoot decapitation stimulus and the onset of bud internode extension was first determined. Estimates were then made of the extent by which cell extension, both during the initial growth phase and subsequently in the developed shoot, could account for the elongation of the bud internode.

Timing of the rapid growth

Measurements of the first trifoliate axillary bud internode length were taken from the enlarged photographic images of the bud made at regular intervals from 2.7 h prior to decapitation to 10 h thereafter. Glass markers were used to facilitate the detection of small increases in internode length, since ink marks tend to become indistinct as internode extension proceeds. Internode length was thus determined from the distance between the insertion of the markers. In practice, the markers were found to be useful, in some instances, in detecting early bud development in another manner. The unopened trifoliate portion of the axillary bud is held at an angle to the internode (Fig. 15a). As development proceeds following decapitation of the shoot, the trifoliate portion bends about the distal end of the internode such that the dorsal surface in the bud stage faces downwards in the expanded leaf (Fig. 15d). This bending movement causes a decrease in the angle at which the upper glass marker is held to the internode, and is enhanced by the internode bending forward from the node (Fig. 15a-d). Since it is difficult to measure small changes in angles, the decrease in distance between the tips of the upper and lower markers was used to monitor the bending process. The use of this method to follow bud development has some inherent

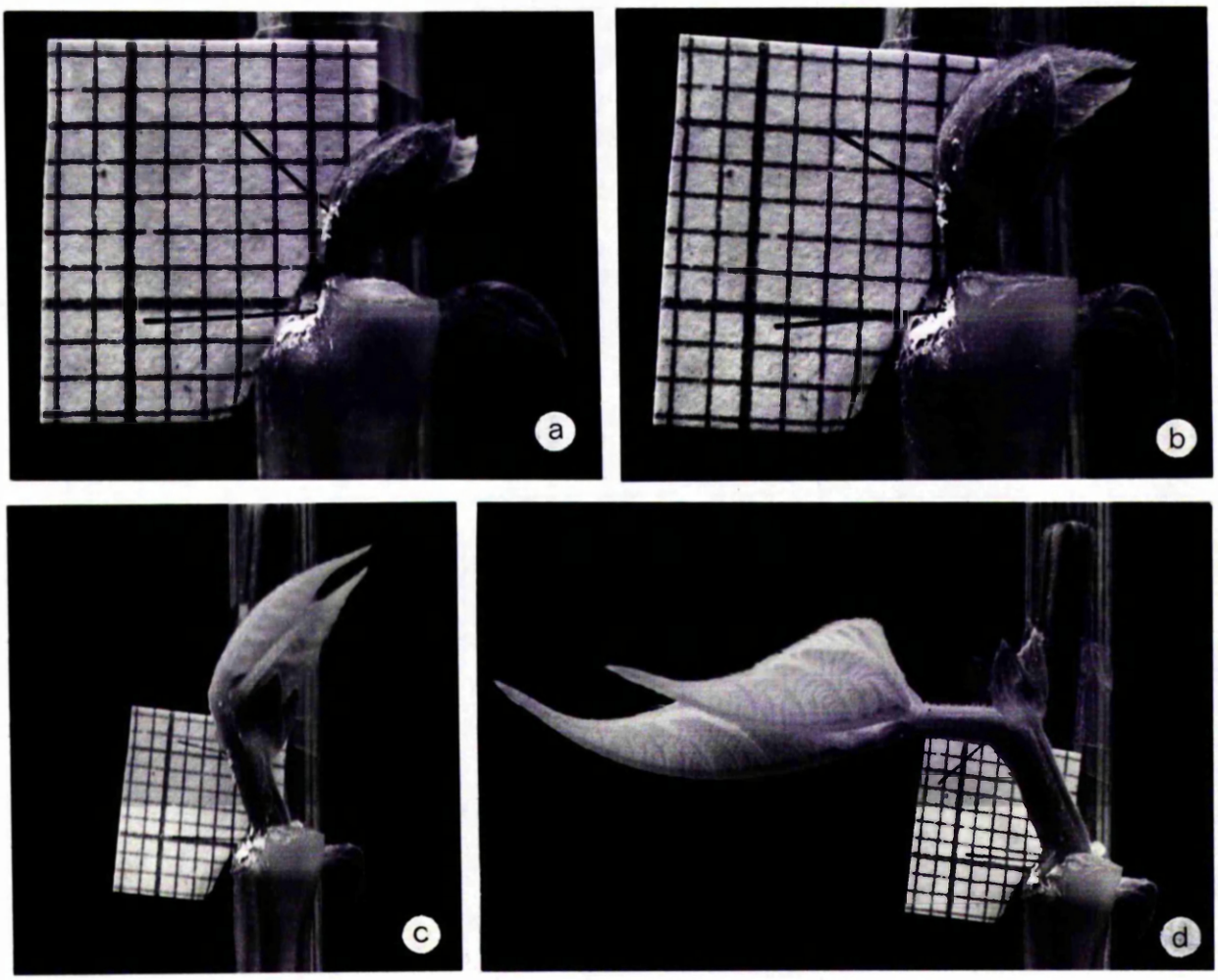


FIGURE 15. 1st trifoliate axillary bud (a) at decapitation of the shoot, (b) at 1 day, (c) 2 days and (d) 3 days after decapitation. Spots of white ink have been painted on the internode of the bud at the insertions of the glass markers to facilitate measurement. The trifoliate leaf arising from the node has been excised to show the orientation of the bud. In the actual experiments, the leaf was left intact.

The internode of the bud bends forward at the node of attatchment as the bud develops. As the trifoliate leaf of the bud expands, it bends about the distal end of the internode such that the dorsal surface faces downwards in the expanding leaf. This results in a change in the angle at which the glass markers (especially the upper marker) are held to the internode. Scale: 1 division = 1 mm.

disadvantages, however. The movement of the marker is influenced by, among other factors, the length of the marker, and the angle and depth at which it is inserted into the internode. For the method to be effective, the response has to occur at an early stage, since otherwise, the extension of the internode would gradually draw the two markers apart and so annul the decrease in distance between the markers caused by bending in the internode. For the same reason, a rapidly extending internode can obscure movement of the marker that is not sufficiently pronounced, and even where a decrease in distance between marker tips is observed, this becomes less evident as the experiment proceeds. It was not unexpected, therefore, that the measured distances between marker tips were sometimes erratic, and the method was less reliable than the direct measurement of internode length as determined by the distance between the points of insertion of the markers. Nevertheless, the former method appeared to be an effective indicator of early response to decapitation in 5 out of 9 decapitated plants, and served to support inferences drawn from direct measurement of internode length.

While active growth of the 1st trifoliate axillary bud was inhibited by the apical shoot in intact *Phaseolus*, the buds in most plants were found to maintain a very slow but measurable growth, confirming the observations of Hall and Hillman (1975) for the primary leaf axillary bud of the same plant. The basal growth rate (estimated from the gradient of the linear regression of bud internode lengths over time) was found to be variable between individual plants. Pre-decapitation recording was therefore necessary to differentiate the inherent basal growth rate from the increased growth activity resulting from shoot decapitation.

The basal growth rate of the bud internode in intact control plants estimated over 12.7 h ranged from -2 to 32 $\mu m\ h^{-1}$ (Table 6). In six out of nine plants, growth rate did not exceed 2 $\mu m\ h^{-1}$ and in eight out of nine plants, growth rate did not exceed 7 $\mu m\ h^{-1}$. In the 9th plant

TABLE 6. Estimated growth rates (μm h^{-1}) of bud internodes at various time intervals in control and decapitated plants. Growth rates were approximated from the gradient of the linear regression of a mean of 27, 8, 5 and 5 internode measurements over time for the time intervals -2.7 h to 10 h, -2.7 h to 0 h, 1 h to 3 h and 5 h to 7 h respectively. Decapitation was carried out at 0 h.

	Control				Decapitated		
	-2.7 h to 10 h	-2.7 h to 0 h	1 h to 3 h	5 h to 7 h	-2.7 h to 0 h	1 h to 3 h	5 h to 7 h
Expt. 1	7	11	8	-2	-3	-20	10
Expt. 2	0	-7	-21	30	0	0	11
Expt. 3	2	-1	0	8	8	0	0
Expt. 4	-2	-22	20	0	-2	0	4
Expt. 5	1	4	-6	-2	3	-2	24
Expt. 6	1	-9	-4	6	3	4	32
Expt. 7	5	2	16	-2	-10	2	-4
Expt. 8	32	31	26	38	20	16	64
Expt. 9	1	0	8	6	9	16	24

(Expt. 8, Table 6), growth was exceptionally vigorous, attaining 32 μm h^{-1}. In one plant, an apparent negative growth rate was obtained, but this was due mainly to a transient but marked decreasing rate in the first 3 h of measurement (Expt. 4, Table 6 and Fig. 16). Generally, therefore, the basal growth rate of the internode of the 1st trifoliate leaf axillary bud was of the order of 1 to 5 μm h^{-1}. While the bud internodes were found to increase in length when observed over a period of 12.7 h, both positive and negative growth rates were encountered when growth rates were estimated over shorter intervals of 2.7 or 3 h (Table 6). These fluctuations might be due to nutational movement of the bud, although measurement errors could have been partly responsible.

A first estimate of the time lag between decapitation of the shoot and

response in the bud was made by inspection of the growth curves (Fig. 16). In the curves for internode lengths, a *sustained* increase over basal growth rate was taken as an actual growth response to decapitation. Similarly, an increase in the rate of change in distance measured between marker tips was considered a response to decapitation.

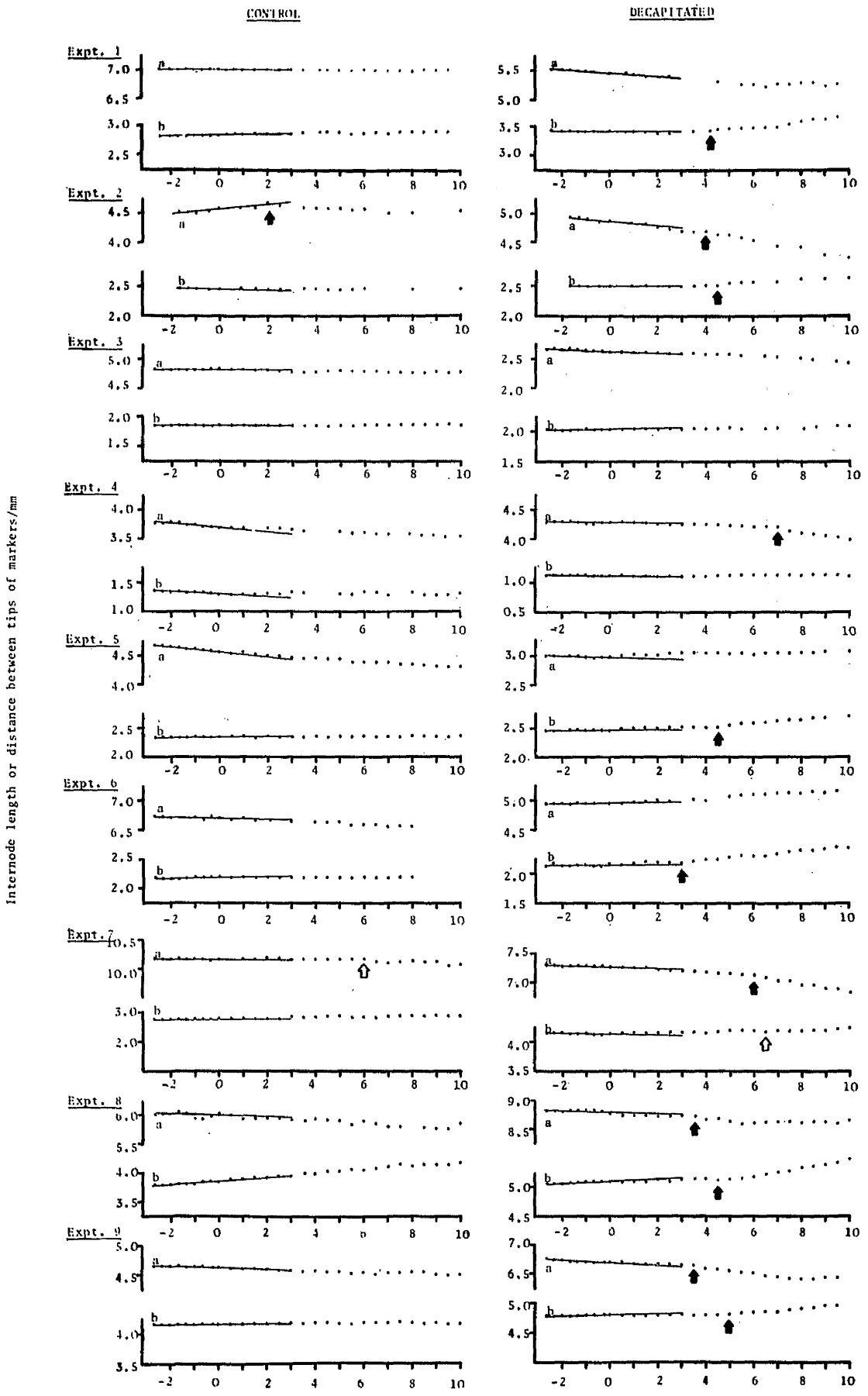
Of nine decapitated plants, a growth response to decapitation was seen in six plants when internode lengths were measured and in five plants when distance between marker tips were measured. In two plants, no response to decapitation by either method was evident in the 10 h following removal of the apical shoot. In the nine control plants which were left intact, none showed any change in growth rate of internode length, while only one showed a change when marker tips were measured. The time lag between decapitation and response varied between 3 to 5 h when internode length was measured, and between 3.5 - 7 h when distance between marker tips was considered. In four plants where a response was evident by either method, the bud bending response occurred consistently earlier (by 0.5 to 1.5 h) than the measurable increase in internode growth (Fig. 16). There was no evidence of any consistent difference in the time lag from decapitation to axillary bud growth response between plants of the two physiological age groups (plants with an expanding 2nd trifoliate leaf or with an expanding 3rd trifoliate leaf).

Having determined tentatively by inspection of the growth curves that visible growth response to shoot decapitation occurred after 3 to 5 h, confirmation was derived from statistical analysis. Estimates of growth rates in bud internodes of control and decapitated plants were made for the time intervals -2.7 to 0 h, 1 to 3 h and 5 to 7 h where 0 h was the time of decapitation (Table 6). The growth rates between each time interval were compared in control plants and in decapitated plants using the Wilcoxon matched-pairs signed-ranks test. No significant difference in decapitated plants was found when the growth rates between -2.7 to 0 h

FIGURE 16. (following page)

Growth of the internode of the 1st trifoliate axillary bud in nine intact and decapitated plants. Curves labelled (a) represent the distance between the tips of the two glass markers and curves labelled (b) represent the distance between the insertions of the markers. Shoot decapitation was carried out at 0 h. The straight line superimposed on each curve is the linear regression of the pre-decapitation measurements over time extrapolated to 3 h after decapitation, and is used as a guide in determining the time lag between decapitation and response.

Filled arrows indicate the time at which a measurable growth response to decapitation is observed. Ambiguous cases are denoted by unfilled arrows.


CONTROL
DECAPITATED
Expt. 1
Expt. 2
Expt. 3
Expt. 4
Expt. 5
Expt. 6
Expt. 7
Expt. 8
Expt. 9
a
b
Internode length or distance between tips of markers/mm
Time/h

and 1 to 3 h were compared. This confirms the finding from visual assessment of the growth curves that detectable growth response did not occur within 1 h from decapitation. The growth rate at 5 to 7 h, however, was significantly higher than that at -2.7 to 0 h or at 1 to 3 h ($p < 0.1$). This is in agreement with the finding from inspection that an increase in axillary bud internode growth occurred between 3 to 5 h following decapitation. No significant difference was found when growth rates at the three time intervals were compared in control plants, again confirming that growth rate in the bud internodes of control plants did not change significantly.

Cell extension during the initial growth

Matching plants were selected into groups of three, and in each group, two plants were decapitated. The internode length of the axillary bud and the length of the pith cells in the internode were measured one day after decapitation in the intact plant and in one of the two decapitated plants. Similar measurements were taken in the axillary shoot (i.e. the developed axillary bud) of the second decapitated plant after six days. The results are presented in Table 7. The proportionate increase in cell length and the proportionate increase in internode length after decapitation are given by $\frac{\Delta C}{C_i}$ and $\frac{\Delta L}{L_i}$ respectively, where

ΔC = Difference in cell length in the bud internode of the intact plant and of the decapitated plant.

C_i = Cell length in the bud internode of the intact plant.

ΔL = Difference in the bud internode length of the intact plant and of the decapitated plant.

L_i = Bud internode length of the intact plant.

When $\frac{\Delta C}{C_i}$ was plotted against $\frac{\Delta L}{L_i}$ for values of ΔC and ΔL obtained one day after decapitation, a significant positive correlation was evident ($r = 0.704^{***}$, Fig. 17). Similar linear regressions were carried between $\frac{\Delta C}{C_i}$ and $\frac{\Delta L}{L_i}$ for values of ΔC and ΔL obtained six days after decapitation, by

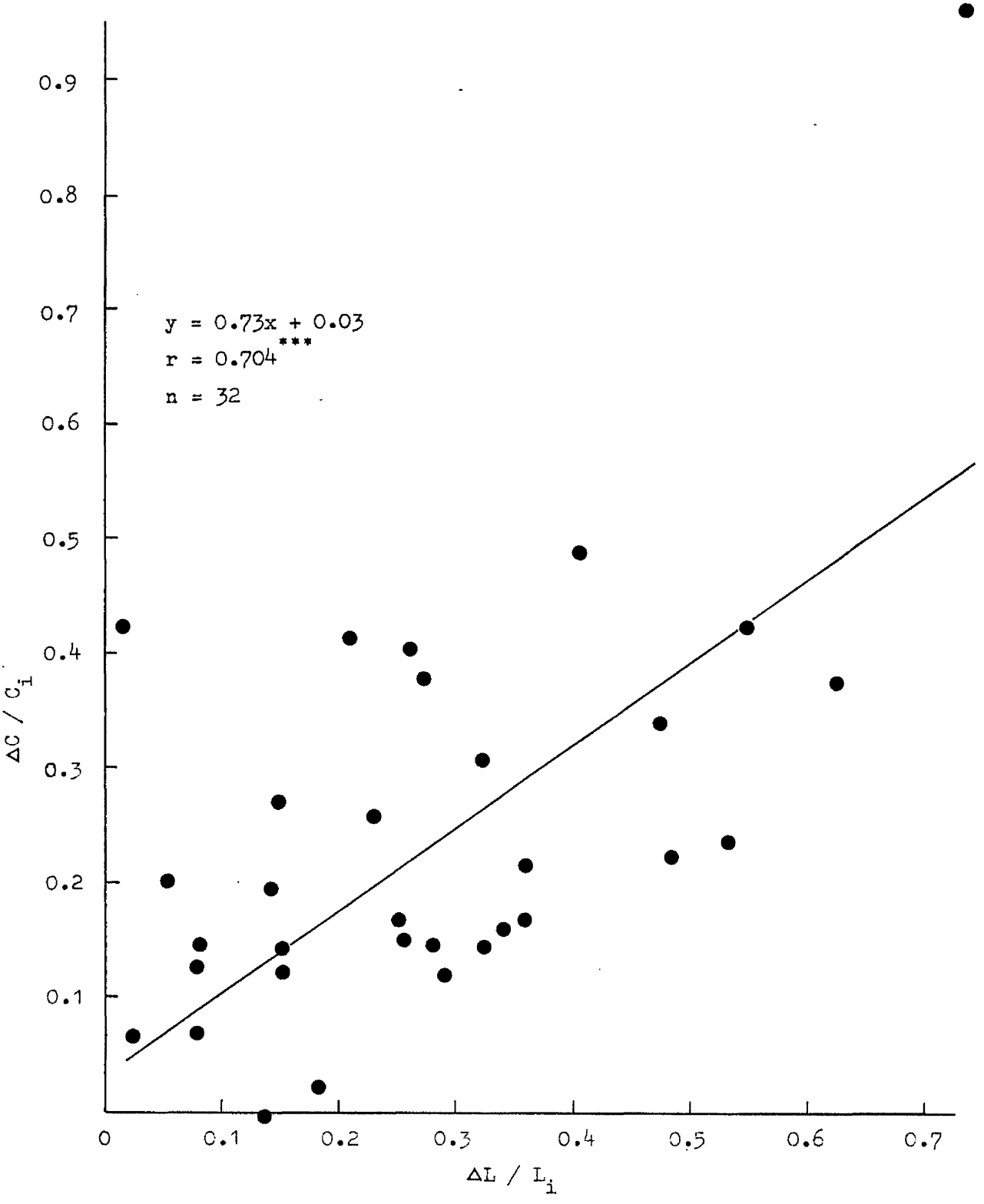


FIGURE 17. Relationship between $\frac{\Delta L}{L_i}$ and $\frac{\Delta C}{C_i}$ in the internode of the 1st trifoliate axillary bud one day after decapitation. $\Delta L, L_i$, ΔC and C_i are defined in the text.

TABLE 7. Extension of pith cells in the internode of the first trifoliate axillary bud and its contribution to the increase in the internode length. The measurements are the means ± standard errors calculated from 32 buds per treatment. Figures in brackets denote the average number of cells measured for each bud. The % increase in internode length due to cell extension is the mean of values calculated individually for each pair of control and decapitated plants.

	DAY 1		DAY 6	
	Intact	Decapitated	Decapitated	
Internode length (mm)	2.33 ± 0.07	2.95 ± 0.10	35.02 ± 2.24	
			base [1]	middle [1]
Cell length (μm)	15.4 ± 0.5 (126)	18.7 ± 0.6 (128)	81.8 ± 3.5 (108)	118.0 ± 6.9 (87)
Increase due to cell extension as % of the total increase in internode length		105.0	37.4	51.2

[1] Measurements of cells from the base of the internode (3-6 mm from the axil) or from the middle of the internode.

which time, growth of the axillary shoot was complete or near completion. When the values of ΔC were based on measurements of cells at the base of the internode no significant correlation between $\frac{\Delta C}{C_i}$ and $\frac{\Delta L}{L_i}$ was found ($r = -0.145$ N.S., Fig. 18a). These two variables were found to be related when ΔC was calculated from the lengths of cells in the middle of the internode ($2 = 0.432$*, Fig. 18b). There was, however, a substantial loss in the extent of correlation when the values of ΔC and ΔL were taken after six days. The variation in $\frac{\Delta C}{C_i}$ could account for 50% of the variation in $\frac{\Delta L}{L_i}$ one day after decapitation, but could explain only 19% of the variation six days later.

Having established that cell extension contributed significantly to the early stages of elongation of the axillary bud internode, estimates

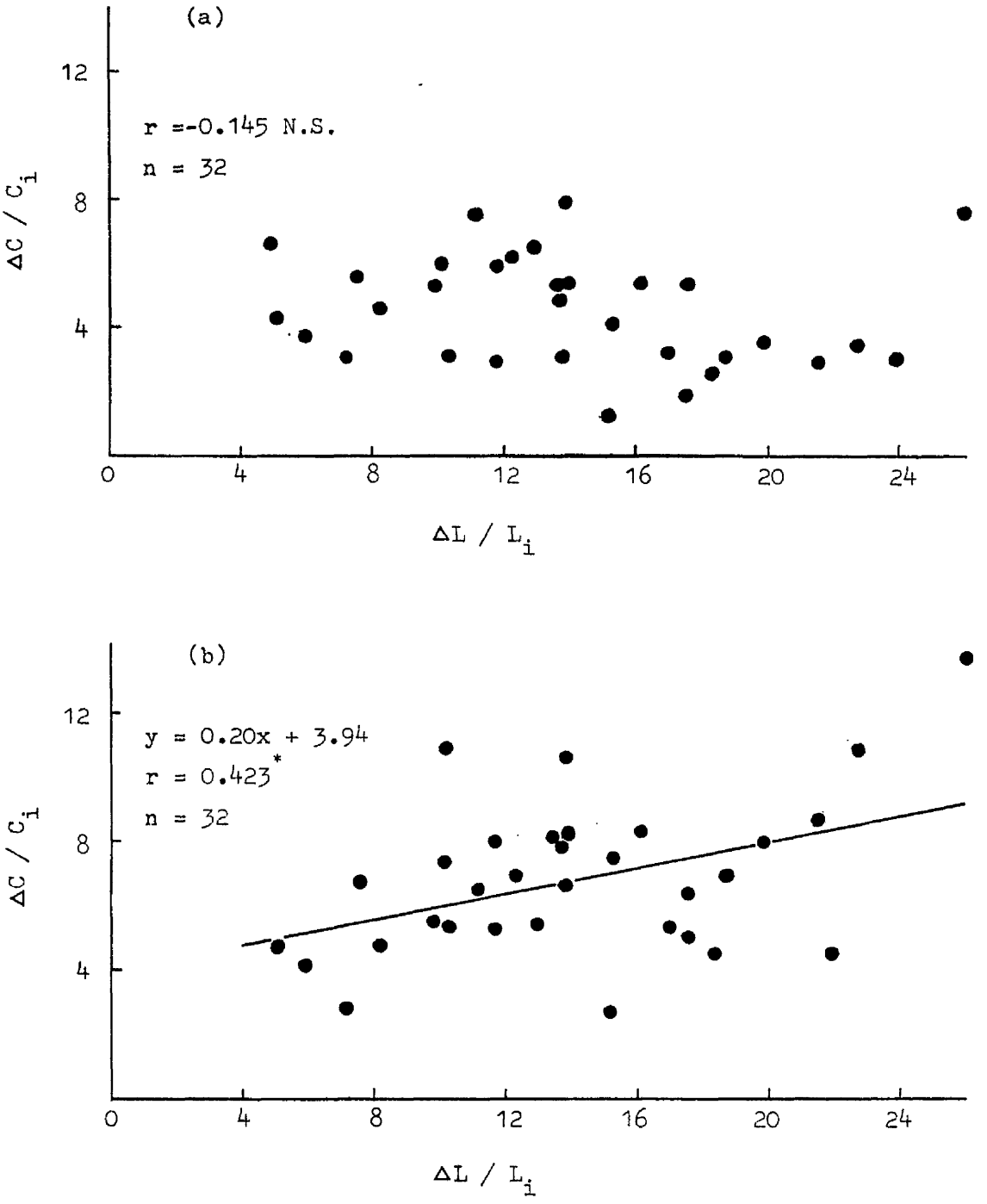


FIGURE 18. Relationship between $\frac{\Delta L}{L_i}$ and $\frac{\Delta C}{C_i}$ in the internode of the 1st trifoliate axillary shoot six days after decapitation. Values of ΔC were calculated from the lengths of cells located at (a) the proximal end and (b) the middle of the shoot internode. ΔL, L_i, ΔC and C_i are defined in the text.

were then made of the increase in internode length that was due to cell extension. This is given by $\frac{\Delta C}{C_i} \times {}^{L}i$, and is expressed in Table 7 as % of the total increase in internode length: $\frac{\Delta C}{C_i} \times \frac{L_i}{\Delta L} \times 100$. A mean close to 100% was obtained for measurements made one day after decapitation. As the axillary bud developed further, the internode elongation due to cell expansion dropped to about 50% after six days.

Discussion

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

Although the basal growth rate of the axillary bud internode was estimated as being of an order of 1 - 5 $\mu m\ h^{-1}$, these values might vary according to environmental conditions. McIntyre (1973) has shown that with favourable light, humidity and nutrient supply, spontaneous growth of axillary buds can occur in intact *Phaseolus* plants.

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

Although basal growth rates vary considerably between axillary buds of individual plants, the time lag between shoot decapitation and bud growth response was fairly consistent, being about 3 to 5 h. As this period represents the interval between stimulus and *measurable* response, the actual

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

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

Much of the deviation of values from the linear regressions between

$\frac{\Delta C}{C_i}$ and $\frac{\Delta L}{L_i}$ (Figs. 17 and 18) arose from two basic assumptions made in the statistical analyses of the data. In the first instance, the buds of the intact plant and decapitated plant were assumed to be identical (with respect to the growth behaviour of the bud internodes) in determining ΔC and ΔL. Secondly, cells from a selected region of the bud internode only were measured. In attempting the correlations between $\frac{\Delta C}{C_i}$ and $\frac{\Delta L}{L_i}$, it was assumed that the remaining cells of the internode underwent extension growth by a proportion similar to that for the cells measured. Estimates of the increase in bud internode length that was due to cell extension are also liable to errors arising from the latter assumption.

3.2.2. Vascular Transport to the Axillary Bud

Transport of basic fuchsin in the xylem

Basic fuchsin in low concentrations is rapidly transported within the plant vascular system, staining red the lignified xylem vessel walls with which it comes into contact (Talboys, 1955). This colouration serves as an indicator of the vascular pathways accessible to the dye from the various points of application. Decolourized basic fuchsin was fed to *Phaseolus* shoots *via* the cut ends of the root, the 1st internode, the petioles of the primary leaf (subtended by the larger axillary bud) and the 1st and 2nd trifoliate leaves. After intervals of 10, 40 and 80 min, hand sections were prepared from the stems, petioles and axillary buds and scored for the presence of stained xylem vessels.

In sections where fuchsin was detected, not all the vessels present were stained Table 8 shows the number of plants out of six where fuchsin was detected in a particular organ after a given time interval. Where more than six plants were examined, the score was adjusted to a maximum of six. Where only one or two vessels were observed to be lightly stained, a score of 1/2 was accorded. From the results, a schematic representation of vascular transport to the lateral buds is compiled (Fig. 19).

Axillary buds subtended by the primary leaf and by the 1st and 2nd trifoliate leaves all had access to fuchsin transported either acropetally or basipetally along the vascular system of the stem (Figs. 19, 20). Fuchsin present at any node, whether having been transported there acropetally or basipetally, had subsequently a greater propensity to travel up the leaf petiole, to the stipules, or (acropetally or basipetally) to the next internode than to move into the axillary bud. Thus, in many cases where fuchsin was found in a petiole end in the internodes on either side of the node, it was not detected in the axillary bud until the above-mentioned organs were supplied. Transport of fuchsin to all the axillary

TABLE 8. Vascular transport of basic fuchsin. Transverse sections were scored for the presence of fuchsin-stained vessels. Scores were adjusted to give a maximum of 6.

HY = hypocotyl; 1I, 2I, 3I, 4I = 1st, 2nd, 3rd and 4th internodes; PL = petiole of primary leaf subtending larger axillary bud; PS = petiole of primary leaf subtending smaller axillary bud; 1T, 2T = 1st and 2nd trifoliate leaf petioles; PB = larger primary leaf axillary bud; 1B, 2B = 1st and 2nd trifoliate axillary buds; R = root.

Point of application	Time of application (min)	Presence/absence of fuchsin stained vessels											
		Stem					Petiole				Axillary bud		
		HY	1I	2I	3I	4I	PL	PS	1T	2T	PB	1B	2B
2T	10	0	0	2	4	1	0	0	0	6	0	0	0
	40	0	1	4.5	6	2	0	0	2	6	0	0.5	1
	80	0	0.5	5	6	3.5	0	0	5	6	0	0.5	3
1T	10	0	4.5	6	5	0.5	0	0.5	6	3.5	0	0	0
	40	2	6	6	6	4	4	4	6	6	1	4.5	1.5
	80	1.5	6	6	6	4.5	5.5	5	6	6	4	5	2
PL	10	0.5	6	2	1	0	6	5	2	1	0	0	0
	40	3	6	6	5.5	2	6	6	6	5	3.5	1.5	0
	80	4.5	6	6	6	4	6	6	6	6	4	3	1.5
1I	10		6	6	6	2	6	6	6	6	4.5	1	0
	40		6	6	6	5	6	6	6	6	6	4.5	2
	80		6	6	6	6	6	6	6	6	5	6	1
R	10	6	5	3	2.5	0	3	3	3	2.5	0	0	0
	40	6	6	6	5.5	2	5	6	6	5	3	1	0
	80	6	6	6	6	3	6	6	6	6	5	2	1

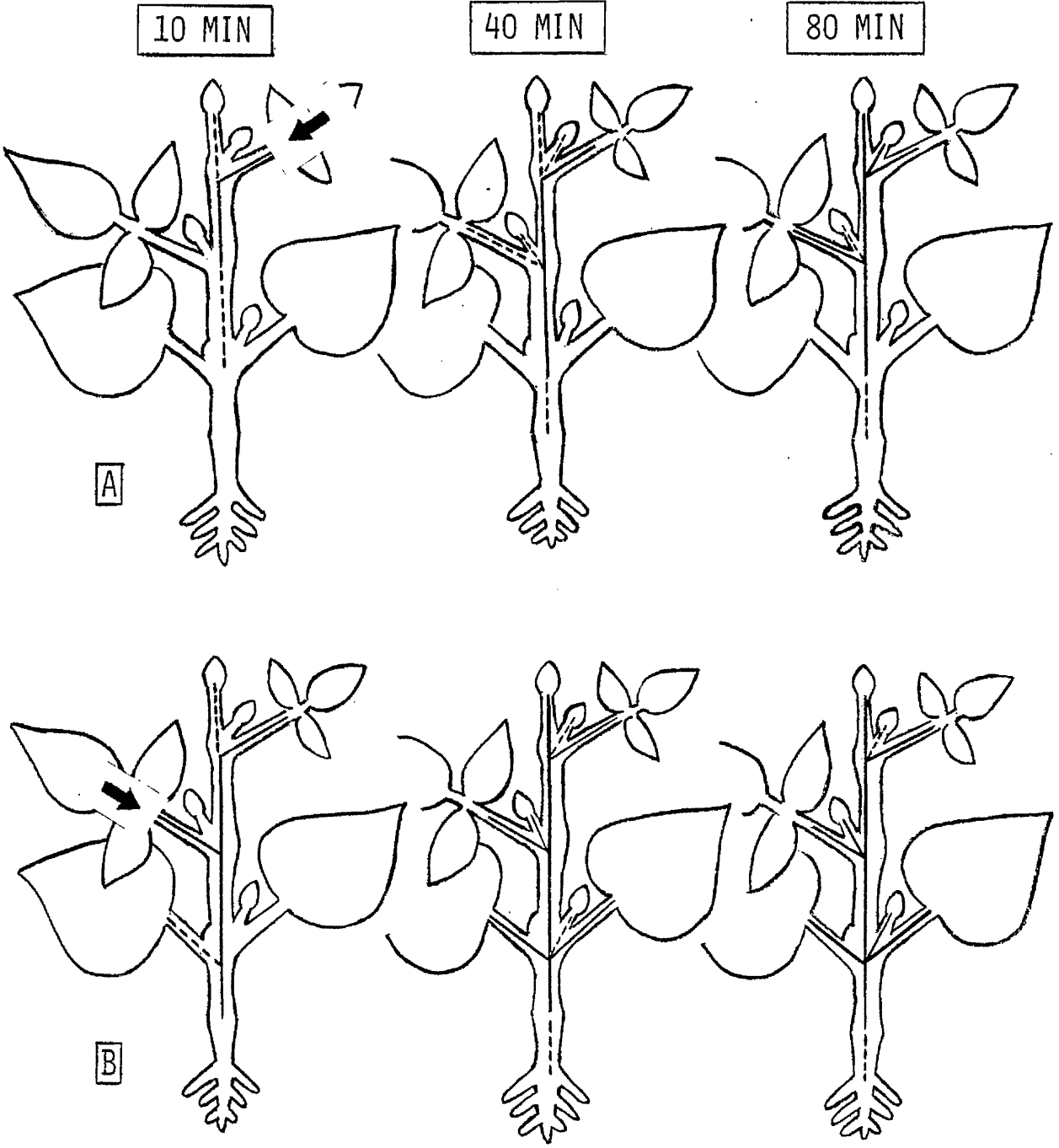
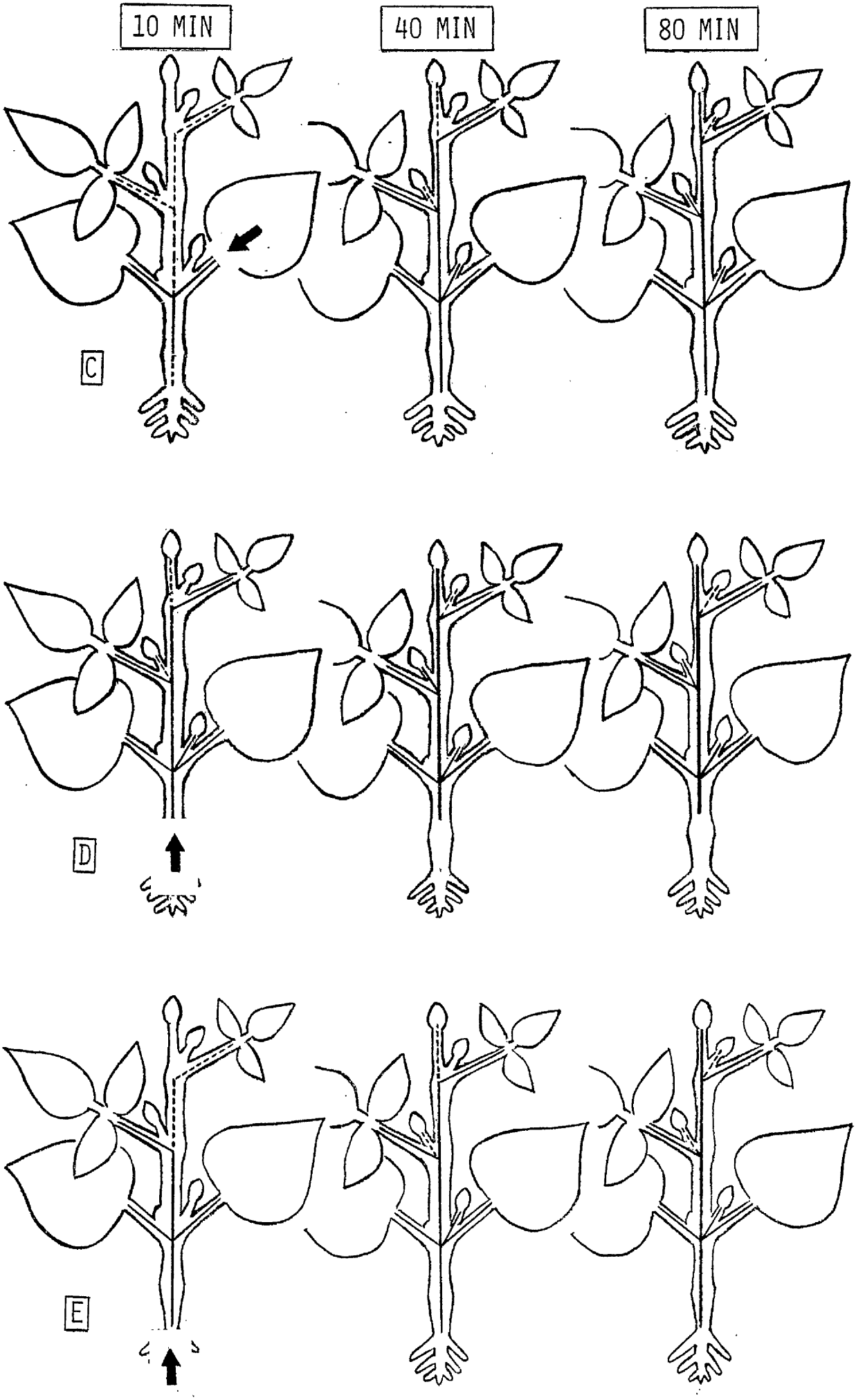


FIGURE 19. (above and following page)

Diagrammatic representation (not to scale) of the transport of basic fuchsin applied to the (A) 2nd trifoliate petiole, (B) 1st trifoliate petiole, (C) primary leaf petiole (adjacent to the larger axillary bud), (D) 1st internode and (E) root over 10, 40 and 80 min. The presence of vascular tissue coloured by fuchsin in the stem, petioles and axillary bud internodes is indicated by firm lines where it was observed in at least three out of six plants and by broken lines where the presence was in fewer than three plants. Arrows denote the points of application of fuchsin.


