ENDOGENOUS CONTROL OF SENESCENCE IN

PISUM SATIVUM L. (THE GARDEN PEA)

Thesis presented by

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SUMMARY

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Early work of the 19th Century suggests that removal of flowers can delay senescence in annuals, an explanation of this phenomenon was not offered until Molisch published his results in 1928. He suggested that the fruit acts as a sink for plant food reserves. An alternative explanation was given by Leopold et al. (1959) who showed that removal of flowers from male spinach plants and defruiting of complete fruits in bean can also delay senescence in them. Lockhart & Gottschall (1961) substantiated Leopold's work in peas.

Wareing & Seth (1967), by applying hormones as a substitute for developing fruit, demonstrated a mobilization of nutrients to the site of hormone application.

Two hypotheses exist to explain senescence of annuals and possibly in herbaceous biennials.

(i) Imbalance in nutrient distribution in whole plant resulting in death of apical meristems.

(ii) Autonomous hormonal control. In the most extreme case this could be a specific senescence hormone.

My work with peas has been designed to confirm the second hypothesis and can be divided into three main headings.

(i) Protein studies.

(ii) Hormone studies.

(iii) Surgical experiments.

(i) Protein studies

In explant studies a frequent correlation is made between proteins and the organ senescence. It was therefore attempted to study protein changes in relation to whole plant senescence, and at the same time try to find an objective method for measuring senescence in plants. Isoelectric focusing of proteins on acrylamide gels was carried out for separating proteins in this and a new staining technique was developed. It reduced the working time from days to hours and was then published.

The results of these studies showed that changes appearing in leaf proteins were independent of flower or fruit development. It was easy to correlate aging in leaves with changes appearing in their proteins, but no correlations between leaf protein at any stage were possible with the aging of whole plant.

(ii) Hormone studies

Quantitative studies of endogenous GAs, ABA and cytokinins were started with a view to correlating their levels with plant senescence. Standard procedure of methanol extraction was followed to extract these hormones. A new method was, however, developed for the initial group separation of GAs and ABA on alkylated sephadex. This column chromatography gave a very good initial purification of the group along with separation of inhibitors. Final purification and separation of individual GAs, ABA and also cytokinins were carried out on TLC, and the amounts were quantified on the basis of their biological activities.

Results from these studies showed that flower and fruit development had very little effect on endogenous levels of leaf hormones as compared with their deflorated controls. The maturity of fruits

however brings distinct changes in leaf hormones. Studies on unknown inhibitors were left over, due to shortage of time.

(iii) Surgical experiments

Both of the nutrient and hormonal explanations to plant senescence were offered from the observations obtained in different kinds of surgical experiments. This kind of experimentation, therefore appeared to be another interesting way for further investigations. Initially the effects of removing flowers, fruits, leaves and apices were studied both individually and in combinations. Later all experiments were conducted to separate the effects of fruit maturity from fruit filling. In these experiments initial observations were taken in an experiment where branching was induced in the plants and then different numbers of pods were allowed to develop at different sites while continuously deflorating rest of the plant. Other experiments were then carried out using grafts. With this grafting technique it was possible to study the effects of fruit development and fruit maturity independently of each other. The main observation in these experiments was the growth of the younger part of the graft as affected by fruit when compared with corresponding defruited controls.

Results obtained from these experiments were very conclusive and for the first time distinct effects of fruit maturity independent of fruit filling were demonstrated. Besides many interesting observations it was clearly shown that fruit development is a nutritional phenomenon but does not exhaust the plant for further growth, and fruit ripening produces a distinct dramatic stimulus that results in senescence of the meristematic region and so cancels any possibilities

of further growth. It was then possible to demonstrate complete development of fruits consecutively for the three times on the same root stock.

Publication

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CONTENTS

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GENERAL INTRODUCTION

When we sow the seed of an annual plant, under favourable conditions it grows, then it flowers and fruits, and thereafter it quickly senesces and dies! But how does it so quickly senesce and die? Is it because the developing flowers and fruits exhaust the remainder of the plant of its organic reserves (Molisch 1928) or because its apex differentiates into final flower and so cannot grow any further (Lockhart & Gottschall 1961) or because its leaves go yellow or roots start rotting or is it because of the levels of abscisic acid, gibberellin, cytokinin, phytoferritin (El-Antably et al 1967, Sitton et al 1967, Fletcher et al 1969, Barton 1970), or just because it is an annual and so is supposed to die after a year - which points to the involvement of genetic apparatus and therefore, is it because of the proteins, RNA or DNA? So which is the cause? In actual fact, at first glance, many correlations can be made between senescence and changes in chlorophyll, proteins, RNA, DNA, hormones or nutrients, but it is not possible to state that these are the causes or results of senescence. A situation of this sort, therefore, can easily lead to suggest that while any one of these may not result in the eventual death of the plant, it is possible that the conjunction of all these events leads to the death.

But before we could go into any more complexities of the cause, an obvious question arises - what is senescence? Senescence in its broad sense means to include some kind of synthetic and other kind of degradative processes that ultimately deteriorate the functional capacity of an organ or organism. This of course is a very general outline, but for a succinct description the difficulty is that the term senescence is frequently used to designate processes of very different nature, such as general decline in vigour in woody plants or the apparently programmed sequence of events leading to the death of a particular organ,

or whole plant in the case of annuals (Wareing & Seth 1967). Therefore it will be useful to describe various types of senescence that are observed in different species of plants.

General types of senescence

Different types of senescence observed in various plant species have been classified by Leopold (1964) in four classes. These are -

(i) Overall senescence

(ii) Top senescence

(iii) Deciduous senescence

(iv) Progressive senescence

Overall senescence is observed in monocarpic plants, which may be annuals or biennials or occasionally perennial (like bamboo) where the whole plant shows a short period of senescence soon after flowering and fruiting, terminating in the death of the plant. Top senescence is exhibited in some perennial herbaceous plants (Aster spp.) where the above-ground portion behaves similarly to annuals and dies every year while the underground portion remains viable for many years. Perennial polycarpic plants (woody plants) flower and fruit for many years while both their underground and above-ground portions remain viable. In some cases, however, all of their leaves shed every year (deciduous senescence) while others show a progressive senescence of leaves every year (progressive senescence).

General symptoms of senescence

The most commonly observed features of senescence are:-

(i) Decline in chlorophyll and photosynthesis

(ii) Decline in protein and RNA levels

(iii) Changes in the levels of different growth regulators

The decline in chlorophyll which is manifest by the yellowing of green tissue, is the most striking feature of senescence that can be easily observed with the naked eye; however, quantitative analyses have also been done to show a sequential loss of chlorophyll during senescence (Osborne 1962, Shaw et al 1965, Woolhouse 1967, Simon 1967). Probably for this reason it has been commonly used as a measure of senescence (Molisch 1928, Leopold et al 1959, Whyte & Luckwill 1966, Goldthwaite & Laetsch 1968, Fletcher 1969, Goldney & Van Stevenick 1970, Back & Richmond 1971). As chlorophyll is essential for photosynthesis (Meyer & Anderson 1939), decline in photosynthesis has also been observed by various workers during the aging of leaves (Freeland 1952, Sorokin & Krauss 1961, Smillie 1962, Leopold 1964, Woolhouse 1967).

It has been known from the works of Yemm (1937) and Chibnall (1948) that proteolysis occurs during senescence of leaves. Bottger & Wollgiehn (1958) showed that decline in RNA content also takes place, parallel to proteins, during senescence. Since then, fall in protein and RNA levels has been observed by various other workers (Wood & Cruikshank 1944, Racusen & Arnoff 1954, Osborne 1962, Shaw et al 1965, Simon 1967, Ecklund & Moore 1968, Atkin & Srivastava 1969, Fletcher 1969, Millikan & Ghosh 1971, Gorsheva 1972, Parisco et al & O'Leary 1972), and therefore it is now generally considered as a significant feature of senescence that can also be used as a measure of this phenomenon (Leopold 1964, Goldthwaite & Laetsch 1967, Osborne 1968, Kurishi 1968, Mizrahi et al 1970, Tung & Brady 1970).

Richmond & Lang (1957) showed that senescence in Xanthium leaves can be retarded by the application of Kinetin. Since then many other workers have shown that different growth promoters, e.g. Cytokinin (see Letham 1967, Sirvastava 1967, Skoog & Armstrong 1970), GAs (Fletcher & Osborne 1965, Beever 1966, Whyte & Luckwill 1966, Goldth-

waite & Laetsch 1968) and auxin (Sacher 1959, Osborne & Hallaway 1964) can retard senescence whereas growth inhibitors, e.g. ABA (El-Antably et al 1967, Aspinal et al 1967, Osborne 1967) can accelerate senescence in detached leaves. It can be assumed from these observations that the normal processes of senescence in plant tissue would also be associated with the changes in the endogenous level of these growth regulators. However, quantitative studies to measure the endogenous levels of different growth regulators have been conducted by various workers (Wheeler 1968, Fletcher et al 1969, Chin & Beever 1970, Mayak & Halvey 1970), which have confirmed that during senescence quantitative levels of different growth promoters decrease while the levels of inhibitors increase.

General theories of senescence

In the overall senescence of annuals, which is the main interest of this study, yellowing spreads from older leaves extending to the younger ones and then to the stem, followed by drying and final death. The striking appearance of yellowing (due to loss of chlorophyll) starts soon after flowering then rapidly becomes intense and is greatest by the time fruit matures. The dramatic appearance of these events leads to the conclusion that flower and fruit development are important in the control of whole plant senescence. Possibly for this reason most of the work carried out in whole plant senescence is in relation to flower and fruit development. Various theories that have been promoted to explain whole plant senescence are:

Nutrient exhaustion theory

Reichart (1821) was probably the first to show that removing flower buds from Vienna wall-flower (annual plant) can considerably prolong the life of the plant (nearly up to three years). Johnson (1862)

suggested that removal of flowers from Nasturtium plants (annual plant) considerably extends their life because the exhaustion of nutrients by developing seeds is checked. Suppression of plant growth by developing fruit was also pointed out by Mason (1922) and Murneek (1926). A detailed study of plant senescence, however, was first carried out by Molisch, who published his results in 1928. He deduced that "when we hinder flower formation by whatsoever means, whether it be through insufficient light, certain temperatures or through greater humidity of the soil and air, we guard the plant against death and defer the latter. But just as soon as it has flowered and fruited, the organic reserves are completely emptied and utilized and a yellowing of leaves or entire plant accompanies this process; at the same time the chloroplasts are destroyed and their proteins move into the seeds. Deprived then of its chloroplast the chlorotic leaf can no longer function normally or serve as an organ of nutrition and for this reason the plant dies after the ripening of its seeds."

Mobilization of nutrients by developing flowers and fruits has been shown by many other workers. Crowther (1934) found that development of cotton fruits can mobilize leaf nitrogen. Marre (1948) observed that the growth of ovary and stamens was associated with a relatively high concentration of starch which degrades and disappears if ovaries are removed. Various other workers have also reported that a number of nutrients, including amino acids, are transported from the lower leaves to the upper regions of growth and development (Williams 1955, Hopkinson 1966, Cockshull & Hughes 1967).