10 MIN
40 MIN
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C
D
E

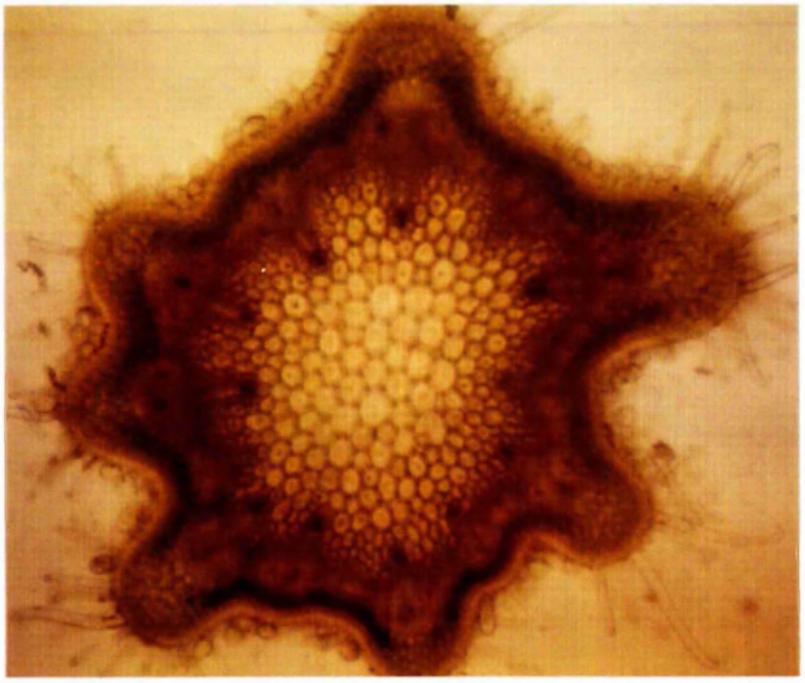

FIGURE 20.(above): Transverse free hand section of the internode of the 1st trifoliate axillary bud 80 min after immersing the cut end of the stem 1st internode into a solution of decolorized basic fuchsin. Several xylem vessels are stained red . X105

FIGURE 21.(right): The stem in the region of the 1st node with the cortical and epidermal tissues ("bark") peeled away. Basic fuchsin was only very sparingly transported basipetally past the 1st node to the hypocotyl when the stain was applied to the primary leaf petiole (left) or 1st trifoliate leaf petiole (centre). In contrast, basic fuchsin applied to the root was rapidly transported acropetally beyond the 1st node (right).

buds was most efficient when applied to the 1st internode.

When fuchsin was applied to petioles, the dye was rapidly transported away from the point of application, and was observed in the stem within 10 min. Transportation from the petiole was initially predominantly basipetal in the case of the 2nd trifoliate leaf and the primary leaf, although this was less distinct with the 1st trifoliate leaf. Fuchsin applied to the petiole of the primary leaf was rapidly transported to the opposite primary leaf.

In plants where the cortical tissue was stripped off, it was evident that basipetal transport was significantly inhibited at the 1st (cotyledonary) node. Typically, when fuchsin was applied to the leaf petioles staining of the vessels stopped abruptly at the 1st node, with only a few vessel bundles proceeding a short distance beyond it into the hypocotyl (Fig. 21). (Although Fig. 19b and c and Table 8 indicate the presence of stained vessels in the hypocotyl, they in fact refer only to the few bundles which were stained.) A similar inhibition of basipetal transport was sometimes noted at the 2nd (primary leaf) node, but this was less consistent and the inhibitory effect much less pronounced. Acropetal transport of fuchsin applied to the root, on the other hand, was completely unaffected by any restriction at the 1st node (Fig. 21).

Lateral transport of ^{14}C-sucrose from the xylem

Experiments were carried out to determine if material translocated in the xylem transferred freely to the phloem and other tissue peripheral to the cambium. Shoots of *Phaseolus* were detached from the plant by cutting at the middle of the 1st internode. The "bark" (tissue peripheral to the cambium) in the portion of the 1st internode attached to the shoot was stripped away. Biddulph *et al.* (1959) have shown that in the stem of *Phaseolus vulgaris*, the "wood" and "bark" (and hence xylem and phloem) are readily and effectively separated at the cambium. The cut end of the

remaining internode tissue, the "wood" (xylem and pith), was dipped into ^{14}C-sucrose solution. The radioactivity in the bark and wood of a 25 mm section of the 2nd internode after 40 and 75 min is shown in Table 9. The results showed rapid lateral transport of ^{14}C-sucrose from the "wood" to the "bark". This was particularly pronounced when radioactivity was expressed on the basis of fresh weight.

Discussion

Photosynthates from the leaves, minerals from the roots and growth factors from the shoot and roots are essential for active growth of an erstwhile inhibited lateral bud. The presence of adequate vascular bundles joining the bud to the principal vascular pathway of the stem greatly facilitates the transport of these materials to the bud, although it has been shown that morphological development of a meristem can occur in the absence of vascular supply (Esau, 1943). Of particular interest in this study is the vascular connections to the primary leaf axillary bud and the first trifoliate leaf axillary bud, since these buds are most frequently used in research on apical dominance in *Phaseolus vulgaris*. Failure to detect fuchsin, however, need not necessarily denote an absence of connected vessels as this could be because of insufficient lignification in the newly developed vessels. This might be particularly true of vascular tissue of the 2nd trifoliate axillary bud which was in its early stages of development. As the movement of fuchsin in the vessels was augmented by the transpiration stream, its rate of transport was influenced by environmental conditions.

The results enable the paths of basic fuchsin transport to be mapped and indicate that the axillary buds probably have adequate access to material translocated from the leaves and roots. When fuchsin was supplied to the leaf petiole, its transport to the subtended axillary bud appeared to be far slower than to the stipules, stem, or other petioles. Two

TABLE 9. Lateral transport of ^{14}C-sucrose from the "wood" to the "bark". Values are means (± standard errors) of material from five internode sections. Results from the two experiments carried out are presented.

Experiment	Period of Uptake and translocation	"Wood" Radioactivity	"Bark" Radioactivity		Radioactivity per unit fresh wt.
	min	Bq	Bq	% of amount in the "wood"	% of amount in the "wood" [1]
1	40	41.8 ± 7.0	21.1 ± 4.5	50.5	91.4
	75	34.8 ± 8.3	35.1 ± 4.1	100.9	140.4
2	40	22.3 ± 5.6	16.1 ± 2.3	72.2	142.7
	75	59.7 ± 12.2	40.6 ± 8.4	68.0	131.7

[1] Calculations based on the ratio of the fresh weight of bark:wood in the sections = 0.33.

possible interpretations are possible. Firstly, vascular connections joining the bud to the stem, while they exist, could be rather poor. Transport to the buds is, therefore, somewhat inefficient. Secondly, fuchsin reaching the node from the petiole does not transfer directly to the vascular system of the bud, but reaches it via an indirect route. An indication of the probable nature of this indirect route may be obtained by a consideration of the results in relation to the published reports of Biddulph and Cory (1965) and Mullins (1970a). Biddulph and Cory made a detailed study of phloem transport of ^{14}C labelled photosynthate from the trifoliate leaves of *Phaseolus vulgaris*, but excluding transport to axillary buds. They reported that assimilates transported from the mature first trifoliate leaf followed three distinct pathways on reaching the node of attachment. A small amount of photosynthate travelled acropetally from the node along cauline bundles. The major portion of photosynthate, however, was transported basipetally down the stem. On reaching the node immediately below, these descending phloem bundles were connected by anastomoses with a series of ascending bundles such that some of the transported materials were re-routed acropetally, while the rest continue basipetally to the lower stem. If the arrangement of xylem bundles in the stem were to be similar to that for phloem bundles, fuchsin applied to the 1st trifoliate leaf petiole could, therefore, reach the subtended axillary bud by two possible routes. It could be supplied by bundles arising directly from the leaf trace at the node of attachment, or it could be supplied, indirectly, by ascending bundles arising from the 2nd (primary leaf) node, at which point, an anastomosis occurred with descending bundles from the first trifoliate leaf. The latter indirect pathway could be the more important in the present study. Biddulph and Cory's study did not extend to the primary leaf, and Mullin's (1970a) work, involving histological and radiotracer techniques, is referred to in this regard. Mullins similarly made no mention of the primary leaf axillary bud. He did state,

however, that no significant direct vascular connection existed between the primary leaves and the shoot above the node of attachment of the leaves. He found that assimilates from the primary leaves descended the stem and, at some point, were transferred to ascending vascular bundles which then supplied the distal tissue. It is thus probable that the primary leaf axillary buds are supplied by this indirect pathway. Mullins did not specify at which point ascending bundles anastomised with descending bundles from the primary leaf, but results from the present study suggest that the anastomosis could be located at the 1st internode (immediately below the primary leaves). Fuchsin applied to the primary leaf petiole was very sparingly transported beyond this node even after the vessels in distal tissues had been stained. An anastomosis located at the first node would also be in agreement with the scheme for trifoliate leaves elucidated by Biddulph and Cory (1965).

The presence of functional xylem connections to the axillary buds indicated that the buds were unlikely to be deprived of mineral nutrients. Since basic fuchsin selectively stains the lignified tissue of the vascular system, however, the results from experiments where fuchsin was used as a tracer do not specifically show the presence of the phloem elements which are important for the transport of photosynthates. The practicability of using the stain sodium fluorescein as a tracer for phloem transport has been considered. Sodium fluorescein gives rise to fluorescence in the callose of sieve plates when viewed in ultra-violet light and has been used to demonstrate the presence of functional phloem in *Glycine* lateral buds (Peterson and Fletcher, 1972). The main problem in the method is the difficulty in limiting uptake of the dye to the phloem alone and preventing rapid translocation taking place in the xylem. Stout and Hoagland (1939) have shown that material translocated in the xylem could transfer rapidly into the more peripheral tissue, i.e. the "bark". Thus, it would be difficult to tell if fluorescence in the phloem

has resulted from the dye diffusing from the xylem or from the dye that has actually been transported in the phloem. The latter case would have to be established to demonstrate that the phloem is functional. The experiments on the lateral transport of ^{14}C-sucrose showed clearly that substances translocated in the xylem could indeed be transferred readily into the "bark". The use of sodium fluorescein as a tracer in the phloem elements was, therefore, not actively pursued. It should also be recognised that inasmuch as material in the xylem could be transferred to the phloem, the reverse could also occur. Thus, the vessels could, in part, have been stained by basic fuchsin translocated in the phloem. This possibility seems remote, however, as the movement in the phloem can be expected to be much slower than that in the xylem, especially when transport in xylem is aided by the transpiration stream.

The problems in demonstrating experimentally the presence of functional phloem connections to the axillary buds remain unresolved. Nevertheless, there are reasons to believe that phloem-transported photosynthate can reach the inhibited buds. In the first instance, differentiation of the phloem and xylem seem to be closely associated with one another. The presence of phloem is probable where the presence of xylem has been established. Secondly, it is possible to infer, indirectly, that photosynthate can be supplied to the tissue reached by xylem vessels *via* the xylem itself.

Biddulph and Cory (1965) showed that in *Phaseolus*, some of the photosynthate from the leaves that was translocated to the stem was transferred laterally from the "bark" (where the phloem was located) to the "wood". It appears, therefore, that a portion of the photosynthate can be translocated in the xylem. As it has been shown in the present study that lateral movement of ^{14}C-sucrose from the xylem to the peripheral tissue occurs readily, it is reasonable to assume that some photosynthate at least can be made available to tissue supplied by the xylem, without direct involvement of the phloem.

3.3. ANALYSIS OF ENDOGENOUS ETHYLENE AND ETHANE

3.3.1. Combined Gas Chromatography-Mass Spectrometry

Mass spectral scans of standard ethylene and ethane gave results closely comparable to the published data of Cornu and Massot (1966) (Figs. 22, 23). However, full mass spectral scans were not carried out on samples extracted from the plants since the concentrations of ethylene and ethane in these samples were below the minimal levels required for discrimination from background. Thus, selective ion detection techniques were employed for the detection of the gases.

When the *Phaseolus* gas extracts were analysed a single peak was obtained after a retention time of 3 min on Channel 2; this Channel had been selectively tuned to correspond to the molecular ion of ethane. Peaks with retention times of 3 and 5 min were noted on Channel 1 which had been tuned to correspond to the molecular ion of ethylene; the earlier response was elicited by a fragment ion of ethane (M-2), while the later response was indicative of the presence of ethylene (Figs. 24, 25). Using standard gases the height of each peak was found to be proportional to the quantity injected (Fig. 26). Concentrations of ethylene and ethane in gaseous samples extracted from non-stressed *Phaseolus* shoots varied between different batches of plants and were found to be 0.4 to 0.7 vpm for ethylene and 0.5 to 1.8 vpm for ethane. These values were comparable to those obtained by GC-FID for similar plant extracts. The ethylene concentrations were close to the lower limit of detectability (peak height = 2 x baseline). Ethane concentrations were much more readily quantified as a result of higher levels in samples coupled with the capacity for greater signal amplification on Channel 2 where the baseline was more stable because of the absence of background ions at m/e 30.

With some *Phaseolus* extracts, a third peak appeared on Channel 1 between the peaks for ethane and ethylene (Fig. 25). A full mass spectral scan

showed this peak to be carbon dioxide ($M^{+\cdot}$ = 43.9898; M-16 = 27.9949) (Fig. 27). The total ion monitor showed carbon dioxide to be present at a concentration of approximately 1% (Fig. 28). Because of its presence in a relatively large quantity, its fragment ion, M-16, was detected on Channel 1.

In conclusion, single and multiple ion detection mass spectrometry confirmed the identity of ethylene and ethane in *Phaseolus* gas extracts. Flame ionization detection, however, remains a far more sensitive (0.005 vpm ethylene can be readily detected in a 1 cm^3 sample) if less specific technique for the quantitative analysis of these gases. Therefore, all quantitative estimates of ethylene and ethane were routinely carried out using GC-FID.

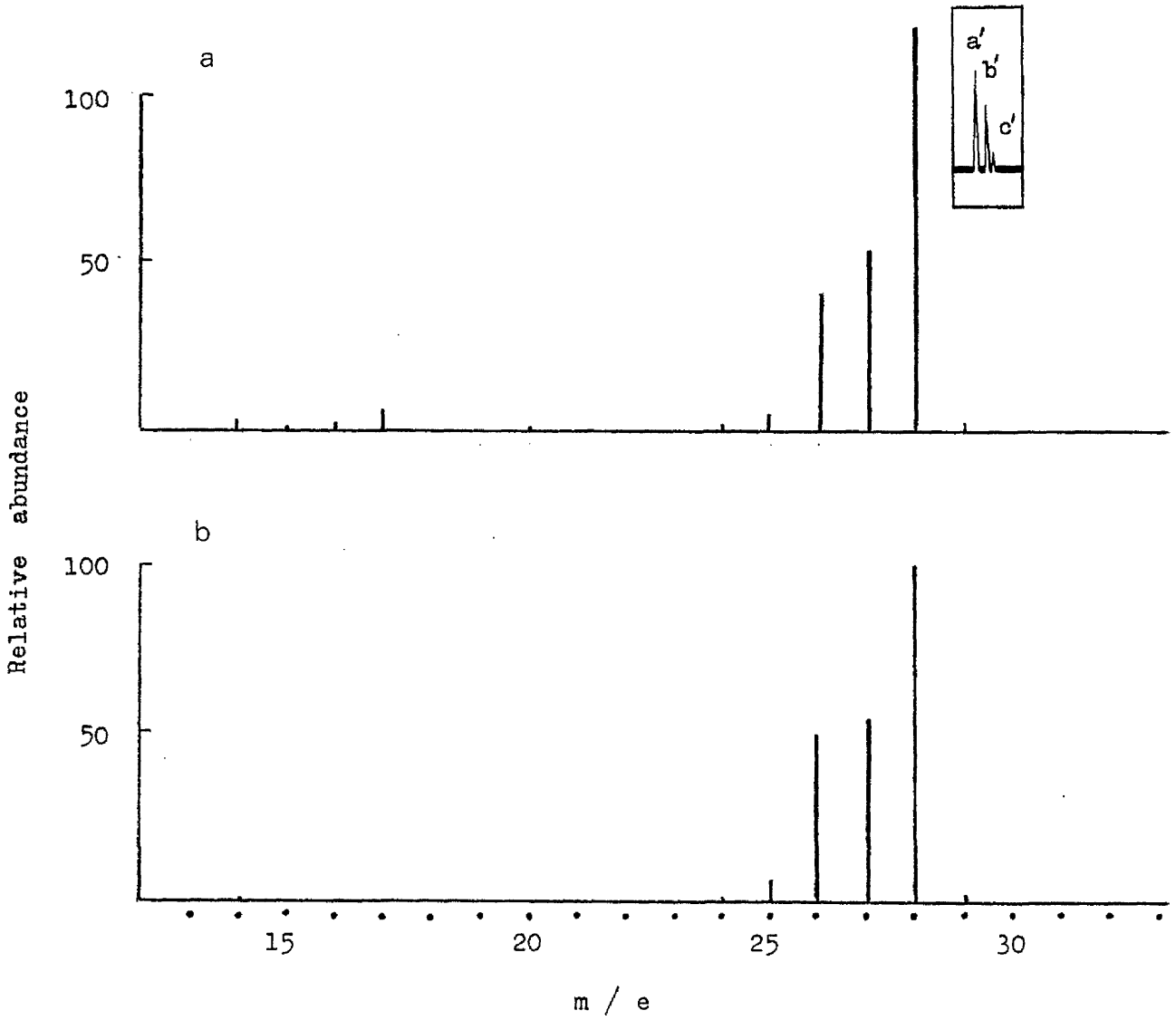

FIGURE 22.

(a) Mass spectrum of ethylene derived from a standard sample.

(b) The published data of Cornu and Massot (1966).

INSET: Resolution of the peaks of (a') ethylene, (b') nitrogen and (c') carbon monoxide in the mass spectrum scan.

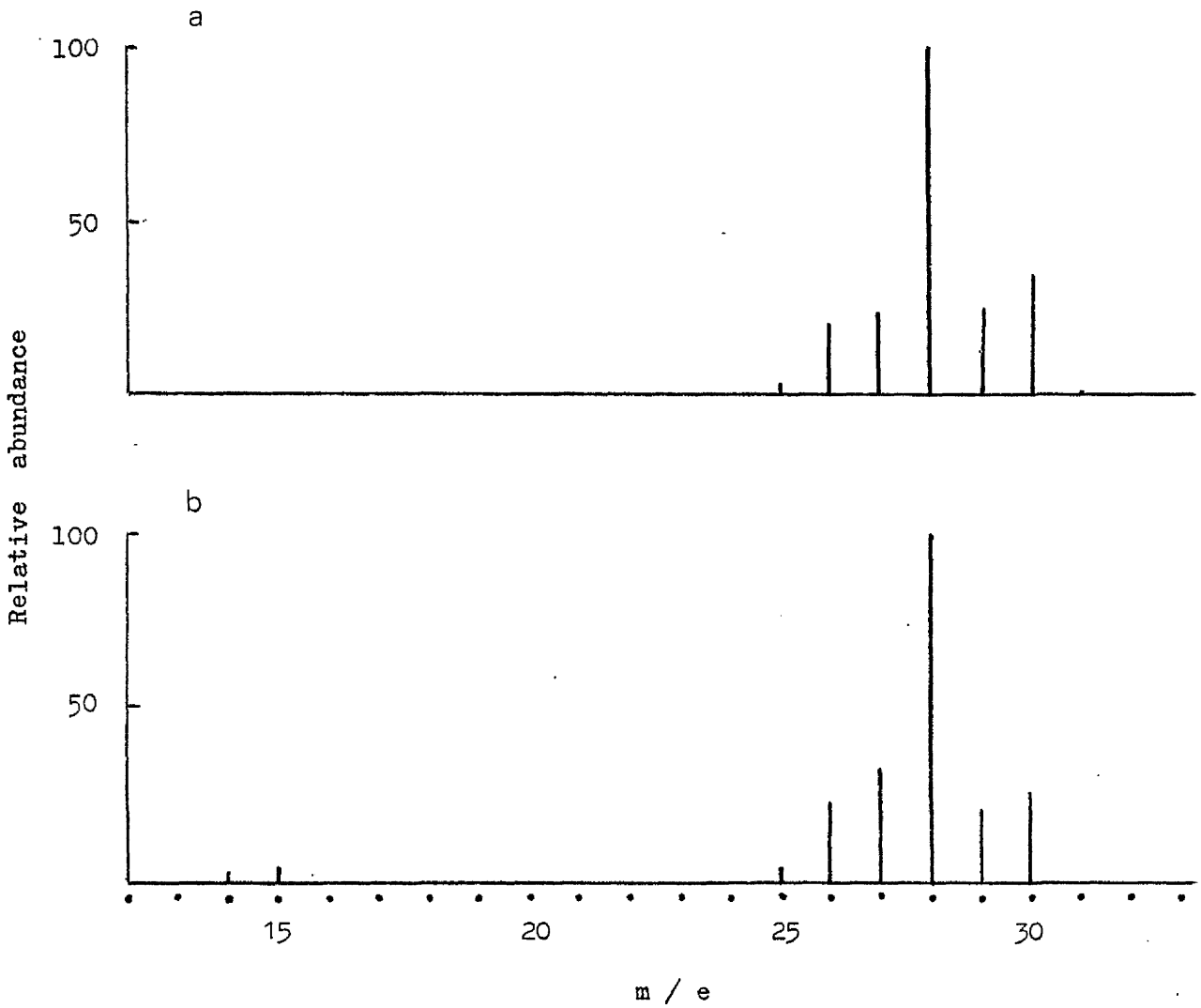

FIGURE 23.

(a) Mass spectrum of ethane derived from a standard sample.

(b) The published data of Cornu and Massot (1966).

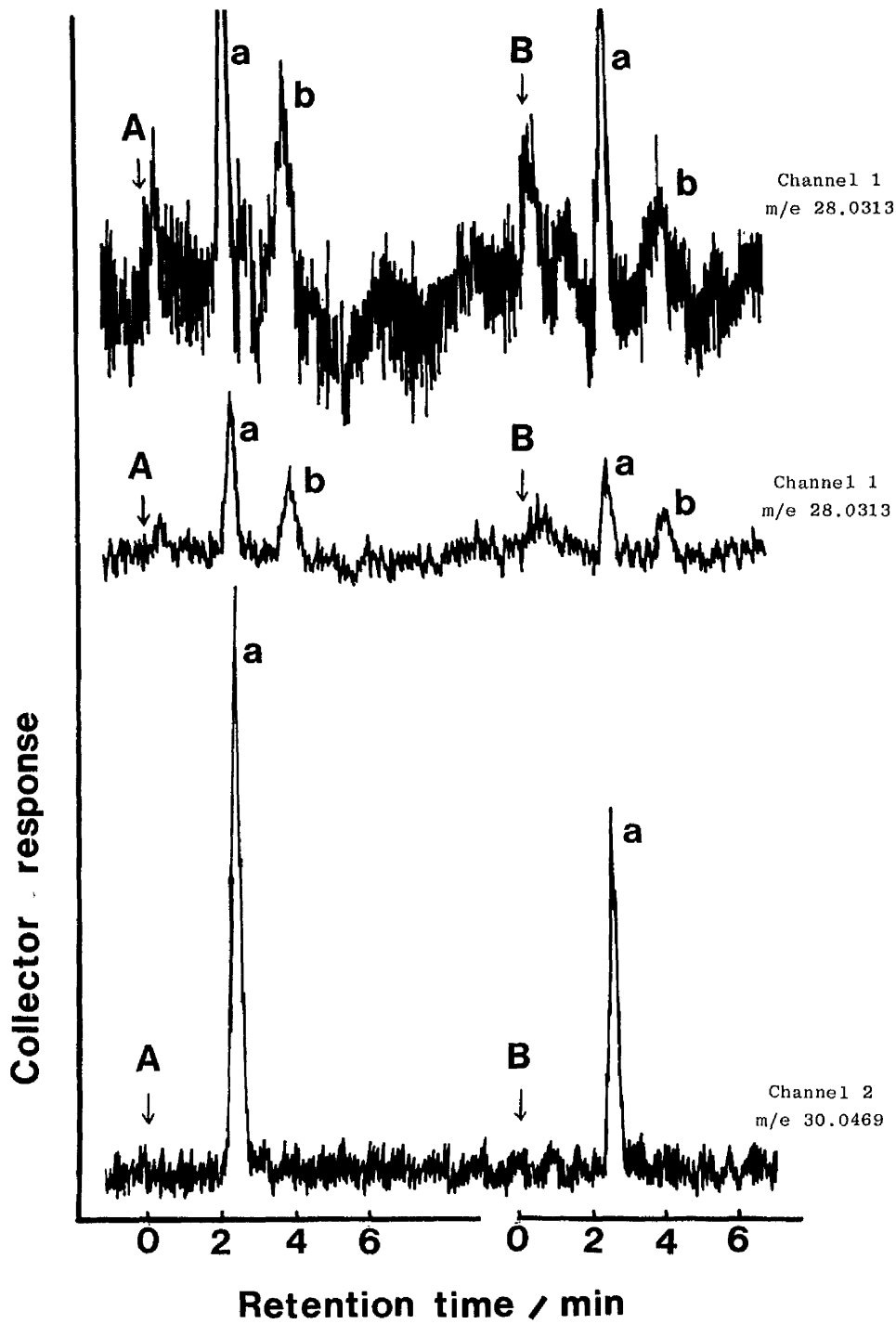

FIGURE 24. GC-MS multiple ion detection.
Top trace: Channel 1 tuned to m/e 28.031; attenuation X3
Middle trace: Channel 1 tuned to m/e 28.031; attenuation X10
Bottom trace: Channel 2 tuned to m/e 30.047; attenuation X1

Key to sample injections (arrows):
A = 2 cm^3 standard gas sample containing 1 vpm ethylene and 1 vpm ethane;
B = 2 cm^3 *Phaseolus* apical shoot gaseous extract.

Key to peaks: a = ethane; b = ethylene.

In this experiment, carbon dioxide is not detected on Channel 1 (compare with Fig. 25).

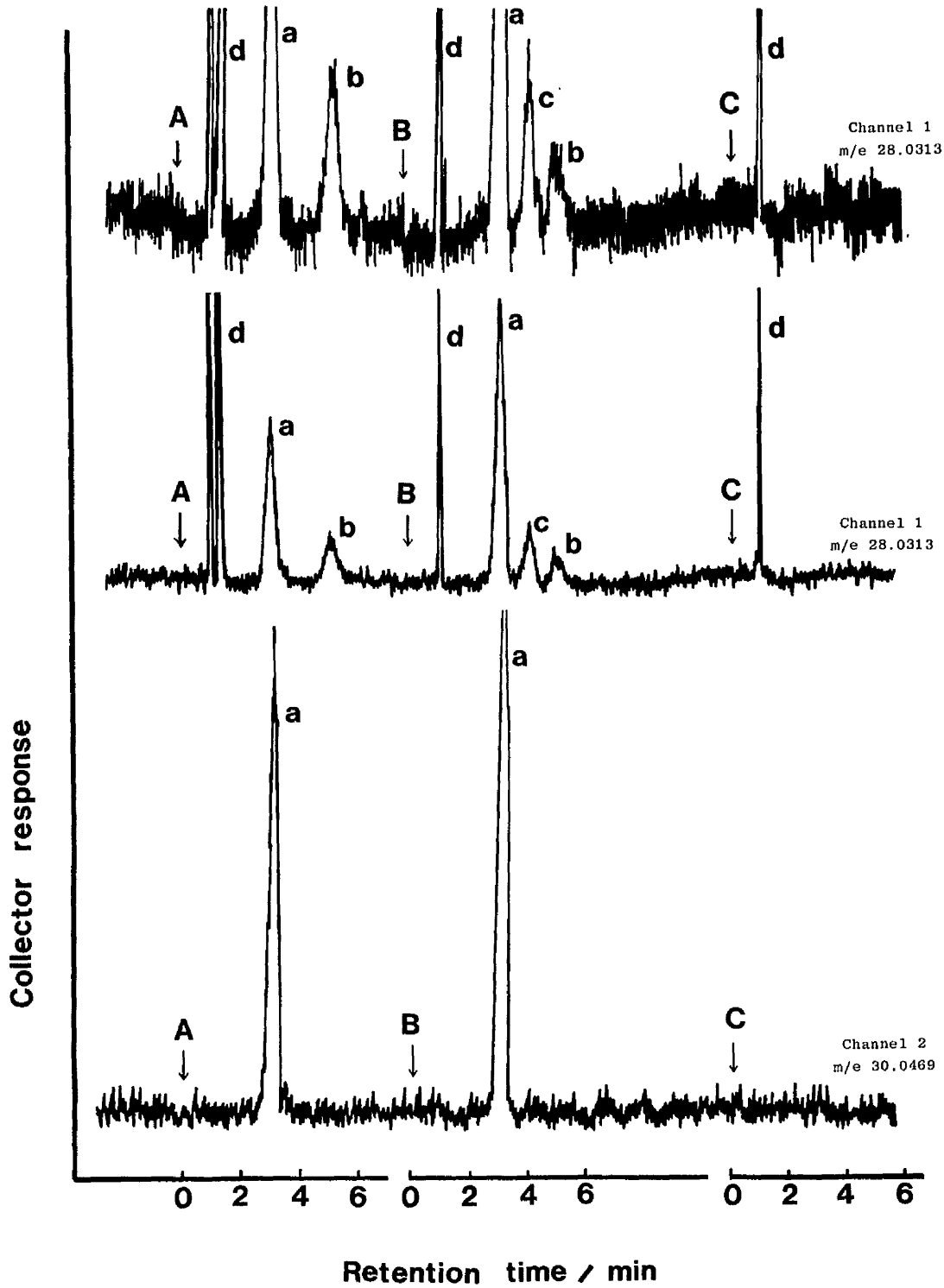

FIGURE 25. GC-MS multiple ion detection.
Top trace: Channel 1 tuned to m/e 28.031; attenuation X3
Middle trace: Channel 1 tuned to m/e 28.031; attenuation X10
Bottom trace: Channel 2 tuned to m/e 30.047; attenuation X1

Key to sample injections (arrows):
A = 2 cm^3 standard gas sample containing 1 vpm ethylene and 1 vpm ethane;
B = 2 cm^3 Phaseolus apical shoot gaseous extract;
C = 2 cm^3 glasshouse air.
Key to peaks: a = ethane; b = ethylene; c = carbon dioxide; d = response due to the air peak being pumped off before reaching the GC-MS separator.

In this experiment, carbon dioxide is detected on Channel 1 (compare with Fig. 24).

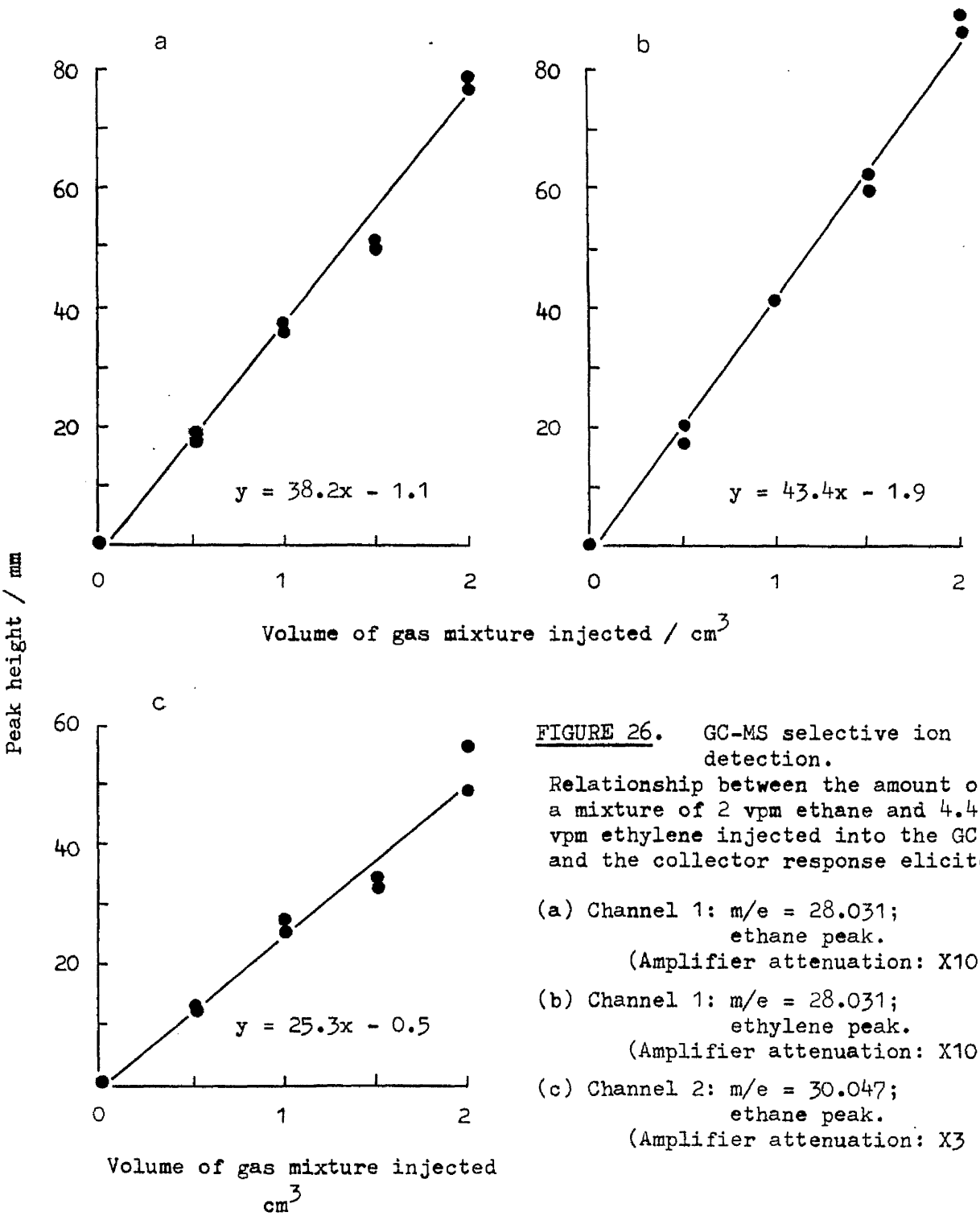

FIGURE 26. GC-MS selective ion detection.
Relationship between the amount of a mixture of 2 vpm ethane and 4.4 vpm ethylene injected into the GC and the collector response elicited.

(a) Channel 1: m/e = 28.031; ethane peak. (Amplifier attenuation: X10)

(b) Channel 1: m/e = 28.031; ethylene peak. (Amplifier attenuation: X10)

(c) Channel 2: m/e = 30.047; ethane peak. (Amplifier attenuation: X3)

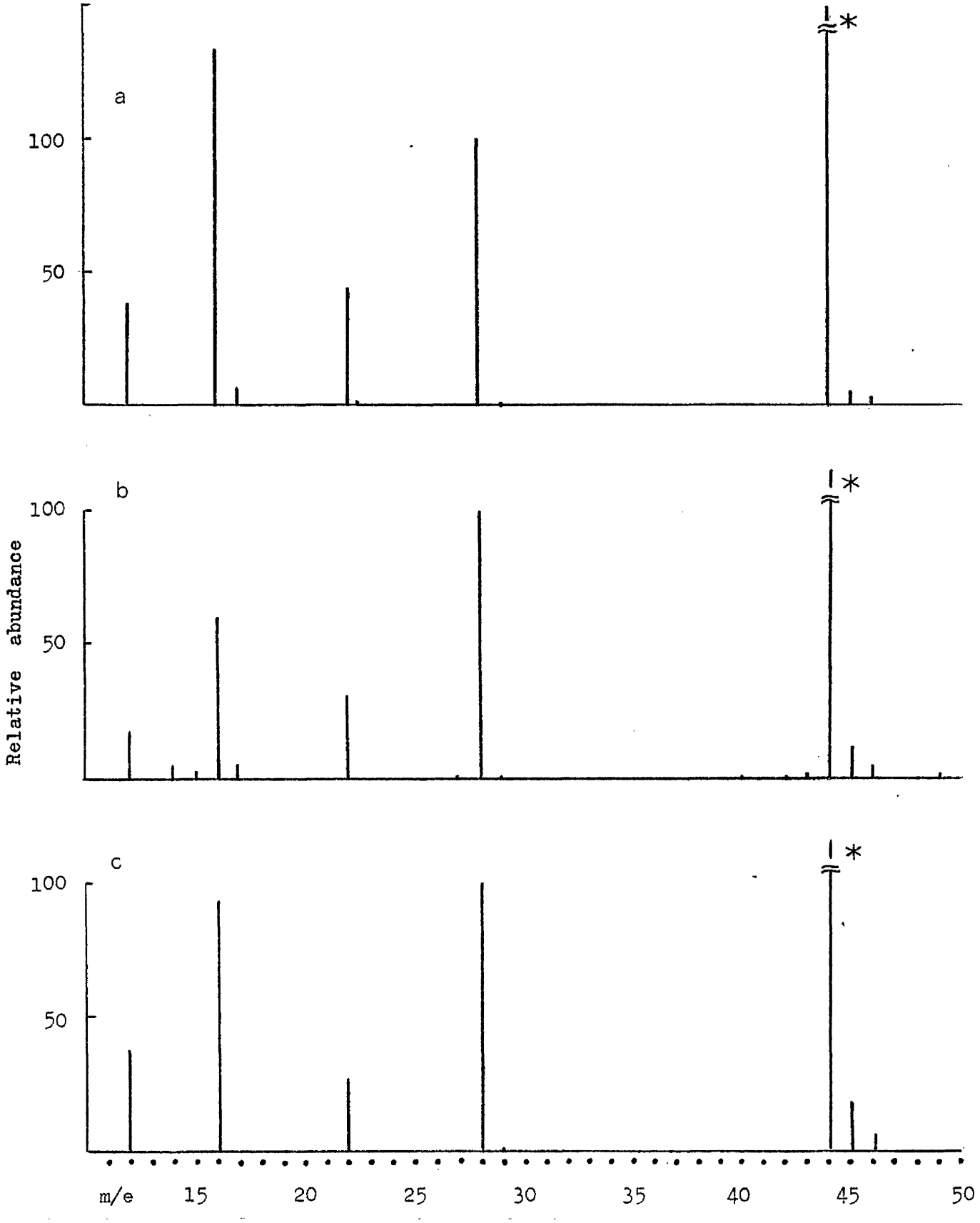

FIGURE 27. (a) Mass spectrum of carbon dioxide derived from Phaseolus apical shoot gaseous extract;
(b) Mass spectrum of carbon dioxide derived from a standard gas mixture;
(c) The published data of Cornu and Massot (1966).