Loomis (1953) suggested that high auxin content of developing seeds may play an important role in nutrient mobilizing activity. High concentration of other plant hormones in developing fruits have also been reported by other workers (Baldev et al 1965, Burrows &

Carr 1970, Wheeler 1972, Jeffcoat & Cockshull 1972). Wareing & Seth (1967) proposed that the nutrient mobilization effect of fruits might be related to their high hormone concentrations. Using high concentrations of hormones (indole acetic acid (IAA), gibberellic acid (GA_3) and kinetin (k)) in lanolin as a substitute fruit, they were then able to demonstrate the mobilization of ^{32}P from lower leaves to the site of hormone application. As leaf proteins remain under continuous turnover (Gregory & Sen 1937, Vickery et al 1940, Chibnall & Wiltshire 1954, Racusen & Foote 1962, Holmsen & Kock 1964), Simon (1967) proposed that it could be that amino acids produced during hydrolysis are rapidly transported to developing fruits and so cannot be utilized again for protein synthesis in leaves. But Wareing & Seth (1967) were of a different view; they suggested that a phytokinin like substance produced in the roots (Seth & Wareing 1965) is diverted away from the leaves into the developing seeds and therefore senescence of leaves occurs due to lack of this factor. But Sitton et al (1967) observed that the production of cytokinin in the roots drops by a factor of ten when plants have reached their final size, and therefore they considered that shoot senescence occurs due to reduced supply of this factor, rather than the diversion by fruits.

Another controversial point with regard to nutrient mobilization effect of fruits was raised by Petrie et al (1939). They observed that although the removal of flowers and fruits can retard the loss of assimilates from the leaves, it did not prevent the shrivelling of leaves. They suggested that senescence breakdown occurs independent of the flower and fruits. These break down products are then translocated to fruits that acts as a sink, but in its absence these are either retained in the leaves or exported to roots. They therefore concluded that developing fruits do not cause leaf senescence by

mobilizing assimilates from it, but in fact it acts as a sink where breakdown products could be deposited. Leonard (1962) has also mentioned various reports that state that the development of fruits in various species is due to their self-synthesis of storage materials.

Therefore it cannot be said, with any certainty, that the whole plant senescence in annuals occurs due to nutrient mobilizing effect of their fruits or *that* nutrient diversion into the fruits is only the result of senescence.

Theory of special senescence stimulus

Thirty years after Molisch (1928), Leopold attempted to provide a quantitative extension of his results. However, after three years experimentation on soyabean and spinach, Leopold et al (1959) proposed the involvement of a special senescence stimulus as distinct from a nutrient exhaustion effect.

In their experiments on soyabean they observed that control plants where fruits were allowed to mature lived for 119 days. If plants were deflorated at the time of first petal-colour formation they lived for 179 days, and if defruited after the pod had elongated but not filled, they lived for 161 days. But even if fruits were removed after they were completely filled, and just starting to show yellowing, the plants lived for 129 days. With spinach their interesting observation was that even the defloration of staminate flowers from male plants delays their senescence. They therefore deduced that although developing fruits play a significant role in whole plant senescence, delay in senescence by removing complete fruits or male flowers cannot be explained in terms of nutrient exhaustion and so there must be a special senescence stimulus involved in the whole plant senescence.

Lockhart & Gottschall (1961) repeated the work of Leopold et al

on peas and reached a similar conclusion, that fruits may not simply act by diverting organic reserves from within the plant, but that reproductive structures cause death by some other mechanism. In Xanthium, Kvĕtek et al (1966) found that the kind of leaf senescence which follows the change from vegetative to reproductive condition canot be attributed to the exhaustion of reserves by flower and fruits. Photoperiodic induction of senescence in leaves of woody plants has been shown by other workers (Olmsted 1951, Osborne and Hallaway 1960, Patricia & Titus 1972). As changes in endogenous growth regulators can appear under different photoperiod (see Evans 1971) as well as due to flower development (Harada & Nitsch 1959), it can be thought that the stimulus of senescence might be a change in the endogenous levels of growth regulators. But indication of a special senescence factor produced in the maturing leaves has also been reported by Osborne (1955). Thus it may be concluded from these reports that a special stimulus, distinct from nutrient exhaustion effect, is involved in the plant senescence, but it is difficult to say that senescence stimulus is a special senescence factor (one or a group of compounds) produced at a certain stage of plant growth, or it is a change in the endogenous levels of growth regulators or .....

Theory of genetic control

The type of senescence exhibited by a plant is characteristic of that species. Some plants may live for the same number of years but show different types of senescence like a perennial monocarpic and a perennial polycarpic, or they may show the same type of senescence but differ in their life span like annuals or a perennial monocarpic plant. The characteristic pattern may be modified by environmental factors to a certain extent but basically they are the fixed properties

of different species and so must be under genetic control (Meyer & Anderson 1939, Butler & Simon 1971).

Apart from theoretical reasoning Lockhart & Gottshall (1961) provided fairly convincing evidence that apical senescence in peas is due to some degenerative changes initiated within the apex itself. Apical senescence in peas would, therefore, seem to be under genetic control. Recently genetic lines which differ in their senescence characteristics relative to fruiting have been produced in Cornell University, U.S.A. (personal communication from Dr P.J. Davies). One of these lines does not undergo senescence after fruiting and continues to grow if the plants are kept under short day conditions. Under long day conditions, however, they do not show any difference from the regular peas and similarly senesce and die after fruiting. It may be said that senescence in the plants of new line is under a photoperiodic control, but there is little doubt that response to photoperiod in the new line is due to induced changes in the genetic apparatus of normal peas.

The genetic programming of senescence in leaves and the possible action of hormone in the de-repression of genes has been discussed by Woolhouse (1967), but very little work has been done to explain the genetic control of whole plant senescence in annuals.

Environmental factors affecting senescence

Various environmental factors such as soil nutrients (Macdougal 1901, Williams 1936), water status (Bakhuyzen 1926, Gates 1955, Shah & Loomis 1965), temperature (Pucher et al 1948) and light (Hopkinson 1966, Goldthwaite & Laetsch 1967) appear to have distinct effects on plant senescence. It would, however, be interesting to point out here that these are the same parameters as the ones that are known to control

the growth of the plant. Thus if growth and senescence are antagonistic to each other then it may be that the effect of these factors in the enhancement of senescence is mainly the limitation of growth. However, we cannot emphasise this point on general terms because in some cases, e.g. photoperiodic effect (as mentioned earlier) could delay senescence without limiting growth.

Ultrastructural aspects of senescence

Molisch (1928) pointed out that due to nutrient mobilizing effect of fruits, the chloroplast in the leaf are destroyed, and thus leaf loses its synthetic function and therefore senesces and dies. In 1957 Sacher found that deterioration of membrane integrity accompanies close to the senescence of bean segments. In 1959 he reported a similar loss of membrane integrity in the leaf discs of Mesembryanthemum sp. and in Rhoeo discolor during their senescence. He found that if the senescence in these plant organs is delayed by the application of auxin, it also prevents the disintegration of membranes and so they remain rigid. Thus he considered that the loss of membrane integrity is due to lower levels of auxin. Das & Leopold (1964) also found that the permeability of bean leaves increases as they approach senescence. They reported that if leaf senescence is prevented by decapitation then it also arrests the permeability increases. They concluded that synthetic efficiencies of leaves or whole plant may be linked with the structural integrity of the cells and their organelles. Changes in other organelles of plant cells during senescence have been described by Varner (1961). Recently a detailed survey on the literature pertaining to ultrastructural aspects of plant senescence has been carried out by Butler & Simon (1971). They have concluded that in the initial stages of senescence protein supply is the key factor, which is under genetic

control directly at a nucleic acid level (either directly due to lower supply of RNA or indirectly through precursors, nutrition and hormone levels). However, when structural and functional degeneration reaches an irreversible phase, then it is characterised by the loss of integrity of membranes and activation of lysosomal enzymes. This means that they do not place any great importance on changes at ultrastructural levels with respect to the cause of senescence.

Martin & Thimann (1972) have also reported that detergents (Triton X 100 and sodium lauryl sulphate) that disrupt membranes, had no effect on either the rate or extent of senescence. They therefore disagreed with any previous suggestion that during senescence protease becomes active after being liberated from lysosomes. They say that proteases must be newly formed.

Chemical regulation of plant senescence

During the last two decades a considerable interest has arisen in chemical regulation of plant senescence mostly in detached organs but occasionally in intact plants. Various studies on proteins and plant hormones have been carried out in relation to senescence. These works as such may not be of any value to explain the mechanism of whole plant senescence but can be useful for the studies on whole plants. A brief account of literature follows.

Protein metabolism

Chibnall & Grover (1926) were first to demonstrate that leaf proteins can be divided into two groups, i.e. soluble and insoluble in water. Since then various separation techniques have been used to study the relationship of different proteins with senescence. For

example, Wood et al (1943) found that during the senescence of sudan grass chloroplast protein decrease more rapidly than cytoplasmic proteins. Dorner et al (1957) with the use of ultracentrifugation, separated water soluble proteins into two fractions and observed that fraction 1 protein disappear more rapidly during senescence. Using acrylamide gel electrophoresis, Carr & Pate (1967) showed a decline in various bands during the aging of leaves. In general a net loss in proteins during senescence is well known (as mentioned earlier).

Simply it may be thought that decline in proteins would be due to their hydrolysis into amino acids. However it has been shown that although protein levels constantly decrease during senescence, old leaves still remain capable of protein synthesis (Mothes 1926, 1931, Walkley 1940, Chibnall & Wiltshire 1954, Deken - Grensen 1954). Gregor & Sen (1937) suggested that synthesis and hydrolysis of proteins are continuous processes of leaf metabolism. However with the use of labelled compounds (^{15}N and ^{14}C) a more direct evidence has been given that protein turnover is a continuous process in the leaves (Vickery et al 1940, Chibnall & Wiltshire 1954, Racusen & Foote 1962, Holmsen & Koch 1964). Thus, considering that synthesis and proteolysis are normal processes of protein metabolism, obvious question arises - is the net decline in proteins during senescence, due to a depression in synthesis or an increase in hydrolysis or both?

Simon (1967) suggested that it may be that nutrient mobilizing effect of growing organ has a decisive role in disrupting the normal protein turnover. He explained that amino acids produced during hydrolysis are rapidly translocated away from the leaf and therefore decline in protein synthesis occurs due to unavailability of amino acids. This can be a strong possibility in attached leaves where nutrient mobilizing effect of fruits and other growing regions is well known (Molisch 1928,

Williams 1955, Leopold et al 1959). But in detached leaves with apparently no mobilizing centres, it becomes difficult to explain that their senescence could be due to nutrient mobilizing effect. Although it has been reported that during the senescence of detached leaves, carbohydrates from the leaf lamina are translocated into the petioles, which can be in response to wounding (Chibnall 1954) or it may be that breakdown in the lamella takes place independently (Petrie et al 1939) and then the breakdown products move into petiole for greater chances of diffusion. Therefore an alternate possibility that senescence in plants could be due to decreasing efficiency of synthesis was also suggested by Simon (1967).

Bottger & Wollgiehn (1958) showed that during senescence loss in proteins parallels the loss in RNA. Osborne (1962) suggested that as it is well established that protein synthesis is closely associated with RNA, the loss of proteins during senescence would be due to reduction of effective sites where amino acids could assemble. Cherry (1967) suggested that in the regulation of protein synthesis mRNA and soluble RNA play an important role. Paranjothy & Wareing (1971), however, showed that inhibition of ribosomal RNA by 5-fluorouracil had no effect on protein synthesis, but they have suggested that soluble RNA could be largely responsible for regulation of protein synthesis.

Chibnall (1954) showed that yellowing of detached leaves is retarded as soon as they form roots. It has been therefore suggested that roots metabolise a factor necessary for protein synthesis (Osborne 1962). And as mentioned earlier Sitton et al (1967) have also pointed out that in intact plant, reduction in the production of cytokinin like substances in roots could be the cause of shoot senescence. Moreover Goldthwaite & Laetsch (1967) have shown that senescence of detached leaves can be retarded by keeping them under

light. They concluded that senescence delaying effect of light would be dependent on photosynthesis. Thus providing another support that senescence is effected by decreased synthesis.

Martin & Thimann (1972) have shown that senescence of oat leaves in the dark can be promoted by the addition of certain amino acids like serine, cysteine, glycine and alanine. They have suggested that these amino acids regulated the new formation of proteases, and therefore the senescence in leaves is controlled by the new formation of proteases. It has also been suggested by other workers that during senescence increase in hydrolytic enzymes like peptidase (Anderson & Rowan 1965) and RNase (Dove 1967, Sarivastava 1968, Udvardy et al 1969, Gabriella & Farkas 1970) occurs.

Hormone action

The interest in growth regulatory control of senescence started when Richmond & Lang (1957) found that kinetin can considerably delay senescence in Xanthium leaves. Later various reports (as mentioned earlier) showed that cytokinin, Gibberellin and auxin can retard senescence in different species, whereas abscisic acid (ABA) has a senescence accelerating effect. Studies on endogenous levels of hormone were also carried out by various workers, which showed that during senescence levels of growth promoters decrease while the level of inhibitor (ABA) increases (Wheeler 1968, Fletcher et al 1969, Chin & Beever 1970, Mayak & Halvey 1970).

Although at present there is little conclusive evidence regarding the mechanism of hormonal control of senescence, however, various attempts have been made (mostly in relation to cytokinin) to resolve this problem.

Mothes & Engelbrecht (1961) observed that senescence in tobacco leaves is retarded only at localized regions where kinetin is applied,

while untreated parts of the leaf start yellowing. Using labelled amino acids they found that an accumulation of amino acids occur in the kinetin treated areas. Therefore, they concluded that kinetin retards senescence by mobilizing metabolites from other regions, rather than enhancing the synthesis. In 1962 Osborne showed that kinetin can retard senescence not only in whole leaf but also in the isolated discs of leaf blade. She deduced that effect of kinetin in delaying senescence of leaf disc cannot be dependent on the accumulation of metabolites from other regions. Thus, using ^{14}C labelled orotic acid, she found that the incorporation of this precursor into RNA declines during senescence, but in the presence of kinetin (while senescence is delayed) the original value is either maintained or surpassed. Similarly incorporation of ^{14}C labelled leucine into proteins was also enhanced by kinetin. She therefore suggested that the effect of kinetin, in retarding senescence of Xanthium leaves, is due to its action in sustaining nucleic acid and protein synthesis, rather than accumulation of metabolites. Anderson & Rowan in 1965 observed that decline in proteins of tobacco leaves with the increasing physiological age and with time after harvest, is correlated with increase in peptidase. In 1966 they found that kinetin which can effectively delay senescence in these leaves, show a significant inhibition of increase in amino acids, but did not inhibit the increase in peptidase. They concluded that since kinetin can inhibit increase in amino acids without preventing the increase in peptidase, therefore, its senescence delaying effect must be through increased protein synthesis rather than the prevention of degradation. However, contrary to above reports, Sacher (1967) reported that in his studies on the senescence of Rhoeo leaf, kinetin had no effect on the incorporation of ^{14}C orotic acid or ^{14}C leucine into RNA and protein respectively. He, therefore, suggested that kinetin delays

senescence by preventing degradation of proteins and RNA and not by increasing their synthesis. Similarly Shibaoka & Thimann (1970) also reported that in pea leaves, kinetin did not increase the incorporation of ^{14}C Leucine into proteins, but it clearly prevented the breakdown. They showed that serine promotes proteolysis and yellowing and it also antagonises the effect of kinetin. They also showed that kinetin strongly inhibits RNase activity. They therefore concluded that the effect of kinetin in retarding senescence is mainly due to the prevention of breakdown. The conclusion that kinetin retards senescence by preventing breakdown has also been drawn by other workers (Karaishi 1968, Mizrahi et al 1970, Tavares & Kende 1970). However Tung & Brady (1970) have suggested that kinetin, besides preventing degradation, also appears to influence cytoplasmic ribosomes.

In an interesting report, Sugiura (1963) described that red light irradiation can retard the senescence in tobacco leaf discs while far-red light has the opposite action. He also mentioned that the effect of red light is similar to the effect of kinetin. Recently Greef & Fredericq (1972) have also shown that far-red light accelerates senescence in mature, green tissues of intact thalli of Marchantia polymorpha, and this effect is reversible on exposure to red light. They have concluded that red far-red reversibility of this phenomena indicates the involvement of phytochrome in the control of plant senescence. Thus if red light can delay senescence, which is similar to the well known effect of kinetin, and as we also know that a relationship between kinetin and red light has been described in other systems, e.g. seed germination (Miller 1956, Berrie & Robertson 1973), then it suggests that the effect of kinetin in delaying plant senescence could be through the phytochrome activation (Woolhouse 1967). However in a report Goldthwaite & Laetsch (1967) had no evidence to suggest that senescence in bean leaves could

be under phytochrome control. Similarly in another report Vorkresenkaya et al (1968) observed that in barley leaves the effect of kinetin is not replaced by red light.

Although kinetin can delay senescence in detached leaves of different species, various attempts failed to show that it could also retard senescence in intact plants (Muller & Leopold 1966, Simon 1967, Srivastava & Atkin 1968). However Fletcher (1969) reported that BA (Benzyladenine - i.e. synthetic cytokinin) can delay senescence in attached primary bean leaves. He said that BA delayed senescence not only in the treated leaf but also in the leaves at upper nodes. He also showed that the delays in senescence due to BA application was associated with the maintenance of chlorophyll protein and RNA levels. In 1970 Adedipe & Fletcher showed that treatment of BA does not mobilize ^{32}P from other parts of the plant. In 1971, in order to provide more conclusive evidence that BA induced delay in senescence is not due to mobilization of nutrients from other parts of the plant, they applied BA or water in various opposite halves of attached primary leaves. Then by applying various labelled compounds on water treated halves, they demonstrated that delayed senescence in BA treated areas was not due to nutrient mobilization. Later, Adedipe et al (1971) observed an increased photosynthesis in the BA treated leaves. They concluded that BA retards senescence in intact bean plants not by mobilization of nutrients from other parts, but by maintenance of chlorophyll, protein and RNA and by enhancing the photosynthesis. Although the literature on the mode of action of cytokinin is far more extensive than described here, however, no well established mechanism is known, but it appears that it is involved in protein and nucleic acid metabolism.

Sacher (1959) showed that an auxin (NAA) can prevent senescence in various fruit and leaf tissue. Later Osborne & Hallaway (1964) showed

that auxin (IAA or 2,4-D) can delay senescence in the leaves of various deciduous trees. The delay in senescence however takes place only in treated parts of the leaves whereas untreated parts show a normal progress of senescence. Using ^{32}P Osborne (1967) observed that application of IAA did not show any mobilization effect on ^{32}P. She suggested that auxin delays senescence by maintaining synthesis rather than mobilization. Sacher (1968) has also reported that the effect of auxin in delaying senescence could be due to RNase suppression.

Osborne (1967) observed that when auxin is applied in a limited area of the leaf blade, senescence in untreated parts always progresses centrifugally from the edges of treated spot. She thought that some product of auxin stimulated metabolism moves outward and induces senescence in surrounding areas. Later she demonstrated that by the application of auxin, ethylene production in the leaves rises by 60-100%. She suggested that the senescence of untreated parts could be due to auxin induced ethylene production. Enhanced production of ethylene due to high levels of auxin and its senescence accelerating effect has been reviewed by Burg in 1968. Fuchs & Lieberman (1968) have shown that kinetin can also enhance ethylene production in Alaska peas, and it also enhances the effect of IAA for ethylene production. However, Goldney & Van Stevenick (1970) have observed that exogenous application of ethylene on the leaves of Nymphoides indica does not increase the breakdown of chlorophyll, soluble proteins, peroxidase and carboxylase as compared with controls. They therefore concluded that ethylene may have some controlling effect but it does not play an important role in the senescence of Nymphoides indica. Lieberman & Kunishi (1970) have reviewed various conflicting reports regarding the action of ethylene and have concluded that action of ethylene is possibly associated with its antagonism to different hormones, IAA, GA, or

cytokinin. Therefore its function may be to modulate the action of hormones.

Although the reports on mode of action of gibberellin in relation to senescence are fewer, the literature pertaining to gibberellin action in various other systems is by far the largest and it is well known that they play a significant role in the synthesis of various enzymes and RNA (Paleg 1965, Varner & Johri 1968, Jacobsen & Knox 1970, Zwar & Jacobson 1970).

Senescence accelerating effect of ABA has also been studied by various workers in relation to decreasing protein and RNA synthesis along with its interaction with other hormones. These reports have recently been reviewed by Addicott & Lyon (1969) and Addicot (1970). In general, Addicott suggested that antagonistic interactions of ABA with other hormones are of non-competitive nature. It promotes the synthesis of cellulase, ribonuclease and invertase. The synthesis and composition of various RNAs are especially sensitive to ABA. However there is little evidence that ABA could inhibit DNA synthesis.

In this study our objective was to investigate the possible causes of overall type of senescence in annuals, with a special emphasis on the theory of special senescence stimulus.

The selection of garden peas as an experimental material was due to various reasons. Firstly, peas are self-pollinating species and therefore even the commercially available seeds give rise to remarkably uniform plant population (Went 1957). As peas do not grow very tall nor become bushy so a large number of plants can be grown in a small area, and therefore were most suited to our growth cabinet conditions. Moreover these peas can be easily grafted (Went 1957), and as we were also interested in some grafting experiments, therefore peas were probably the best plant material for our use.