Relative abundance of the ions is compared against the peak height of m/e 27.995 (due to $CO^{+\cdot}$) which is the second highest peak in the scan. The highest peak is that of m/e 43.990 (due to $CO_2^{+\cdot}$, asterisked). This peak is at saturation in (a)and (b).

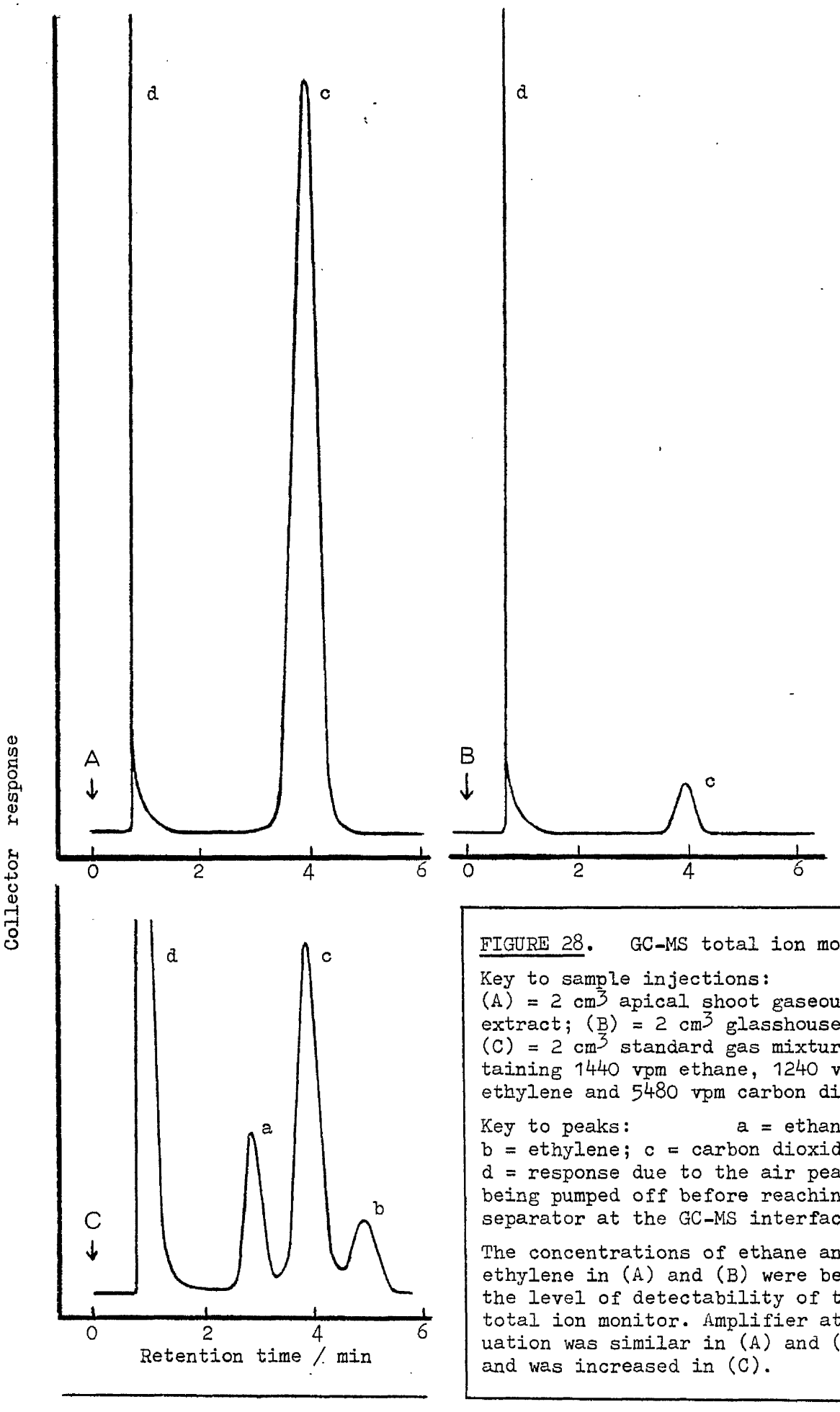

FIGURE 28. GC-MS total ion monitor.

Key to sample injections:
(A) = 2 cm^3 apical shoot gaseous extract; (B) = 2 cm^3 glasshouse air; (C) = 2 cm^3 standard gas mixture containing 1440 vpm ethane, 1240 vpm ethylene and 5480 vpm carbon dioxide.

Key to peaks: a = ethane; b = ethylene; c = carbon dioxide; d = response due to the air peak being pumped off before reaching the separator at the GC-MS interface.

The concentrations of ethane and ethylene in (A) and (B) were below the level of detectability of the total ion monitor. Amplifier attenuation was similar in (A) and (B) and was increased in (C).

3.3.2. Internal Concentration and Emanation of Ethylene and Ethane

Quantitative analyses of endogenous ethylene from *Phaseolus* were routinely employed in the study of the involvement of ethylene in correlative inhibition of lateral buds. Endogenous ethylene was expressed either as the internal concentration in the tissue or as the rate of emanation of the gas. The experiments in this section are aimed at clarifying some of the ambiguities that surround the different approaches and variations in the quantitative analysis of ethylene levels in plant tissues. In the course of the experiments, the levels of endogenous ethane from *Phaseolus* tissue were also recorded and are examined together with ethylene.

Vacuum extraction of small amounts of plant tissue

As in many other studies in the literature, the internal ethylene in *Phaseolus* tissue was sampled by the vacuum extraction method of Beyer and Morgan (1970b). The basic procedure in this method has been outlined in section 2.6.1. (Materials and Methods). The test tissues that were analysed in the present study were usually small and some additional manipulations on top of the basic procedure had to be carried out to recover the small amounts of gases extracted.

An adaptation of the apparatus was necessary where short stem sections were to be extracted. When placed in the extraction bottle in the desiccator (Fig. 5), the sections rose into the neck of the bottle where they became tightly packed, making it difficult to free the air bubbles from the surfaces of the sections after the vacuum treatment. To overcome this problem, a rubber trap attached to a release line was used to prevent entry of the stem sections into the bottle neck (Fig. 29a). After the sections were extracted by vacuum, they were agitated so that the air bubbles were dislodged, and these then coalesced into larger bubbles. The

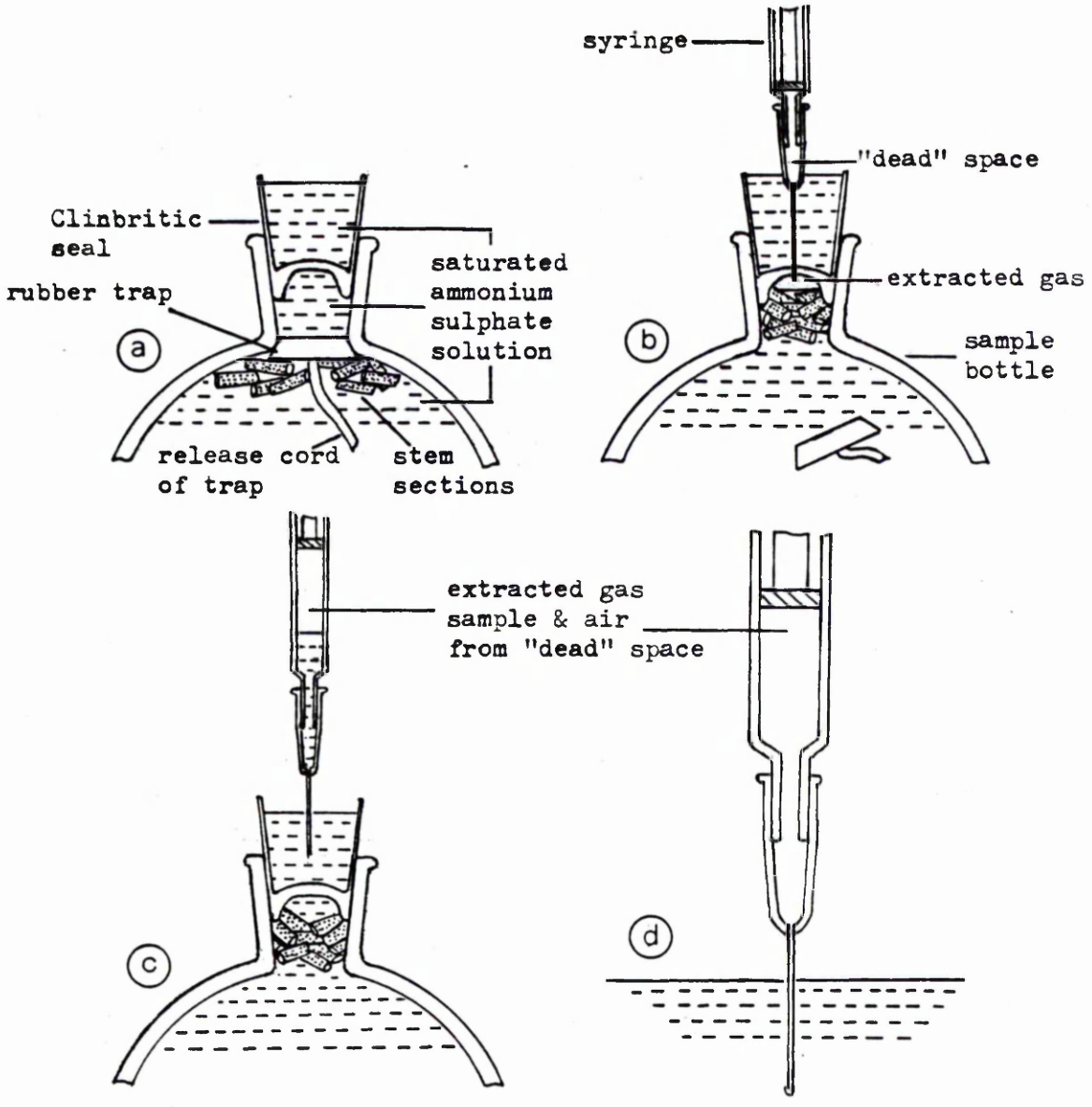

FIGURE 29. Extraction and recovery of internal gases from small samples of plant tissue.

(a) The rubber trap is fitted to prevent the short stem sections packing into the neck of the sample bottle. The desiccator (not shown above; see Fig. 5 for diagram of the entire apparatus) is evacuated using a vacuum pump.

(b) After vacuum extraction, the stem sections are agitated to dislodge the adhering bubbles of gas. The trap is displaced and the gas bubbles rise to the lower surface of the Clinbritic seal. The needle of a syringe is inserted through the seal.

(c) The plunger of the syringe is withdrawn, and the needle eased out of the Clinbritic seal at the same time. The tip of the needle is always kept below the surface of the salt solution in the Clinbritic seal reservoir.

(d) Salt solution taken into the syringe together with the extracted gases is expelled with the tip of the needle held in the salt solution in the desiccator. Salt solution in the syringe is completely expelled when a small gas bubble just appears at the tip of the needle.

trap was removed using the release line and the air bubbles allowed to rise upwards to collect beneath the Clinbritic seal (Fig. 29b). The extracted gases were recovered using an airtight plastic syringe and injected into the GC.

The final manipulation - that of recovering the extracted gas - required another adaptation of the basic procedure of Beyer and Morgan when small tissue samples were extracted. When the volume of the extracted gas was small (as little as 0.15 cm^3), it was desirable to recover all of the gas to enable the maximum amount of sample to be injected into the GC. Complete recovery of the extracted gas also allowed its volume to be determined, this information being useful in some experiments.

The flange of the Clinbritic seal fitted to the bottle in the extraction desiccator was not turned down (as would be the usual practice when using the seal) but was left intact to form a reservoir which was filled with saturated ammonium sulphate solution (Fig. 29). The needle of the syringe was pushed through the seal and the plunger of the syringe withdrawn to draw in the bubble of extracted gas lying beneath the seal. As the bubble shrunk in size, the needle was drawn out of the seal before the pressure inside the syringe had equilibrated. At the moment the tip of the needle was flush with the lower surface of the Clinbritic seal, the small bubble of gas remaining adpressed to the seal was drawn into the syringe. Once the needle had been removed from the seal, care was taken so that the tip of the needle remained in the reservoir of salt solution (Fig. 29c). Thus, equilibration of the pressure inside the syringe was completed by the intake of solution from the reservoir without contamination of atmospheric air. By repeating the needle-withdrawal procedure several times, all of the extracted gas could be recovered in the syringe. With the needle of the syringe immersed in saturated ammonium sulphate solution, the plunger was then depressed to expel the salt solution that had been taken into the syringe together with the gas sample. Expulsion of the salt solution was complete when a small gas bubble just appeared at the tip of the needle

(Fig. 29d). The needle was quickly pushed into a rubber stopper which had been wrapped in layers of tissue paper to absorb the small amounts of salt solution adhering to the needle. The volume of the gas sample recovered was read from the calibration on the syringe.

Since there was a "dead" space of 0.05 cm^3 in the syringe between the surface of the plunger and the tip of the needle (Fig. 29b), this had to be allowed for in calculating the concentration of ethylene and ethane in the gas sample. Dilution of the extracted gas by the "dead" space was only marginal where the volume extracted was large. Where small volumes were involved, however, correction for the "dead" space was essential to avoid an underestimate. Ethylene concentration in the extracted gas sample was given by:

$$\text{Ethylene concentration (vpm)} = \frac{(V + 0.06)}{V^2} \times \frac{H_e}{H_s}$$

where V = Volume of the gases extracted (cm^3)

H_e = Height of the ethylene peak elicited on the recorder trace by the extracted gas sample

H_s = Height of the ethylene peak elicited on the recorder trace by 1 cm^3 1 vpm ethylene.

To facilitate repeated calculations, the formula was programmed into the Hewlett Packard HP67 calculator. The same basic formula was used to determine ethane concentrations. Note that although the "dead" space was approximately 0.05 cm^3, a value of 0.06 cm^3 was used as the correction factor as this was found in preliminary trials to give a better estimate of the ethylene or ethane concentration in the sample. The trials, described below, had been carried out to verify the appropriateness of the formula in practice.

Varying quantities of standard 1 vpm ethylene were bubbled into the extraction bottle in the desiccator and the ethylene samples were recovered by the method described above. The concentration of ethylene in the samples estimated applying the formula is given in Fig. 30. For samples

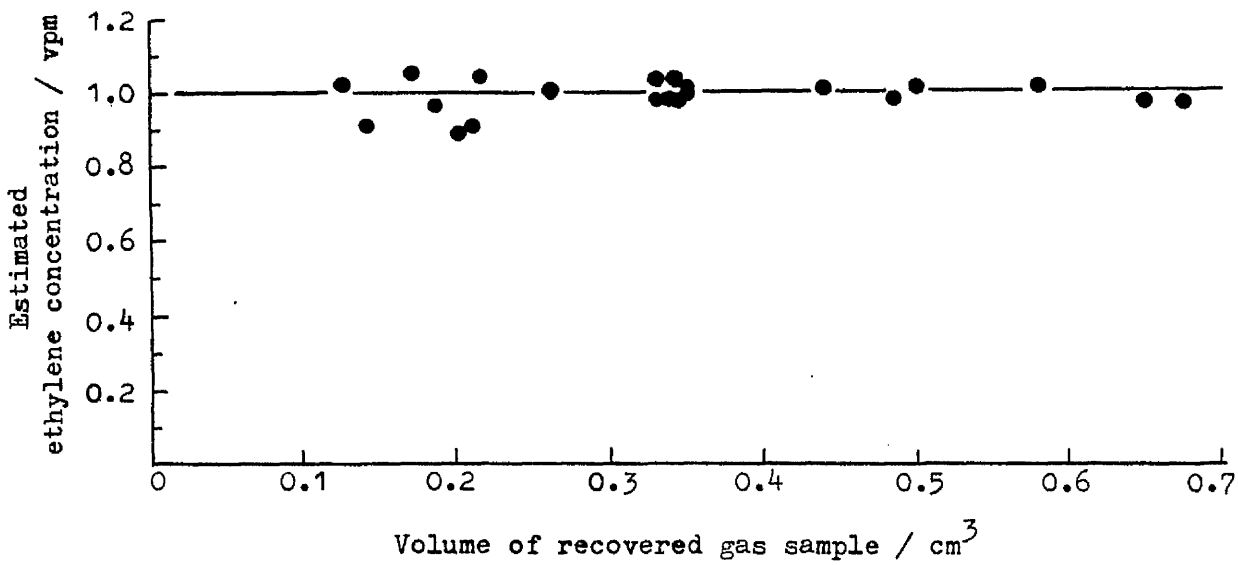

FIGURE 30. Estimation of the ethylene concentration in small gas samples. Various volumes of standard 1 vpm ethylene were bubbled under saturated ammonium sulphate solution into the apparatus used to extract the internal gases in plants (see Fig. 5). The ethylene samples were then recovered by the procedure illustrated in Fig. 29 and injected into the GC. The values presented are the apparent ethylene concentrations after correcting for the syringe "dead" space in the calculations as described in the text. The straight line indicates the actual ethylene concentration (1 vpm).

greater than 0.25 cm^3, the error in the estimate did not exceed ± 4%. As expected, the margin of error increased when smaller samples were injected into the GC. This loss in accuracy stemmed from the fact that lower responses of the FID were obtained from the smaller samples and that small errors which occurred when the samples were handled were more critical in small samples. Nevertheless, ethylene concentrations estimated from samples 0.15 - 0.25 cm^3 were still generally within 10% of the actual value. A potential source of error in the analysis of small volumes of vacuum extracted gas samples was the ethylene artifact arising from the Clinbritic seal during vacuum extraction. Small amounts of ethylene were released by old seals or seals which were not ventilated properly into the gas bubble lying in contact with the seal during vacuum extraction. As a precaution, therefore, air was injected into the extraction bottle, subjected to vacuum and then checked for ethylene artifacts at the beginning of each set of analyses.

Effects of evacuation conditions

Plants with an expanding 3rd trifoliate leaf were used to compare the effects of applying a vacuum of 13.3 kPa for 2 min with a vacuum of 66.7 kPa for 3 min in the extraction of the internal gases from *Phaseolus* leaves. The 2nd trifoliate leaf was just fully expanded or nearing full expansion while the primary leaves were fully developed but not showing visible signs of senescence. The paired primary leaves or paired leaflets of the 2nd trifoliate were assigned one of a pair to either evacuation treatment, thus minimizing the effects of variation between plants. Eight leaflets of the second trifoliate or four primary leaves were used in each extraction. The order in which the two evacuation treatments were carried out was alternated between successive extractions to eliminate any temporal artifact. The effect of the two evacuation treatments on the volume of gases recovered and on the concentrations of ethylene and ethane in the extracted gases are

presented in Table 10. Ethylene and ethane content in the extracted gases were observed to be substantially modified by changes in the evacuation conditions. The manner and extent of the modifications were dependent on the leaf being examined and were not always the same for ethylene and ethane. In the extraction of the leaflets of the 2nd trifoliate, intensification of the ethylene extraction increased the volume of the internal gases recovered, the ethane concentration in the extracted gases and the total ethylene and ethane per unit weight of leaf tissue. (Total ethylene or ethane = Concentration x volume of gas recovered per unit weight of leaf tissue.) Ethylene concentration in the gases was not significantly changed. When the primary leaves were subjected to the stronger of the two evacuation treatments, the volume of gases recovered was again increased as compared with the weaker extraction treatment while both the ethylene and ethane concentrations were significantly reduced. Increases were noted in the total ethylene and ethane contents. Although the differences were not statistically significant, the increase in total ethylene was nevertheless observed in nine out of twelve extractions while the increase in total ethane was observed in ten out of twelve extractions.

The concentration of the internal ethylene and ethane in the leaves decreased with the age of the leaves; being highest in the 2nd trifoliate leaves, lowest in the primary leaves and intermediate in the 1st trifoliate leaves. There were strong correlations between the concentration of ethylene and that of ethane ($r = 0.862^{***}$, Fig. 31a) and between their total contents ($r = 0.771^{***}$, Fig. 31b). The relationship between the volume of extracted gases per g tissue and the concentrations of internal ethylene and ethane are given in Figs. 32 and 33 respectively. When the concentrations of these two gases were plotted against the reciprocal of the volume per g tissue, linear correlations were observed for both ethylene ($r = 0.805^{***}$, Fig. 34) and ethane ($r = 0.629^{***}$, Fig. 35).

Two series of experiments involving vacuum extraction at 66.7 kPa were

TABLE 10. Effect of evacuation conditions on the volume of gas recovered and the concentrations of ethylene and ethane therein. Values are means and standard errors of 12 readings. Significance in the differences of the readings between the two evacuation treatments is indicated.

Leaf/ leaflet	Vacuum applied	Volume of gases recovered (mm^3 per leaf or leaflet)	Fresh weight (g per leaf or leaflet)	Vol. of gases per unit wt. of leaf ($mm^3 g^{-1}$)	Ethylene concentration (vpm)	Ethane concentration (vpm)	Total ethylene per unit wt. of leaf ($10^3 \mu m^3 g^{-1}$)	Total ethane per unit wt. of leaf ($10^3 \mu m^3 g^{-1}$)
2nd trifoliate	13.3kPa; 2 min	27.2 ± 3.4	0.30 ± 0.02	90.4 ± 9.2	0.16 ± 0.01	0.25 ± 0.03	14.3 ± 1.6	24.2 ± 4.6
	66.7kPa; 3 min	33.4 ± 4.2	0.30 ± 0.02	110.3 ± 12.2	0.18 ± 0.02	0.34 ± 0.03	18.2 ± 1.6	37.3 ± 5.3
		**	N.S.	**	N.S.	**	**	**
Primary	13.3kPa; 2 min	242 ± 23	1.16 ± 0.07	207 ± 14	0.053 ± 0.004	0.076 ± 0.009	10.7 ± 0.9	15.3 ± 1.9
	66.7kPa; 3 min	314 ± 24	1.15 ± 0.06	274 ± 15	0.042 ± 0.004	0.066 ± 0.008	11.7 ± 1.1	17.2 ± 2.2
		***	N.S.	***	***	*	N.S.	N.S.

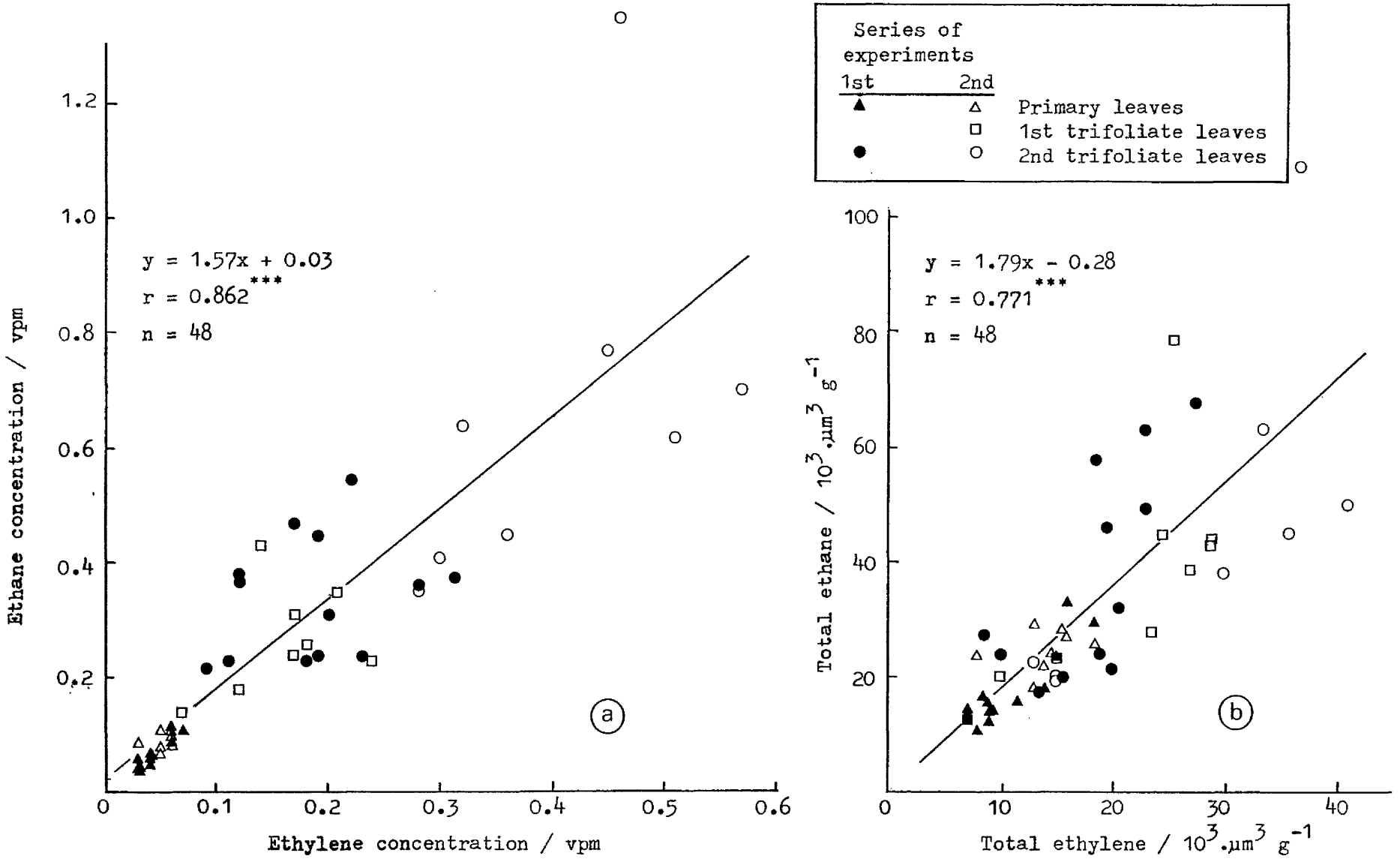

FIGURE 31. Relationship between endogenous ethylene and ethane in Phaseolus leaves. Ethylene and ethane levels are expressed as (a) concentration in the gaseous extract and (b) total ethylene or ethane per unit weight of tissue. The results presented are based on vacuum extraction of the gaseous content of leaves at 66.7 kPa for 3 min. When extractions were carried out at 13.3 kPa for 2 min, significant correlations were simlarly obtained between ethylene and ethane concentrations ($r = 0.824^{***}$) and between total ethylene and ethane ($r = 0.863^{***}$) (data not presented).

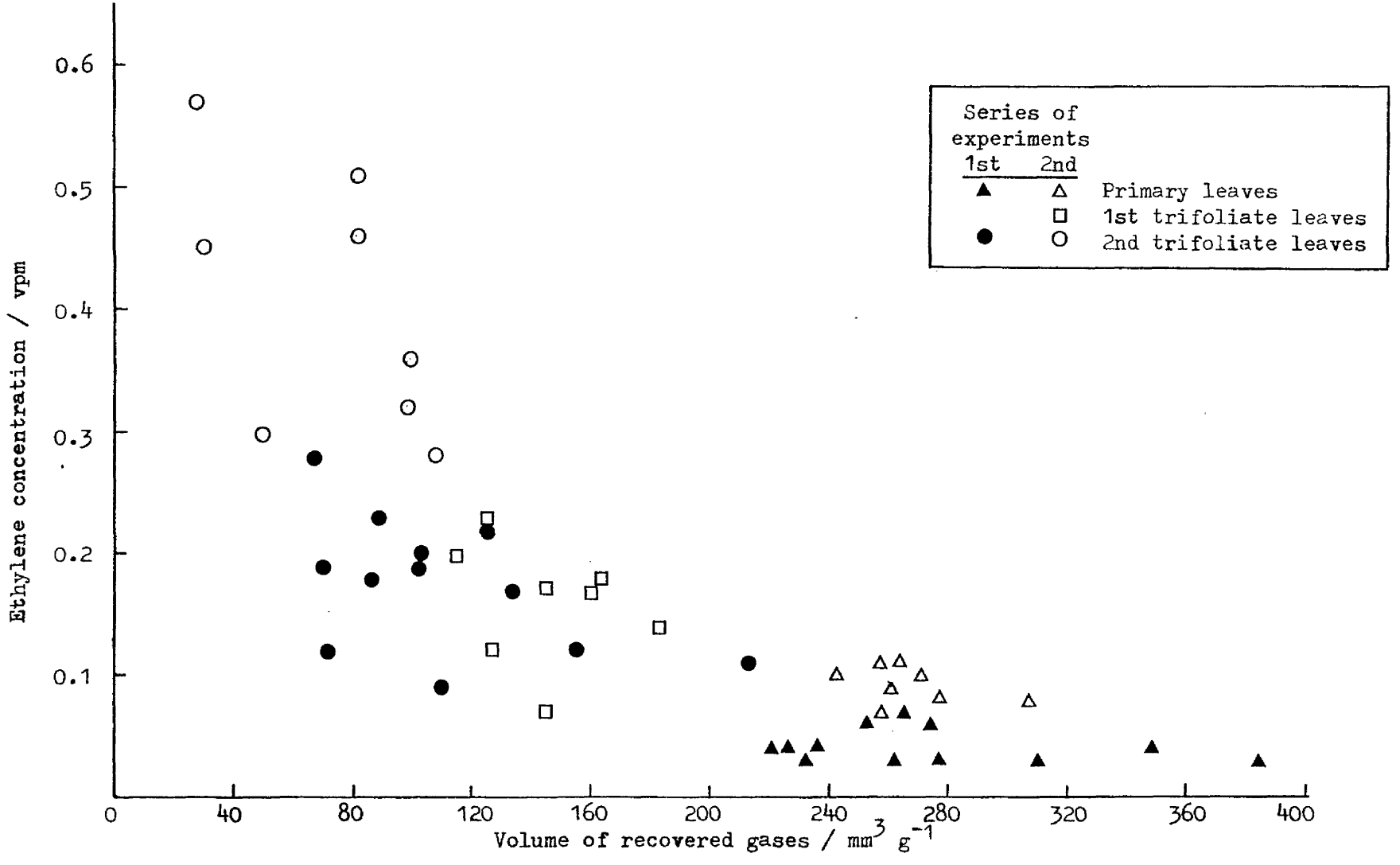

FIGURE 32. Relationship between the volume (per unit weight of tissue) of gases extracted from Phaseolus leaves by applying a vacuum of 66.7 kPa for 3 min and the concentration of ethylene in the gases.

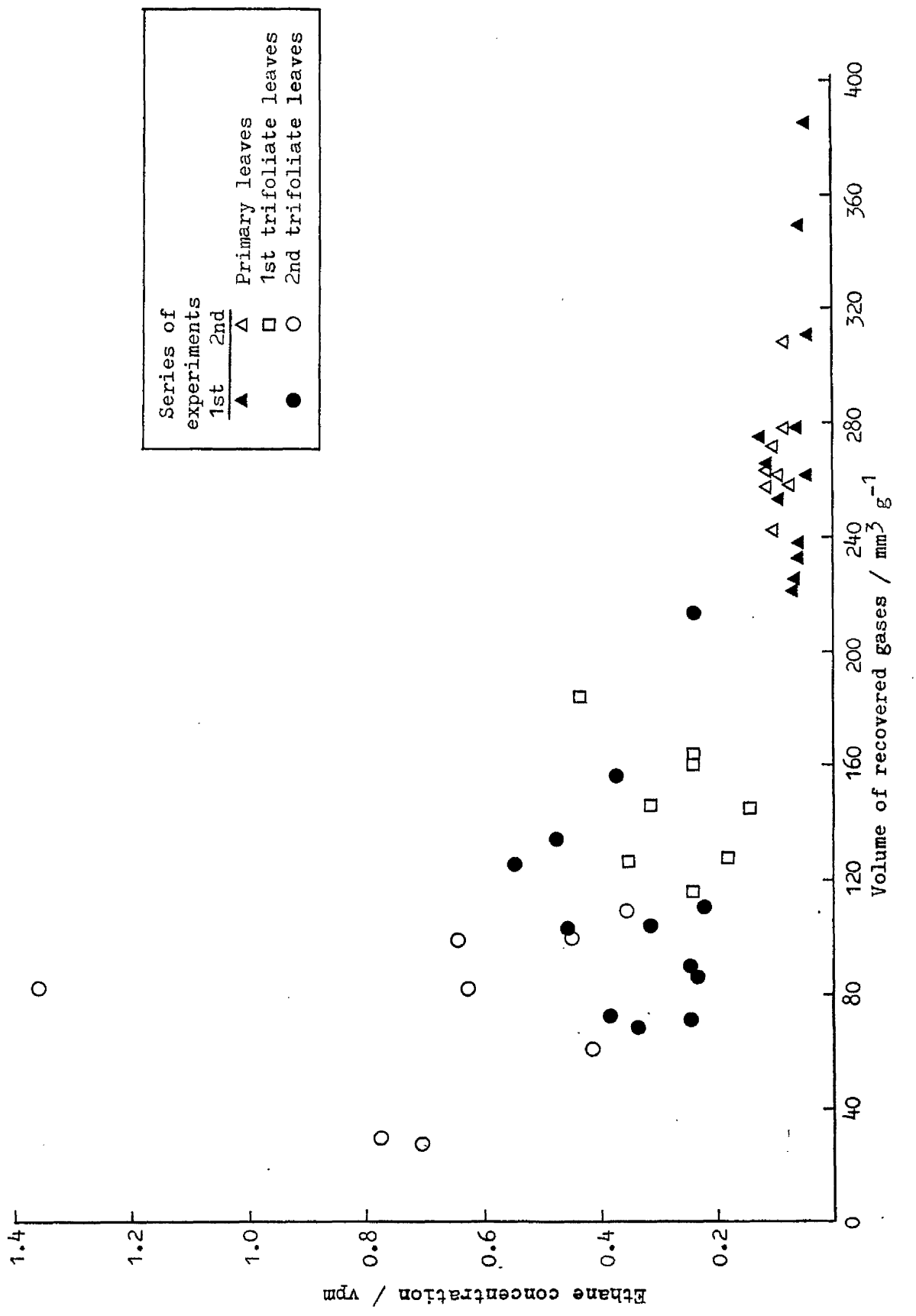

FIGURE 33. Relationship between the volume (per unit weight of tissue) of gases extracted from Phaseolus leaves by applying a vacuum of 66.7 kPa for 3 min and the concentration of ethane in the gases.

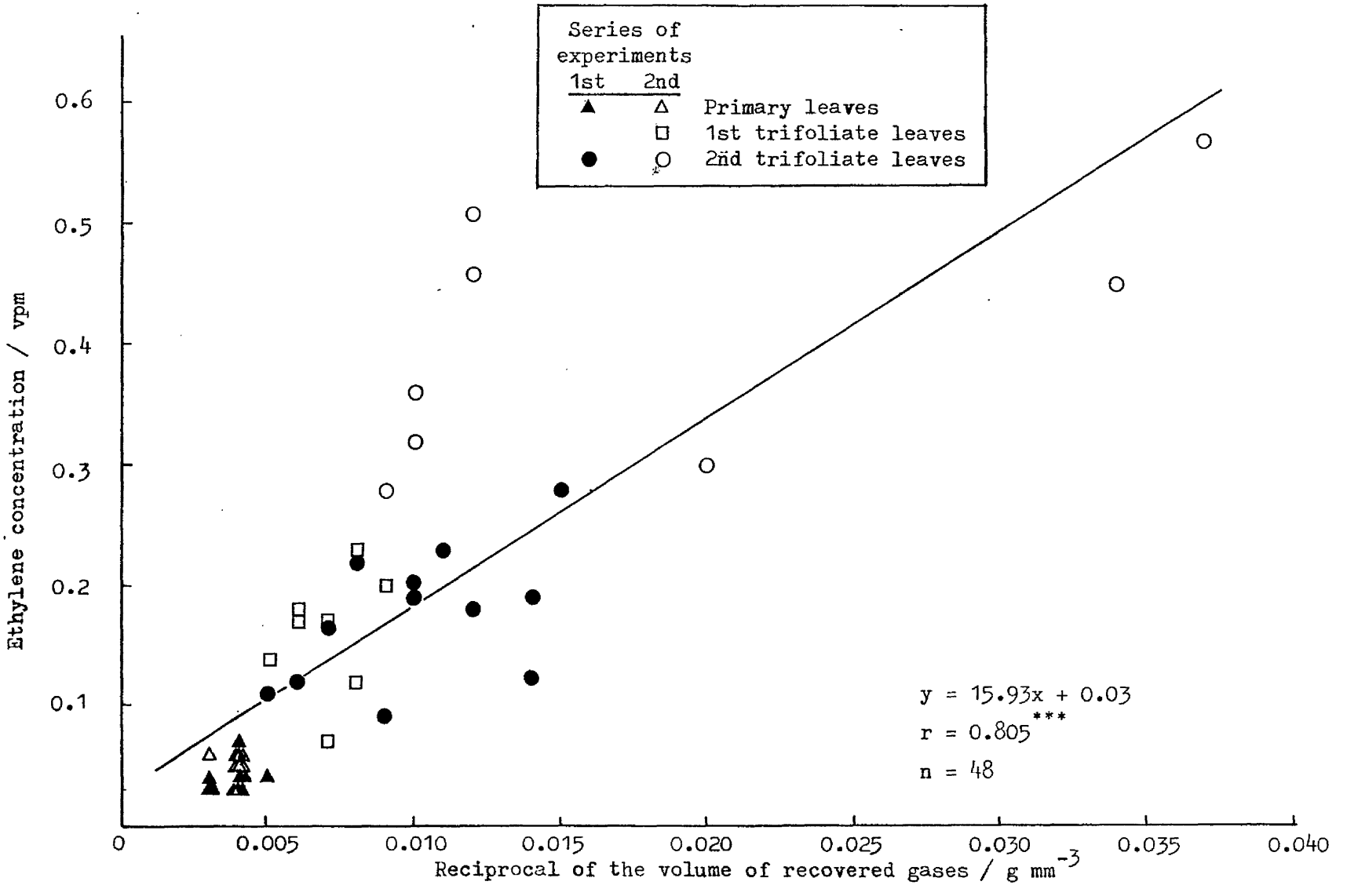

FIGURE 34. Relationship between the reciprocal of the volume (per unit weight of tissue) of gases extracted from Phaseolus leaves and the concentration of ethylene in the gases. Extraction was carried out using a vacuum of 66.7 kPa for 3 min. A significant correlation ($r = 0.711^{***}$) was similarly obtained when the extraction was carried out at 13.3 kPa for 2 min (data not presented).