MATERIALS AND METHODS

CULTURAL TECHNIQUES

Pisum sativum L. cvs Alaska and Greenfeast were used as experimental plant material for all studies described in this thesis. Plants for all experiments, excepting for bulk hormone extraction studies, were grown in growth cabinets under controlled conditions. Initially, trials (not described in this thesis) were conducted to find the best method of producing suitable plants in the growth cabinets available. A standard procedure was then followed, unless otherwise stated, for all experiments. Soaking of seeds prior to sowing (which is generally recommended) was found unsatisfactory as poor germination and growth of plants results, which was in accordance with the reports of Berrie (1960). For good and uniform plant growth, seeds were germinated in moist perlite in 2" deep plastic trays, without prior soaking, at $20^{\circ} \pm 2^{\circ}C$ in darkness. A week after germination, healthy and uniform seedlings were selected and transplanted to 4" diam. pots filled with soil, peat and perlite (25:25:50) mixture. At the time of transplanting excess water was given so that seedlings could be picked out with minimum injury to their root system. Pots with young seedlings were then transferred to the growth cabinet adjusted to $18^{\circ} \pm 2^{\circ}C$ and 18 hr. photoperiod. The light energy (17.02 Watt/Meter^2) in the growth cabinet was provided by alternating tubes (5 ft. long, 65W) of WWX and daylight arranged at a distance of 1" between and 5 ft. above the pots. A total of 19 tubes were used per cabinet. Full strength modified Hoagland's nutrient solution (details of which are given on page 21) was applied twice per week and on other occasions water.

For field experiments seedlings were raised as described above and after selection individual seedlings were transplanted in 2' apart rows

Nutrient solution

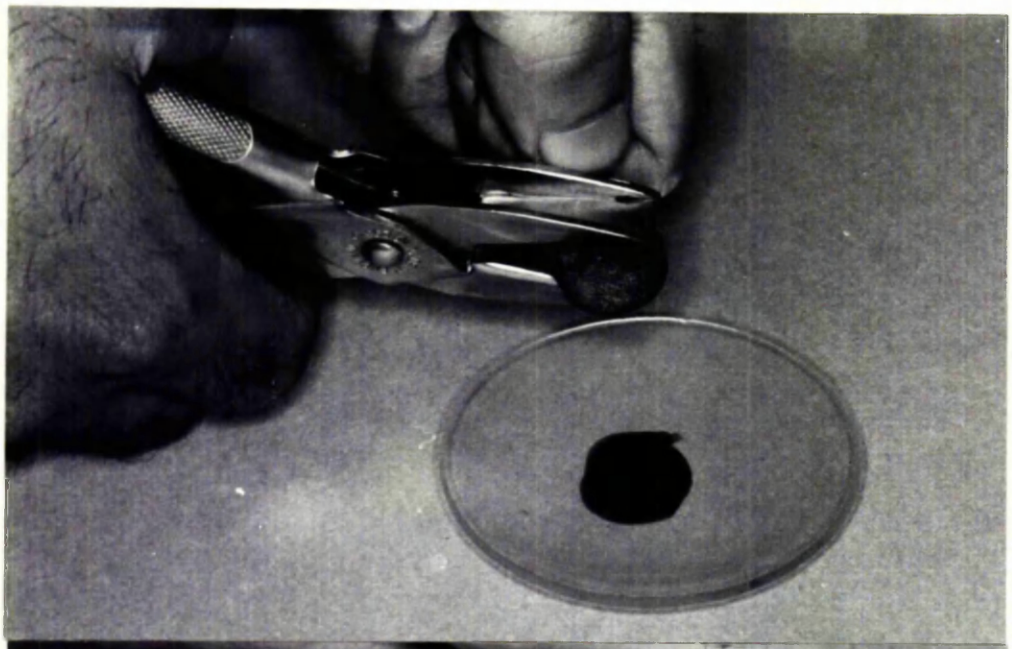
49%	$MgSO_4.7H_2O$	1 ml/litre
20%	KNO_3	2.5 ml/litre
14%	K_2HPO_4	1 ml/litre
	Iron solution	1 ml/litre
	Trace element solution	2.5 ml/litre

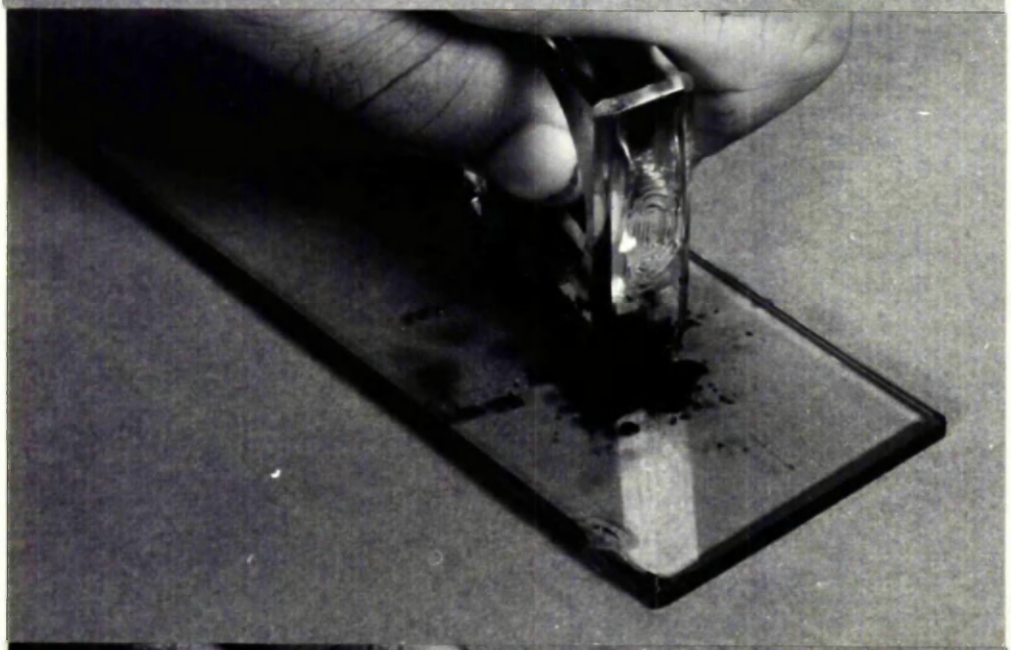
Iron solution

2.35 gms sequestrene 138Fe (Geigy Products Ltd) per litre of solution

Trace element solution

	Gms/litre in stock solution
$CuSO_4.5H_2O$	0.0354
$MnSO_4$	0.609
$ZnSO_4$	0.0974
H_3BO_3	1.269
$H_2MoO_4.H_2O$	0.0398





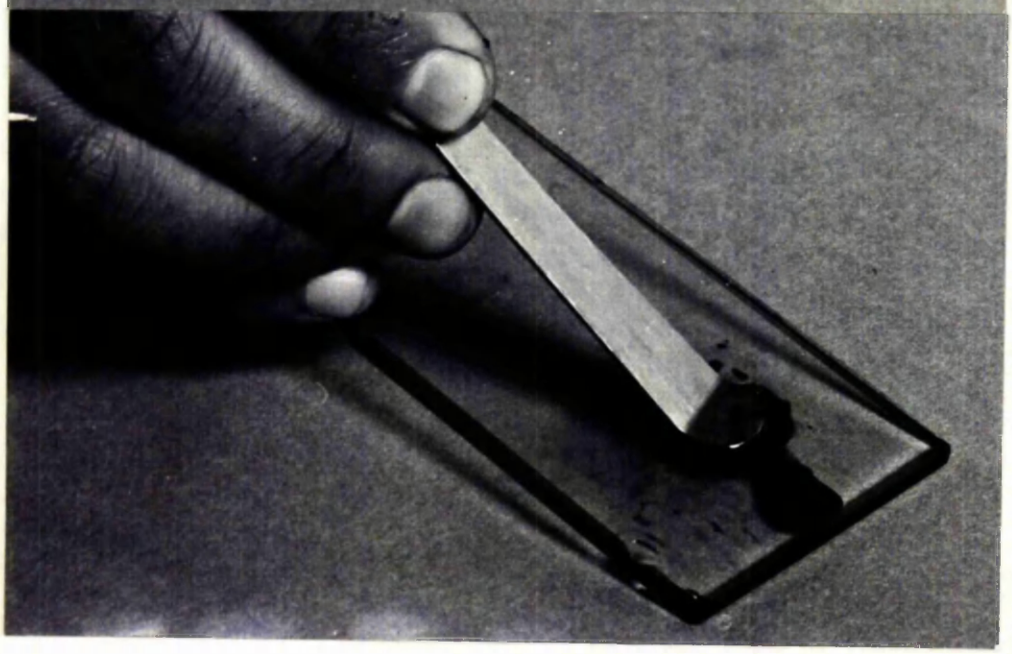


Plate 1. Methods of sap extraction from pea leaves.

(1) Direct expression of leaf sap using blunt-nosed plier.

(2) Extraction of leaf sap using glass blocks and spatula.

(a) Crushing of the leaf.

(b) Extract of the sap.

at a distance of 9" between plants. Weeding between the plants in a row was carried out by hand pulling or by uprooting with a hand trowel. Weeding between rows was carried out with a hoe. At the time of flowering, healthy and uniform plants were staked, labelled and given their first treatment.

PROTEIN STUDIES

(1) Extraction procedure

Proteins from individual pea leaves were extracted by direct expression with a blunt nose plier (see Plate 1). The extract obtained this way was generally enough for running 2-3 replicate analyses. A 10 µl sample of the crude extract was found satisfactory for electrophoretic analysis of proteins. In some cases where leaves were very small (leaves at node 3 and 4), the sap was expressed by crushing the leaf on a glass plate with a glass block and then squeezing out sap from crushed leaf material by means of a small spatula (see Plate 1). Samples from this crude extract were then directly laid over the acrylamide gel column. Further purification of this crude sap (before putting on gel) by centrifugation, dialysis and other usual ways of protein preparation did not improve the separation. It was therefore decided to use crude extract directly as it proved quite satisfactory for comparative studies and was far quicker (½ minute) compared to conventional ways of sample preparation (at least 2 days).

(II) Disc electrophoresis

The procedure adopted for the electrophoretic separation of pea leaf proteins was essentially that of the original method devised by Ornstein & Davis (1964). It consisted of three main steps: 1) gel preparation; 2) electrophoresis; 3) staining and destaining of gels to locate proteins.

Gel preparation:

Acrylamide gel columns, prepared in glass tubes 6.5 cm long with 4 mm diam., consisted of a lower layer of small pore gel (4.5 cm deep) and an upper layer of large pore gel (1 cm deep). A concentration of 7.5% acrylamide was used for small pore gel and 3.75% for large pore gel; pore size depending on acrylamide conc.

Appropriate volumes of stock solutions (described on page 26) were mixed (first for small pore gel) and thoroughly degassed to avoid any bubble formation in the gel. Degassed gel solution was then poured with a pasteur pipette in to glass tubes, vertically held, having their bottoms firmly sealed with plastic stoppers. The solution in the tube was carefully overlaid with deionized water to avoid the formation of meniscus at the gel surface. This was done by using a layering pipette so that mixing of the layers was avoided. Tubes with gel solution were kept undisturbed for 30 minutes at room temperature to allow chemically induced polymerization. After gelation the overlaid water was removed and a 1 cm deep layer of large pore gel mixture (thoroughly degassed) was laid over the small pore gel. This was also overlaid with deionized water to avoid meniscus formation. Large pore gel was photopolymerised using a 20W 240V fluorescent tube for 30 minutes, at a 5 cm distance from the gel tubes.

Electrophoresis:

After gelation of the large pore gel, the overlaid water was removed and gels (8) were used for an electrophoretic run within 2-3 hours of polymerization. Keeping the gel column for longer periods or overnight prior to electrophoresis was found unsatisfactory as tailing of bands occurred with poor resolution.

Samples (10 µl) from crude extract were introduced at the top of gel columns and overlaid carefully with the buffer solution. The plastic stoppers were removed from the tubes soon after sample application, taking care not to introduce air bubbles, and tubes (8 at a time) were fitted vertically in the upper reservoir (cathodic) of a locally constructed apparatus. This buffer pH 8.3 was poured in the lower reservoir (anodic), and the upper reservoir with gel column tubes was placed over it so that the bottom of the tubes were well immersed in the buffer solution and there were no air bubbles between the gels and buffer. Same tris buffer was poured gently into the upper reservoir avoiding any mixing with samples. After this was set up, the gels were subjected to a constant current at 32 mA/8 tubes (i.e. 4mA/tube) supplied by a Vokam Power Pack, for 40 minutes. (Initially the voltage was 100 volts but over the period of the run it rose to 190 volts.) This time was generally sufficient to allow the front to progress about 3 cm down the small pore gel which gave an optimum balance between separation and kinetic diffusion of the proteins.

Staining:

On completion of electrophoresis, current supply was switched off and the upper reservoir was removed and its buffer was poured off. Immediately the gels were removed from the tubes using a 5 ml hypodermic syringe filled with water. This was done by rimming the gel in the tube with the needle of the syringe while continuously pushing water

through it. This was continued until the gel slipped out, pushing or sucking was not practised as it resulted in breaking of the gel. On removal from the tube the gel was instantly placed in 12.5% Trichloroacetic acid (TCA) solution for at least ½ hr. to fix proteins and so to prevent diffusion. Later staining was carried out by placing gel in a solution of Coomassie Brilliant Blue R250 (1% stock soln. diluted 20 times with 12.5% TCA) for 30 minutes. Destaining of the gel was carried out by washing with 5% TCA for several times until clear protein bands distinct from a faint background were obtained. Gels were stored in deionized water and scanning of protein bands was carried out using a Joyce Loebl chromoscan.

(III) Isoelectric focusing

Protein separation by isoelectric focusing on acrylamide gels was carried out according to the method of Malik & Berrie (1972, see Appendix IV). However, with new batches of Coomassie Brilliant Blue R250 a lower yield of green supernatant was obtained. To obtain satisfactory preparation of stain fixative from those batches it was necessary either to:

(i) Increase the concentration of Coomassie Blue soln. to 4%, or -

(ii) Concentrate the supernatant in vacuo until it was noticeably grass green.

Or both.

It was found that efficiency of stain depended on the intensity of green colour, and so care must be taken that stain should not turn blue while adding alkali.

Results obtained in these studies were recorded by scanning the gels on a Joyce Loebl chromoscan.

Stock solutions

Tris buffer solutions

1(a)		1(b)	
Tris	= 36.0 g	Tris	= 5.7 g
N/1 HCl	= 48.0 ml	N/1 H_3PO_4	= 25.6 ml
Temed	= 0.46 ml	H_2O	= to 100 ml
H_2O	= to 100 ml		

Acrylamide solutions

2(a)		2(b)	
Acrylamide	= 30.0 g	Acrylamide	= 10.0 g
Bis	= 0.8 g	Bis	= 2.5 g
H_2O	= to 100 ml	H_2O	= to 100 ml

Initiators

3(a)		3(b)	
Ammonium per sulphate	= 0.14 g	Riboflavin	= 0.004 g
H_2O	= to 100 ml	H_2O	= to 100 ml

Gel solutions

Large pore gel		Small pore gel	
1(b)	= 1 part	1(a)	= 1 part
2(b)	= 2 parts	2(a)	= 2 parts
3(b)	= 1 part	3(a)	= 4 parts
H_2O	= 4 parts	H_2O	= 1 part

Reservoir buffer solution

Glycine	= 28.8 g
Tris	= 6.0 g
H_2O	= to 1 litre

STUDIES ON ENDOGENOUS PLANT HORMONES

Sampling

Leaf samples contained in polythene bags were stored in a deep freeze at -20°C. Frozen material was freeze dried at -60°C and dried material was powdered in a coffee mill. Powdered material was kept in tinfoil bags at 4°C before use.

Extraction

A known weight of powdered leaf material was extracted for 72 hrs. in cold methanol (1:10 w/v) at 4°C in the darkness. It is necessary that extraction and purification should be carried out in darkness in order to minimize the risk of isomerisation in ABA. After 24 hrs. the plant material was re-extracted with fresh methanol after removal of the first extracting liquid by filtration. Re-extraction was done again. The extracts were combined and the residue discarded. The combined extract was then evaporated to dryness under reduced pressure at 30-32°C. The residue was taken up in 0.5M phosphate buffer (pH 8.5). Any pigments sticking on the sides of the flask were dissolved in pet. ether. Pet. ether solution was added to the buffer and the solvent was evaporated in vacuo and the buffer was allowed to cool for ½ hr. at 4°C. The cold buffer extract was filtered through glass wool packed in the stem of a filter funnel. The flask was rinsed 3 times with a little fresh buffer and each rinse was filtered through glass wool. Most of the chlorophyll pigments (precipitated suspension) was retained over glass wool and was discarded. The flow diagram of extraction procedure is given on page 40).

Initial purification

Buffer filtrate (pH 8.2) was partitioned three times with pet. ether and then pet. ether was discarded. The buffer was then acidified with 1N H_2SO_4 to pH 2.5 and partitioned four times, each time with half of its volume of ethyl acetate, in a separatory funnel.

The organic phase layers were combined and stored overnight in a deep freeze to freeze out water. Ice crystals were removed from ethyl acetate by filtering through glass wool. Cold ethyl acetate (-20°C) was used to wash the ice and the washings were added to the filtrate. This filtrate was then evaporated to dryness in vacuo at 30° ± 2°C in the dark. The dried material, marked acid fraction, was then further processed for separation of GAs and ABA.

The aqueous phase left after partitioning with ethyl acetate was then adjusted to pH 3.3, and 10 N $AgNO_3$ was added. The precipitate which formed was allowed to age for 24 hrs. at 4°C. The supernatant was then decanted and the precipitate was washed 4 times with 1% $AgNO_3$. Finally precipitates were centrifuged down at 6,000 r.p.m. for 15 minutes and clear supernatant silver nitrate solution was decanted. Cytokinins were then extracted by continuously shaking the precipitate in .2N HCl at 50°C for 2 hrs. After acid extraction, solids were removed by centrifugation and were discarded. The acid soluble portion was evaporated in vacuo and the residue washed repeatedly with water which was also evaporated. The residue (marked "kinin" fraction) was finally taken up in a small volume of ethanol for further chromatographic separations.

Chromatography

Further purification and separation of individual hormones from both acid fraction and "kinin" fraction was carried out using different

chromatographic techniques.

Purification and separation of individual GAs and ABA from acid fraction

Column chromatography

A new method of group separation of GAs and ABA for the initial purification was developed on a hydroxyalkoxy-propyl-sephadex (here for convenience it will be briefly termed "alkylated sephadex"). The separation of authentic GAs on alkylated sephadex by column chromatography had been reported by Brooks & Keates (1969). It was therefore decided to find out if it could be used for GAs and also for ABA purification from plant extracts (acid fraction). Initially the chromatographic behaviour of authentic GAs and ABA on alkylated sephadex columns was investigated with slight variation in the polarity of the solvent system given by Brooks & Keates (Benzene : Isopropanol 75:25). It was interesting to note that ABA ran close to GAs in most solvent systems used. With biological extracts group purification of GAs and ABA was found to give best results when a solvent system, Benzene : Isopropanol (90:10), was used. This therefore was used for routine work and was found highly reproducible, convenient and indeed an excellent way of purifying the acid fraction from leaf extracts.

Preparation of Alkylated Sephadex

Hydroxy alkyl derivative of sephadex LH20 (here termed alkylated sephadex) was prepared by Dr Alam in this department following the method of Ellingboe et al (1968). The olefine oxide used, Nedox 1114, was obtained from Ashland Oil & Refining Co., Minneapolis, Minn., USA. The alkylated sephadex produced after the reaction was double the weight of starting material (sephadex LH20), due to 50% content by

weight of hydroxyalkyl groups, and therefore may be designated as N1114-50%-LH20. Finally washed and dried sephadex (washed consecutively with chloroform, ethanol and acetone and dried in vacuo), had a hydrophobic and waxy appearance.

Packing of column

Appropriate amounts of alkylated sephadex (ca 20-22 g for a 75 ml bed volume of the column) were allowed to swell overnight in excess amounts of the appropriate solvent system (most commonly used solvent system was Benzene : Isopropanol, 90:10). From the swollen gel excess of solvent was decanted and a thick gel slurry was poured into a 43 x 2 cm column (Fischer & Poster Ltd). At the time of pouring slurry care was taken that the column was absolutely vertical and also that bubbles were not included in the gel. Slurry was allowed to settle under gravity without any solvent flowing through. If solvent was allowed to flow through the column while slurry was settling, it resulted in a very compact bed with a slow flow rate. After 6 hrs. of gravitational sedimentation, gel was washed with appropriate solvent (3-4 bed volumes) and the column was ready for use.

Standardization and routine use of the column

The column (filled with alkylated sephadex) was standardized by applying authentic samples of GAs and ABA in a test mixture containing 0.1 - 0.2 mg of each compound in 0.5 ml of eluting solvent.

At the time of sample application excess of eluting solvent was carefully drained and elution was stopped just before the gel surface started drying. The sample was then applied on the top of the gel surface and was carefully washed below the surface by drop-wise addition of eluting solvent (0.1 - 0.2 ml). Elution of sample was carried out at a flow rate

of 2 ml/hr. and 200 drops/fraction were collected using a Gilson 'Microcol' fraction collector. Authentic compounds in different fractions were identified by their colour reaction or UV absorbance on TLC. Retention data were recorded as the standard elution volume (S.E.V.). This is defined as the ratio between measured elution volume and total column volume multiplied by 100. S.E.V. therefore is numerically equal to percentage of total column volume. It served to manipulate the elution volume if a different sized column is to be used, and was found fairly reproducible. S.E.V. values of different GAs ($GA_{3, 4, 7, 5, 13}$) and ABA were found to be very close to each other in the solvent system (Benzene : Isopropanol, 90:10) used, and it therefore appeared that group collection of GAs + ABA in one fraction can be the best use of this technique for plant extracts.

For group separation of GAs + ABA in a big fraction (marked fraction "G"), the fraction volume was calculated which was the total volume of all fractions in which different authentic compounds (GAs and ABA) were detected. The elution volume for this fraction was taken as the volume of the solvent eluted through the column before any compound (GAs + ABA) could be detected in the eluent, after which the calculated volume of fraction"G"was collected. To separate fraction"G"for routine work a simple fraction collector was designed (which was made by the departmental mechanic (see Appendix I)) and was found quite satisfactory. This fraction collector collected first elution volume (in 1st flask) then fraction"G"(in 2nd flask) after which eluting solvent started collecting in a beaker placed below.