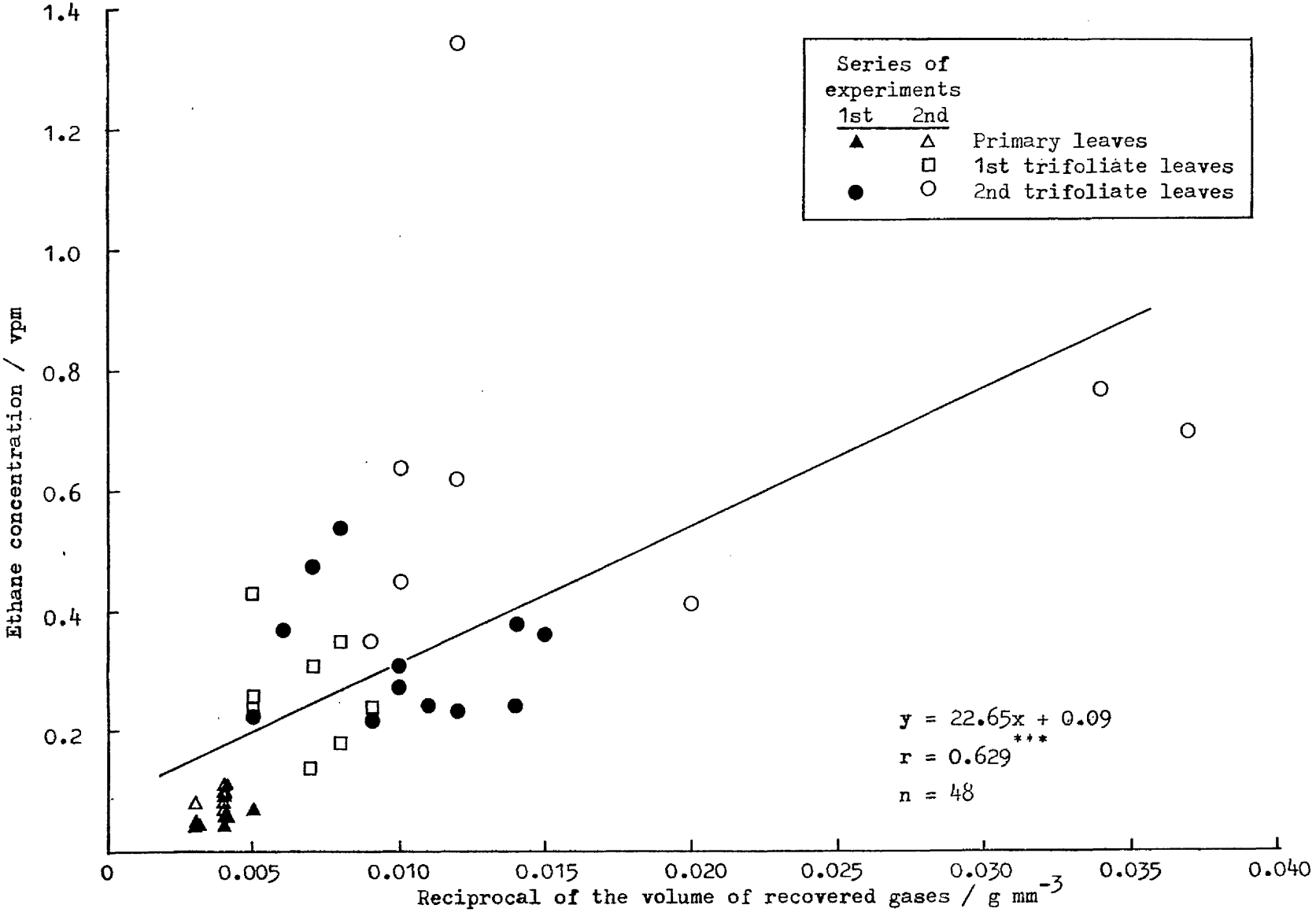

FIGURE 35. Relationship between the reciprocal of the volume (per unit weight of tissue) of gases extracted from Phaseolus leaves and the concentration of ethane in the gases. Extraction was carried out using a vacuum of 66.7 kPa for 3 min. A significant correlation ($r = 0.387^{*}$) was also obtained when the extraction was carried out at 13.3 kPa for 2 min (data not presented).

carried out. The results presented in Table 10 are from the first series while a second series of extractions was made to study the relation between internal ethylene and ethylene emanation (see next section). The internal concentrations of ethylene and ethane were consistently higher in the second series while the volumes of extracted gases were lower.

Relation between internal ethylene and ethylene emanation

Plants with an expanding 3rd trifoliate leaf were used to determine the relationship between the internal ethylene in leaf tissue and its rate of ethylene release. Either of the paired primary leaves or paired leaflets of the 1st and 2nd trifoliates were assigned one to vacuum extraction (66.7 kPa; 3 min) and the other to the estimation of ethylene emanation. For the latter analysis, twelve lateral leaflets or eight primary leaves were enclosed in a bell jar (235 cm^3) lined with moist filter paper. The order in which the two analyses were carried out was alternated between successive determinations.

Although ethylene was readily detected after the 20 min incubation period, ethane was found only in minute amounts, usually insufficient for accurate quantification. The relationship between internal ethylene and ethylene emanation in the primary leaves and the 1st and 2nd trifoliate leaves is presented graphically in Fig. 36. A linear correlation existed between the two methods by which endogenous ethylene from *Phaseolus* was quantitatively expressed ($r = 0.827^{***}$).

Discussion

In the analysis of the internal ethylene content of plant tissue, it is desirable to use large samples of plant material in the extraction procedure. Sufficiently large amounts of extracted ethylene or ethane are required for accurate quantification by GC-FID and errors arising from

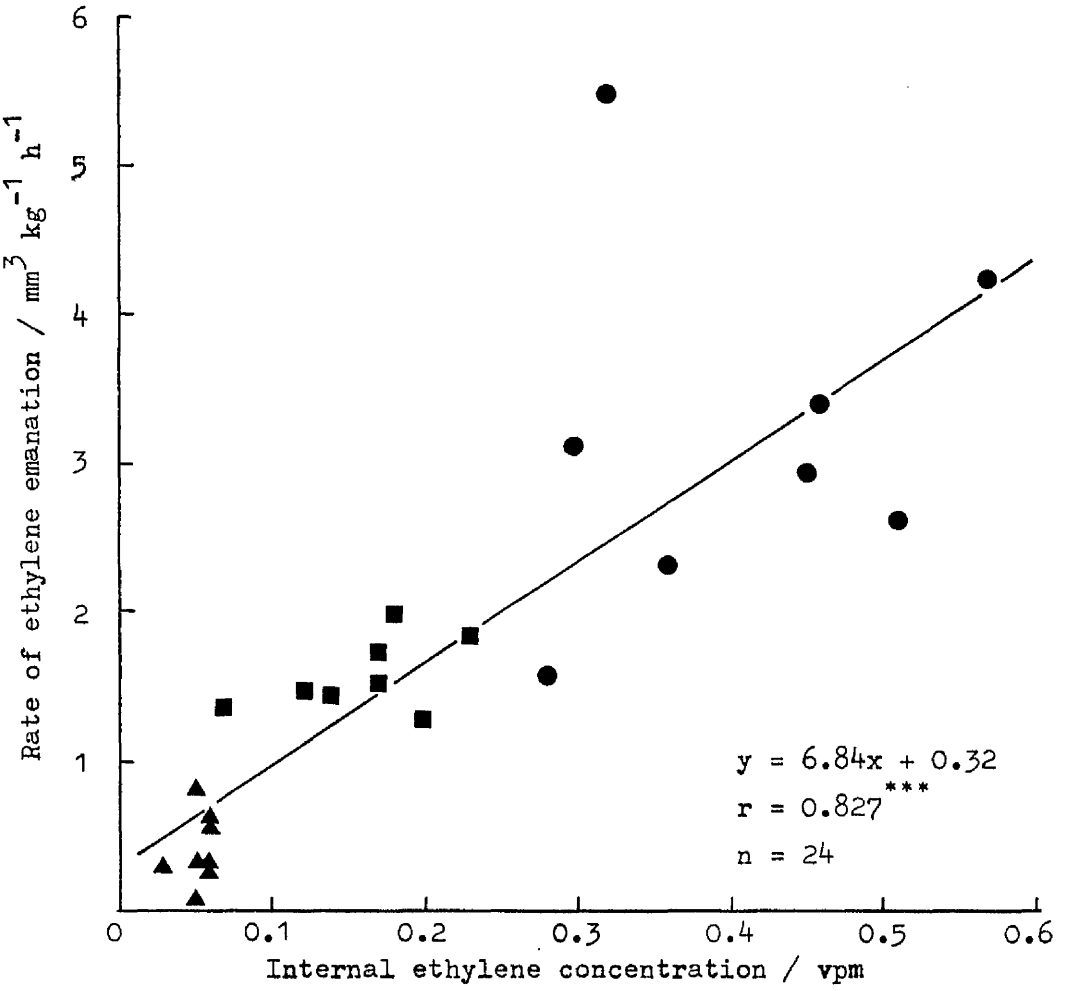

FIGURE 36. Relationship between the internal ethylene concentration in Phaseolus leaves and the rate of ethylene emanation. A vacuum of 66.7 kPa was applied for 3 min to extract the gaseous content of the leaves.

▲ = Primary leaves; ■ = 1st trifoliate leaves;
● = 2nd trifoliate leaves.

handling the gas samples (e.g. the diffusion loss from the syringe during injection into the GC) are likely to be less significant. However, it is not always possible to have large amounts of plant material for extraction. In some experiments, the test plants are first subjected to experimental treatments which require specialised apparatus and equipment and which are necessarily limited in number. In other instances, it might be desirable to analyse only a small selected portion of the test plant. The method described above for the extraction and recovery of gas samples from small amounts of plant tissue is simple and requires only minimal adaptation of the basic method of Beyer and Morgan (1970b).

A notable characteristic of the concentrations of ethylene and ethane for the same evacuation conditions was the strong correlation between the two gases over the range of concentrations (Fig. 31a). This trend is consistent with similar observations in the diurnal variation of internal ethylene and ethane concentrations in unstressed *Faba vulgaris* leaves (El-Beltagy and Hall, 1974). The relationship between the concentrations of ethylene and ethane suggests that the concentrations were dependent on one or more over-riding factors common to both gases. An obvious factor that could affect both ethylene and ethane concentrations simultaneously and by the same extent is the amount of other gases that are extracted together with ethylene and ethane: the greater their dilution by other gases, the lower their concentrations. The involvement of a dilution factor is confirmed by plotting ethylene and ethane concentrations against the reciprocal of the volume of extracted gas per unit weight of leaf tissue, whereby positive linear correlations were obtained (Figs. 34, 35). The relationship between the volume of gases extracted and the internal ethylene and ethane concentrations was apparent between readings from the three different leaves (primary, first and second trifoliate). This relationship was not obvious when data from each leaf were considered separately. The dilution factor is thus a relevant consideration when

the concentrations of internal ethylene and ethane between different leaves are considered. Accordingly, the differences in the internal content of ethylene and ethane per unit weight of the tissue between the different leaves might be far less than is suggested by their concentrations in the samples of extracted gases. For example, in Table 10, the concentration of ethylene in gases extracted using a vacuum of 66.7 kPa was 4.3 times higher in the 2nd trifoliate leaves than in the primary leaves. When internal ethylene was expressed as the quantity per unit weight of plant tissue, the difference was only 1.6 fold. In the same manner, the differences in the volumes of gases (per unit weight of tissue) recovered from leaves in the first and second series of extractions accentuated the differences in the internal ethylene and ethane concentrations between the series.

It is not clear how much of the gases extracted by vacuum originated from the inter-cellular air spaces and how much was intra-cellular air. Nevertheless, the proportion of inter-cellular air spaces in a given volume of plant tissue can be expected to be an important determinant of the internal ethylene and ethane concentration. In this context, it is conceivable that the decrease in the internal concentrations of these two gases with the age of the leaves (as determined by the vacuum extraction method) is due to a considerable extent to the increase in inter-cellular spaces in the tissues as they mature.

Inasmuch as the dilution by other extracted gases is an important determinant of the internal concentrations of ethylene and ethane, the correlation between the two is not adequately explained by a common dilution factor. The relationship persists strongly even when the amounts of gases present in the tissues are expressed as total ethylene and total ethane (Fig. 31b). It is possible that the correlation between the two gases stemmed from factors governing their synthesis. Although the biosynthesis of ethylene from methionine does not involve ethane, production of the two compounds in the tissue could be subject to a common indirect

metabolic control. It is possible also that the extraction procedure itself was responsible for an apparent correlation between ethylene and ethane content. Perhaps the propensity towards extraction from the plant tissue under different conditions was similar in endogenous ethylene and ethane.

Although intensifying the vacuum treatment increases the volume of gases extracted, the resultant effect is not one of simple dilution alone; the effect being different in the primary leaves and 2nd trifoliate leaves. Nevertheless, the results from the vacuum extraction of *Phaseolus* leaves by the two evacuation treatments provide useful clues regarding the status of ethylene and ethane in the leaf tissues.

To simplify explanations, endogenous ethylene and ethane may be envisaged to occur in a single homogeneous pool of freely accessible and freely diffusible inter-cellular gases as "free" ethylene and ethane. Emanations of ethylene and ethane from the cells would pass first into these gases in the inter-cellular spaces before diffusing out of the plant tissue. In addition, ethylene and ethane might also occur in "bound" forms which are retained at intra- and/or extra-cellular sites. Drawing from indirect experimental evidence, Beyer and Morgan (1970b) proposed that vacuum extraction of vegetative plant tissue in various species, including *Phaseolus vulgaris*, at 13.3 kPa for 2 min gave an accurate assessment of ethylene concentration in the "gas phase" (i.e. inter-cellular gases) with negligible interference from dissolved or bound ethylene. If this proposition is tenable even when the intensity of the extraction is increased to 66.7 kPa for 3 min, the additional gases extracted by the latter procedure should still be entirely inter-cellular gases. Assuming that a single homogeneous pool of inter-cellular gases exists, there should be no difference in ethylene concentration in the gases extracted by the two procedures. The fact that a difference in ethylene concentrations was observed in extractions of the primary leaves

and differences in ethane concentrations were observed in both the 2nd trifoliate and primary leaves indicate that the gases recovered in the two treatments were not all derived from the same pool.

Further circumstantial evidence for the existence of separate pools of extractable gases lies in the observation that only trace amounts of ethane emanated from the leaves, whereas there was free outward diffusion of ethylene. This is in contrast to the fact that ethane was found in vacuum-extracted gases from the leaves in concentrations higher than those of ethylene. Although release of ethane can arise from tissue injury (Elstner and Konze, 1976), any suggestion that the ethane recovered by vacuum extraction was an artifact of wounding during the extraction procedure would require ethane to be formed within the 2 or 3 min the extraction was carried out. The meagre ethane emanation indicates that the freely accessible inter-cellular spaces in the leaf tissues contain only very small amounts of the gas. This implies that ethane occurs in a "bound" state from which free diffusion into the air is restricted. The large amounts of ethane contained in the gaseous extracts obtained by using a vacuum of 13.3 kPa showed that "bound" ethane was recovered even by this relatively weak vacuum treatment.

The amounts of extracted ethylene and ethane (expressed as the total per unit weight of tissue) were generally greater when the vacuum was increased from 13.3 to 66.7 kPa. However, the decrease in internal ethylene and ethane concentrations in the primary leaves as a result of intensifying the vacuum contrasts with the effects on the 2nd trifoliate leaves. Internal ethane concentration increased in the 2nd trifoliate leaves while internal ethylene concentration remained unchanged. The fall in ethylene and ethane concentrations in the primary leaves may be explained by postulating that the additional gases recovered by the stronger evacuation treatment included large amounts of intra-cellular gases which contained relatively little ethylene and ethane; hence, a dilution in the inter-cellular gases (with respect to ethylene and ethane

concentrations) resulted. This explanation does not presuppose the occurrence of "bound" ethylene (although its presence would not invalidate the reasoning). An alternative explanation for the decrease in ethylene and ethane concentrations could be that a large proportion of the extractable "bound" ethylene and ethane was already effectively recovered using a vacuum of 13.3 kPa. Increasing the vacuum to 66.7 kPa did not increase substantially the amount of "bound" ethylene and ethane extracted but did increase the volume of inter-cellular gases recovered; hence the dilution effect. The two explanations proposed are not mutually exclusive and both could have contributed to the experimental results.

The mechanisms responsible for the dilution effect in internal ethylene and ethane concentrations in the primary leaves when the vacuum was intensified might also be operating in the 2nd trifoliate leaves. The effect could, however, be less significant in the 2nd trifoliate leaves and accordingly did not result in a net decrease in ethylene and ethane concentrations.

The results from this study indicate that whereas the amount of ethylene (expressed as the total per unit weight of leaf tissue) increased when vacuum extraction was intensified, the ethylene concentration in the recovered gases was either unchanged or was reduced. This is in contrast to the observation by Beyer and Morgan (1970b) of an increase in internal ethylene concentration in cotton vegetative and fruit tissue when the evacuation was intensified. Although there is strong circumstantial evidence from the present study to suggest the presence of "bound" ethane in the vacuum-extracted gases, conclusive evidence that "bound" ethylene was also present is lacking. The existence of "bound" ethylene is compatible with the experimental results but other interpretations of the results, which do not implicate "bound" ethylene, are possible.

The finding that the internal ethylene concentration in *Phaseolus* leaves declined with maturation, and that the rate of ethylene emanation showed a corresponding change, is in agreement with the observations of

Aharoni and Lieberman (1979b) for tobacco leaves.

The reference made above to ethane being in a "bound" state (since there was little emanation) might be comparable to the concept of "compartmented" ethylene (Jerie *et al.*, 1978b, 1979). Compartmentation of ethylene in plant tissue allows for the accumulation of the gas in a bound form in excess of the amount expected to have dissolved in the cell fluids. This phenomenon has the potential ability to modify ethylene emanation which is otherwise regulated by the rate of ethylene synthesis and metabolism and the physical resistance to outward diffusion of ethylene from the tissue. The above discussion makes no reference to the compartmentation of ethylene in *Phaseolus* leaves as this would not have materially altered the main points of the discussion. There is, in any case, little evidence to show that compartmentation occurs in normal, unstressed plant tissue. In the instances cited above where the phenomenon has been demonstrated, ethylene levels in the test sample had always been initially increased by experimental treatment.

3.4. INVOLVEMENT OF ETHYLENE IN APICAL DOMINANCE

In this section, the effects of ethylene, ethephon and ethylene inhibitors applied to the apical shoot or directly to the inhibited bud are examined with respect to changes in endogenous ethylene and ethane levels and the release of apical dominance. An appraisal is also made of experimental treatments relating to bud growth where exogenous ethylene is not involved, but where the action of induced ethylene is suspected. The promotion of axillary bud growth by physical restriction of the apical shoot and by TIBA are two such treatments evaluated in this study. Another aspect pertaining to this consideration is the possible involvement of IAA-induced ethylene in treatments where the auxin is applied to plant tissue.

3.4.1. Ethylene in the Apical Shoot in relation to Axillary Bud Growth

As the apical portion of the shoot plays an essential role in the correlative inhibition of axillary bud growth, experimental treatments which alter the physiology of the shoot apex and expanding leaves might modify the apical control of growth in the buds. The results of experiments carried out on this theme enable an assessment to be made of the relative importance of ethylene production, internal ethylene content and ethylene emanation on the outgrowth of axillary buds.

Effects of physical constriction

Enclosure of the shoot within a sealed glass tube above the 3rd node was found to induce vigorous growth of the 1st trifoliate axillary bud (Figs. 37, 40) and, frequently, growth of the larger primary leaf axillary bud. Corresponding axillary buds in control plants showed very little internode extension and the unexpanded leaf in the bud remained folded. During the first two days of treatment the enclosed shoot appeared healthy

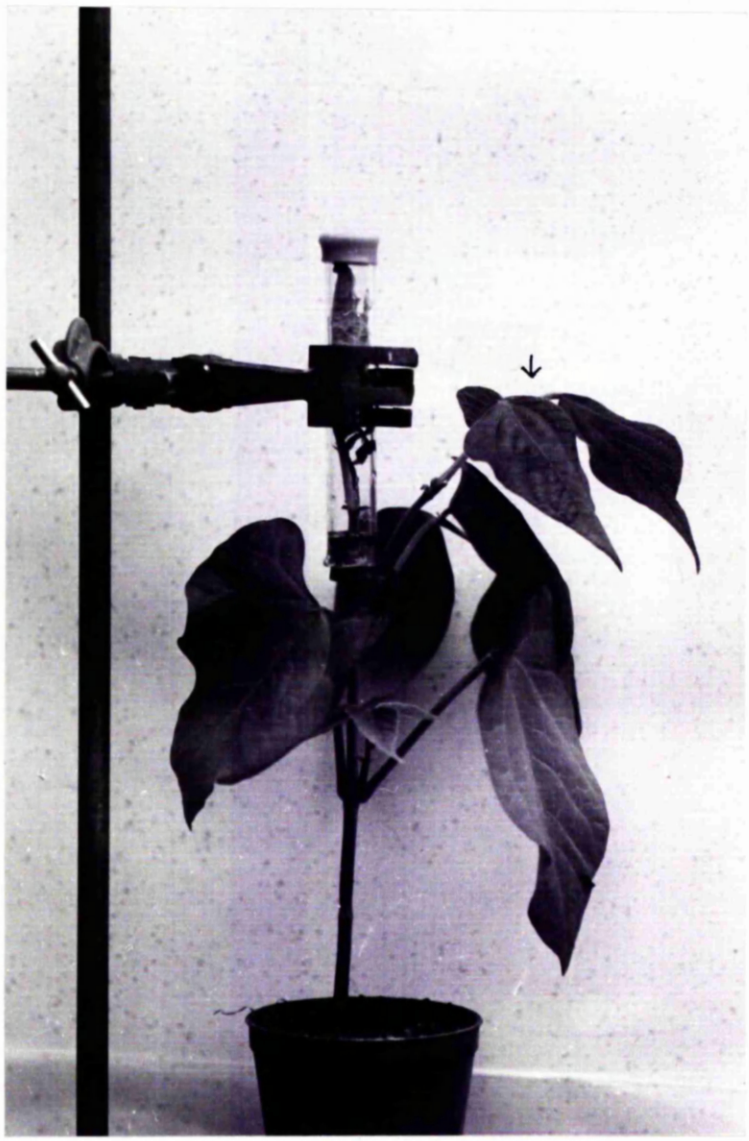

FIGURE 37. Enclosure of the shoot above the 3rd node by a sealed glass tube. The 1st trifoliate axillary shoot is indicated by the arrow. (Treated 8 days.)

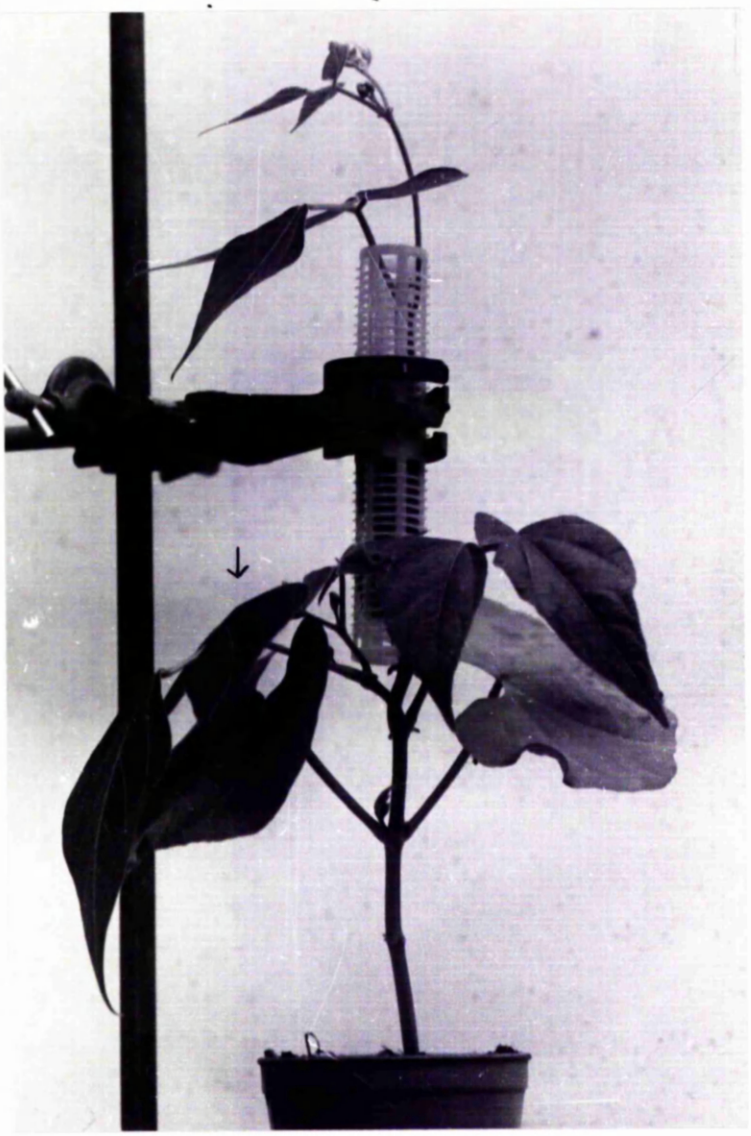

FIGURE 38. Enclosure of the shoot above the 3rd node by a plastic mesh tube. The 1st trifoliate axillary shoot is indicated by the arrow. (Treated 10 days.)

FIGURE 39. Enclosure of the shoot above the 3rd node by a paper tube. The 1st trifoliate axillary shoot is indicated by the arrow (Treated 7 days.)

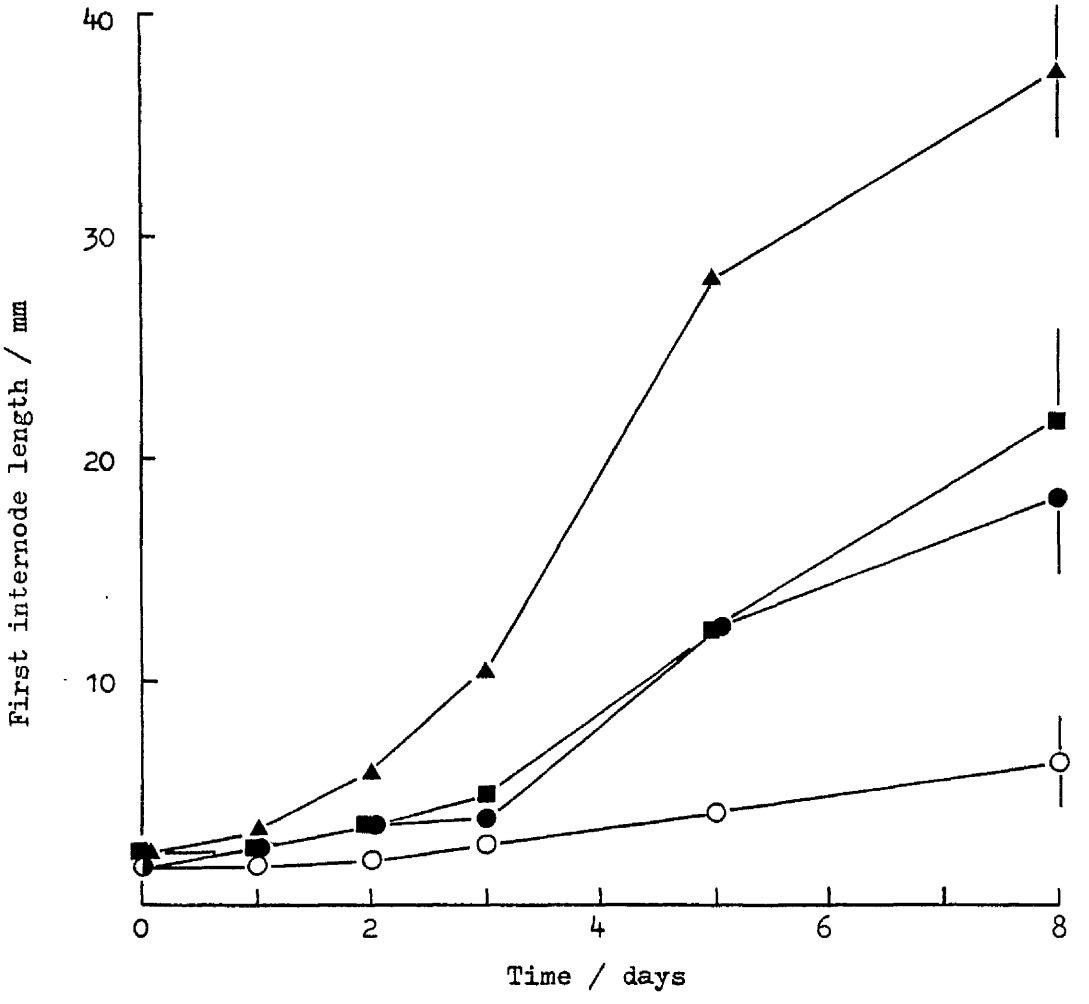

FIGURE 40. Effect of physical confinement of the shoot above the 3rd node on the growth of the 1st trifoliate axillary bud. Values are the means ± std. errors of nine plants per treatment.

○ = Intact controls; ■ = Shoots confined in plastic mesh tubes; ● = Shoots confined in unsealed glass tubes; ▲ = Shoots confined in sealed glass tubes.

with no overt signs of adverse effects. On prolonged treatment (9-12 days), abscission of the terminal bud typically occurred and in about half the instances the enclosed leaves senesced; but these developments occurred after axillary bud development had been well established. The enclosed shoot was severely retarded in growth, both in leaf expansion and internode elongation (Table 11). Convolutions developed in the enclosed

TABLE 11. Lengths of leaves and internodes and dry weight of the apical shoot nine days after the plants were enclosed above the 3rd node in plastic mesh tubes, unsealed or sealed glass tubes. Means of five plants per treatment. Values in each column bearing the same letter are not significantly different ($p < 0.05$).

Treatment	Length of central leaflet (mm)		Length of 4th internode (mm)	Dry weight of shoot above 4th node (mg)
	2nd trifoliate	3rd trifoliate		
Control	109.8 [a]	99.0 [a]	50.1 [a]	589 [a]
Plastic mesh tube	73.8 [b]	50.2 [b]	58.0 [a]	337 [b]
Unsealed glass tube	53.8 [c]	59.0 [b]	52.0 [a]	304 [b]
Sealed glass tube	44.2 [d]	24.0 [b]	20.6 [b]	65 [c]

leaves, giving a crinkled appearance (Fig. 41). This was apparently caused by the growth of the veins (vascular bundles) being inhibited to a greater extent than the inter-venal tissue of the lamina. The axillary bud on the enclosed 4th node would usually sprout, but further development was always curtailed. The concentration of ethylene in the sealed tubes during the first four days of treatment were of the order of 0.1 vpm . The tubes were found not to be completely ethylene-tight and so the ethylene present did not represent the cumulative total produced.

When the shoot was enclosed above the 2nd or 4th nodes, similar axillary bud development basipetal to the enclosure and inhibition of the

FIGURE 41. Crinkling of the leaf lamina caused by physical constriction.

enclosed shoot was observed (Tables 12, 13). Thus, this response was not confined to the 1st trifoliate axillary bud alone, but was common to other buds as well.

TABLE 12. Lengths (mm) of bud internodes, leaves and stem internodes six days after the plants were enclosed above the 4th node in sealed glass tubes.

Readings are means of five plants. All measurements are significantly different between control and treated plants ($p < 0.01$).

	Length of 1st internode		Length of central leaflet		Length of 5th stem internode
	1st trifoliate axillary bud	2nd trifoliate axillary bud	3rd trifoliate	4th trifoliate	
Control	5.1	3.5	114.2	44.2	79.6
Treated	29.0	20.4	44.2	12.8	26.0

TABLE 13. Lengths (mm) of bud internodes, leaves and stem internodes six days after the plants were enclosed above the 2nd node in sealed glass tubes.

Readings are means of five plants. All measurements are significantly different between control and treated plants ($p < 0.05$).

	Length of 1st internode			Length of central leaflet		Length of 3rd stem internode
	Larger cotyledonary axillary bud	Larger primary leaf axillary bud	Smaller primary leaf axillary bud	1st trifoliate	2nd trifoliate	
Control	0	1.3	0.1	119.8	103.4	45.4
Treated	4.4	19.8	11.3	46.0	31.6	23.2

When the experiments were repeated with the shoot enclosed in glass tubes but with either end left open, or enclosed with a tube of plastic

meshing, a similar response in axillary bud development was observed (Figs. 38,40). However, the axillary buds grew less vigorously than when the tubes were sealed and no growth of the primary leaf axillary buds occurred. The reduction in growth of the 2nd trifoliate leaf was less marked while there was no inhibition of internode growth and the shoot normally emerged from the upper open end of the tube (Fig. 38, Table 11). Generally similar observations were made in the 1st trifoliate axillary bud and the apical shoot when the latter was enclosed in perforated paper tubes except that growth of the shoot internode was also inhibited (Fig. 39, Table 14).

TABLE 14. Lengths (mm) of bud internodes, leaves and stem internodes four days after the plants were enclosed above the 3rd node in paper tubes.

Readings are means of ten plants. All measurements are significantly different between control and treated plants ($p < 0.05$).

	Length of internode of the 1st trifoliate axillary bud	Length of central leaflet		Length of 4th stem internode
		2nd trifoliate	3rd trifoliate	
Control	5.8	115.9	53.7	91.0
Treated	14.5	70.3	23.9	64.6

In experiments where the 2nd trifoliate leaf alone, but not the newly expanding 3rd trifoliate or terminal bud, was enclosed in a sealed tube, inhibition of growth and crinkling of the lamina were again seen in the treated leaf. Analysis of the air in the tube showed a level of ethylene comparable to tubes where the entire shoot had been enclosed (*ca.* 0.1 vpm). However, no axillary buds development occurred.

The internal ethylene content of shoots enclosed in plastic mesh tubes was found to be about 2.4 times that in controls after treatment for three

days (Table 15). There was no difference between ethane levels.

TABLE 15. Ethylene and ethane in constricted apical shoots and in the 1st trifoliate leaf and 2nd internode portions where no mechanical stimulus was applied. For the determination of internal ethylene content, apical shoots above the 3rd node were enclosed in plastic mesh tubes for three days while the shoots were enclosed in paper tubes for four days before the rates of ethylene emanation were determined. Figures in brackets denote the number of determinations on which the readings are based. Three apical shoots were used for each determination of internal ethylene and ethane and a single shoot used for each determination of ethylene emanation. Significance of the differences in the readings between control and treated plants is indicated.

	Internal ethylene concentration (vpm)		Internal ethane concentration (vpm)		Rate of ethylene emanation from the apical shoot (10) ($mm^3\ h^{-1}\ kg^{-1}$)
	Apical shoot (19)	1st trifol. and 2nd internode (16)	Apical shoot (19)	1st trifol. and 2nd internode (16)	
Control	0.34	0.27	2.75	0.71	1.81
Treated	0.82	0.27	2.45	0.88	8.28
	***	N.S.	N.S.	N.S.	***

Internal ethylene and ethane in the 1st trifoliate and 2nd internode (which were not enclosed) were not significantly different between treated and control plants, and were appreciably lower than in the younger apical shoots of either treated or control plants. The difference in the rates of ethylene emanation between control and constricted apical shoots was even more pronounced. Release of ethylene from apical shoots enclosed in perforated paper tubes was 4.6 times that from untreated shoots (Table 15).

Effects of ethylene

When apical shoots were enclosed in large glass vessels injected with ethylene, a loss of ethylene from the vessels sometimes occurred. Ethylene concentration fell from 0.5 vpm to 0.2 - 0.3 vpm by the fourth day in some

experiments, but the loss was variable and was balanced to an extent by the natural ethylene emanation from the enclosed shoots (Fig. 42). Vessels which were flushed daily had ethylene levels ranging from 0.02 vpm to about 0.1 vpm. Since air in the vessels was sampled just before a flushing was due, the concentration of ethylene for most of the time between flushings would have been considerably lower. Ethylene present in the glass vessels was effectively absorbed by mercuric perchlorate contained in dishes (Fig. 43) to form 2-hydroxyethylmercuric perchlorate ($HOCH_2CH_2HgClO_4$) (Rakitin and Rakitin, 1977). Ethylene was either not detected or just barely detectable (0.005 vpm) in glass vessels with mercuric perchlorate and the vessels were, for all practical purposes, ethylene-free. There was apparently a balance between the turnover of oxygen and carbon dioxide in respiration and photosynthesis by the enclosed shoot as the oxygen concentration in the air within the vessels, determined by GC, was not depleted during the course of the experiment. (The oxygen peak on the GC, identified by absorption with alkaline pyrogallol, was probably not a true flame ionization response but arose from oxygen affecting the FID flame. The peak height increased progressively, but not linearly, with the amount of air injected (Fig. 44). Where an air sample from the glass vessel gave a similar peak height as an identical volume of laboratory air, the oxygen concentrations were considered not significantly different in the two cases.)