For purification of plant extract on alkylated sephadex column, the acid fraction was taken in a small volume (ca 0.5 ml) of eluting solvent and applied on the top of a standardized column as described above, and the elution of the sample was carried out at a flow rate of 2 ml/hr. The

column was kept in the dark by wrapping with tinfoil until fraction"G" had been separated.

Fraction"G"was reduced to dryness in vacuo in the dark and was further processed for separating individual GAs and ABA on T.L.C., using different solvent systems.

Thin layer Chromatography

Final purification and separation of individual GAs and ABA from fraction "G" and cytokinin from "kinin" fraction was carried out by thin layer chromatography.

The technique of thin layer chromatography which was first developed by Ismailof and Schraiber in 1938 has now become a most prominent and widespread method of chromatography because of its greater speed, efficiency, resolution and convenience than the analogous columns. TLC involves the use of thin layer of finely powdered material, evenly distributed over the surface of a suitable support, upon which separation may be effected by adsorption, partition, exclusion or ion exchange processes.

Thin layer chromatography for fraction "G" was carried out using silica gel G (Merck) as coating material, and for "kinin" fraction silica gel HF 254 (Merck)was used. Thin layer plates were prepared by mixing silica gel with water (1:2 w/v) for 2 minutes; the slurry thus obtained was immediately distributed over 20 x 20 cm glass plates using Quickfits. TLC spreader. For analytical work 0.2 mm thick layers were found suitable but preparative work was carried out on 0.75 mm thick layers. Thin layer plates were activated at 120^{o}C and prep. plates were pre-run in both eluting and developer solvents. After pre-runs silica gel plates were reactivated before use.

For preparative work a sample was applied in 15 cm long narrow band

at a 1" distance from the edge of the plate. Initially application of samples on prep. plates was carried out by intensively spotting the sample, with a capillary tube, so close to each other that finally spots merged into each other and appeared as a straight line. This was an extremely time consuming and inconvenient method, therefore a new and simple machine was designed (this was also made in the departmental workshop) for sample application on preparative plates (see Appendix II) and was found extremely useful for routine work. Along each side of the sample band, authentic samples were spotted at a 1 cm distance from sample line. A narrow line was drawn with a spatula between sample and authentic compound, across the whole plate on both sides. The plates were then run in an appropriate solvent system which was allowed to run up to 10 cm above the line of sample application. After the run, plates were dried under stream of (a gentle) air in the fume cupboard. For the detection of standards (on the side strips) the plates were carefully covered with tinfoil, keeping only side strips exposed. Authentic samples on side strip were detected either directly by their absorbance under a UV lamp (wave length 254 m U) or by their colour reaction. For colour reaction side strips of the plate were sprayed with 0.5% H_2SO_4 in methanol (taking care that no spray reagent should reach inside plate) which were then heated with a hair dryer for a few minutes, after which standards could be detected either visibly by their colour or under UV light by their fluorescence. After detecting standards, tinfoil was removed from the plate and zones corresponding to standards were marked with a needle. Side strips were then scraped off completely and the sides of glass plate underneath the standard strips were thoroughly cleaned with tissue paper soaked in ethanol and then dried either with a dry tissue or simply in air. Silica gel zones, corresponding to standards, were then scraped into pyrex eluting tubes, having a sintered glass disc

at the bottom, and were eluted with ethanol for 3 times with 3 ml volumes. The eluates were collected in 10 ml conical flasks and afterwards evaporated to dryness in a vacuum desiccator at room temperature, and then were used either for purification or separation on another TLC, with different developer solvent system and or finally for bioassay or GLC or GCMS.

Final purification and separation of individual GAs and ABA

Preparative thin layer chromatography on silica gel G, for the separation of GAs and ABA was carried out according to Cleland & Zeevart (1970).

Fraction 'G' was taken in a small volume of ethanol and was applied on a preparative plate (as described above) which was developed in a solvent system consisting of chloroform : ethyl acetate : acetic acid (60:40:5). Two zones, first corresponding to GA_1 and GA_3 and second corresponding to $GA_{4,7,5}$ and ABA were eluted separately. The eluate from the first zone was dried and then used directly for bioassay while the eluate from the second zone was further chromatographed on TLC for the separation of ABA from $GA_{5,4}$ and 7 using a different solvent system. To prepare the second solvent system, carbontetrachloride : acetic acid : water (8:3:5 v/v) were shaken in a separatory funnel and separated into two phases. The plate was equilibrated with the upper phase overnight and then developed with a mixture of lower phase (5 parts) and ethyl acetate (1 part). Plate developed in second solvent system was dried overnight under a gentle stream of air to get rid of acetic acid as much as possible. Next day four zones corresponding to $GA_{4,5,7}$ and ABA were scraped and eluted separately as described earlier. The eluates were completely dried in vacuum desiccator and used for bioassay.

Purification and separation of cytokinin from "kinin" fraction

The crude kinin fraction was taken up in a small volume of 35% ethanol and spotted on to a preparative plate of silica gel HF254 (Merck) and the plate was developed in solvent system consisting of n-butanol:ammonia (4:1 v/v) Dekhuijzen & Overseem 1971). Authentic Zeatin was run on side strips of the plates and was detected directly under UV light. Zones corresponding to Zeatin and a 1.5 cm zone above Zeatin zone were scraped and eluted separately (in initial experiments other zones on the plate showed no cytokinin like activity and so were not assayed in routine work). The eluates were dried in a vacuum desiccator and were directly used for bioassay.

BIOASSAYS

Bioassays for GA like activity

(i) Lettuce hypocotyl bioassay

The bioassay technique described by Frankland and Wareing (1960) was used to measure GA like activity. Lettuce seeds (Grand Rapids) were germinated in moist filter paper in the dark (with slight exposures to light to break the dormancy) at 25°C for 24-36 hours. Seedlings in which the hypocotyl had not started elongating and the length of the radicles was almost similar (6-8 mm) were selected. Similar seedlings (9) were then placed in a pyrex glass petri-dish lined with filter paper containing aqueous eluate or standard or blank (i.e. eluates from a blank plate subject to whole procedure) or control (only deionized water). The dishes were then kept in growth cabinets at 20° ± 1°C and an 18 hr. photoperiod. After 3 days the length of hypocotyl was measured to the nearest mm.

(ii) Dwarf pea bioassay

Brian & Hemming (1955) have described the use of dwarf pea (var. Meteor) as a bioassay for measuring GA like activity. They suggested that best responses were obtained 22 days after treatment. However, Pegg (1966) reduced the treatment-to-measurement time to 3 to 5 days; he suggested that shorter time period should be preferred when activity is low. It was therefore decided to take measurement after 5 days as it was a quicker method and also the activity in our extracts was low.

Meteor peas were sown in moist perlite and kept at $20^{\circ} \pm 2^{\circ}C$ in the dark for germination. After germination when the fourth internode was elongating, uniform seedlings were selected and transferred to 2.4 x 18 cm test tubes, held in wooden test tube stands and filled with tap water. Test tubes containing seedlings were labelled and then transferred to a growth cabinet maintained at $20 \pm 1^{\circ}C$ and 18 hr. photoperiod. Next day plants (5 for each treatment) were treated with a 20 µl drops of test solution (in 35% ethanol) twice, giving a total dose of 40 µl in the axil of the leaf at the third node. After 5 days measurements were made of the internode between third and fourth node to the nearest mm.

Bioassay for ABA like activity

Wheat coleoptile bioassay

Seeds of wheat (Triticum sativum) were soaked in running tap water for 6 hrs. and were sown on moist filter paper in a big plastic tray. The tray was kept in darkness at $25^{\circ}C$ for three days till the coleoptiles had reached the required length (12-18 mm). Uniform seedlings (ca 15 mm coleoptile) were selected and 10 mm segments were chopped off using a special cutter (having two blades held at 10 mm distance), discarding the 3 mm apical portion. Segments (10 mm) were floated on deionized water prior to treatments. Nine segments were then placed in each pyrex glass petri-

dish (4.5 cm diameter) containing test solution (in 0.01 M sodium phosphate buffer at pH 7.4). The dishes were then incubated in the darkness at 25°C for 48 hours. To prevent evaporation during incubation, a filter paper was stuck in the lid of the petri-dish and was moistened with water to keep atmosphere moist and thus reduce evaporation. After the incubation period segments were measured to the nearest mm.

Bioassay for Cytokinin like activity

Radish cotyledon bioassay

The bioassay technique used for cytokinin was followed according to the method described by Letham (1968). However, Radin & Loomis (1971) prepared their phosphate buffer (2 mM, pH 5.9) in 10 mM KCl which in our condition was found to improve results and so was followed in routine work. Radin & Loomis also used larger cotyledons of 5 days old seedling compared to small cotyledon of 35 hour old seedling suggested by Letham, but in our conditions large cotyledons of older seedling (5 days) were found to give inferior response and so Letham's procedure with regard to cotyledon age was followed.

Uniform Radish seeds (cv White Icicle) were left at 25-26°C in darkness on moistened filtered paper (having its ends continuously dipped in water) in special plastic tray made for seed germination work. After 36 hours, the smaller cotyledons were excised from each seedling, taking care to remove the hypocotyl completely, because the presence of a hypocotyl reduces the response to a marked extent. Cotyledons (5) of uniform size were selected and placed in pyrex glass petri-dish lined with filter paper containing test solution made in 2 mM phosphate buffer (pH 5.9) in 10 mM KCl. The petri-dishes were then placedon wet filter paper in plastic tray (in which seeds were germinated) which was then transferred to growth cabinet adjusted at 20° ± 1°C and 18 hour photoperiod. After

3 days cotyledons were blotted dry and weighed.

SURGICAL TECHNIQUES

Defloration was carried out by removing flower buds (3-4 mm) with a fine tipped forcep. Care was taken that while removing flower buds damage to the young apical leaves was avoided.

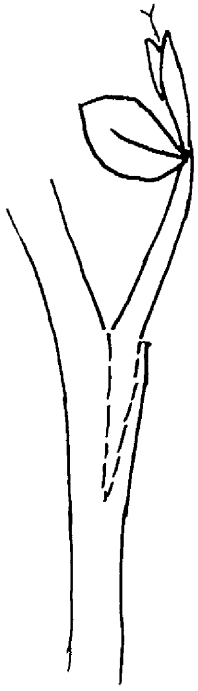
In defoliation studies the leaves were removed by cutting the petiole, with a pair of scissors, close to the stem but taking care that axillary buds should not be damaged. In peas stipules are modified to a leaf like structure, and these were removed along with the lamina and petiole.

De-apexing or decapitation was carried out by cutting apical portion just above the last visible internode.