Rapid growth of the 1st trifoliate axillary bud directly basipetal to the enclosure, and, occasionally, growth of the larger primary node axillary bud was observed in the ethylene-treated plants. Development of similar buds in control plants with shoots enclosed in vessels that were flushed or in vessels where ethylene was absorbed by mercuric perchlorate was not significantly different from intact controls (Table 16, Fig. 45a). Addition of 20 vpm ethane to vessels containing mercuric perchlorate or 0.5 vpm ethylene did not affect the trends in the growth of the axillary buds or

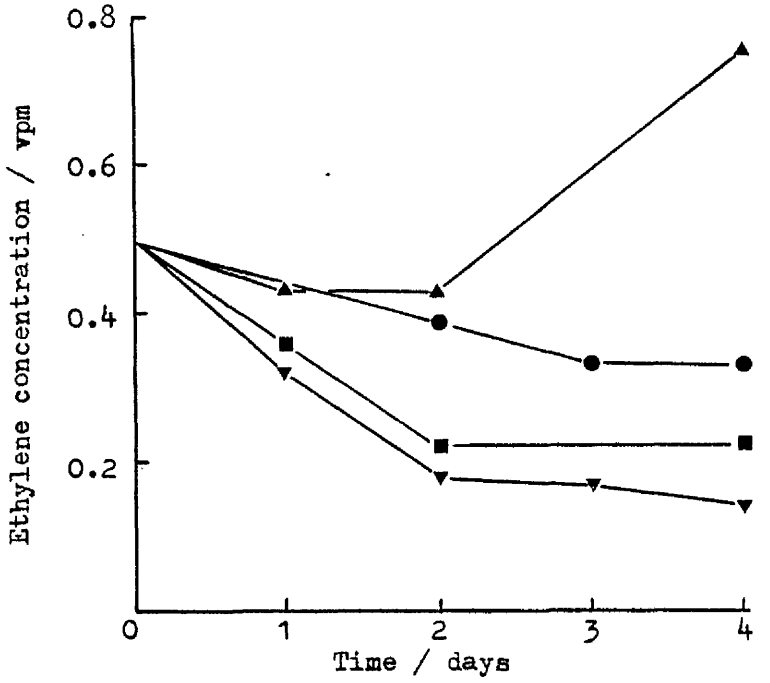

FIGURE 42. Changes in ethylene concentration of the air within glass vessels enclosing apical shoots. Ethylene was injected into the vessels at the beginning of the experiment to give a concentration of 0.5 vpm. Four representative curves are presented and the values are the means from three vessels.

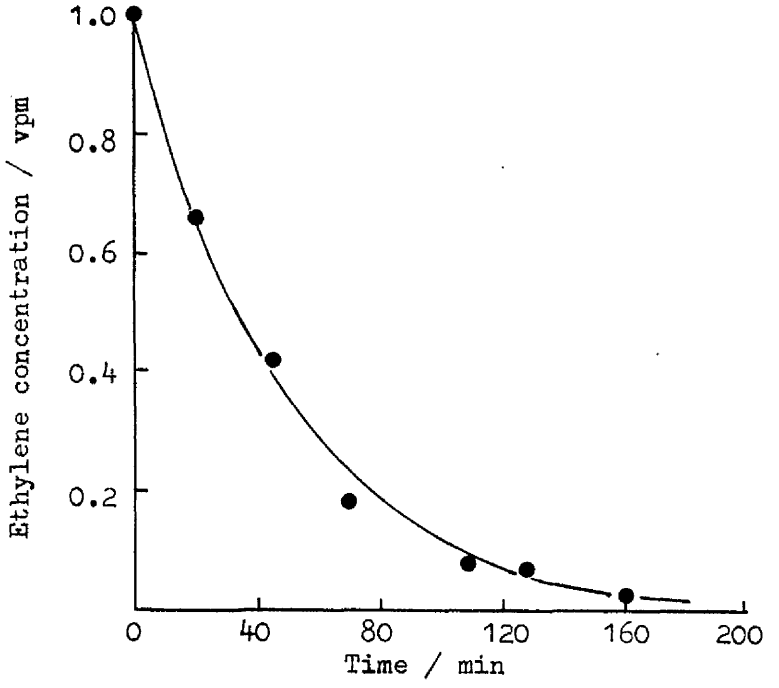

FIGURE 43. Absorption of 1 vpm ethylene from a glass vessel (used to enclose apical shoots) by 250 mol m^{-3} mercuric perchlorate in 2 kmol m^{-3} perchloric acid. The solution (6 cm^3) was dispensed into a shallow dish in the vessel giving a surface area of absorption of 1800 mm^2.

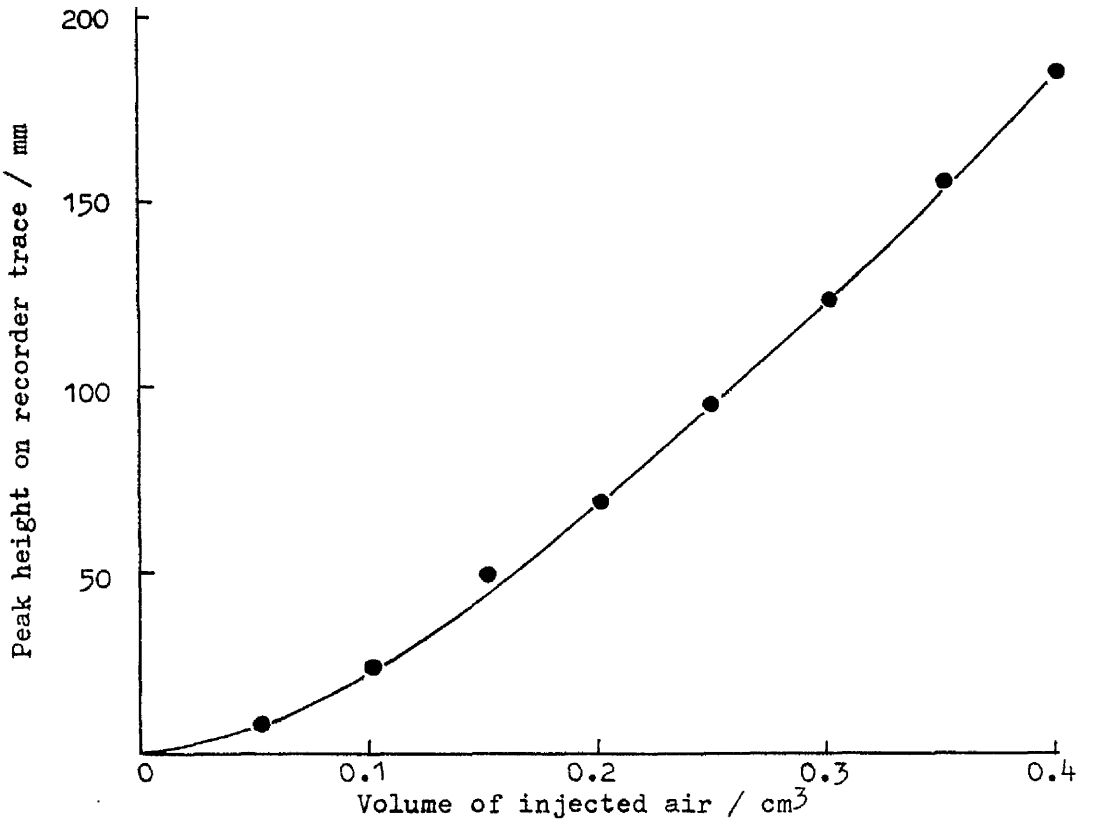

FIGURE 44. Relationship between the volume of air injected into the GC and the collector response due to oxygen.

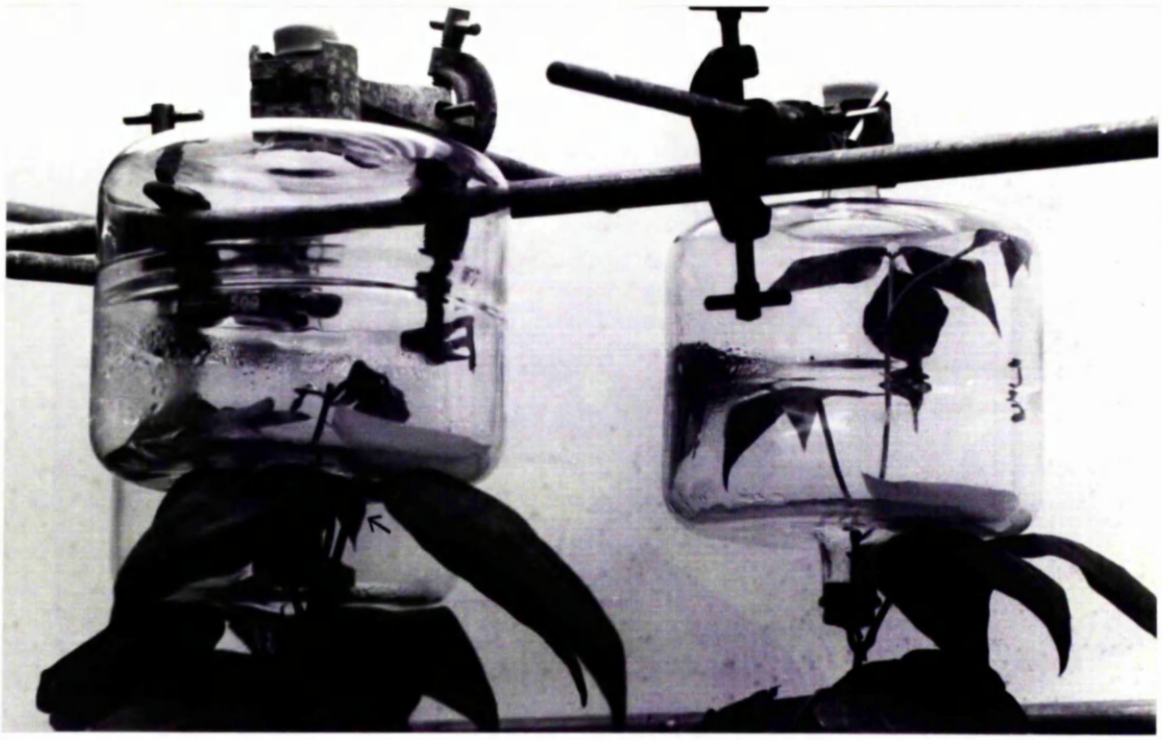

FIGURE 45a. Enclosure of the shoot above the 3rd node by large glass vessels containing ethylene-enhanced air (left) or ethylene-depleted air (right). The 1st trifoliate axillary shoot in the plant supplied with ethylene is indicated by the arrow. The corresponding axillary bud in the plant not treated with ethylene remains undeveloped. Dishes used to contain mercuric perchlorate (or control solutions of perchloric acid or water) are visible on the floor of either vessel. (Treated 4 days).

FIGURE 45b. The enclosed shoots removed from the glass vessels. The 2nd trifoliate leaf which has been enclosed in an ethylene-depleted atmosphere shows slight chlorosis; it appears light green with darker green regions around the veins (plant on the left). In the shoot supplied with ethylene, a loss of geotropic control in the sub-apical internode is evident.

the enclosed shoots as compared to similar treatments without supplementation of ethane (Table 17).

TABLE 16. Lengths (mm) of bud internodes, leaves and stem internodes four days after the plants were enclosed above the 3rd node in glass vessels. Air in the vessels was enhanced with ethylene (0.5 vpm), depleted of ethylene using mercuric perchlorate or flushed daily.

Means of five plants per treatment. Values in each column bearing the same letter are not significantly different ($p < 0.05$).

Treatment	Length of 1st internode of the 1st trifoliate axillary bud	Length of central leaflet		Length of 4th stem internode
		2nd trifoliate	3rd trifoliate	
Control	3.5 [a]	116.7 [a]	54.1 [a]	72.3 [a]
Ethylene-depleted	2.3 [a]	89.7 [a]	52.3 [ab]	65.0 [a]
Flushed	3.1 [a]	78.6 [b]	45.3 [b]	61.2 [a]
Ethylene-enhanced	11.8 [b]	74.9 [b]	22.9 [c]	37.3 [b]

TABLE 17. Effect of 20 vpm ethane on the modification of shoot and axillary bud growth by 0.5 vpm ethylene applied to the apical shoot.

Means of six plants per treatment. Measurements (mm) were made after four days and values bearing the same letter are not significantly different ($p < 0.05$).

	Length of 1st internode of the 1st trifoliate axillary bud	Length of central leaflet		Length of 4th stem internode
		2nd trifoliate	3rd trifoliate	
Control	3.1 [a]	106.2 [a]	41.3 [a]	80.7 [a]
20 vpm ethane + mercuric perchlorate	2.7 [a]	77.5 [b]	45.2 [a]	75.0 [a]
20 vpm ethane + 0.5 vpm ethylene	15.9 [b]	60.8 [c]	12.2 [b]	24.7 [b]

The shoots of ethylene-treated plants showed typical responses to ethylene: a marked reduction in leaf growth and internode extension (Table 16) and a nyctinastic reaction in the enclosed leaves. However, unlike leaves grown under conditions of physical confinement, the lamina was not crinkled. There was a loss of normal geotropic control in the sub-apical (5th) internode which tended to grow horizontally (Fig. 45b). The terminal bud (which included the unexpanded 4th trifoliate leaf) abscinded in about half the cases after 3-4 days of treatment. Unenclosed controls with the terminal bud excised one day after the beginning of the experiment did not show any significant increase in axillary bud growth within the experimental period, indicating that the abscission of the apical bud did not contribute appreciably to the growth response of the axillary buds. This is in agreement with the finding (White, Medlow, Hillman and Wilkins, 1978) that even in the absence of the terminal bud, the presence of an expanding trifoliate leaf exerted effective correlative inhibition on the basipetal axillary buds.

Shoots enclosed in ethylene-free air in control vessels showed no significant inhibition of internode extension (Table 16). Although there was a decrease in the growth of the 2nd trifoliate leaf as compared with intact controls, this was not as marked as in the ethylene-treated shoots. Growth of the 3rd trifoliate leaf was not affected. A slight chlorosis in the 2nd trifoliate leaf was sometimes observed, the leaf then appearing light green with darker green regions around the veins (Fig. 45b). Development of shoots that were enclosed in vessels which were flushed daily was intermediate between the ethylene-enhanced and ethylene-depleted treatments (Table 16). Growth inhibition in the 2nd trifoliate leaf was practically as severe as in ethylene-treated shoots. However, the 3rd trifoliate leaf was much less affected as was the 4th internode, which was not significantly different from that in the intact control. The chlorosis in the 2nd trifoliate leaf observed in shoots grown in an ethylene-depleted

atmosphere was not seen in shoots contained in vessels which were flushed.

Vacuum extraction of the apical shoots showed that all three treatments - ethylene enhancement, ethylene depletion and daily flushing of air in the vessels - gave rise to an increase in the internal ethylene concentration. The increase persisted even after the plants had been removed from the vessels and ventilated for 2.5 h or one day (Fig. 46). Internal ethane concentration was consistently increased in the ethylene depletion treatment, but was unchanged when ethylene was added to the vessels. The result of the flushing treatment on the internal ethane concentration was inconclusive (Fig. 46).

In contrast to the internal ethylene content, the rate of ethylene emanation was not increased by any of the three treatments (Fig. 47). It was notable, however, that when the treated plants were removed from the vessels and ventilated for 1.5 h before determining the rate of ethylene emanation, an approximately four to five fold increase in the rate was observed. The probable reason for this increase is that the removal of the apical shoots from the high humidity (due to transpiration) inside the vessels resulted in the shoots being water-stressed. Certainly, the leaves of enclosed shoots appeared more flaccid than those of control plants. The effects of water-stress on ethylene release are dealt with in section 3.5.

Effects of ethephon

Ethephon (2-chloroethylphosphonic acid) applied to the plant surface decomposes readily to release ethylene. The mechanisms relating to the decomposition of the compound have been summarised by Klein *et al.* (1979). Ethephon (50 or 100 ppm) applied to the apical portion of the shoot induced outgrowth of axillary buds below the point of application. Treatment of the abaxial lamina surface of the 2nd trifoliate leaf promoted growth in the 1st trifoliate axillary bud and, frequently, to the larger primary leaf axillary bud. Ethephon painted on the terminal bud (which included the

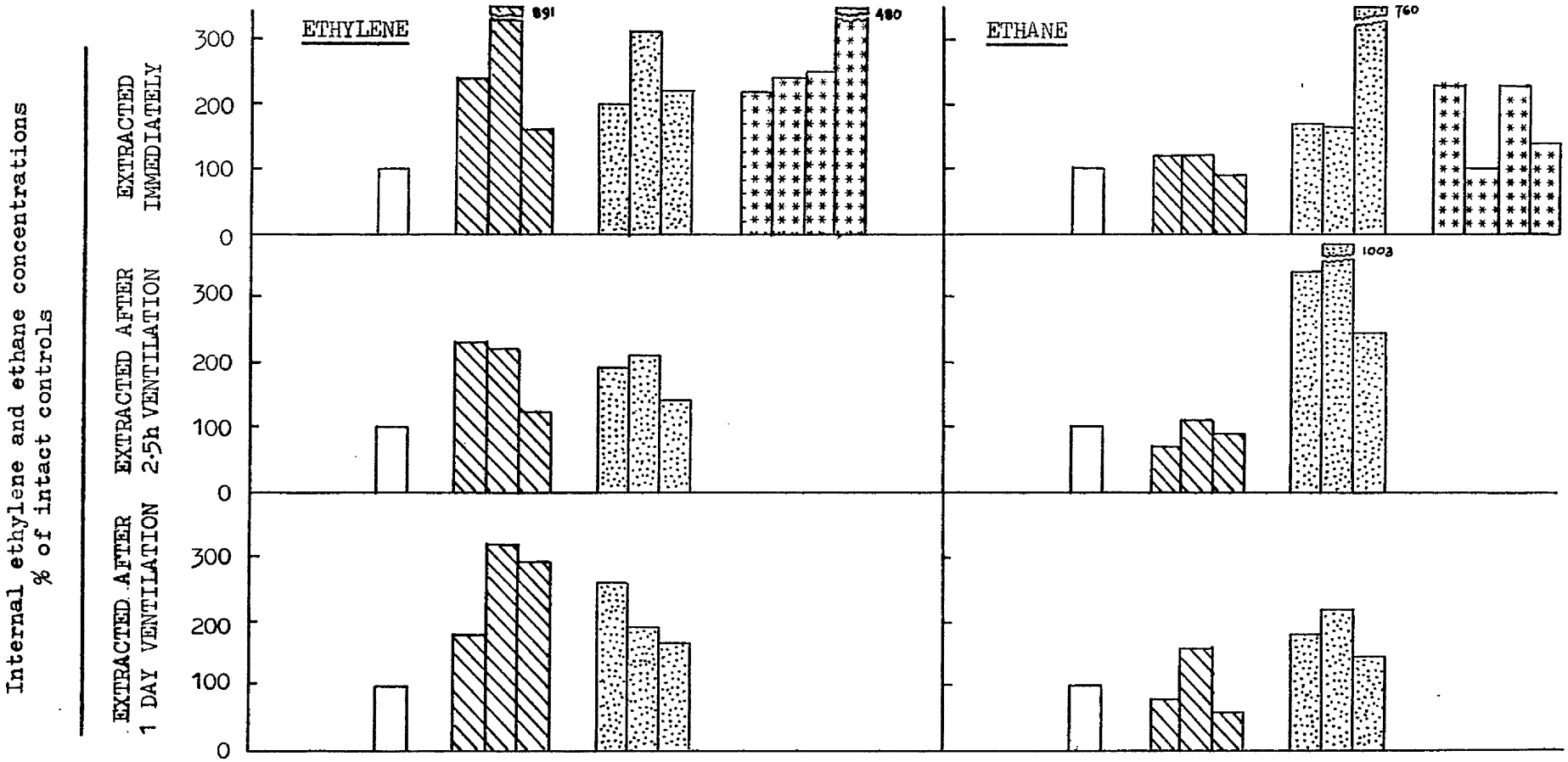

FIGURE 46. Internal ethylene and ethane content in apical shoots after enclosure in glass vessels for four days. Air in the vessels was enhanced with ethylene (0.5 vpm), depleted of ethylene by absorption with mercuric perchlorate, or the vessels were flushed daily. Vacuum extractions of the shoots were carried out immediately after their removal from the vessels, or extractions were delayed for 2.5 h or one day. Concentrations of ethylene and ethane in the gases recovered from the shoots varied between experiments, and were particularly influenced by the medium in which the plants were grown. Typical ethylene concentrations in gas samples from intact control shoots of plants grown in loam-based compost and in Levington compost were 0.5 and 0.25 vpm respectively; typical ethane concentrations were 1.8 and 0.8 vpm respectively. In this figure, the concentrations of gases recovered from the treated shoots are expressed as % of the concentrations in the respective intact controls. Results from three or four experiments involving each treatment are presented. Material from three plants were used for each extraction of internal gases. □ = Intact control; ▨ = Ethylene enhanced ⁝⁝ = Ethylene depleted; ⁘ = Vessels flushed.

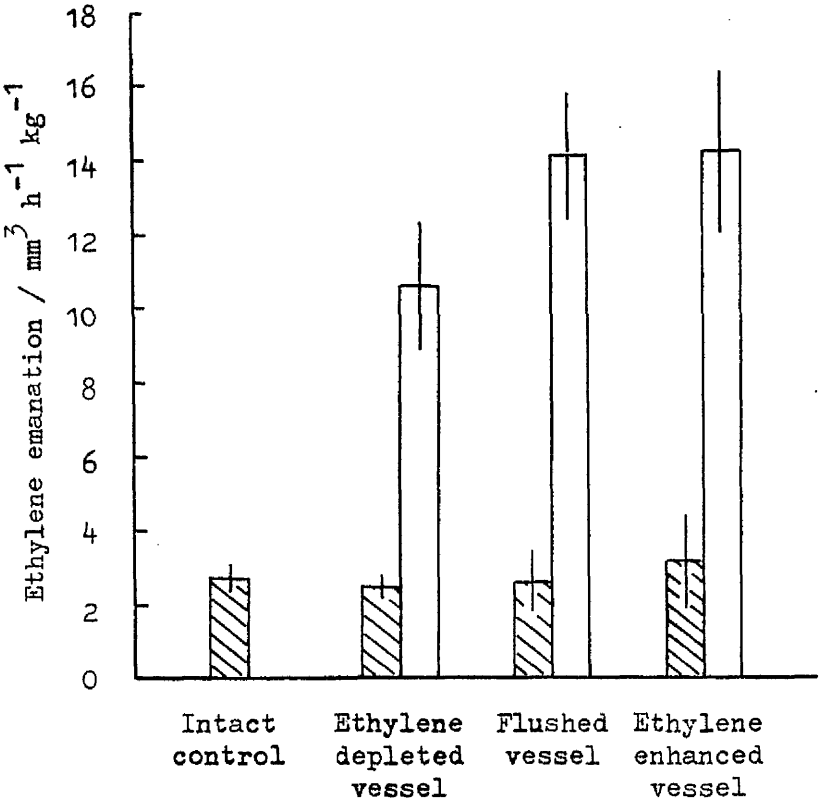

FIGURE 47. Ethylene emanation from apical shoots enclosed above the 3rd node in glass vessels. Air in the vessels was enhanced with ethylene (0.5 vpm), depleted of ethylene by absorption with mercuric perchlorate, or the vessels were flushed daily.

Shaded bars: Readings taken immediately after the shoots were removed from the glass vessels, or readings from intact controls.

Unshaded bars: Readings taken after the shoots had been ventilated for 1.5 h following removal from the vessels.

Values are the means ± std. errors of at least five determinations made after four days of treatment.

folded 3rd trifoliate leaf) induced outgrowth of the 1st trifoliate axillary bud. Growth of the 2nd trifoliate axillary bud was often enhanced as well. The effects of applications to other parts of the apical shoot such as the sub-apical 4th internode or 2nd trifoliate leaf petiole were usually only marginal (Fig. 48a). Leaflets on the treated leaf, and often also those on adjacent leaves, displayed a nyctinastic response to the treatment. A reduction in the growth of the treated leaf and developing leaves and internodes acropetal to it was observed. The terminal bud frequently abscinded in the later part of the experiment when 100 ppm ethephon was applied, whether to the 2nd trifoliate leaf or terminal bud. However, this was observed after outgrowth of the axillary buds had occurred. When ethephon concentration was reduced to 50 ppm, abscission of the terminal bud was rare and the inhibition of shoot growth reduced (Fig. 48b). Results from the application of 50 ppm ethephon are presented in Table 18.

TABLE 18. Effect of 50 ppm ethephon on the growth of the apical shoot and axillary bud after seven days.

Means of ten plants per treatment. Values (mm) in each column bearing the same letter are not significantly different ($p < 0.05$).

Tissue treated	Length of 1st internode of the 1st trifoliate axillary bud	Length of central leaflet		Length of 4th stem internode
		2nd trifoliate	3rd trifoliate	
Control	6.0 [a]	90.7 [a]	50.9 [a]	53.7 [a]
2nd trifoliate leaf	14.4 [b]	71.3 [b]	57.6 [a]	37.8 [b]
Terminal bud	17.3 [b]	88.3 [a]	49.0 [a]	30.6 [b]

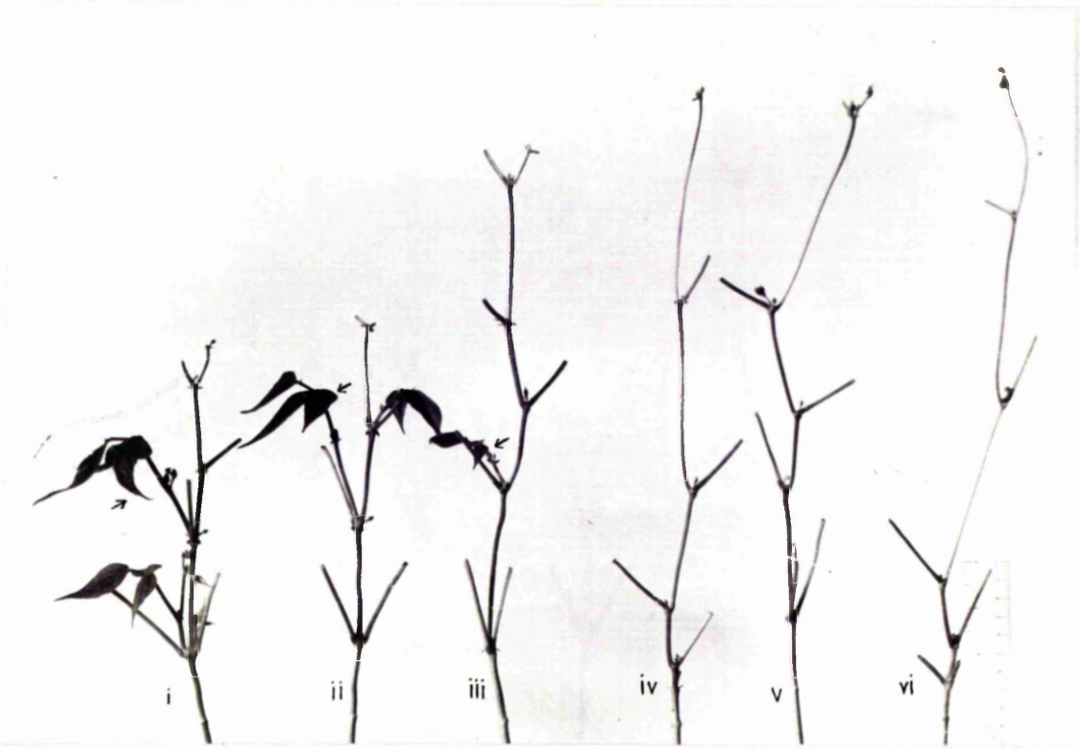

FIGURE 48a. Treatment with ethephon.(Leaf laminae have been removed to show the axillary buds and shoots clearly.)
Development of axillary shoots nine days after treatment with 100 ppm ethephon.

(i) Ethephon applied to the abaxial surface of the 2nd trifoliate leaf lamina;
(ii) Ethephon applied to the terminal bud (including the folded 3rd trifoliate leaf);
(iii) Ethephon applied to the 5th internode;
(iv) Ethephon applied to the petiole of the 2nd trifoliate leaf;
(v) Untreated control;
(vi) Control with the surfactant Triton X-100 applied to the 2nd trifoliate leaf.

The 1st trifoliate axillary shoots are indicated by arrows.

FIGURE 48b. Development of axillary shoots eight days after treatment with 50 ppm ethephon.

Left: Ethephon applied to the abaxial surface of the 2nd trifoliate leaf lamina;
Middle: Ethephon applied to the terminal bud (including the folded 3rd trifoliate leaf).
Right: Control.

The 1st trifoliate axillary shoots are indicated by arrows.

Effects of ethylene inhibitors

The expanding 2nd trifoliate leaf and terminal bud were treated with 0.1 mol m^{-3} aminoethoxyvinyl glycine (AVG) or 0.5 mol m^{-3} silver nitrate at the beginning of the experiment and two days later. AVG and Ag^{+} are known to inhibit ethylene biosynthesis and action respectively in plants.

Both ethylene inhibitors had no significant effect on the growth of the axillary buds or the treated shoot itself (Table 19). Ag^{+}, but not AVG, caused a slight crinkling of the treated leaves.

TABLE 19. Effect of 0.1 mol m^{-3} AVG and 0.5 mol m^{-3} silver nitrate applied to the 2nd trifoliate leaf and terminal bud on the growth of the apical shoot and axillary bud. The plants were treated on day 0 and day 2, and the measurements (mm) were taken on day 4. Values are the means of eight plants per treatment and all measurements are not significantly different between treatments ($p < 0.05$).

Treatment	Length of 1st internode of the 1st trifoliate axillary bud	Length of central leaflet		Length of 4th stem internode
		2nd trifoliate	3rd trifoliate	
Control	4.8	107.8	40.4	89.1
AVG	4.1	110.0	37.7	70.3
Silver nitrate	4.8	96.1	41.1	63.4

Ethylene levels in the shoots of the treated and control plants were analysed four days from the beginning of the experiment. AVG reduced ethylene emanation and internal ethylene content by roughly the same proportion as compared with the untreated control (Fig. 49). Ag^{+} increased internal ethylene, but had no significant effect on ethylene release. There was a tendency towards an increase in internal ethane in both treatments.

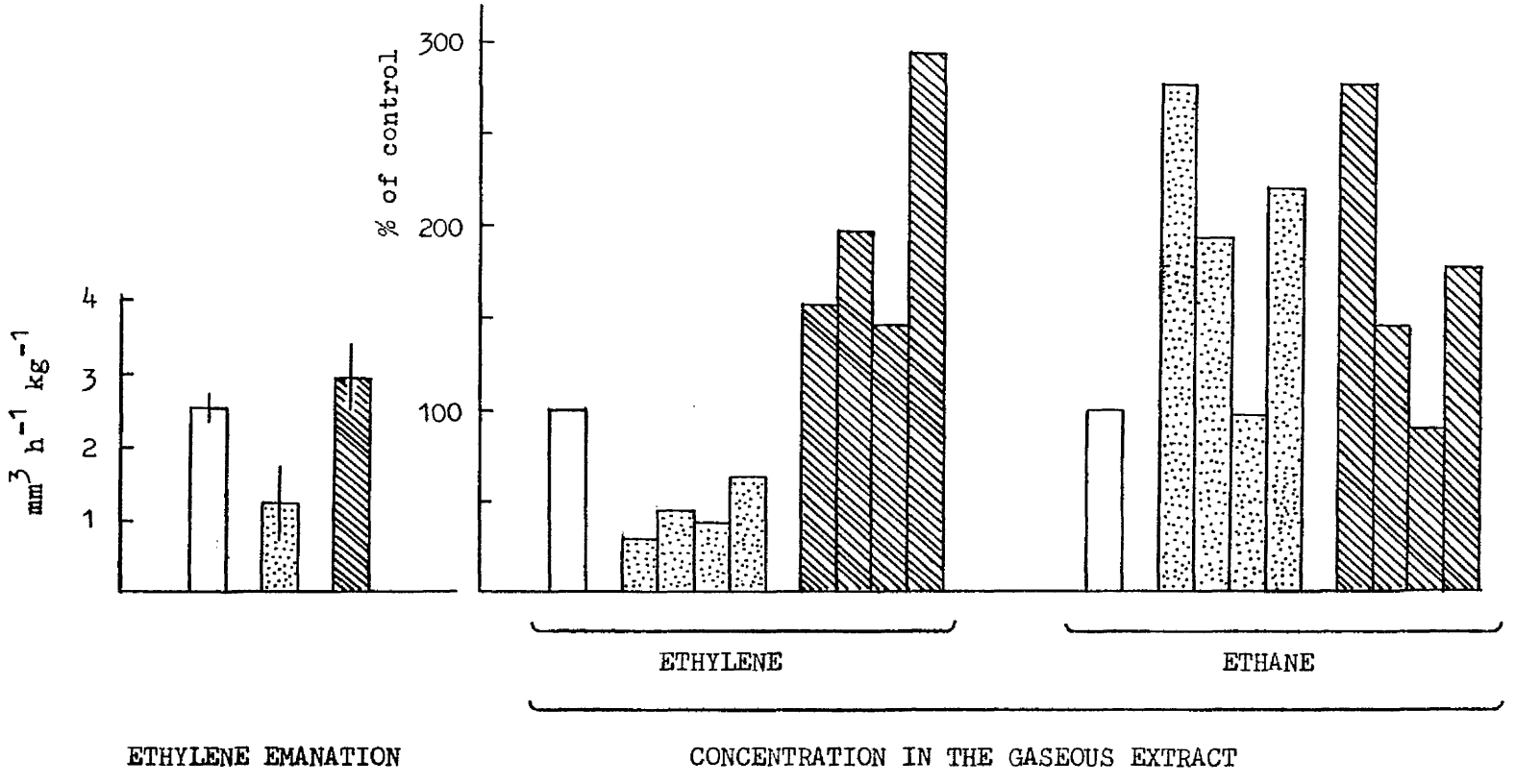

FIGURE 49. Effect of 0.1 mol m^{-3} AVG and 0.5 mol m^{-3} silver nitrate applied to the apical shoot on its rate of ethylene emanation and on its internal ethylene and ethane contents. Values for ethylene emanation are the means ± std errors of seven apical shoots. Results from four determinations of internal ethylene and ethane are presented individually and are expressed as % of the values from intact controls. □ = Intact control; ▨ = AVG-treated; ▨ = Silver nitrate-treated.

Effects of tri-iodobenzoic acid

To avoid the chemical pruning effects of tri-iodobenzoic acid (TIBA), a relatively low concentration of 500 μg g^{-1} prepared in hydrous lanolin was applied as a ring around the 3rd internode and then wiped away two days later. The general responses of the axillary buds, internodes and leaves to TIBA treatment were similar to those described by White and Hillman (1972), but without the propensity of young plant organs to abscind.

Outgrowth of the 1st trifoliate axillary bud followed TIBA treatment (Table 20, Fig. 50). Development of the larger (and sometimes, also the smaller) primary leaf axillary bud was initially slow, but in some experiments, its growth outstripped that of the 1st trifoliate axillary bud in the later stages of the experiment (4-7 days).

TABLE 20. Effect of 500 μg g^{-1} TIBA applied as a ring to the 3rd internode (and removed after two days) on the growth of the apical shoot and axillary buds. Measurements (mm) were taken after six days and the values presented are the means of five plants. Significance of the differences in the readings between control and treated plants are indicated.

	Length of 1st internode		Length of central leaflet		Length of 4th stem internode
	Larger primary leaf axillary bud	1st trifoliate axillary bud	2nd trifoliate	3rd trifoliate	
Control	0.9	5.7	121.4	80.0	77.6
Treated	13.8	16.3	107.0	20.4	28.2
	***	**	N.S.	**	**

TIBA inhibited the growth of the shoot acropetal to the point of application. Only the very young leaves were significantly affected in this way. The 2nd trifoliate leaf which was about one-third expanded at the beginning of the experiment and lying immediately above the point of TIBA application was only slightly inhibited in its growth. The effect

FIGURE 50. Development of axillary shoots six days after treatment of the 3rd internode with TIBA. The 1st trifoliate axillary shoot is indicated by the arrow.

on the 3rd trifoliate leaf which had just begun its expansion, on the other hand, was much more pronounced (Table 20). Besides a marked inhibition in growth, the leaf showed an epinastic bending of the petiole, infolding of the leaflets and inrolling of the margins of the leaflets. Growth of the 4th and especially the 5th (sub-apical) internodes was reduced by TIBA and in some plants, the internodes showed a loss of normal geotropic control (Fig. 50). Further development of the apical bud was restricted.

As mentioned above, abscission of plant organs was uncommon, but there were isolated cases of the 3rd trifoliate leaf, 4th internode or the apical bud abscinding after an extended period of observation (about 6-8 days). This implies that physiological changes which potentially lead to abscission might be taking place, even though they were not severe enough to actually cause abscission in the vast majority of the plants. Thus, in determining whether TIBA stimulates production in the apical shoot, analyses of ethylene emanation were carried out one day after TIBA treatment to avoid interference from ethylene production associated with abscission. A slight curling of the 2nd and 3rd trifoliate leaves was observed indicating that the apical shoot was responding to TIBA within one day of application. The analyses of ethylene emanation showed that TIBA did not promote the release of ethylene, but in fact reduced it ($p < 0.05$). Ethylene emanation from control shoots was 1.92 $mm^3\ h^{-1}\ kg^{-1}$ compared with 1.02 $mm^3\ h^{-1}\ kg^{-1}$ from TIBA-treated plants.

Discussion

Various mechanical stimuli are known to influence shoot growth and development in the plant (Goeschl, Rappaport and Pratt, 1966; Neel and Harris, 1971; Turgeon and Webb, 1971; Jaffe, 1976; Catchpole and Hillman, 1976; Beardsell, 1977; Mitchell, 1977). While ethylene emanation as a wound reaction is well known (Abeles, 1973), the responses to many mechanical effects not involving tissue laceration have been attributed

to ethylene (Goeschl *et al.*, 1966; Turgeon and Webb, 1971; Jaffe, 1976; Catchpole and Hillman, 1976; Mitchell, 1977). Unlike most previous studies where plants were subjected to a single or an intermittantly repeated mechanical stimulus, physical confinement of apical growth imposed a continuous stimulus to the shoot system.

The results obtained from this study suggest that the development of the axillary buds basipetal to the shoot enclosures was related to increased ethylene production in the treated shoot. The observed retardation of shoot growth is a common response to ethylene, although a more complex explanation cannot be ruled out. For example, Suge (1978) observed a decrease in gibberellin activity in shoots of *Phaseolus* which showed a reduction in growth following repeated mechanical stress. Analysis of the internal gaseous content of shoots in the present study provided supporting evidence for ethylene involvement, with the confined shoots showing enhanced ethylene content. The increase in the rate of ethylene emanation in treated plants was even more pronounced.

Since the inhibition of leaf growth and release of apical dominance also occurred when shoots were confined in ventilated tubes, these effects were not dependent on the accumulation of ethylene in the surrounding atmosphere, although presence of ethylene in the air (as in the sealed tubes) did enhance these effects. Ethylene, either accumulated in the air or induced endogenously, appeared to be a common feature of the observed inhibition of shoot growth. If it is a causal agent, then the lack of inhibition of internode growth in ventilated tubes could be readily explained. As the enclosed internodes extended vertically along the axes of the tubes, they encountered little mechanical resistance and no significant increase in endogenous ethylene occurred. Nevertheless, induction of ethylene in the expanding leaves alone was apparently sufficient to bring about the release of apical dominance. Thus, axillary bud development was observed in plants enclosed in ventilated tubes despite

the absence of internode growth inhibition. The critical role of developing leaves in maintaining apical dominance is in agreement with the report of White *et al.* (1975) which showed, by excision treatment, that young growing leaves were responsible for the correlative inhibition of lateral bud growth in *Phaseolus*.

As the expanding leaves of the apical shoot exerted an inhibiting influence on the basipetal axillary buds (Snow, 1929; Shein and Jackson, 1971; Tucker and Mansfield, 1973; White *et al.*, 1975), it is conceivable that ethylene synthesised in the restricted leaves could be acting directly on the leaves themselves to cause the release of apical dominance. When the expanding 2nd trifoliate leaf alone was enclosed in a sealed tube such that the newly expanding 3rd trifoliate and apical bud were allowed to develop normally, no axillary bud development occurred despite growth inhibition and lamina crinkling symptoms appearing in the treated leaf. This might indicate that mechanical stimulation of the expanding leaf, and thereby enhanced ethylene in the leaf tissue, did not produce a factor promoting lateral bud growth; but rather, it prevented the action or synthesis in, or transport from, the treated leaf of a factor inhibiting lateral bud growth. Accordingly, where the 3rd trifoliate leaf and apical bud were left untreated, no lateral bud development occurred because the inhibitory factor continued to be available from the unenclosed expanding tissue.

The inference that the induction of axillary bud growth by shoot confinement is mediated through ethylene presupposes that ethylene acting on the apical shoot can induce such a correlative response. This presumption is justified by the results of experiments where the shoot, while not physically restricted, was supplied with ethylene. The axillary bud lying below the treated portion of the shoot sprouted in response to the ethylene treatment. Loss of correlative inhibition was again observed when ethylene, in the form of ethephon, was supplied to the expanding leaf or terminal bud.

When AVG was applied to the apical shoot, the resultant decrease in internal ethylene and ethylene release was as expected. On the other hand, it is not surprising that application of silver nitrate had no significant effect on the rate of ethylene release since Ag^+ is generally regarded as an inhibitor of ethylene action rather than ethylene synthesis. The increase in internal ethylene in the silver nitrate-treated shoot tissue in the absence of increased ethylene emanation is noteworthy, particularly since similar occurrences were encountered in shoots enclosed in large glass vessels, irrespective of whether ethylene was added to or depleted from the vessels or if the vessels were flushed daily. In the case of the silver nitrate-treated shoots, this might be due to the negative feedback reaction induced by Ag^+ de-sensitizing the tissue to ethylene action, although a chemotoxic reaction to silver nitrate cannot be ruled out. With regard to shoots enclosed in glass vessels, the concept of a feedback response is applicable only where ethylene in the vessels was actively depleted by absorption with mercuric perchlorate. On the other hand, the increase in internal ethylene content appeared likely to have resulted directly from enclosure by the glass vessels. An increase in temperature within the vessels was not the cause, since this was only of an order of 0.5 K. Two other probable factors are the high humidity within the vessels and the light filtration effect of the glass leading to a change in light spectral quality in the non-visible wavelengths. The departure from the relationship of proportionality between internal ethylene and the rate of ethylene emanation is comparable to a similar situation in *Faba vulgaris* where the internal ethylene concentration, but not the rate of emanation, was increased by experimental treatments with ethylene (Zeroni *et al.*, 1977; Jerie *et al.*, 1978a). These observations might involve the phenomenon of ethylene "compartmentation" (Jerie *et al.*, 1978b; Jerie *et al.*, 1979 and see section 3.3.2.) in which ethylene sequestered in the tissue is not readily lost from the tissue through

emanation.

Although the action of TIBA in releasing lateral buds from apical dominance has been commonly ascribed to the restriction of basipetal transport of inhibitory amounts of auxins from the apical shoot, at least two other explanations are possible. Firstly, TIBA is known to induce abscission of young leaves and the terminal bud shortly after application (Whiting and Murray, 1948; White and Hillman, 1972). The loss of these organs is as effective in inducing axillary bud growth as decapitation of the plant (White *et al.*, 1975). Secondly, the abscission and growth inhibition effects on the shoot, the nastic response in the leaves and the loss in geotropic control in the sub-apical internodes all resemble the effects of ethylene on the apical shoot of *Phaseolus*. As described above, ethylene action on the apical shoot can lead to the outgrowth of axillary buds basipetal to the treated portion of the shoot.

The proposition that growth of axillary buds in TIBA-treated plants was due to abscission of the terminal bud and young leaves was first examined. In preliminary experiments using TIBA, it appeared that abscission of the sub-apical leaves and internodes resulted from the cumulative effects of excessive amounts of the chemical slowly infusing into the plant tissues. In subsequent experiments, therefore, the applied dosage of TIBA was very much reduced; the concentration in lanolin was only half, and the volume applied just over one tenth of that used by White and Hillman (1972). Whereas White and Hillman renewed the lanolin pastes every two days, the TIBA dispersion in the present study was removed after two days and not replaced. Even so, this quantity of TIBA was sufficient to partially inhibit growth of the 1st trifoliate axillary bud during the later stages of the experiment, causing increased compensation growth of the primary leaf axillary bud. Nevertheless, the experiments showed that the release of buds from correlative inhibition was not a direct result of the abscission of the young leaves and terminal bud.

The possibility of the effects of TIBA being mediated through ethylene

has been put forward by Webster and Leopold (1972). Analyses of ethylene emanation showed that this was not increased in the apical shoot one day after TIBA application, even though the effect of the chemical in the shoot was already evident from the slight curling of the 2nd and 3rd trifoliate leaves. On the contrary, there was a decrease in the rate of ethylene release. The diminution of ethylene emanation by TIBA might be compared to a similar effect in *Phaseolus* seedlings reported by Abeles and Rubinstein (1964). In the latter case, however, plant tissue basipetal to the point of application was analysed and the response was attributed to the reduction of auxin transport from the apex and hence a reduction of auxin-induced ethylene. The results from the present study show that despite similarities to the effect of ethylene, the initial effects of TIBA treatment did not arise from increased ethylene levels in the apical shoot, although ethylene could be important in the later stages. The internal ethylene level in TIBA-treated shoots was not assessed, but this is not considered to be an important factor in the induction of axillary bud growth, as explained below. There is, therefore, no evidence from this study that is contrary to the commonly held contention that TIBA induces lateral bud growth by blocking basipetal transport of auxin originating from the apical portion of the shoot.

Taking the various shoot treatments together, an assessment of the relation between the ethylene status of the shoot and the release of axillary buds from apical dominance might be made by considering three ways by which ethylene levels may be expressed: internal ethylene, ethylene emanation and "free" ethylene. Internal ethylene refers to the concentration of ethylene in the gaseous extract of the apical shoot tissues. As mentioned in section 3.3.2., it might include both intercellular and "bound" ethylene. A proportion of the compartmented ethylene (where it is present) might also be a constituent of internal ethylene. "Free" ethylene refers essentially to ethylene that is not

compartmented or otherwise bound, but which is freely diffusible within the inter-cellular air spaces. No differentation is made between exogenous and endogenous "free" ethylene. Thus, tissues which showed an increase in ethylene emanation were deemed to have corresponding increase in "free" ethylene. At the same time, applied ethylene or ethephon would also increase the "free" ethylene in the treated tissue. The involvement of ethylene in the apical shoot in relation to apical dominance following various experimental treatments is summarized in Table 21. It is clear

TABLE 21. Summary of the involvement of ethylene in the apical shoot in relation to the growth of the first trifoliate axillary bud.

+ = Significant increase over intact control plants.
0 = No significant increase over intact control plants.
ND = Not determined.
I = Inconclusive

Treatment	Growth of 1st trifoliate axillary bud	Internal ethylene	Internal ethane	Ethylene emanation	"Free" ethylene
Shoot constriction	+	+	0	+	+
Ethylene-enhanced air	+	+	0	0	+
Ethylene-depleted air	0	+	+	0	0
Ventilated vessel	0	+	I	0	0
Ethephon	+	ND	ND	ND	+
Silver nitrate	0	+	+	0	0
AVG	0	0	+	0	0
TIBA	+	ND	ND	0	0

that the internal ethylene content is not critical to enhanced axillary bud growth since this was increased both in treatments which released apical dominance and treatments which did not. Outgrowth of axillary buds also did not depend on the rate of ethylene emanation, since rapid bud growth occurred without any significant change in ethylene emanation when the

apical shoot was treated with ethylene. With the exception of the TIBA treatments, where the promotion of axillary bud growth could be explained by mechanisms not involving ethylene (e.g. the inhibition of auxin transport), the release of correlative inhibition in axillary buds was always associated with an increase in "free" ethylene. It appears, therefore, that the outgrowth of axillary buds was brought about by the availability of freely diffusible ethylene in the tissues of the apical shoot.

In considering ethylene levels, the internal ethane concentrations might also be examined. Shoots which were constricted or supplied with ethylene to promote axillary bud growth showed an increase in internal ethylene, but no increase in internal ethane. In contrast, in the three treatments where internal ethylene was increased but where there was no loss of apical dominance (i.e. in silver nitrate-treated shoots and shoots enclosed in vessels with the air depleted of ethylene or flushed daily), an increase in internal ethane occurred at the same time. To determine if internal ethane was "protecting" the shoots from the effects of internal ethylene, ethylene and ethane was supplied to the shoot at the same time. As was evident from Table 17, 20 vpm ethane had no effect on the induction of axillary bud development by 0.5 vpm ethylene.

It is difficult at this stage to assign a specific mode of action to ethylene in the shoot in relation to the release of apical dominance. As ethylene is not normally transported in the plant at significant rates (Zeroni *et al.*, 1977), it seems improbable that the increased ethylene in the shoot is actually transferred basipetally to the axillary buds. Nevertheless, changes in ethylene concentration in one part of the plant may influence concentrations in other parts (Zeroni *et al.*, 1977; Jerie *et al.*, 1978a) and the ethylene status in the bud may be indirectly influenced in this manner. The rôle of ethylene in the axillary bud in regard to its release from correlative inhibition is examined in the following section.

3.4.2. Ethylene in the Axillary Bud and Adjacent Tissue in relation to Bud Growth

Whereas the apical portion of the shoot has a major role in maintaining lateral buds in their inhibited state, the correlative signal that is transmitted might act on the buds themselves. The possible involvement of ethylene at the axillary bud and adjacent tissue in the control of bud development - either in growth promotion or growth inhibition - was examined in intact and decapitated plants. IAA applied to the cut stem of the decapitated plant or directly to the lateral bud in an isolated node segment has been found to suppress lateral bud outgrowth (see Rubinstein and Nagao, 1976). This response might conceivably have been brought about by the action of auxin-induced ethylene production in the treated plant tissue, a proposition that has been examined experimentally.

Effects of ethephon applied to the bud and decapitated stem

In the *intact* plant, ethephon was applied to the first trifoliate axillary bud using a paintbrush. Treatment with approximately 30 mm^3 of 20, 50 or 100 ppm ethephon did not stimulate development of the bud. Zobel (1973, 1974) has pointed out that the physiological requirements of ethylene in plants may be satisfied by exceedingly minute concentrations of the gas - of the order of 0.005 vpm, for example, in the case of the ethylene-requiring mutant tomato. Lower doses of ethephon were therefore also attempted. Nevertheless, treatment of the bud with 5 ppm ethephon, or application of 5 ppm ethephon which was then rinsed away after varying time intervals ranging from 20-240 min produced no significant effect on its outgrowth.

The effect of ethephon applied to the axillary bud of the *decapitated* plant was examined using 5, 50, 100 and 200 ppm aqueous solutions of ethephon. Solutions of 50, 100 and 200 ppm were applied with a paintbrush

as before, while 5 ppm applications were made by dispensing 0.3 cm^3 of the solution on to cotton wool wrapped around the node and bud, sealed with adhesive tape and removed the following day (see section 2.7.3. : Materials and Methods for details).

Decapitation of the plants resulted in the rapid outgrowth of the third node axillary bud in controls. Treatment of the node and bud with 100 or 200 ppm ethephon effectively inhibited its development following decapitation, while 50 ppm ethephon was partially effective in suppressing bud growth. Ethephon (5 ppm) applied in a cotton wool wad almost completely inhibited axillary bud growth (Fig. 51a). In each treatment suppression of growth of the third node axillary bud was compensated by development of the axillary bud basipetal to it; i.e. the primary leaf axillary bud (Fig. 51b).

The outgrowth of the primary leaf axillary buds did not cause the suppression of growth in the inhibited buds. In subsequent experiments when the primary leaf axillary buds were excised at the beginning of the experiments, similar growth inhibition of the third node axillary buds was observed.

In another series of experiments, the effects of applying 5, 50, 100 and 250 ppm ethephon to the decapitated stem stump (rather than to the node and bud) were compared. Ethephon solution (0.1 cm^3) was added to a wad of cotton wool wrapped around the cut end of the stump and sealed with adhesive tape. Treatment with 50, 100 and 250 ppm ethephon was found to retard growth of the third node axillary bud to varying degrees (Fig. 52), while 5 ppm was ineffective. With 250 ppm ethephon, however, abscission of the inhibited buds occurred from the third day.

In all of the above experiments no apparent injury or aberrant development to the inhibited buds seemed to have resulted from ethephon application. Even in cases where the buds abscinded after a few days, they remained green until abscission: the buds in such cases were not

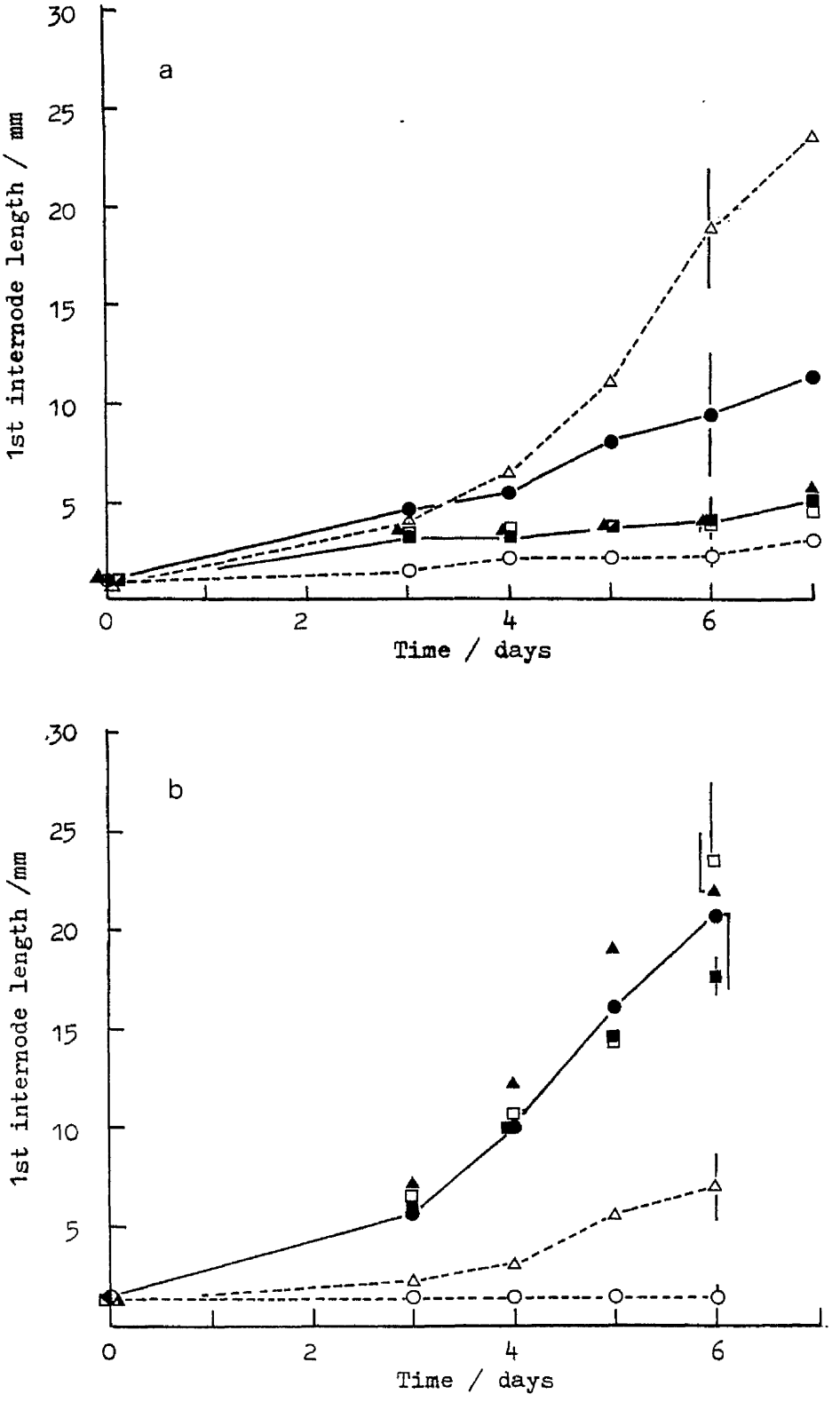

FIGURE 51. Application of ethephon to the 3rd node and axillary bud of the decapitated plant. Effect on the growth of (a) the treated (1st trifoliate) axillary bud and (b) the larger primary leaf axillary bud. Ethephon in concentrations of 50 - 200 ppm was painted on the node and bud; 5 ppm ethephon was applied to cotton wool wrapped around the node and bud. Values are the means ± std. errors of five plants per treatment. ○ = Intact control; △ = Decapitated control; ■ = 200 ppm; ▲ = 100 ppm; ● = 50 ppm; □ = 5 ppm (in cotton wool).

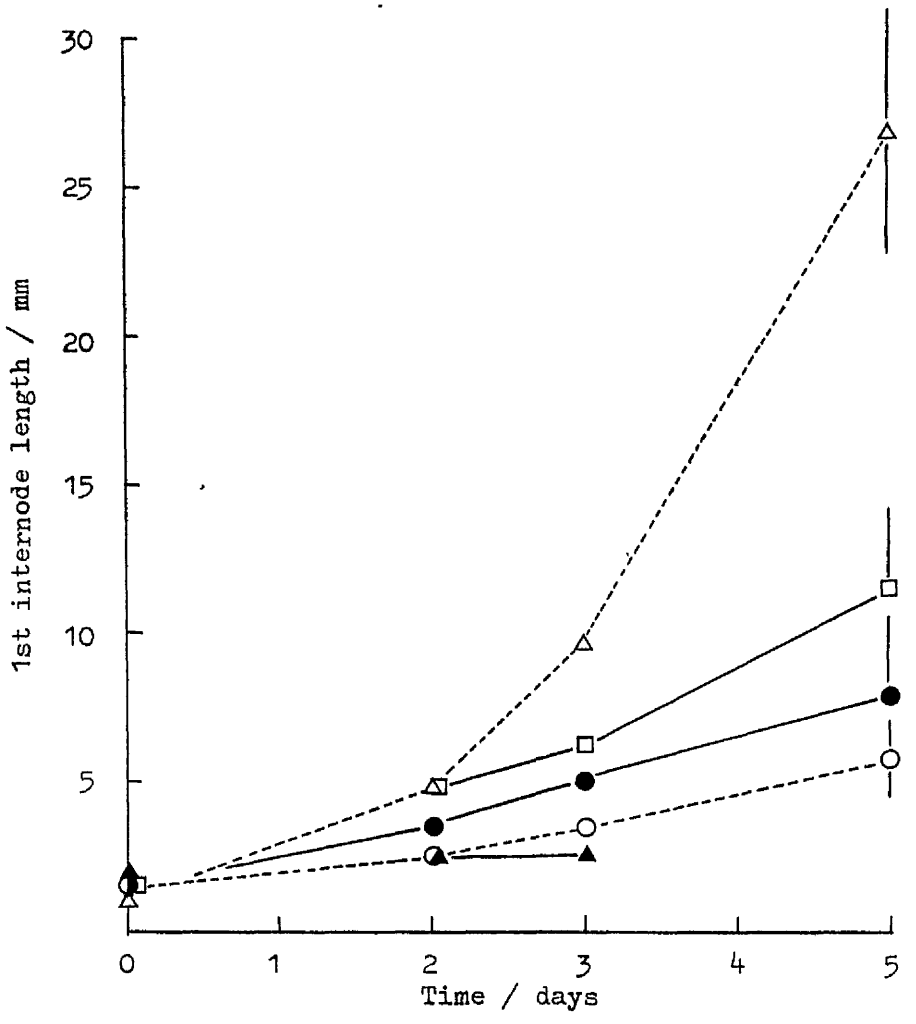

FIGURE 52. Application of 50, 100 and 250 ppm ethephon to the cut end of the 3rd internode of the decapitated plant. Effect on the growth of the 1st trifoliate axillary bud. Treatment with 250 ppm ethephon caused abscission of the buds from day 3. Values are the means ± std. errors of five plants per treatment.
○ = Intact control; △ = Decapitated control; ▲ = 250 ppm;
● = 100 ppm; □ = 50 ppm.

senescent, but were detached from the plant with the formation of an abscission layer in the internode. The limited development of the inhibited axillary buds showed no visible abnormalities. However, as some expansion of the folded leaf in the bud often occurred even where the bud internode was strongly inhibited, growth increment of the bud expressed as dry weight was often greater than apparent from the bud internode length. This was also true of bud inhibition by ethylene inhibitors which is described below.

Effects of ethylene inhibitors applied to the bud and decapitated stem

The effects of ethylene inhibitors on the regulation of axillary bud growth were investigated along the lines adopted above for ethephon. Two ethylene inhibitors were tested: Ag^+ in the form of the nitrate salt or silver-thiosulphate complex, and AVG. The former blocked ethylene action in plant tissues while the latter inhibited the biosynthesis of ethylene. As in the case of ethephon, application of ethylene inhibitors (1 and 0.1 mol m^{-3} Ag^+ and 0.01 mol m^{-3} AVG) to cotton wool wrapped around the node and bud in the *intact* plant did not promote bud development. Indeed the primary effect of the direct application of ethylene inhibitors to the bud is that of the inhibition of bud growth. Thus, most of the experiments with Ag^+ and AVG deal with the suppression of axillary bud growth in the *decapitated* plant.

Silver nitrate inhibited growth of the 1st trifoliate axillary bud of the decapitated plant when 0.4 cm^3 of a 4 mol m^{-3} solution was added to cotton wool wrapped around the abraded node and bud. The treatment was less effective when silver nitrate concentration was reduced to 1 mol m^{-3} (Fig. 53). As with ethephon-induced bud inhibition, the reduction in growth of the 1st trifoliate axillary bud varied inversely with the growth of the (untreated) primary leaf axillary buds on the same plant (Fig. 53).

Silver nitrate caused the bracts of the treated axillary buds to open

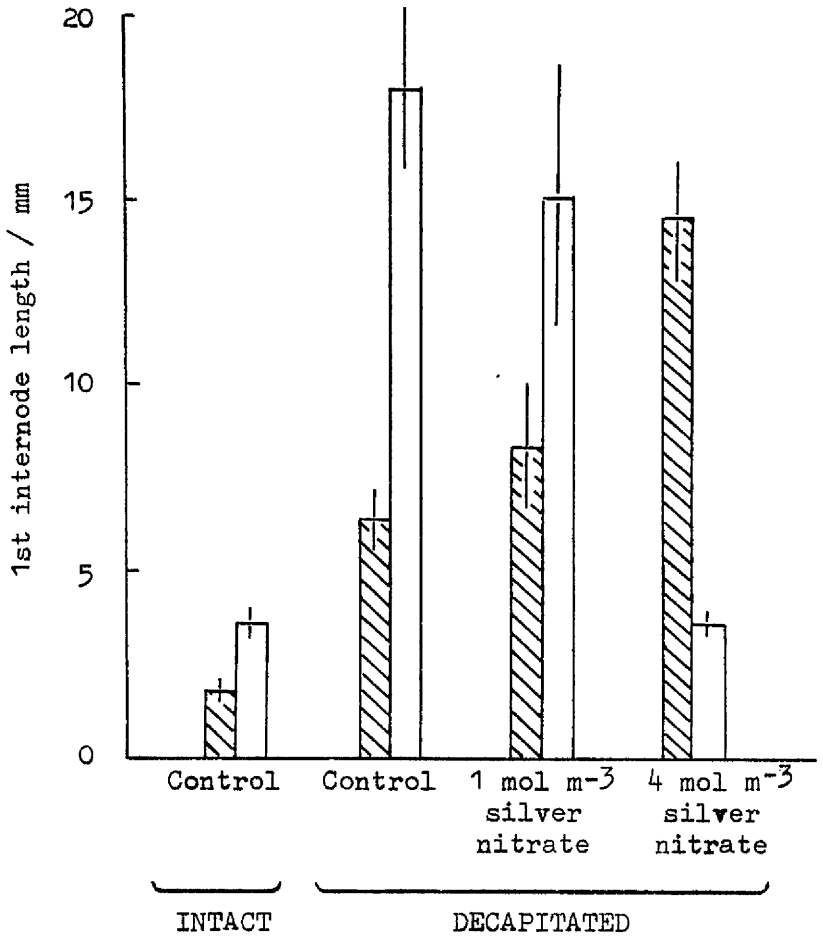

FIGURE 53. Effect of silver nitrate applied to the 3rd node and axillary bud on the growth of the treated (1st trifoliate) axillary bud and the larger primary leaf axillary bud. Measurements were taken after six days and the values presented are the means ± std. errors of five plants per treatment.

Shaded bars: Larger primary leaf axillary bud
Unshaded bars: 1st trifoliate axillary bud

after one or two days, giving the buds a swollen appearance. Outgrowth and development of the axillary buds were frequently characterized by various morphological abnormalities. The leaflets of the young trifoliate developed from the bud were often short and wide, thus appearing rounded rather than angular in outline. Sometimes, leaflet development in a trifoliate was unequal with one or two leaflets stunted in growth, or even with one lateral half of a leaflet growing more than another. An abnormally short first internode was frequently encountered in the axillary shoot.

It appeared, therefore, that silver nitrate, while partially achieving the desired effect of inhibiting bud growth in decapitated plants, also produced undesirable secondary effects on the buds. Veen and van de Geijn have demonstrated the poor transport properties of silver nitrate in plant tissue, and it was suspected that this could have contributed to some of the abnormalities seen in bud growth. For example, non-homogeneous distribution of Ag^+ to various parts of the treated bud could have led to certain portions of the bud being inhibited to a greater extent than others; hence the sometimes unequal growth of leaflets in the developing bud. Veen and van de Geijn further showed that Ag^+ in the form of silver nitrate solution mixed into an excess of sodium thiosulphate solution was transported in plant tissue much more efficiently than silver nitrate alone, while retaining its ethylene-inhibition properties. The complex formed is represented by the general formula $Na_{2n-1}\ (AgS_2O_3)_n$ and a mixture of related complexes with more than one value of n may occur (Jaenicke and Hauffe, 1949). This mixture was subsequently used in experiments and is henceforth referred to as Ag^+. (Mol concentration of Ag^+:thiosulphate = 1:5)

In preliminary experiments, sodium thiosulphate in low concentrations was found to have only a slight inhibitory effect on bud growth. Penetration of the applied Ag^+ solutions into the tissues of the node and bud was poor when the node was left unabraded and only slight inhibition of bud growth was achieved (Fig. 54a). Abrading the node before application

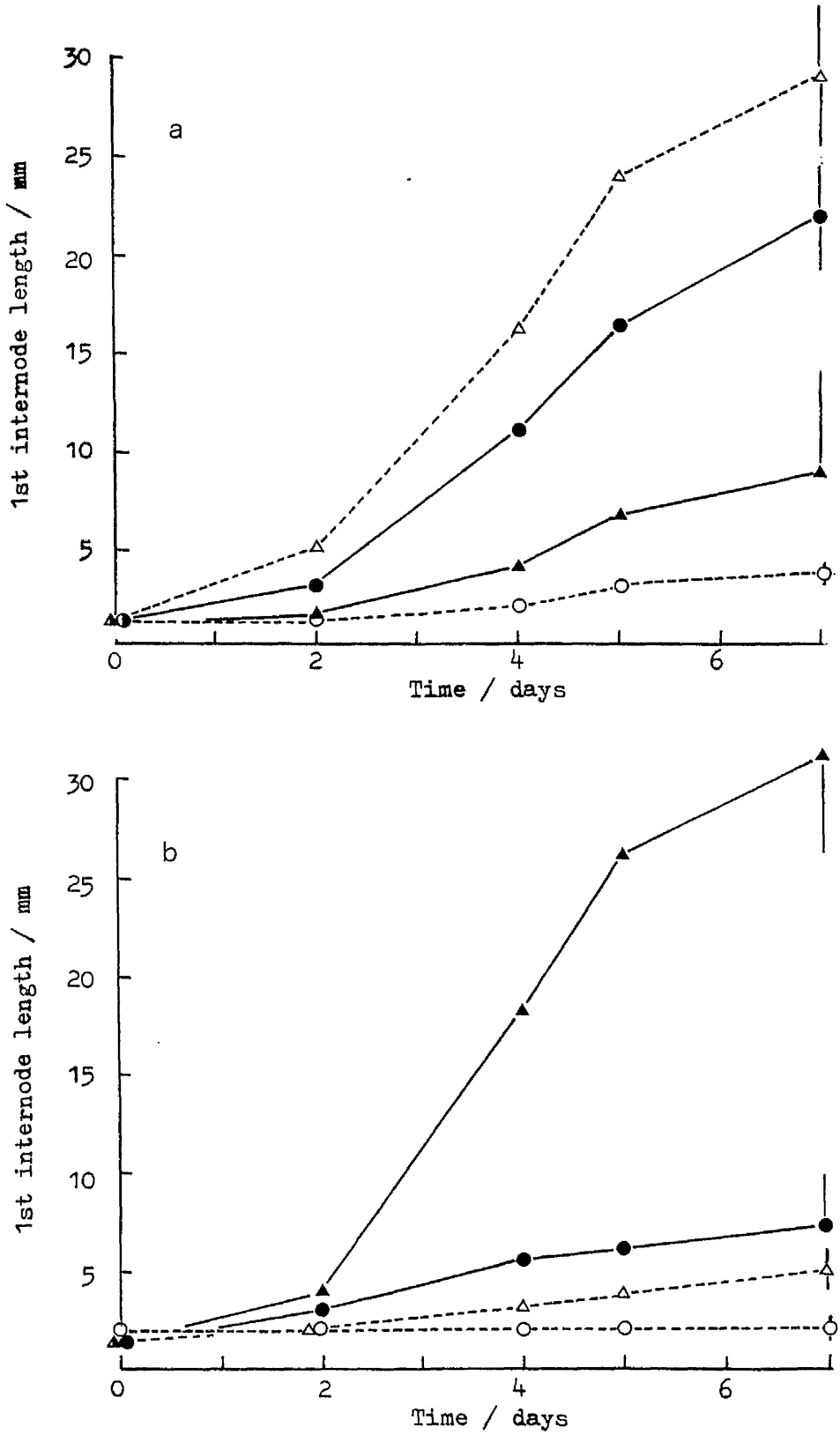

FIGURE 54. Application of Ag^{+} (0.3 cm^{3}; 4 mol m^{-3}) to the 3rd node and axillary bud of the decapitated plant. Effect of abrading the 3rd node before Ag^{+} application on the growth of (a) the treated (1st trifoliate) axillary bud and (b) the larger primary leaf axillary bud. Values are the means ± std. errors of six plants per treatment.

○ = Intact control; △ = Decapitated control; ▲ = 3rd node abraded; ● = 3rd node not abraded.

of Ag^+ markedly improved its effectiveness and this was therefore carried out in subsequent experiments. As expected, the primary node axillary buds developed vigorously in the absence of active growth in the treated buds (Fig. 54b).

Application of Ag^+ to the decapitated stem (1 ml added to a wad of cotton wool wrapped around the cut end) was inefficient in suppressing axillary bud growth. Ag^+ concentrations of 4 or 8 mol m^{-3} gave only a slight inhibiting effect (Fig. 55). While increasing the concentration to 20 mol m^{-3} enhanced inhibition, this was found to be phytotoxic, causing the stump to darken and wither.

Ag^+ applied to the first trifoliate leaf of decapitated plants or to the axillary buds of intact plants produced no visible effects on bud growth.

Unlike treatment with silver nitrate, Ag^+ in sodium thiosulphate did not cause any abnormal development in the treated buds. Whatever limited outgrowth was attained in the inhibited buds was well formed except that inhibition in the bud internode was somewhat stronger than in the foliar portion of the bud.

It was apparent from the preceding results and from the experiments involving ethephon that both the presence of ethylene and the inhibition of ethylene action could bring about the suppression of axillary bud growth in decapitated plants. One approach to resolve this apparent contradiction is to regard ethylene requirement as being essential only in an early stage of a sequence of physiological processes leading to the removal of apical dominance. The latter stages require no ethylene and would indeed be inhibited by its continued presence. Thus, in this argument, a "pulse" of ethylene would be required to initiate growth of the dormant bud. The presence of Ag^+ obstructs this initial ethylene-dependent step while direct application of ethephon to the bud inhibits the processes subsequent to this step: hence the inhibition of bud growth in either case. If this

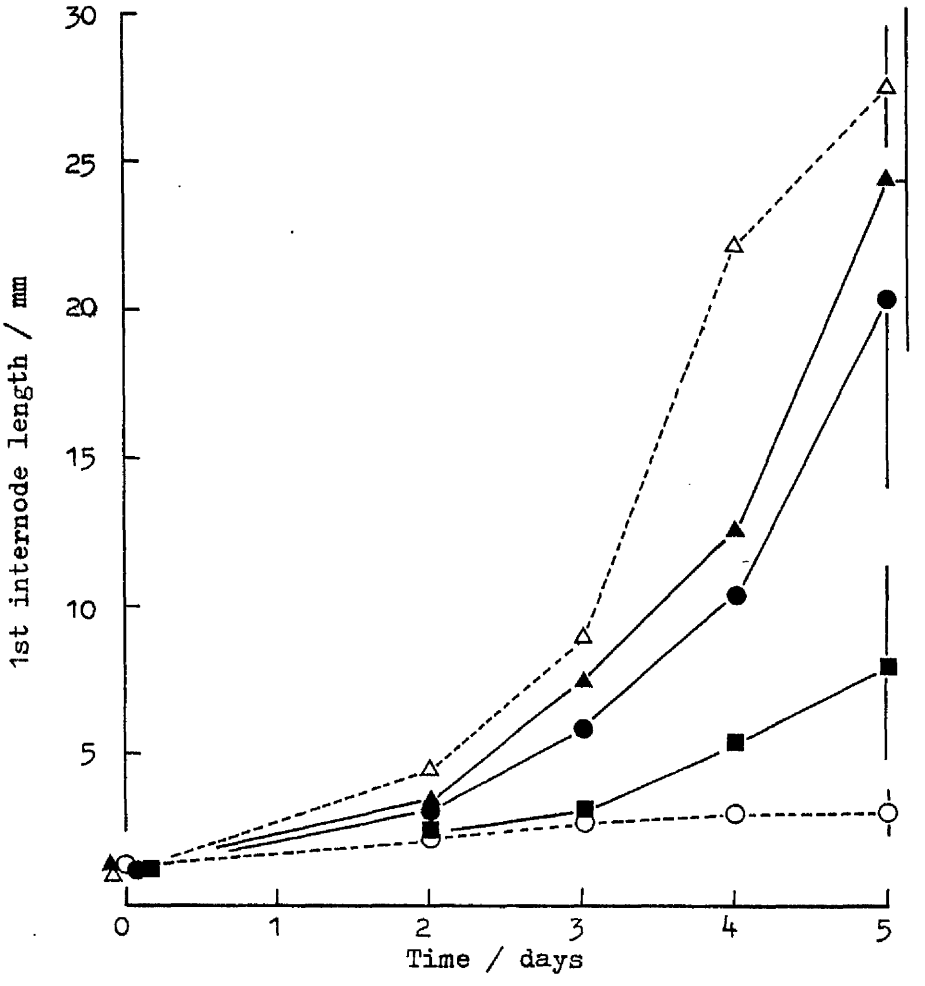

FIGURE 55. Application of 0.1 cm^3 Ag^+ to the cut end of the 3rd internode of the decapitated plant. Effect on the growth of the 1st trifoliate axillary bud. Values are the means ± std. errors of five plants per treatment.

○ = Intact control; △ = Decapitated control;
■ = 20 mol m^{-3} Ag^+; ▲ = 8 mol m^{-3} Ag^+; ● = 4 mol m^{-3} Ag^+.

hypothesis were correct, then treatment with Ag^+ some time *after* decapitation should not inhibit bud growth since the initial ethylene requiring step would have been able to proceed. In an experiment where axillary buds were treated with Ag^+ at various periods before and after decapitation, all treatments were found to be equally effective in suppressing bud growth. Buds treated 24 h after decapitation were as strongly inhibited as buds treated 24 h before (Fig. 56).

The effect of applying both ethylene (in the form of 5 ppm ethephon) and the inhibitor of ethylene action (in the form of 4 mol m^{-3} Ag^+) together to the axillary bud of the decapitated plant was examined. The presence of both reagents at the node did not mutually alleviate the inhibiting effects of each reagent when applied separately (Fig. 57).