In grafting experiments only side grafting of the scion was carried out in all cases excepting once where approach grafting was tried but without success. The method of side grafting was however very successful. In this procedure, prior to grafting massaging of the root stock was carried out very gently in such a way that phloem sap is diverted to the site where the graft is to be inserted. Massaging was carried out for one to two minutes and then soon after an oblique cut was made on the root stock (at the site where phloem sap was expected to be diverted by the massaging (personal communication from Dr. J. Milburn)) and then an immediately cut scion was inserted into the cut of the root stock. The joint was then quickly tied or fixed by wrapping with self-adhesive tape. All operations from the first cut in the root stock to the final wrapping of scotch tape were carried out as quickly as possible (approx. 1-2 minutes) as with longer periods, freshly cut

ROOT STOCK

SCION

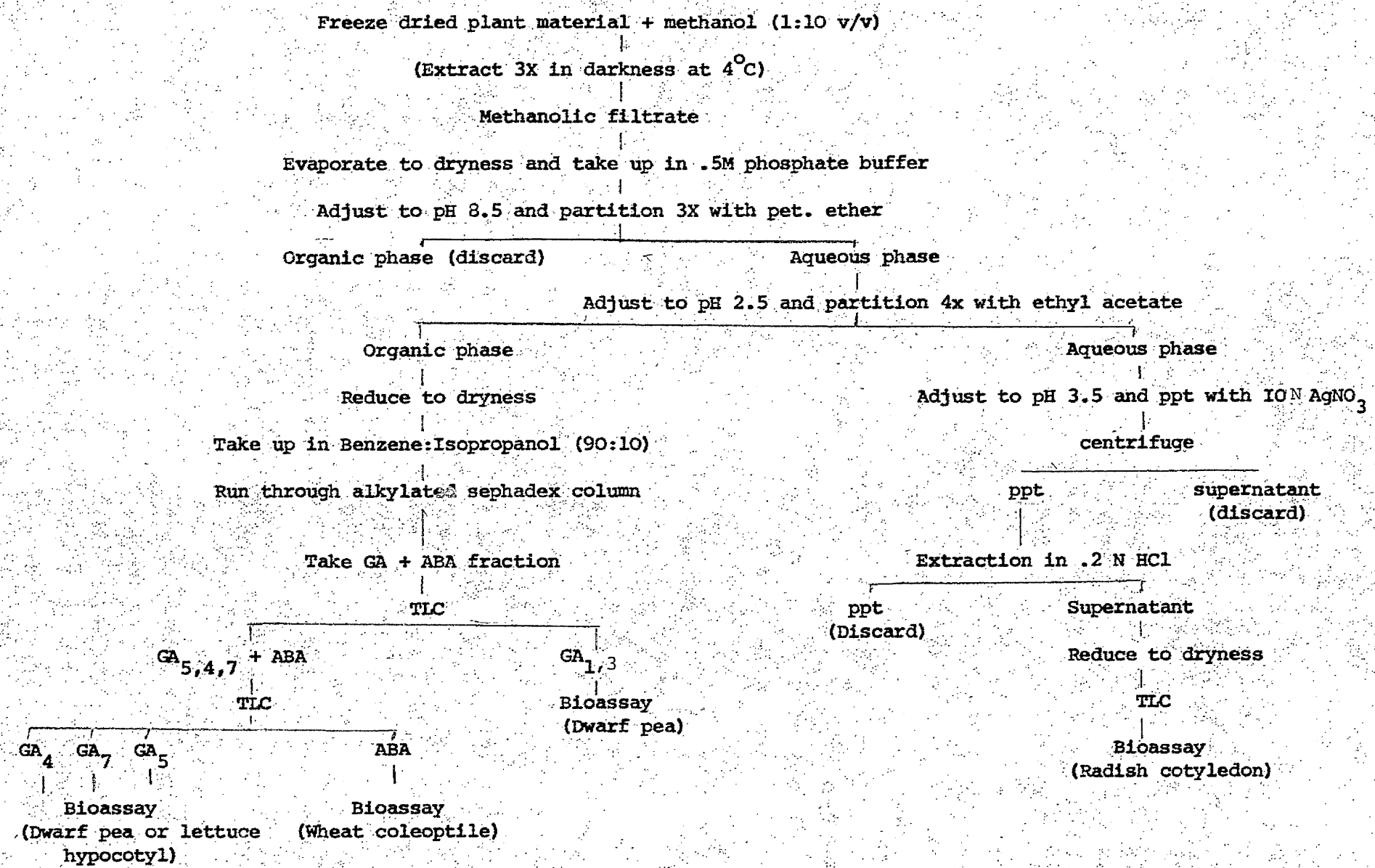


GRAFT JOINT

Fig. 1. The diagramatic presentation of the angles at which the scion is excised from the seedling plant, and corresponding to which a cut is given in the root stock. The dotted lines show the cut surfaces.

surfaces dry out and thus chances of successful union of the graft are reduced. While cutting the scion care was taken that the angle of cut should be similar to the angle of cut given in the root stock, because marked differences in the two angles result in either breaking of the scion or root stock while joining, or some air spaces are left over in the joint, thus minimising the chances of successful union. After fixing the joint with self-adhesive tape, the plant was covered with a clear polythene bag into which a wet piece of polyurethane foam was introduced to keep 100% humidity, as desiccation of the graft was found to have an adverse effect on the success of the union. Polythene bags, however, were removed soon after the union had taken place, as longer periods of high humidity caused fungal growth over the plant. The growing scion was staked separately and scotch tape from the joint was removed.

Extraction and separation method for GAs, ABA and Cytokinin



EXPERIMENTAL

LEAF PROTEINS

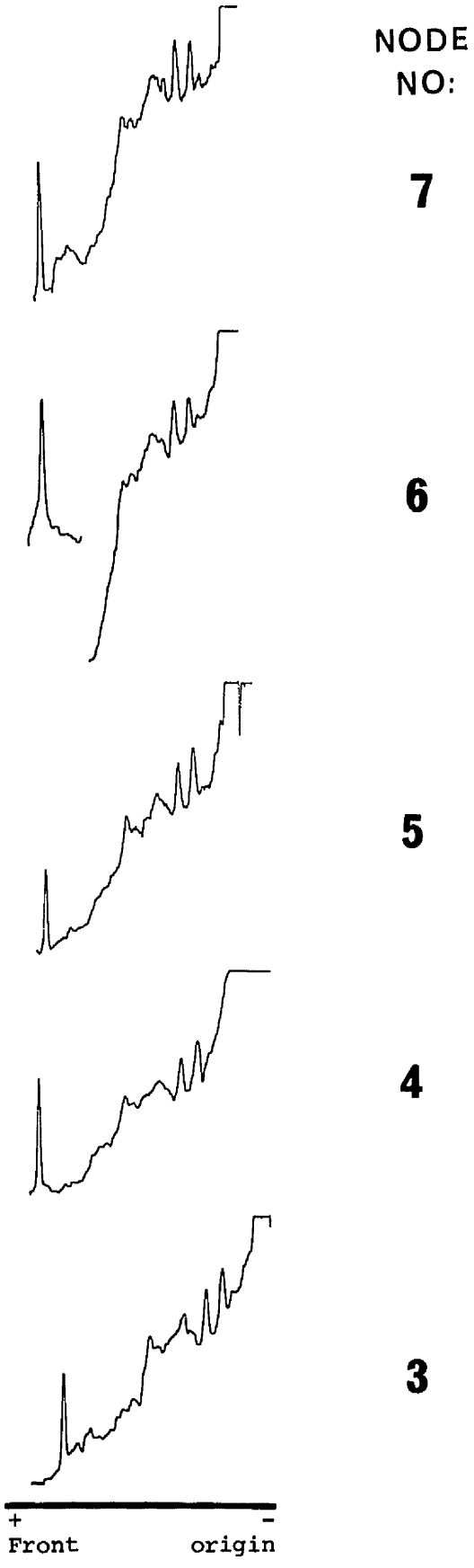


Fig. 2. Densitometer tracings of acrylamide gels showing the pattern of leaf sap proteins of Alaska peas after separation by electrophoresis and staining with Coomassie brilliant blue. Leaf node numbers are from base upward. There were eight visible nodes on the plant and flowers had not appeared.

Section A PROTEIN STUDIES

Study of leaf sap proteins by electrophoresis in Alaska peas prior to flowering

This study was conducted as a preliminary investigation to determine soluble leaf protein patterns in peas. The objective was to try to establish a consistent technique which would allow the recognition of any variation in leaf protein analysis by electrophoresis that could be correlated with leaf age and experimental treatments.

Initially proteins were extracted from leaves by direct expression with the blunt nosed pliers and then the expressed sap was centrifuged and purified, using conventional methods of dialysis and $(NH)_2SO_4$ precipitation, prior to electrophoresis. But soon it was found that crude extracts used directly could give equally good results. It was therefore decided to use crude extract without further purification in our studies. 10 µl of expressed leaf sap were found to be an appropriate sample size. For the detection of protein bands on acrylamide gels, staining was carried out by Amido Black (Smith 1960) and Coomassie Brilliant Blue R250 (Groth et al 1963).
The latter gave results more quickly and with the same degree of sensitivity as Amido Black; it was therefore adopted as the standard procedure.

To have any confidence in the separation from the standpoint of biological reproducibility, comparison in protein patterns of leaves of the same node number was made between at least three plants of the same chronological and physiological age. For technical reproducibility each sample was run in triplicate on different occasions. However, no problems were encountered in obtaining biological or technical reproducibility. The selection of plants, however, was done

LEAF PROTEINS

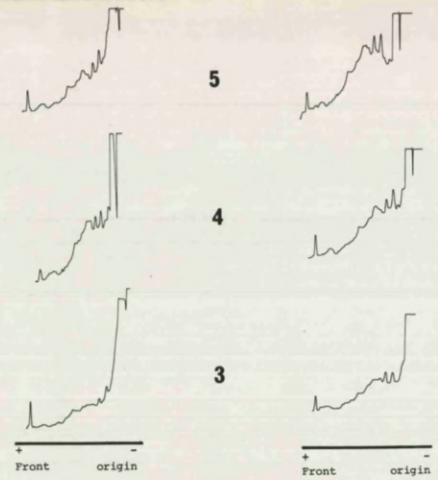


Fig. 3. Densitometer tracings of acrylamide gels showing the pattern of leaf sap proteins of Alaska peas after separation by electrophoresis and staining with Coomassie brilliant blue. Right hand series is from deflowered (total nodes 13) and left hand from untreated (total nodes 12) plants. The plants were of the same chronological age at the time of sampling. Leaf node numbers are from base upward.

with great care to keep alike plants in one set of experiment as much as possible.

Fig. 2 shows typical densitometer traces of acylamide gels after the staining of protein bands with Coomassie Brilliant Blue, following the electrophoresis. The separation of different proteins on acrylamide gels was obtained from the crude extracts of leaf saps. The numbering of the scans (in Fig. 2) corresponds to the node number of the plant from which the sample of leaf proteins was taken. Node numbers on the plant were counted from base upward. At the time of sampling none of the leaves had any visible signs of senescene.

From the scans shown in Fig. 2 there is hardly any difference that could be picked up among the leaves of different nodes. It therefore appears that in Alaska peas, before flowering, there is very little variation in the leaf sap proteins separated by disc electrophoresis.