Treatment of the 1st trifoliate axillary bud or cut stem of the decapitated plant with AVG produced results similar to those with Ag^+ treatment. The inhibitory action of AVG was stronger and inhibition of bud growth could be effected by 0.4 and 4 mol m^{-3} AVG applied to the node and bud and to the cut surface of the stem respectively (Figs. 58a, 59a). As in the inhibition of bud development in decapitated plants by ethephon or Ag^+, there was a limited expansion of the folded leaf in the bud even when bud internode elongation was strongly suppressed by the above-mentioned concentrations of AVG. This slight growth in the foliar portion of the bud was prevented by 4 mol m^{-3} AVG applied to the bud and node and by 20 mol m^3 AVG applied to the cut stem. Compensating growth was again seen in the untreated primary leaf axillary buds (Figs. 58b, 59b), although this was reduced when high concentrations (4 mol m^{-3} to the bud and node and 20 mol m^{-3} to the cut stem) of AVG were applied, presumably because of basipetal translocation of the inhibitor to the primary leaf axillary buds. Treatment with AVG caused the leaf tips in the inhibited buds to curl outwards and induced chlorosis in the treated buds. In one experiment where AVG was applied to the node and axillary bud, chlorophyll contents

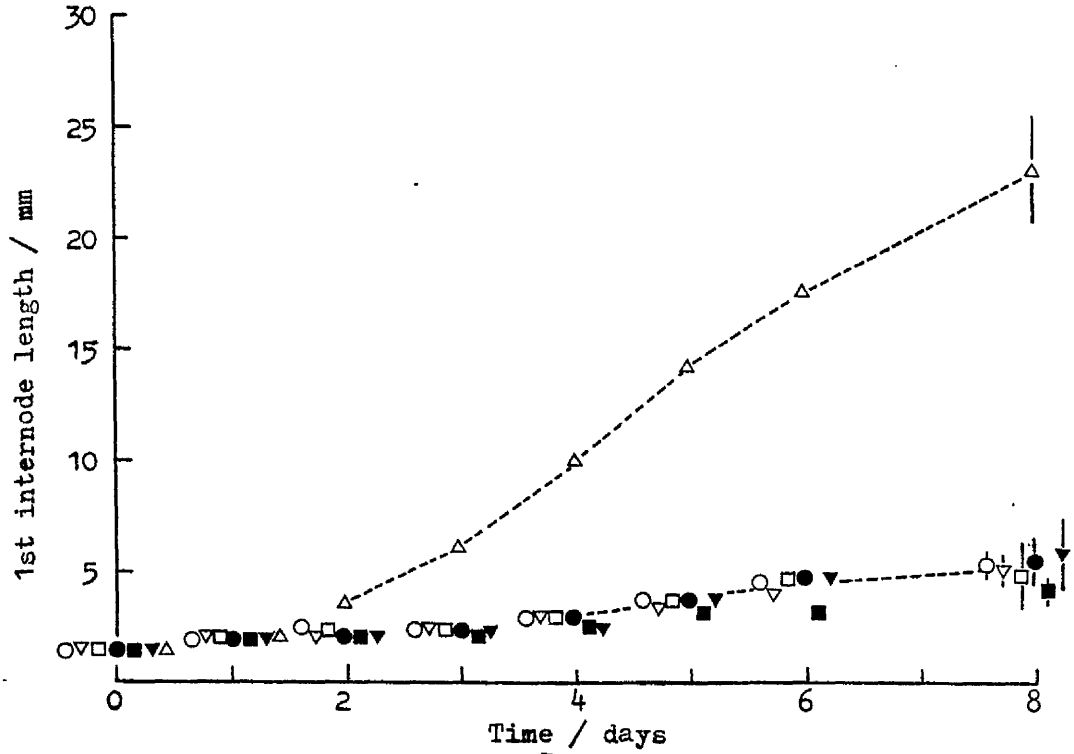

FIGURE 56. Application of Ag^+ (0.3 cm^3; 4 mol m^{-3}) to the 3rd node and axillary bud of the decapitated plant. Effect of the timing of Ag^+ application with respect to decapitation on the growth of the treated (1st trifoliate) axillary bud. Values are the means ± std. errors of five plants per treatment. ○ = Intact control; △ = Decapitated control; ▽ = Applied 1 day before decapitation; □ = Applied 5 h before decapitation; ● = Applied at decapitation; ■ = Applied 5 h after decapitation; ▼ = Applied 1 day after decapitation.

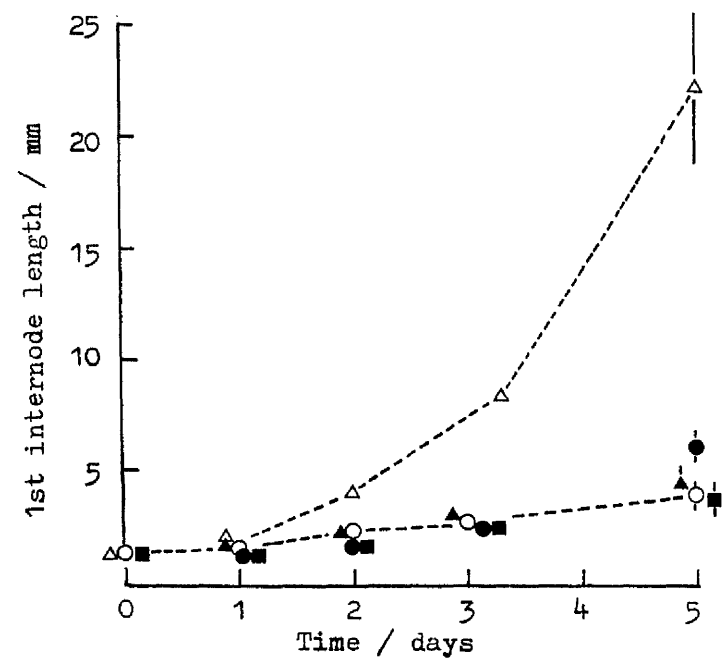

FIGURE 57. Application of ethephon and Ag^+ (0.3 cm^3) to the 3rd node and axillary bud of the decapitated plant. Effect on the growth of the treated (1st trifoliate) axillary bud. Values are the means ± std. errors of five plants per treatment.
○ = Intact control; △ = Decapitated control; ● = 4 mol m^{-3} Ag^+; ▲ = 5 ppm ethephon; ■ = 4 mol m^{-3} Ag^+ & 5 ppm ethephon.

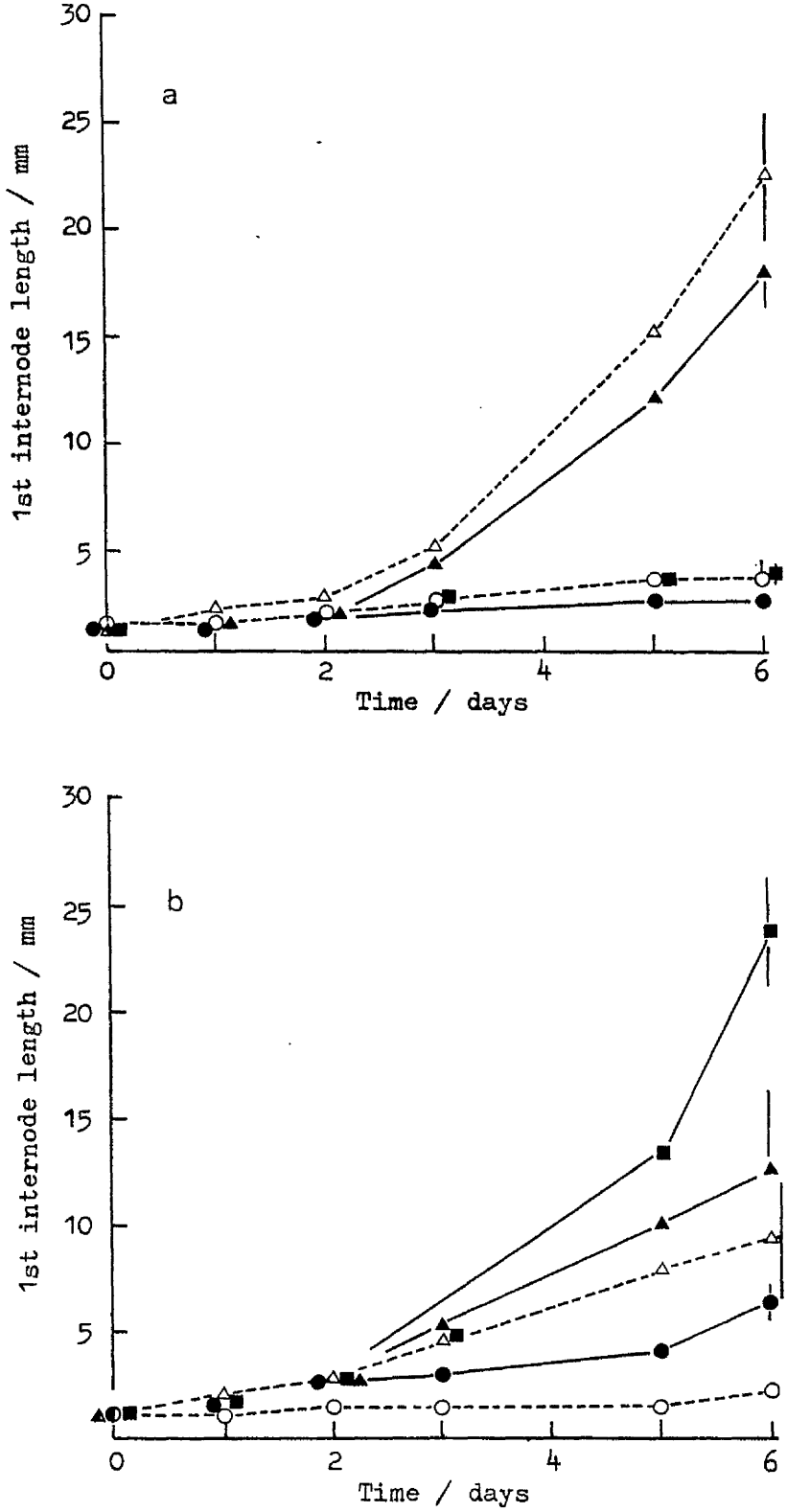

FIGURE 58. Application of 0.3 cm^3 AVG to the 3rd node and axillary bud of the decapitated plant. Effect on the growth of (a) the treated (1st trifoliate) axillary bud and (b) the larger primary leaf axillary bud. Values are the means ± std. errors of five plants per treatment.

○ = Intact control; △ = Decapitated control; ● = 4 mol m^{-3} AVG;
■ = 0.4 mol m^{-3} AVG; ▲ = 0.04 mol m^{-3} AVG.

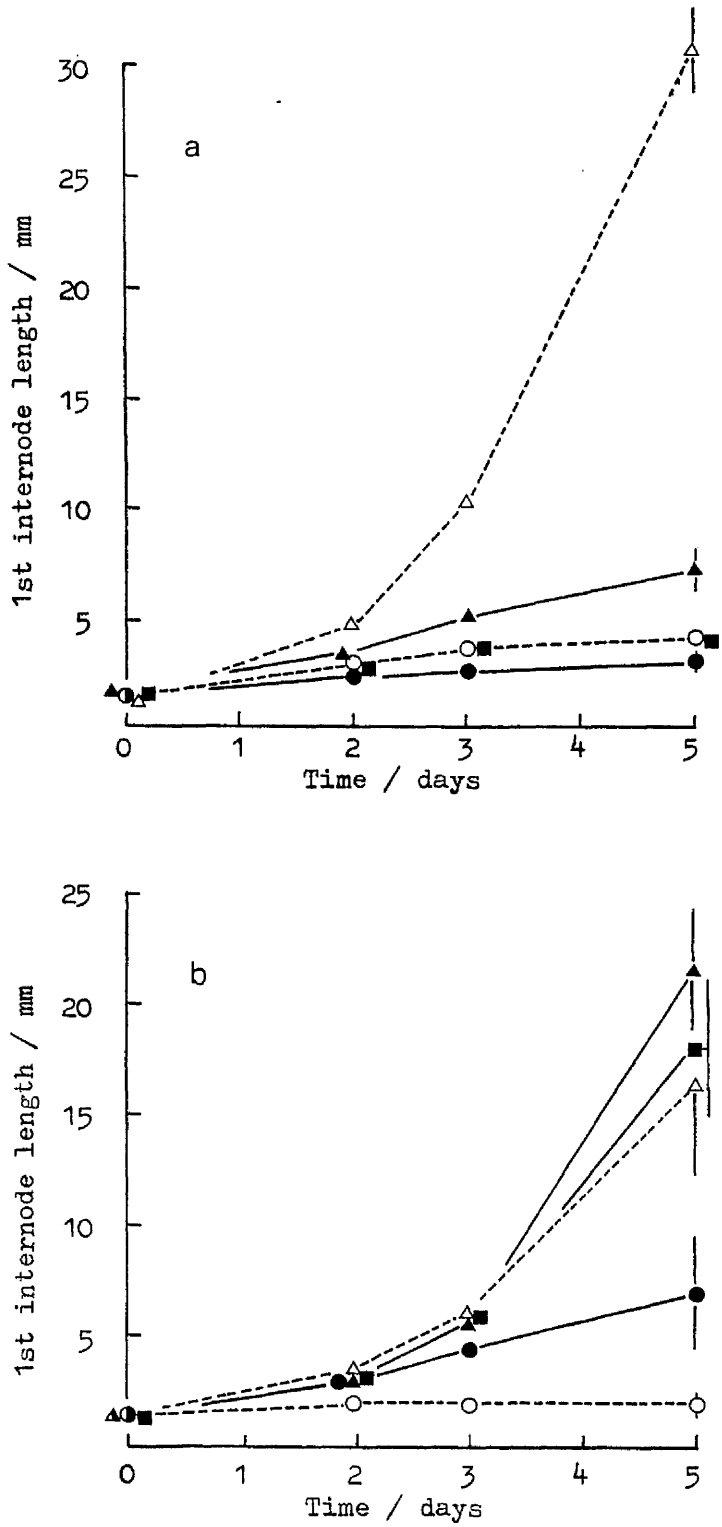

FIGURE 59. Application of 0.1 cm^3 AVG to the cut end of the 3rd internode of the decapitated plant. Effect on the growth of (a) the 1st trifoliate axillary bud and (b) the larger primary leaf axillary bud. Values are the means ± std. errors of five plants per treatment.

○ = Intact control; △ = Decapitated control;
● = 20 mol m^{-3} AVG; ■ = 8 mol m^{-3} AVG; ▲ = 4 mol m^{-3} AVG.

in treated and control buds were found to be 0.33 ± 0.06 and 0.56 ± 0.04 mg g^{-1} fresh weight respectively.

Ethylene and ethane in the node and internode

To determine if high ethylene levels at the nodal region of the stem were responsible for the inhibition of the axillary bud situated at the node, a 6 mm section of the 3rd node (from which the first trifoliate leaf arises) was excised from the intact plant. At the same time 20 mm internodal sections immediately above and below the nodal section were also excised. The nodal section included part of the bud internode (but not the foliar portion of the bud) and a small portion of the base of the 1st trifoliate leaf petiole (Fig. 60). Internal ethylene and ethane and the rate of ethylene emanation were determined in intact control plants and in plants 2.5 and 5.5 h after decapitation. These time intervals were selected as it had been found earlier that accelerated growth of the 1st trifoliate axillary bud was first detected 3-5 h following decapitation (see section 3.2.1.). Thus, 2.5 and 5.5 h after decapitation would be the time just before and just after bud growth first occurred. To gauge the effects of IAA on ethylene levels in the node and internode, 0.02 cm^3 plain lanolin or lanolin containing 10 mg g^{-1} IAA was applied to the cut stem (3rd internode) of the decapitated plant. Analyses of ethylene emanation and internal ethylene and ethane were carried out two days later. A small number of treated plants were set aside for an extended observation to ascertain that the applied IAA was indeed effective in inhibiting axillary bud growth as intended.

The results supported, to a certain extent, the contention that a high ethylene level in the nodal region is responsible for sustaining the inhibition of bud growth. In intact plants, there was no significant difference between the rates of ethylene emanation in the nodal and internodal sections (Fig. 60c). There was, however, a fall in emanation rates

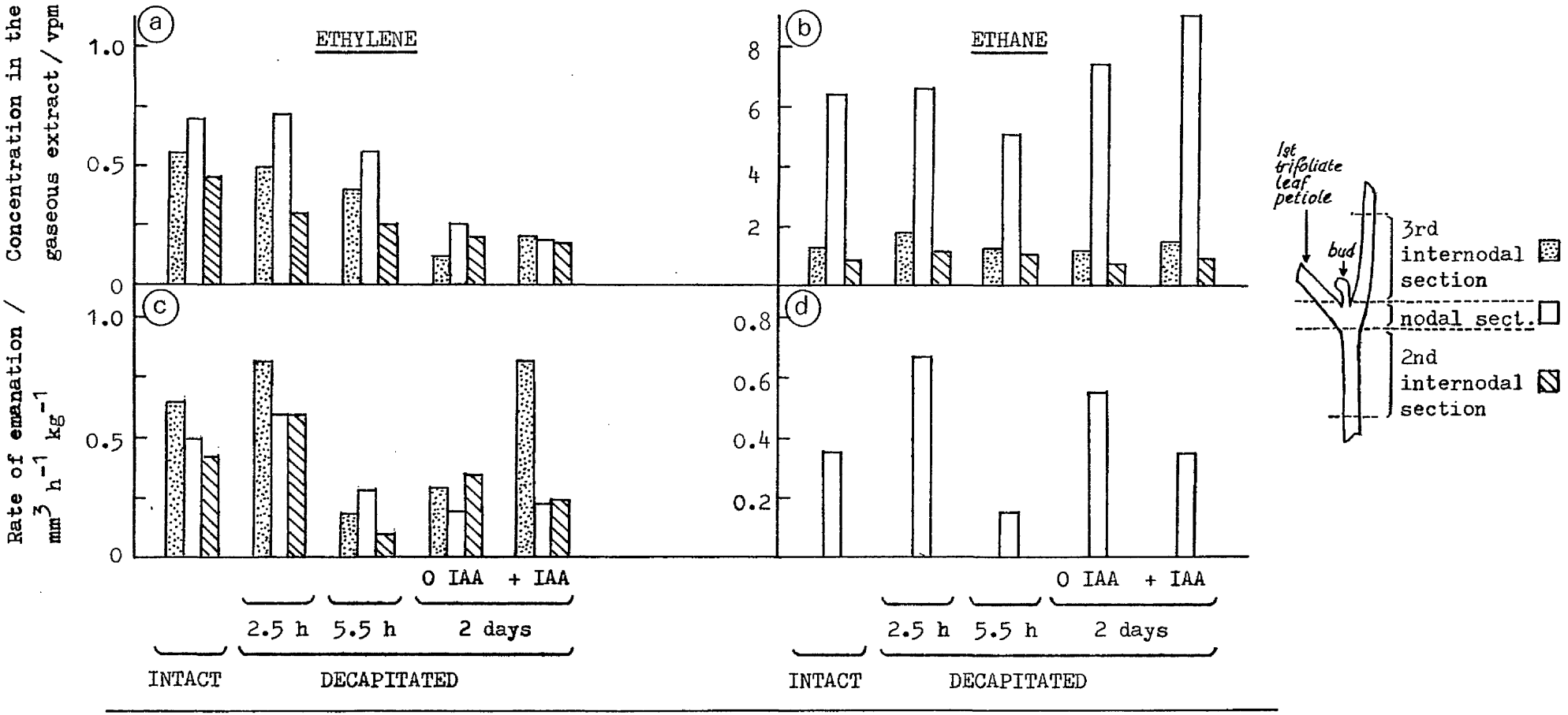

FIGURE 60. Endogenous ethylene and ethane in the 3rd node and in the 2nd and 3rd internodes of intact and decapitated plants. (a) Internal ethylene concentration; (b) Internal ethane concentration; (c) Rate of ethylene emanation; (d) Rate of ethane emanation. Decapitated plants were harvested 2.5 h, 5.5 h or 2 days after decapitation at the 3rd internode. Approximately 100 plants were used per treatment to determine internal ethylene and ethane contents. The plants were divided into two batches so that the excision and vacuum extraction procedures could be completed within 26 min and thus obviate any interference from wound-induced ethylene. The gases recovered from the two extractions were then combined for GC analysis. Twelve plants per treatment were used in the determination of ethylene and ethane emanation. Representative results are presented. [0 IAA] = Plain lanolin applied to the cut end of the 3rd internode of the decapitated plant. [+ IAA] = 10 mg g^{-1} IAA in hydrous lanolin applied to the cut end of the 3rd internode of the decapitated plant.

in the nodes and internodes following decapitation; this being especially evident after two days. Where IAA was applied to the cut end of the 3rd internode of the decapitated plant, ethylene release was enhanced in the 3rd internodal section (which included tissue near the site of IAA application), but IAA did not increase ethylene emanation in the nodal section (Fig. 60c). The internal ethylene content in nodal and internodal sections similarly showed a decrease two days following decapitation (Fig. 60a). The increase in ethylene emanation in the 3rd internodal section was, however, not reflected in the internal ethylene concentration. In most of the experiments, the nodal section had a slightly higher internal ethylene concentration than the internodal sections. This may be largely explained by the amount of gas that can be extracted from the different tissue. The mean volumes of gases recovered by vacuum extraction from the 3rd internodal section, the nodal section and the 2nd internodal section were respectively 46.2, 13.2 and 63.0 $mm^3\ g^{-1}$ of fresh weight of the tissue. Thus, the small amount of gases extractable from the nodal section favours a higher ethylene concentration through a smaller "dilution effect" (see section 3.3.2.).

There was no significant difference in the relative proportions of internal ethane in nodes and internodes between the different treatments (Fig. 60b). What was remarkable, however, was the very high internal ethane levels in the nodal sections. These were some five times as high as the internal ethane concentrations in the internodal sections or in leaf or apical shoot tissues reported elsewhere in this thesis. The high internal ethane levels in the nodal sections were matched by significant ethane emanation (Fig. 60d). Release of ethane from the internodal sections was only barely detectable. Growth conditions appeared to have some influence in this respect. The results presented in Figs. 60b and d are from plants grown in the loam-based compost. Material from plants grown in Levington compost gave relatively lower internal ethane concen-

trations in the nodal sections and low but detectable ethane emanation from the internodal sections. Nevertheless, the levels of both internal ethane and ethane emanation were always significantly higher in the nodal sections, and so the basic trend was not different under the two growth conditions.

Effects of indole-3-acetic acid applied to the bud

Preliminary experiments showed that IAA applied to cotton wool wrapped round the first trifoliate axillary bud and 3rd node promoted bud growth over the first two days, but that this increased growth was not sustained thereafter. It was thought that the growth-promoting effects of IAA were curtailed by the antagonistic effects of IAA-induced ethylene production from the treated tissue. To test this proposition, treatments with 0.3 cm^3 of 0.1 mol m^{-3} IAA and IAA combined with 0.01 mol m^{-3} AVG were compared with intact and decapitated controls. The results, presented in Table 22, show that IAA had significantly increased the growth of the 1st trifoliate axillary bud after two days, the increment approaching that brought about by

TABLE 22. Effect of 0.1 mol m^{-3} IAA and 0.01 mol m^{-3} AVG on the growth of the 1st trifoliate axillary bud. 0.3 cm^{-3} of either chemical or a mixture of both was applied to cotton wool wrapped around the 3rd node and axillary bud. The readings presented are the increases in the lengths (mm) of the 1st internodes of the buds on day 2 and day 4 over the lengths on day 0. Values are the means of fourteen readings per treatment, and those in each column bearing the same letter are not significantly different (tested at $p < 0.05$ and $p < 0.01$ with similar results).

Treatment	Increase in length of the 1st internode of the 1st trifoliate axillary bud	
	Day 2	Day 4
Control	0.93 [a]	2.07 [a]
Decapitated	2.64 [b]	8.82 [b]
IAA	2.11 [c]	3.04 [a]
AVG	1.18 [a]	2.54 [a]
IAA + AVG	0.82 [a]	1.79 [a]

shoot decapitation. However, while buds in the decapitated plants continued to develop vigorously, growth of the IAA-treated buds was not sustained. After four days, the internode lengths of IAA-treated buds were not significantly different from those of intact control plants. Treatment with AVG did not affect the normal slow growth increments in the inhibited buds, but eliminated completely the additional growth due to IAA. On the other hand, AVG had no effect on the growth of the axillary buds in decapitated plants (Table 23). The latter observation was expected

TABLE 23. Effect of 0.01 mol m^{-3} AVG on the growth of the 1st trifoliate axillary bud in the decapitated plant. 0.3 cm^{-3} of AVG was applied to cotton wool wrapped around the 3rd node and axillary bud. The readings presented are the increases in the lengths (mm) of the 1st internodes of the buds on day 2 and day 4 over the lengths on day 0. Values are the means of nine readings per treatment, and those in each column bearing the same letter are not significantly different (tested at $p < 0.05$ and $p < 0.01$ with similar results).

Treatment	Increase in length of the 1st internode of the 1st trifoliate axillary bud	
	Day 2	Day 4
Control	0.72 [a]	1.50 [a]
Decapitated	2.33 [b]	8.61 [b]
Decapitated & AVG	2.61 [b]	9.33 [b]

since earlier studies (see Fig. 58) had shown that 0.04 mol m^{-3} AVG did not inhibit axillary bud growth in the decapitated plant.

Discussion

In experiments on the methods of applying aqueous solutions of plant hormones to *Pisum* axillary buds, Sachs and Thimann (1967) found two conditions that had to be satisfied before efficient uptake of the applied substances was possible. Firstly, the test chemical had to be in close

contact with the plant tissue and, secondly, it must not be allowed to dry out. Sachs and Thimann used alcohol as a surfactant to meet the first condition and carbowax, which is hygroscopic, to satisfy the second. In the present study, the method of applying the test chemical to cotton wool wrapped around the bud and node is simple and complies with the conditions set out by Sachs and Thimann. The use of carbowax was avoided since Sachs and Thimann found that it had a slight inhibitory effect on bud growth.

Of the two ethylene inhibitors examined in this study, AVG was the more potent with respect to the inhibition of axillary bud development in the decapitated plant. Almost complete inhibition was achieved by 0.4 mol m^{-3} AVG applied to the bud and unabraded node while 4 mol m^{-3} Ag^{+} applied to the bud and abraded node was required for the same effect. AVG suppressed axillary bud growth when applied to the cut end of the stem of the decapitated plant, whereas Ag^{+} was only partially effective. The latter observation could, however, be more indicative of the relative efficiency of basipetal translocation of either compound to the axillary bud.

Although the growth inhibition effects of ethylene inhibitors on the axillary buds in decapitated plants suggest that bud outgrowth was ethylene-dependent, application of ethephon directly to the bud did not promote its growth. In fact, ethephon inhibited bud development in decapitated plants. The general inference that can be drawn from these observations is that while development of the inhibited bud is ethylene-dependent, lack of ethylene is not the reason for the absence of active bud growth in the intact plant. The requirement for ethylene probably follows the release of bud correlative inhibition.

Burg and Burg (1968b) first raised the interesting possibility that high ethylene emanation at the nodes of the stem was responsible for the inhibition of axillary bud growth. In a subsequent paper (Burg and Burg, 1968a) they reported that although ethylene emanation from the node

decreased upon stem decapitation, this decrease was not observed when scale leaves at the node were removed. As no quantitative data of ethylene emanation from nodes without scale leaves were presented in the paper, there is some ambiguity regarding the precise meaning of the results. In the introduction to this thesis, the results have been interpreted to mean that high ethylene emanation was associated with the scale leaves, the removal of which eliminated the difference in ethylene emanation from the nodes of intact and decapitated plants. Other interpretations are possible; e.g. the removal of scale leaves could increase ethylene emanation and offset the decrease due to decapitation. Nevertheless, it is clear that the results of Burg and Burg's second paper do not support their earlier proposition of ethylene-induced bud inhibition at the node. In spite of this, it seems profitable to carry out further investigations along similar lines to explore the involvement of internal ethylene and the effects of IAA applied to the cut end of the stem in the decapitated plant. Internal ethylene needs to be determined in addition to ethylene emanation since the relative levels of internal ethylene in various tissues are not necessarily in agreement with those of ethylene emanation. In excising the nodal sections for analyses, the major portion of the axillary bud itself was excluded. It was felt that active ethylene production from the bud would accompany its development once the plant was decapitated, and this would mask the decrease (if there was one) in ethylene level in the node which enabled the bud to develop in the first instance. Moreover, the foliar portion of the axillary bud trapped air in the bracts and unopen leaflets, presenting problems in the vacuum extraction of internal gases.

The comparison of ethylene levels in the node and internode gives a measure of support to the contention that a relatively high ethylene level in the node suppressed bud development in the intact plant. While Burg and Burg (1968a) found no decrease in ethylene emanation from the node (with scale leaves removed) when pea plants were decapitated, both ethylene

emanation and internal ethylene content dropped when the bean plants in this study were decapitated. However, there is some doubt if much significance should be placed on this observation since ethylene levels in the internodes also fell at the same time. The decrease in ethylene in the node reflected the decrease in the stem of the decapitated plant as a whole; this development being comparable to the decrease in ethylene emanation found in *Phaseolus* hypocotyls when the seedlings were decapitated (Abeles and Rubinstein, 1964). In the present study, no decrease in ethylene levels has been observed that could be associated with changes in the node specifically.

The significance of the high internal ethane concentration in nodal sections is not clear. It is nevertheless noteworthy that the nodal sections are the only *Phaseolus* tissues found in this entire study to release consistently ethane in readily detectable quantities.

Using excised pea nodal sections, Burg and Burg (1968 a,b) demonstrated a distinct relationship between auxin-induced ethylene and the inhibition of bud growth. Bourbouloux (1978) has shown that IAA applied to the cut stem of decapitated *Faba vulgaris* had a tendency to accumulate at the nodes. There was therefore a basis to suppose that the inhibition of axillary bud growth in the decapitated plant by IAA applied to the cut stem could be essentially an effect of auxin-induced ethylene at the nodal regions of the stem. However, the experimental results in the present study do not substantiate this hypothesized function of IAA.

Sachs and Thimann (1967) reported that IAA induced shoot growth, mainly in the internodes, when applied to the inhibited shoot of a two-shoot pea plant or the axillary shoot developed from a cytokinin-treated bud. IAA applied to the undeveloped bud, however, only produced increased inhibition. Libbert (1954) had earlier noted that auxin could protect a weak shoot from inhibition if it was applied only at the shoot apex. Sachs and Thimann, on the other hand, found that application to the apex or

internode were both effective. In the present study, IAA applied directly to the entire inhibited axillary bud and to the node from which it arises gave rise to an initial growth promotion in the bud (as measured by bud internode length). This result is in agreement with the observation of a transient enhancement in *Phaseolus* axillary bud growth by application of a 1 mm^3 drop of 1 mol m^{-3} aqueous solution of IAA to the bud (H. Nonhebel, pers. comm.). It is uncertain if the increase in bud growth due to IAA is essentially similar to that arising from shoot decapitation. There are reasons to suspect that bud growth in the two instances is brought about by different mechanisms. Firstly, IAA-induced growth did not normally proceed beyong 2-3 days. Unlike bud development due to decapitation, IAA-induced bud growth was completely annulled by 0.01 mol m^{-3} AVG. It is significant to note that this concentration of AVG did not block the growth processes altogether. The small amount of bud growth found in untreated plants was not affected and only the additional growth due to IAA was prevented.

The results have not established any grounds to suggest that accelerated development of the bud resulting from IAA treatment beyond about two days was prevented by the build-up of IAA-induced ethylene since supplementation with AVG did not extend the period of growth stimulation. On the contrary, growth promotion was prevented by AVG, suggesting that the response was ethylene dependent and perhaps brought about by IAA-induced ethylene in the first instance.

3.5. STRESS-INDUCED ETHYLENE

In quantitative analyses of endogenous ethylene from plants, one potential source of error is the inflation of estimates by ethylene artifacts arising from the effects of stress. The production of ethylene induced by wounding and by water-stress is examined and the extent to which it affects the results of ethylene analyses reported in this thesis is assessed.

Ethylene induced by wounding

The effects of ethylene enhancement or depletion in the atmosphere on the shoot and on apical dominance have been described in section 3.4.1. After treatment for four days, 5 mm sections were cut from the 4th internode and 4.7 mm diameter discs punched from the 2nd trifoliate leaf to study the effects of wounding in relation to ethylene evolution. The average weight of internode sections and leaf discs was generally similar between treatments, except that ethylene-treated internode sections were marginally lighter than those of controls. Lateral thickening did not, therefore, accompany the ethylene-induced inhibition of internode elongation.

Enhanced ethylene release induced by wounding of the fourth internode of control plants began after a lag period of about 25 min (20 - 30 min) (Fig. 61). Ethylene production peaked 90 - 100 min from the time of sectioning. Internodes from shoots enclosed in an ethylene-depleted atmosphere showed a similar pattern of ethylene release, but the wound reaction was less intense. In sections of internodes pre-treated in an ethylene-enhanced atmosphere, the wound ethylene released was similarly depressed. In addition, the maximal release rate was advanced, and occurred 50-70 min from the time of sectioning. An indication of a slight decrease in the time lag preceding enhanced ethylene production was inconclusive

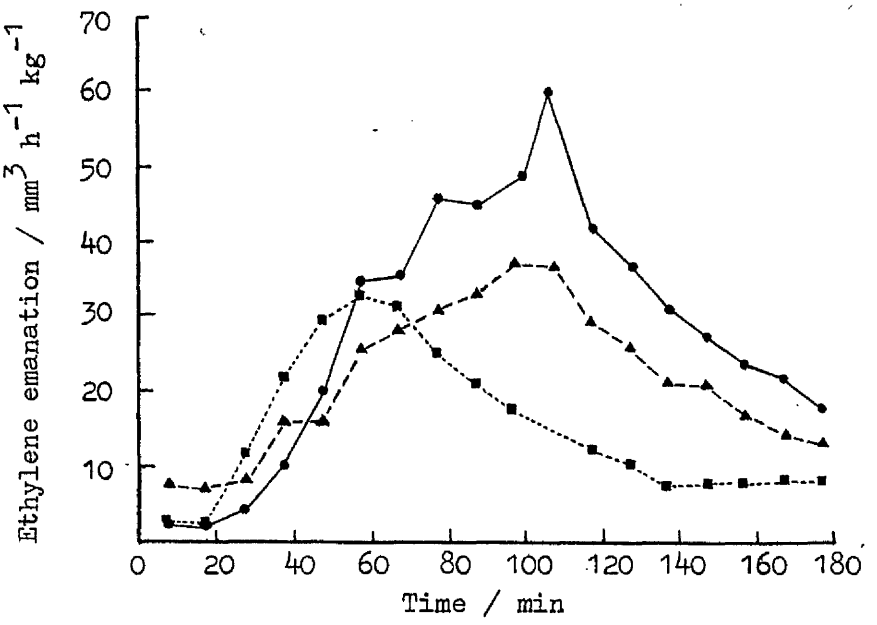

FIGURE 61. Ethylene emanation induced by wounding in sections of the 4th internode. ● = Sections from intact shoots; ▲ = Sections from shoots enclosed in an ethylene-depleted atmosphere; ■ = Sections from shoots enclosed in an ethylene-enhanced atmosphere. Results from a representative experiment are presented.

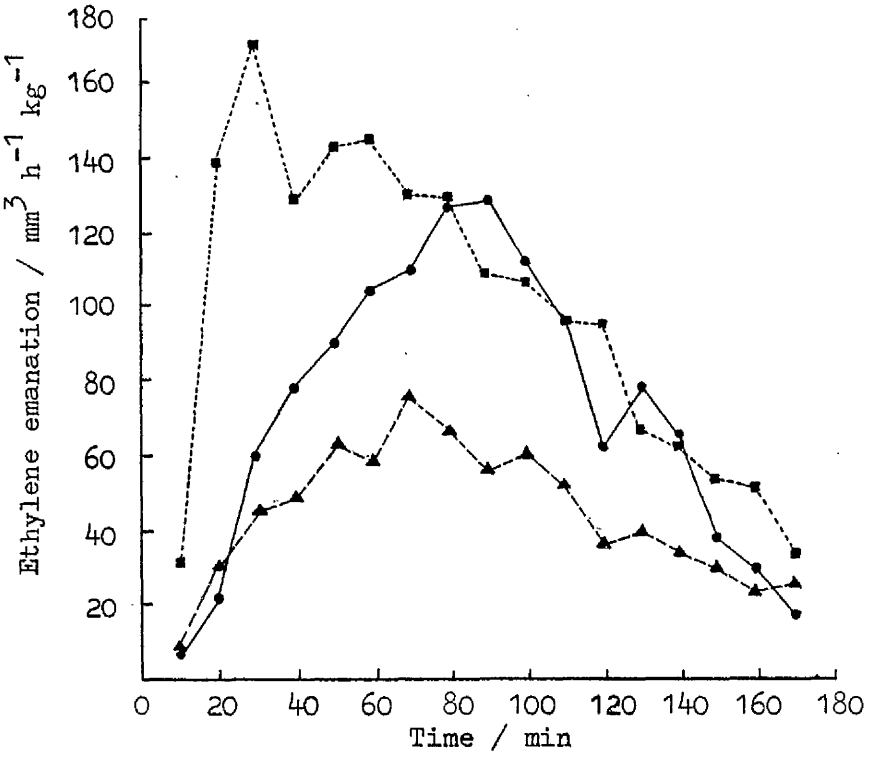

FIGURE 62. Ethylene emanation induced by wounding in discs of the 2nd trifoliate leaf. ● = Discs from intact shoots; ▲ = Discs from shoots enclosed in an ethylene-depleted atmosphere; ■ = Discs from shoots enclosed in an ethylene-enhanced atmosphere. Results from a representative experiment are presented.

from the available data.

The wound reaction in the second trifoliate leaf showed no significantly consistent lag period in any of the treatments and the surge in ethylene production could have begun within 10 min of cutting (Fig. 62). With leaf discs from control plants, the time to the peak rate of ethylene release, 80 - 100 min from sectioning, was comparable to that for the fourth internode. As was the case for internodes, pre-treatment of leaves in an ethylene-depleted atmosphere reduced the wound reaction. In three out of four experiments, no distinct ethylene peak was observed. The effect of applied ethylene on the second trifoliate leaf was quite different from that on the fourth internode. The wound reaction was greatly enhanced by the ethylene pre-treatment but no consistent ethylene peak was observed.

In all treatments, the rate of ethylene declined slowly from the time of peak production, and had not reverted to pre-wounding rates after 3 h. Evolution of ethane remained low throughout the ethylene wound reaction and only trace quantities were detected from the internode sections and leaf discs.

Ethylene induced by water-stress

Ethylene production resulting from water-stress was stimulated by treating the 4th internode and the central leaflet of the 2nd trifoliate leaf (both excised the day before) with a stream of warm air. Of the three experiments carried out, two showed a slow increase in the rate of ethylene emanation from the leaf up to 60 min from treatment. Thereafter, the increase in ethylene emanation was accelerated, reaching a peak 100 - 110 min from the time of treatment (Fig. 63a). The third experiment showed a rapid increase in emanation rate from the time the leaves were stressed. However, the timing of the peak rate was unchanged. Ethylene release from the internode was twenty to thirty times lower than from the

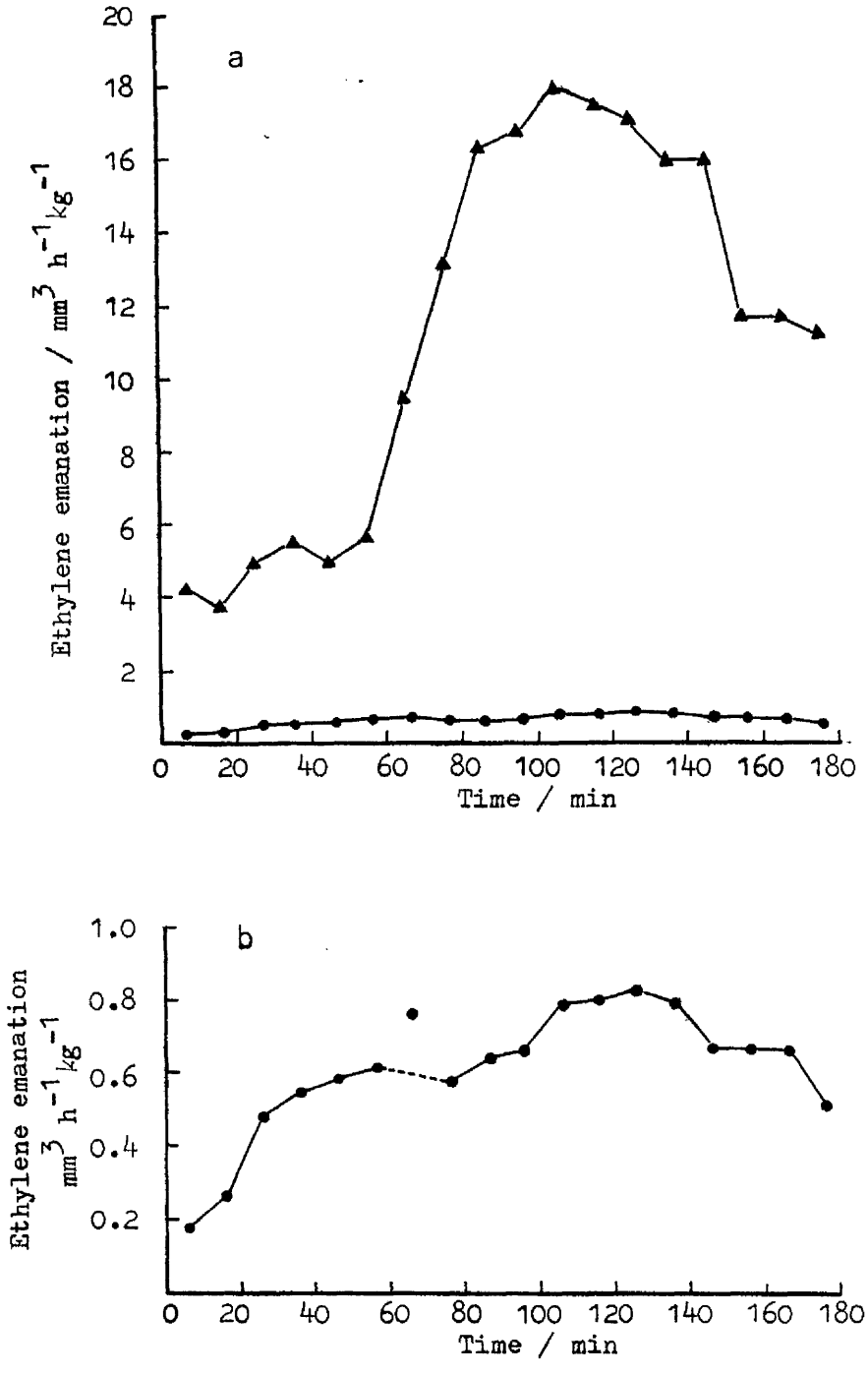

FIGURE 63. (a) Ethylene emanation induced by water-stress in the 2nd trifoliate leaf and in the 4th internode.

(b) Ethylene emanation induced by water stress in the 4th internode: data presented on an enlarged scale.

Results from a representative experiment are presented.

▲ = Ethylene emanation from the 2nd trifoliate leaf.
● = Ethylene emanation from the 4th internode.

leaf (Fig. 63a). Because of the small amounts of ethylene evolved, accurate measurement of small changes in ethylene emanation was difficult. Nevertheless, the maximum rate of ethylene emanation seemed to occur about 130 min from the stress stimulus (Fig. 63b).

To determine if the induction of ethylene production by wounding and by water-stress were one and the same, ethylene emanation was analysed in 5 mm 4th internode sections treated to modify the water potential in the tissue. Dehydration of the cut surfaces of the internode sections was inhibited by immediately covering the surfaces with silicone grease, or by cutting the sections under water so that the cut surfaces were covered with a film of water. Saltveit and Dilley (1979) reported that *Pisum* stem sections that were pre-treated in water showed a substantial reduction in wound-ethylene production. To test if this response occurred in *Phaseolus* and if it was due to high water potential being maintained in the tissues, a comparison was made between ethylene emanating from internode sections immersed in water for 0.5 h and immersed in 0.7 kmol m^{-3} mannitol.

The results, presented in Fig. 64, showed that a diminution of wound ethylene by pre-treating the tissue in water occurred in internode sections. A reduction in the rate of ethylene emanation by 55-70% was recorded. However, a comparable reduction also took place when the internode sections were immersed in mannitol solution, despite the low water potential of the solution (*ca.* -19×10^{-5} Pa). Treatment with silicone grease did not decrease wound ethylene production. This is in agreement with the observation in *Pisum* stem sections (Saltveit and Dilley, 1979). Internode sections cut under water showed a reduction in ethylene emanation of 20%.

Discussion

The wound reaction in the internodes of control shoots or shoots pre-treated by enclosure in an ethylene-depleted atmosphere was generally similar to that reported by Jackson and Osborne (1970) for petioles of the

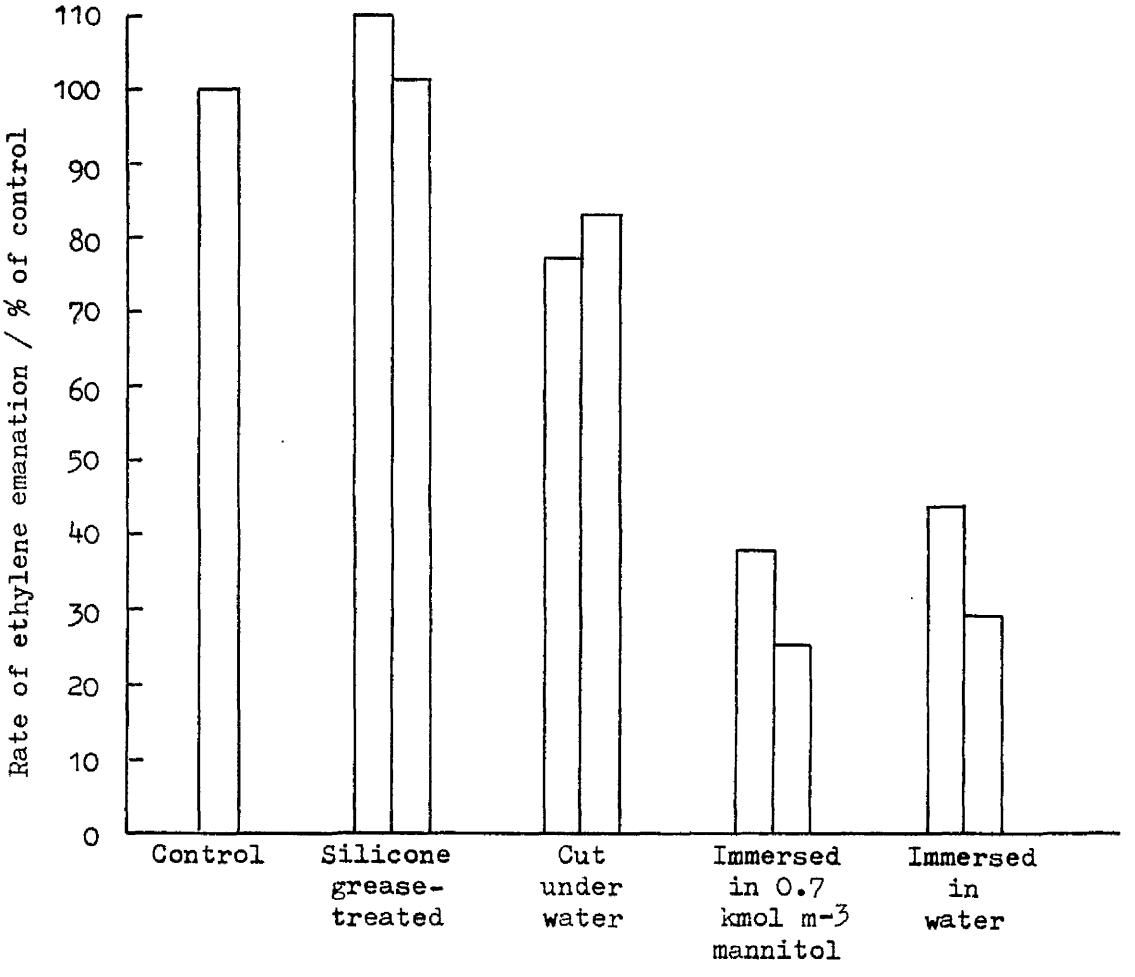

FIGURE 64. Ethylene emanation induced by wounding in 4th internode sections. Effect of modifying the tissue water potential. Rates of ethylene emanation are expressed as % of the rate in untreated sections. Results of the two experiments carried out are presented.

same species. As with *Phaseolus* petioles, a single peak was observed, and the lag period as well as the time to maximal ethylene production were comparable.

The lag period appeared to be very short (or absent) in the wound reaction of the second trifoliate leaf. This would imply that the precursors and enzymes necessary for wound-ethylene production were already present in the leaves and the time lag for wound-ethylene synthesis was, therefore, minimal.

Pre-treating the shoot in an ethylene-depleted atmosphere de-sensitized it to the wound reaction, this effect being more marked in the leaf than in the internode. The effect of applied ethylene, on the other hand, appears to be more complex, having opposite effects on the leaf and internode in regard to the intensity of the wound reaction. In the internode, the time to maximal ethylene production was also altered. It is improbable that the high ethylene production from discs cut from ethylene pre-treated leaves could be due to the accumulated exogenous ethylene being released. Had this been the case, an exponential decay in the rate of ethylene release rather than an initial increase would have been observed. Furthermore, the treated shoots were given adequate ventilation in ambient air prior to wounding.

In shoots pretreated in an ethylene-depleted atmosphere, the possibility exists that the effects observed could be due to other effects of enclosure in the glass vessel rather than to ethylene depletion of the atmosphere specifically.

Although the release of ethane can be induced by freezing injury in *Beta vulgaris* leaves (Elstner and Konze, 1976) or chemical (sulphur dioxide) injury in *Medicago sativa* seedlings (Peiser and Yang, 1979), the absence of any significant ethane involvement in the present investigation is in agreement with the findings of Saltveit and Dilley (1978) for the wound reaction of *Pisum sativum* internodes.

The observation that water-stress ethylene was far more significant

in leaves than in internodes is not surprising because of the larger surface area to volume ratio and the presence of stomatal openings in the former. It is possible that in the wounding treatments, a proportion of the ethylene thought to have been produced by wounding could in fact have arisen from water loss from the freshly cut surfaces. This effect would be minimal in the internode since ethylene resulting from water-stress is relatively very low. In the leaf discs, however, ethylene apparently induced by wounding could well have been supplemented by ethylene induced by water-stress. Water-stress might effect wound-ethylene analysis in plants with shoots enclosed in glass vessels in another way. As has been shown in section 3.4.1. (see Fig. 47), shoots which were removed from the vessels for 90 min before ethylene analysis showed a large increase in ethylene emanation over shoots which were analysed immediately after removal from the vessels. This was thought to be due to water-stress triggered by transferring the shoot from the highly humidified air in the vessels. In the wound-ethylene experiments, the wounding treatments were carried out 1.5 h after the shoots were removed from the glass vessels. By this time, the putative water-stress ethylene would have reached its peak emanation rate and any subsequent increase in ethylene emanation should not have been an artifact of removing the shoots from the bottles. Nevertheless, the induction of wound ethylene immediately following a water-stress stimulus could still lead to other interactions which might perhaps explain the erratic wound-ethylene emanation pattern in leaves which had been enclosed in glass vessels.

As wounding and dehydration of the tissues are both forms of stress stimuli, it might be expected that the mechanisms involved in inducing ethylene production could be identical in the two cases. The relation between ethylene brought about by wounding and by water stress may be further explored by hypothesizing that wound-ethylene arises entirely from the drop in water potential in the tissue through the freshly exposed cut (or otherwise lacerated) surfaces. There is circumstantial evidence in

support of this proposition. Both stimuli give rise to characteristic set patterns of ethylene emanation. Aharoni (1978) reported that ethylene production from lettuce leaves induced by water-stress conformed to a consistent pattern despite being subjected to varying degrees of dehydration. The timing of the maximum emanation rate was unchanged even though the amount of ethylene evolved increased with the severity of water-stress. Jackson and Osborne (1970) found that the amount of wound-ethylene released increased with the area of cut surfaces in the test tissue. This observation is in agreement with the proposition that wound-ethylene arises from water loss through the cut surfaces. Yet another characteristic of wound-ethylene which suggests the implication of a water-stress relationship is the finding by Saltveit and Dilley (1979) that pre-treating pea stem sections with water markedly reduced the production of wound-ethylene. Although Saltveit and Dilley explained their observation by proposing that a water-soluble factor was lost in the pre-treatment, it is possible that the water had acted simply to maintain water potential. The fact that the putative factor was stable to heat, and that Saltveit and Dilley had attempted but failed to isolate it only served to strengthen the possibility that this factor might not actually exist.

In the present study, however, experimental treatments designed to modify water potential in internode sections did not support the hypothesis that wound-induced ethylene was mediated through water-stress. Treatment of the sections with 0.7 kmol m^{-3} mannitol, which had a low water potential (*ca.* -19×10^5 Pa), did not increase wound-ethylene as compared with treatment with water. There was no diminution of wound-ethylene when water loss from the cut surfaces of the sections was reduced by covering with silicone grease. Cutting the sections under water gave only a small decrease, but this could be due to the momentary immersion of the internode sections in water. The results as a whole indicate that wounding and water-stress are quite separate stimuli with respect to the induction of

enhanced ethylene emanation.

The account of stress-induced ethylene production given in this section is not intended to be an exhaustive treatment of the subject. It is primarily aimed at characterizing the rapidly induced production of stress ethylene in *Phaseolus* with the view of assessing its effect on the analyses of endogenous ethylene. Detailed accounts of stress ethylene may be obtained from the reviews by Abeles (1973) and Yang and Pratt (1978).

It is clear that ethylene emanation arising from wounding and/or water-stress can lead to sizeable errors in estimating the rate of ethylene evolution. In the quantitative analyses of ethylene emanation from *Phaseolus* tissue reported in this thesis, the incubation of the test tissues was completed within 25-28 min from the time the tissue was excised from the plant. Ethylene artifacts from the stem due to wounding were avoided since there was a lag period of about 25 min from excision before enhanced ethylene emanation occurred. As ethylene induced by water-stress in the stem is limited, this would not have posed a problem during routine analyses. Ethylene emanation from the leaves arising from water-stress was encountered in this study mainly in shoots that had been enclosed in glass vessels. "Wounding" of the leaves pertained essentially to the handling of the leaves in the course of preparation for ethylene analysis. The effects of ethylene induced either by wounding or water-stress in the leaves were minimized in this study by carrying out ethylene analyses within 25-27 min following their excision from the plant.

4. GENERAL DISCUSSION

Several theories of the correlative inhibition of lateral bud growth have been proposed and a variety of experienced findings in support of them have been described in the literature. There can be little argument that the morphological observations and physiological phenomena thought to control the expression of correlative inhibition do exist and operate at least to a certain extent, at least in some species and at least under specific experimental conditions. What requires critical evaluation is whether these observations and phenomena exert *sufficient* effect on the plant to bring about the characteristics of apical dominance attributed to them. In this regard, it is not always easy to ascertain that the observed changes in the status of apical dominance have actually arisen from corresponding morphological and biochemical changes, and not *vice versa*. To illustrate this point, the nutrient theory of apical dominance may be considered. Elements of the nutrient theory are very often drawn into experiments on apical dominance although they are not always acknowledged as such or incorporated into the discussion of experimental results. There is no question that very little organic and inorganic nutrients are transported to the inhibited bud, or that in decapitated plants, nutrients are diverted to the re-activated buds. There is also little doubt that when shoots were physically constricted or treated with ethylene, ethephon or TIBA in the present study, the active development of axillary buds which followed was augmented by compensation growth arising from the inhibition of shoot growth. However, while nutrient supply to the buds is essential to their development and that nutrient diversion contributes to this development, it does not necessarily follow that the lack of nutrient supply is directly responsible for the inhibition of bud growth in intact plants. That nutrients are not actively *supplied* to the inhibited bud does not necessarily mean that they are *unavailable*. If bud development is suppressed by other regulatory factors, there would

consequently be no demand for nutrients even if the buds have access to them.

Elucidating the regulatory factor(s), i.e. the correlative signal(s), is obviously a fundamental aim of research on apical dominance towards which much effort has been channelled. While the identity of the factor remains elusive, the research findings have revealed useful clues regarding its nature and probable mode of action. In one approach towards characterizing the mechanisms of the apical control of bud growth adopted in the present study, axillary buds of *Phaseolus* were examined in relation to their rapid growth following decapitation of the shoot. Bud growth was first detected 3-5 h after decapitation of the shoot. This interval is obviously too short for any substantial amount of new vascular tissue to be formed between the bud and stem. The studies on the vascular supply to the axillary bud showed that functional vascular continuity was already well established even in the inhibited bud and so confirmed that the lack of vascular connection to the stem was not the cause of growth inhibition. It would appear that cell expansion is an important feature of the initial growth of the axillary bud in *Phaseolus*, at least in the bud internode. In other species, cell division stimulated by the removal of apical dominance might contribute significantly to the initial bud growth. The increase in bud internode length in *Faba vulgaris* 12-48 h following decapitation was accompanied by a comparable increase in the number of epidermal cells in the bud internode (Couot-Gastelier, 1978). Further studies into the kinetics of bud growth appear to be a promising approach to the understanding of the correlative control on inhibited buds. Future work could encompass investigations into simultaneous and sequential changes in cell elongation, division and differentiation both in the foliar and internode portions of the bud. The kinetics of metabolic changes, including the importation patterns of metabolites, need also to be further investigated. Comparisons might be made between the early events in bud growth induced by shoot decapitation and by cytokinin

application. Work on the early growth activity of cytokinin-stimulated buds in *Cicer arietinum* has already been initiated by Usciati *et al.* (1969) and in *Pisum sativum* by Nagao and Rubinstein (1976); the bud growth having been first detected after 6 and 8 h respectively. The latter authors found the timing of bud growth and the onset of mitotic activity to coincide approximately and that they were comparable to observations in the buds of decapitated plants. It would also be interesting to look into the inhibition of lateral bud growth by auxins in relation to the kinetics of rapid bud growth.

A large part of this thesis is concerned with the involvement of ethylene in the control of axillary bud growth. A number of ancillary investigations were carried out on ethylene methodology in order that the results of experiments in which ethylene was analysed might be appraised more objectively. Analyses of endogenous ethylene are at present carried out in plant physiology research frequently as a matter of routine. Yet investigations into the methods of quantitative analyses themselves are scant. Ward *et al.* (1978) have collated information on the techniques of ethylene analysis currently available. The two most common methods of analysing endogenous ethylene are to determine the rate of emanation or the internal concentration in the gaseous extract of the test tissue. Both methods are liable to variability in the results to the extent that strict comparisons between the different treatments in an experiment might not be fully valid. Variability arises from errors during analyses, changes in experimental conditions or differences in gross morphology of the test tissues being analysed.

The errors which occur during ethylene analyses are likely to arise not from GC analysis - which is carried out readily and accurately - but from the procedures used in preparing the gas sample for GC. The potential sources of errors in the determination of ethylene emanation are first considered. Ethylene artifacts brought about by wounding and water-stress in *Phaseolus* have been described in this thesis. Wound-induced ethylene,

especially, is well known and provisions are commonly made in experiments to allow for this. One common method of circumventing the effects of wound ethylene is to carry out the analysis a few hours after excision of the test tissue to allow the extraneous ethylene to dissipate (e.g. Burg and Burg, 1968 a,b). This is not at all a satisfactory method since ethylene emanation increased by wounding does not rapidly return to the basal rate, and as evident from the results in this study, but remain well above the basal rate 3 h from excision. In addition, with the test tissue detached from the plant, the effects of ethylene substrate exhaustion and water-stress become more significant with time. In fact, substrate exhaustion and water-stress become potential sources of error with the extension of the period of incubation in the sealed container. An extended incubation period can lead to errors in other ways. Under certain circumstances, accumulation of ethylene in the container can lead to autocatalytic ethylene production (see Lieberman, 1979) or, conversely, autoinhibition of ethylene release (Jerie *et al.*, 1979). The latter effect - the reduction of ethylene emanation - need not even involve a biochemical response at the cellular level; the reduction of the ethylene concentration gradient between the internal air within the tissue and the external (atmospheric) air results in a reduction in the rate of outward diffusion of endogenous ethylene in accordance with Fick's first law of diffusion. The concentrations of oxygen and carbon dioxide present in the air can also critically affect the estimated rate of ethylene production. Ethylene synthesis from methionine requires oxygen (Lieberman *et al.*, 1966) while high concentrations of carbon dioxide have been shown to increase endogenous ethylene production (Aharoni and Lieberman, 1979a; Aharoni *et al.*, 1979). One way by which changes in the proportions of oxygen and carbon dioxide in the air within the container can be slowed down is by carrying out the incubations in clear glass containers in the light so that the respiratory and photosynthetic processes can balance one another, at least

to a limited extent. In this regard, determination of ethylene emanated from photosynthetic tissue in the dark (e.g. Wright, 1980) could be disadvantageous. Since the turnover of oxygen and carbon dioxide are unlikely to match one another exactly, an imbalance of the two gases is likely to increase with the time of incubation. The sources of error in the determination of the rate of ethylene emanation, as outlined above, are varied. However, they all have a common characteristic, namely that the chances of the error obscuring the true basal rate of ethylene release increase from the time the test tissue is excised from the plant. Quite obviously, the one experimental precaution that should mitigate the probability of error arising from the different sources is to carry out the analysis immediately after the tissue is excised from the plant and to avoid an excessively long incubation period. The incubation of test tissue in this study - 20 min immediately following tissue excision and carried out in the light - had been adopted with this consideration in mind. Wound-ethylene in the stem was thus avoided (because of the lag period) and wound-ethylene in the leaves, ethylene induced by water-stress, the effects of ethylene accumulation and the effects of oxygen-carbon dioxide imbalance would all be minimized. The main disadvantage of this short incubation period was that the small amount of ethylene accumulated did not allow for as accurate a determination by GC as where a higher concentration of ethylene was present. However, this slight loss of accuracy is far more acceptable than the various errors - often unpredictable and possibly additive - which accompany longer periods of incubation, hitherto the rule in reports in the literature (e.g. Burg and Burg, 1968a: 18 h; Aharoni and Lieberman, 1979: 18-24 h).

In the vacuum extraction of internal ethylene, many of the above precautions apply and the extraction procedure was normally carried out within 20 min of excision of the test tissue. The potential source of variability in determining internal ethylene does not lie in errors arising

from the extraction procedure alone, but also from variation in evacuation conditions and gross morphology of the tissue extracted. It is clear from this study that significant changes in internal ethylene and ethane concentrations occur in gaseous extracts when the evacuation conditions are changed. Furthermore, the internal ethylene and ethane concentrations are influenced to a considerable extent by the amount of extractable gases in the tissue (which is influenced by the relative proportion of intercellular air spaces in the tissue). An increasing "dilution" effect occurs with the increase in volume of extracted gases, leading to a corresponding diminution in the concentrations. It is obvious then that comparisons of internal ethylene between differently treated tissues should be made only if they are roughly similar in gross morphology. The same can be said of determining ethylene emanation since the outward diffusion of ethylene produced in the tissue is influenced by the physical resistances it encounters. In this regard, the disparity in the sizes between ethylene-treated or physically constricted shoots and their controls in the present study need to be borne in mind although it seems unlikely that the differences are serious enough to alter materially the interpretation of the results.

In describing the method of vacuum extraction, Beyer and Morgan (1970b) regarded the recovered gases as being inter-cellular in origin. They cautioned against the use of a vacuum stronger than 13.3 kPa as the gases might then include "bound" or dissolved ethylene. In the absence of any detailed understanding of the functions and relative importance of inter-cellular ("free") and "bound" ethylene in the plant, there appears little justification in arbitrarily assuming "free" ethylene to be the more important and so regarding "bound" ethylene as an undesirable contaminant. It is conceivable that both "free" ethylene and "bound" ethylene (where it is present) have essential functions in their respective rôles in the physiology of the plant even though the precise nature of these functions

remains to be elucidated. In comparing plant tissues of roughly similar morphology, an indication of the inter-cellular ethylene content may be obtained by measuring the rate of ethylene emanation. If an estimate of the ethylene concentration in the vacuum extract is to be made *in addition* to measuring the emanation rate, it would logically be more purposeful to apply a strong vacuum in order that "bound" ethylene might also be assessed. It is with this consideration in mind that 66.7 kPa was used in vacuum extractions in this study.

The presence of large amounts of ethane in the gaseous extract of *Phaseolus* shoot tissue requires closer scrutiny. Although the lack of active emanation of the gas has been ascribed to its occurrence in a "bound" form, the possibility of it being an artifact of the vacuum extraction procedure cannot be ruled out. Notwithstanding the fact that the extraction lasted only 2-3 min, the ethane recovered could have resulted from a rapid response to tissue wounding. Konze and Elstner (1978) have pointed out that ethane production may represent a sensitive marker for membrane damage.

In the discussion of the putative "bound" ethylene in ethylene analyses, the concept of ethylene "compartmentation" (Jerie *et al.*, 1978b, 1979) might be taken into account when relating the concentration of ethylene to its functions in the tissues. Other than the reference made to internal ethane being in a "bound" state, further discussions on compartmentation have been restricted in this thesis. The reason for this is that very little is known about the nature and function of compartmented ethylene. It is not clear what proportion of compartmented ethylene is recovered by vacuum extraction, or even whether it exists at all under natural conditions. Since compartmentation, if it occurred, could result in misleading values in ethylene emanation or the internal ethylene content, both methods of ethylene analysis were employed in this study.

The experiments carried out on ethylene methodology enabled a more thorough assessment of the relative significance of internal ethylene,

ethylene emanation and freely diffusible ethylene in the apical shoot in relation to the release of correlative inhibition on axillary bud growth. Apparently, the promotion of axillary bud development depended only on the availability of freely diffusible ethylene in the tissues of the treated apical shoots. Treatment of the apical shoot with ethylene also inhibited its growth. This may be compared with the effects of other experimental treatments which induce lateral bud growth, but where decapitation is not carried out. Treatment of the shoot with TIBA has been described in this thesis. Inhibition of apical growth was very evident even though ethylene is not thought to be implicated. Bark ringing (White, 1973) and treatment of the shoot with "M & B 25-105" (May & Baker Ltd.: information pamphlet) or abscisic acid (Aung and Byrne, 1978) are other methods of promoting lateral bud growth in non-decapitated plants, and all three inhibit growth of the apical shoot. It might be argued therefore that the inhibition of apical growth is the underlying cause of lateral bud outgrowth, and that ethylene treatment is merely one of several methods of achieving the inhibition of apical growth. It is conceivable that with the reduction of shoot growth, nutrients and other growth factors are then channelled to the lateral buds where compensation growth subsequently occurs; a mechanism essentially conforming to the nutrient theory. This proposition must remain a possibility since at least some retardation of shoot growth was always observed when ethylene or ethephon were applied to the shoot or when the apical shoot was physically constricted. On the other hand, treatment with low concentrations of ethephon (50 ppm) was effective in inducing outgrowth of the lateral bud whereas the reduction in shoot growth was slight. Conversely, enclosing the shoots in glass vessels, but without adding ethylene, significantly inhibited the growth of the apical shoot without promoting axillary bud growth.

If, in promoting axillary bud development, it is supposed that ethylene plays a more specific rôle in the apical shoot than in inhibiting apical growth, what would this rôle be? With the physiology of ethylene action

still a subject of much debate, a proposed rôle of ethylene in this instance is largely a matter of conjecture. It would appear that the absence of physiologically significant transport of ethylene (Zeroni *et al.*, 1977; Jerrie *et al.*, 1978) renders it improbable that ethylene applied to the shoot is transferred to the bud. Similarly, ethephon applied to the shoot tends to be translocated to the apex (Weaver *et al.*, 1972) rather than basipetally towards the axillary buds. Any assertion of the lack of ethylene transport should, however, be qualified by the amount of ethylene that is regarded as physiologically significant. The concentration of ethylene required to restore normality to the ethylene-deficient mutant tomato plant (Zobel, 1973) and that required for cyto-differentiation in lettuce pith cells (Zobel and Roberts, 1978) are reported to be of an order of 0.005 vpm. Thus, while it can be demonstrated that mass transport of ethylene does not take place in the plant, movement of small, yet physiologically active, quantities of ethylene or ethylene metabolites can certainly pass undetected by presently available analytical techniques. Even assuming the absence of ethylene transport, high ethylene concentration at one location on the plant is able to induce an increase in ethylene production elsewhere (Zeroni *et al.*, 1977; Bradford and Yang, 1980), possibly through the translocation of ethylene precursors such as ACC (Bradford and Yang, 1980). Nevertheless, the negative effects on bud growth when ethephon was applied directly to the bud argues against a direct involvement of ethylene at the bud. Zobel (1974) encountered a comparable situation in his work with the ethylene-requiring mutant tomato seedling. He found that while treatment of the entire plant with a low concentration of ethylene induced normal development of the otherwise non-branching roots, ethylene treatment to isolated roots in culture did not produce a similar effect. He concluded that (a) ethylene induced the production of an active "substance" that was produced in the shoot and transported basipetally to the roots and (b) the seedling roots did not produce this substance themselves. Drawing a parallel in the present study, ethylene

in the apical shoot may be modified in some manner - perhaps metabolized - or may itself modify other factors to bring about a change in the correlative signal to the basipetal axillary buds. Taking a hypothetical stance, ethylene can act indirectly through its effect in reducing auxin transport. Auxin plays a central rôle in both the "Direct" and "Indirect" theories of auxin-induced bud inhibition. Auxin also forms the basis of the "Hormone-Directed Transport" theory, and a paper by Patrick and Wareing (1978) proposes that a continuous auxin "stream" from apex to inhibited bud is necessary for the maintenance of IAA-promoted acropetal transport of metabolites. Application of ethephon to the shoot could cause the breakdown of these auxin-mediated mechanisms of correlative inhibition that are in operation.

Direct application of ethephon to the inhibited bud showed that bud outgrowth was not stimulated by ethylene, even when steps were taken to avoid donating a supra-optimal concentration. The application of the ethylene inhibitors Ag^+ and AVG to the axillary bud of the decapitated plant also inhibited development of the bud. In most of the buds, a limited development occurred before further growth was curtailed. It appears that the requirement for ethylene at the bud was not so much associated specifically with the onset of axillary bud outgrowth, but with the processes relating to subsequent tissue growth and development (e.g. cell division). In general, therefore, ethylene is found to be essential in maintaining normal axillary bud growth in the decapitated plant (which is to be expected) but the absence of bud outgrowth in the intact plant is not due to a lack of "free" ethylene.

Burg and Burg (1968b) took the diametrically opposite view in suggesting that high ethylene concentration at the node was responsible for the suppression of lateral bud growth. Direct application of Ag^+ and AVG to the bud and node did not bear out this proposition; instead, as mentioned above, growth inhibition resulted. However, it might be argued

that the ethylene inhibitors effected a drastic non-selective inhibition in all ethylene-requiring processes, including those essential to bud development. It was decided, therefore, to repeat - with additional precautions - Burg and Burg's experiment to determine ethylene levels at the node and internodes of *Phaseolus*. The main objection against Burg and Burg's procedure was that ethylene emanation was measured 5 h after the test tissues had been excised from the plant to allow for the subsidence of wound-ethylene. Moreover, the subsequent incubation period was 16 h. The disadvantages of such a time lapse between isolation of the test tissue and the sampling of endogenous ethylene have been outlined earlier in this discussion. There is, in addition, a further consideration which could critically affect the interpretation of the experimental results. Whereas Burg and Burg were attempting to estimate ethylene levels in the node bearing the inhibited bud, the act of isolating the bud from the plant apex effectively releases the bud from inhibition. Several researchers (e.g. Wickson and Thimann, 1958; Peterson and Fletcher, 1975) and, in fact, Burg and Burg (1968b) themselves in the same paper have used the "isolated node system" in experiments to demonstrate release of buds from apical dominance. This point is particularly relevant as ethylene was sampled 21 h after excision of the test tissues, whereas the increment in growth and mitotic activity in *Pisum* buds released from inhibition can take place within 8-12 h (Nagao and Rubinstein, 1976). Thus, while Burg and Burg were of the opinion that ethylene plays a growth inhibition rôle in apical dominance, their results can equally well be interpreted to indicate ethylene involvement in the *release* of buds from inhibition. In the present study, analyses of ethylene emanation were carried out within 27 min of excision of the nodal and internodal sections from intact and decapitated plants, before the onset of wound-ethylene release. The internal ethylene levels in the node and internode were also assessed and the effect of IAA applied to the cut stem of the decapitated plant was examined with respect to internal ethylene and ethylene emanation. As the results showed,

decapitation of the plant decreased endogenous ethylene in the stem. However, it is not conclusive that this decrease is responsible for the release of axillary buds from inhibition. The contention that auxin-induced ethylene suppresses axillary bud growth in the decapitated plant where the apical shoot is replaced by auxin is not borne out in this study.

When IAA was supplied directly to the inhibited axillary bud, a transient promotion - rather than the expected increased inhibition - of its growth was recorded. Growth of the bud was enhanced despite the fact that a relatively high concentration of IAA (10^{-1} mol m^{-3}) was used. It is possible that the uptake and subsequent transport of IAA was inefficient and that only a small proportion of what was applied actually reached the site of action in the bud tissue. The increased growth could have resulted from cell expansion, a response known to be induced by auxins. The finding earlier in this study that the initial growth of the axillary bud internode following decapitation is largely due to cell expansion adds credence to such a proposition. This aspect requires verification in further experiments.

There is, nevertheless, a degree of doubt as to whether growth promotion in IAA-treated buds, even in the initial phase, is similar to that in decapitated plants. In the first instance, IAA-promoted growth of the axillary buds was not sustained beyond 2-3 days, although this could be due to a depletion of the auxin as IAA application was not repeated. Secondly, the enhancement of growth by IAA was completely annulled by AVG treatment while that due to decapitation was unaffected. The transient outgrowth of IAA-treated buds is perhaps more comparable to the promotion of extension growth in axillary shoots newly developed from cytokinin-treated buds in *Pisum* or in shoots that are inhibited by other more dominant shoots, as described by Sachs and Thimann (1967). While in the present case, the IAA-treated buds in *Phaseolus* were not pre-treated with cytokinin, they were nevertheless well developed, possessed defined internodes and showed a slow but continuous growth. These buds, even though inhibited, might therefore be responding to IAA in the same way as weakly developing

axillary shoots in *Pisum*. Some preliminary experiments carried out in the present study on the combined effects of IAA and the cytokinin, zeatin, on *Phaseolus* bud growth were inconclusive (results not presented). What was evident, however, was that treatment of the buds with both hormones did not give rise to a growth promotion effect markedly greater than that due to zeatin alone.

Irrespective of the mechanisms responsible for the observed growth enhancement by IAA, it is clear that the effect of auxins in general, and IAA in particular, on the inhibited lateral bud is not always one of growth inhibition. The enhancement of axillary bud growth by IAA is compatible with the report by Hillman *et al.* (1977) that IAA levels of *Phaseolus* lateral buds rise when released from inhibition.

Because of the extensive range of physiological activities which are mediated by ethylene, several aspects of ethylene-related physiology which could have a bearing on apical dominance have not been investigated in this thesis. For example, red light and far-red light treatments affect apical dominance in some plants such as *Nicotiana* (Kasperbauer, 1971) and *Xanthium* (Tucker and Mansfield, 1972). Apical dominance in *Pisum* is diminished by increasing the carbon dioxide content of the air (Andersen, 1976). Both the treatment with red/far-red light (Goeschl *et al.*, Samimy, 1978) and with carbon dioxide (Aharoni *et al.*, 1979) are known to modify ethylene production and/or action in plants. The phenomenon of gravimorphism, in which growth is promoted in the upward orientated lateral buds on stems and branches trained into a horizontal position, might be mediated through stress-induced ethylene. Abeles and Gahagan (1968) reported that *Coleus* plants placed in a horizontal position produced more ethylene. Branches of *Pinus strobus*, *Pyrus malus* and *Prunus persica* tied in arcs showed significant increases in their internal ethylene content (Leopold *et al.*, (1972). Hormone-directed transport is another aspect in which the possibility of ethylene involvement could be investigated. Some work in

this area has been carried out by Mullins (1970b) who showed that ethylene increased the transport of radioactive-labelled photosynthate in *Phaseolus* internodes. However, the concentration of ethylene used was very high (undiluted or 500 vpm) and the results were not corroborated when internodes were treated with ethephon to observe its effect on the transport of labelled sucrose. An insight into the mechanisms of hormone-directed transport might be obtained from experiments designed to study the effects of ethylene, ethephon and ethylene inhibitors, and their interactions with other growth regulators, on the phenomenon. Besides Ag^+ and AVG, the two ethylene inhibitors used in this study, future experiments on ethylene physiology could also incorporate the use of new ethylene inhibitors such as aminooxyacetic acid (Amrhein and Wenker, 1979) to ascertain that the responses observed have actually arisen from ethylene antagonism rather than from other metabolic effects.

Various aspects of the correlative control on lateral bud growth which are not necessarily related to ethylene also require detailed study. Research into the action of proprietary compounds such as "M & B 25-105" which act on the apical shoot to induce development of inhibited buds could reveal various similarities and differences in plant responses to this compound and to other treatments which promote lateral bud growth in non-decapitated plants (e.g. treatment with ethylene or TIBA). This information might be useful in providing leads in identifying the specific responses that are primarily related to the release of lateral bud inhibition. Some preliminary work with "M & B 25-105" was carried out on *Phaseolus vulgaris* but the effective dosage required for the promotion of bud growth also caused considerable chemical scorching of the treated young leaves; this line of work was not, therefore, actively pursued.

Although considerable research has been carried out to relate cytological and (gross) morphological changes in the lateral bud to the onset of their development, very limited work has so far involved the use of the electron microscope. There appears to be considerable potential in

studying the changes in fine structure of lateral buds when they are released from inhibition.

There is still extensive scope in the employment of classical methods in research into the apical control of lateral bud growth. With the rapid advent of new laboratory techniques, novel approaches could be expected to generate added impetus towards the understanding of the phenomenon of apical dominance.

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