The effect of flower development on the patterns of leaf sap proteins

Alaska peas were used in this experiment and were grown under controlled conditions. At the time of flowering nearly identical plants, i.e. similar in the total number of nodes, the node of the first flower development and in general appearance, were selected. Half of the selected plants were deflowered and labelled. Removal of young flower buds of these plants was continued until sampling time. Ten days after defloration when there were at least three complete flowers on the flowering plants, protein samples from individual leaves (of all nodes) of flowering and deflowered plants (three replicate plants in each treatment) were taken separately. Extraction of proteins of all leaves from both flowering and deflowered plants was carried out at the same time. Individual samples were then labelled and stored in the deep

freeze at -20°C. These samples were then thawed just before starting electrophoresis, and after taking the appropriate volume (10 µl for each gel) the remaining portion was discarded. The effect of freezing and thawing on the protein patterns of the samples was tested in early trials and was found not to result in observable changes. After the electrophoretic run the protein bands on the gel were first fixed in trichloroacetic acid (TCA) and then stained with Coomassie Brilliant Blue for location and scanning. Protein patterns of leaves of the same node number of both flowering and deflowered plants were then compared to see if any differences existed and the experiment was repeated three times to minimize experimental error.

Typical densitometer scans of acrylamide gels showing electrophoretic patterns of sap proteins of leaves of different physiological age in both flowering and deflowered plants are compared in fig. 3. At the time of sampling, the leaves at the third node were nearly yellow while leaves at the fourth and fifth nodes were starting to show yellowing. Comparing the leaf proteins patterns of corresponding leaves of flowering and deflorated plants leads to the conclusion that there is little difference, if any, between the control and treated plants. Neither in young nor old leaves is there manifest any change and it must be concluded that flower development does not in any way influence the leaf sap proteins, as extracted by us.

It is however fairly easy to observe a general quantitative depression in protein bands of old leaves compared to young leaves in each treatment. From these results it therefore appears that with the aging of leaves on intact plant, changes in protein bands appear quite prominently but development of flower does not seem to produce any specific changes in electrophoretically different components of leaf sap proteins at any node.

LEAF PROTEINS

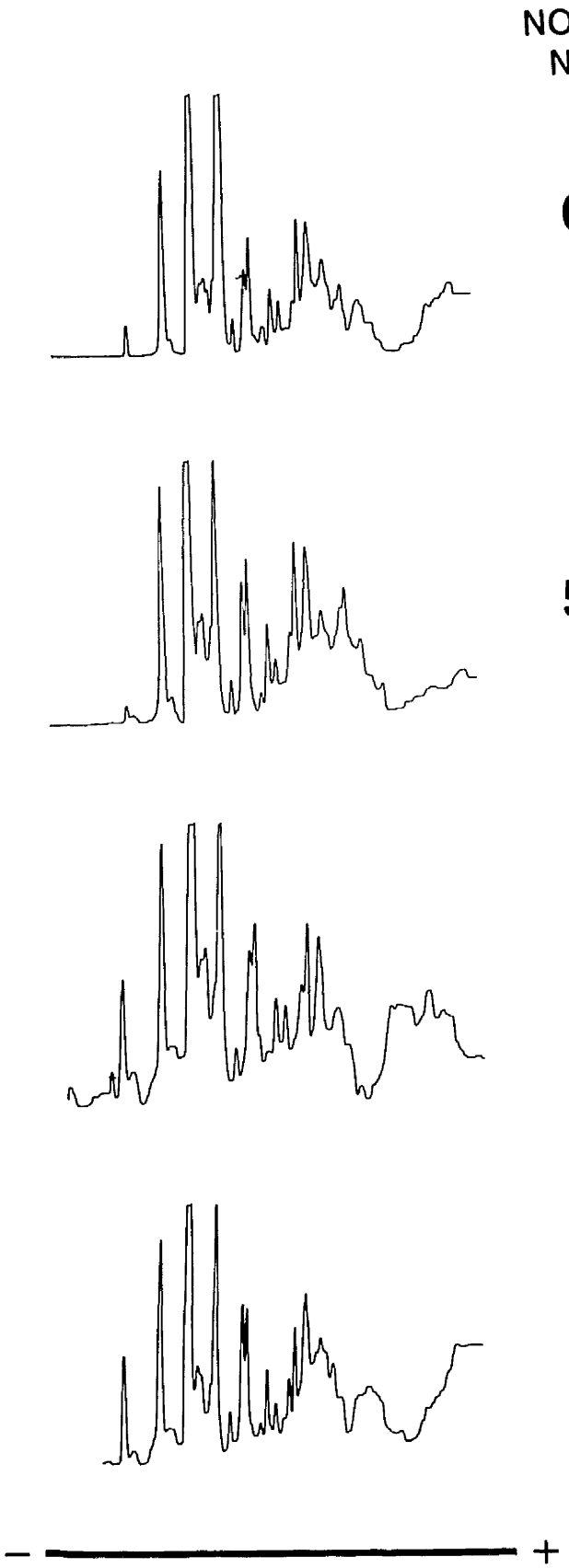


Fig. 4. Densitometer tracings of acrylamide gels showing the pattern of leaf sap proteins of Alaska peas after separation by isoelectric focusing and staining with Coomassie brilliant blue. Leaf node numbers are from base upward. There were seven visible nodes on the plant and flowers had not appeared.

Study of leaf sap proteins of Alaska peas by gel isoelectric focusing

In our previous studies we were unable to observe any changes in leaf sap proteins that could be related with the flower development. But it was realized that the technique of electrophoresis does not resolve the sample of the leaf saps into a wide variety of different proteins, and therefore it may be that our technique is not sensitive enough to show any changes in the leaf proteins due to the flower development. Various authors (Wrigley 1969, Florini et al 1971, Salaman & Williamson 1971) however, claimed that isoelectric focusing of the proteins was the most powerful technique for protein separation. It was therefore decided to see if this technique could also be used in our studies. The present study was conducted to observe the feasibility and separation capacity of the isoelectric focusing technique for the analysis of our leaf sap samples.

Initially Wrigley's analytical method of isoelectric focusing on 7.5% Acrylamide gels was followed as such. In this procedure, leaf proteins were first purified from the crude extract and then were mixed in the gel solution prior to polymerization. But mixing of sample in gel solution was found to be an unsatisfactory procedure not only because it was inconvenient when a number of different samples was to be analysed but also for the dark stained streaks that appeared in the gel, thus masking the proteins bands. However, the alternate method of sample application on the top of polymerized gels was found to overcome the problems of streaking. This method was also quite convenient for multiple analysis, particularly because the use of crude extract without prior purification could also be used very satisfactorily. Therefore, sample application on the top of polymerized gels was adopted as the standard procedure.

The separation of proteins achieved by this technique was certainly quite encouraging. But great difficulties were encountered in obtaining technical reproducibility. Therefore various modifications in the analytical method were tried and eventually a highly satisfactory procedure was developed (see Appendix IV). No difficulties, however, appeared in obtaining biological reproducibility.

During the course of initial trials for obtaining technical reproducibility of the technique, a new method of staining was also developed which eliminated the need of otherwise necessary steps of fixing, de-ampholyting and destaining of the gels. It was then used in all later studies.

Fig. 4 shows the densitometer traces of acrylamide gels after the isoelectric focusing of 10 µl crude leaf sap. The experimental plants, their stage of development and the volume of leaf sap per gel, were nearly the same as in the first experiment. Comparing the protein separations of these two experiments, it can be easily noticed that the number of protein bands obtained with isoelectric focusing (Fig.4) is far more than the ones obtained with simple disc electrophoresis. Moreover, Fig. 4 also shows a progressive appearance of two prominent bands (last two peaks toward the cathodic end of pH 10) with the increasing physiological age of the leaves (older leaves are at lower node numbers). It therefore appears that some changes also appear in leaf proteins among the leaves at different nodes, even prior to flowering. This difference, however, was not observed with the disc electrophoresis. The technique of isoelectric focusing, therefore, seems to be a superior method of protein separation as compared with electrophoresis.

LEAF PROTEINS

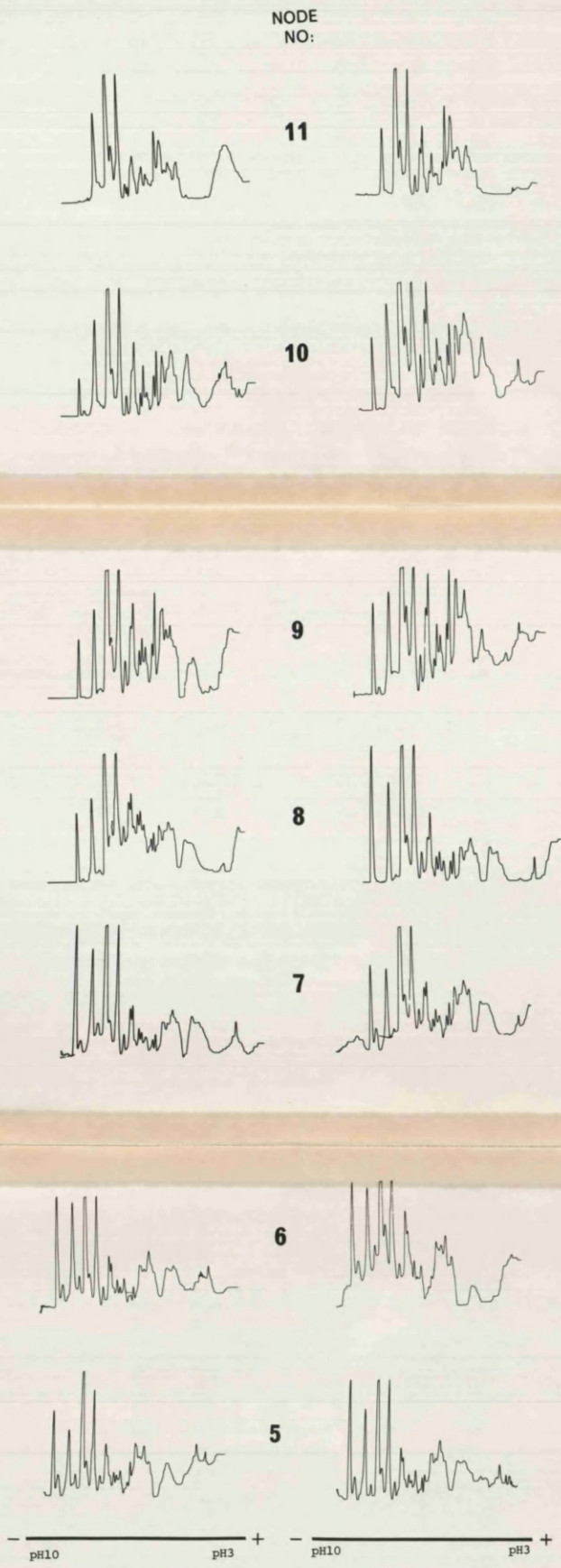


Fig. 5. Densitometer tracings of acrylamide gels showing the pattern of leaf sap proteins of Alaska peas after separation by isoelectric focusing and staining with Coomassie brilliant blue. Right hand series is from deflowered (total nodes 13) and left hand from untreated (total nodes 12) plants. The plants were of the same chronological age at the time of sampling. Leaf node numbers are from base upward.

The effect of flower development on the patterns of leaf sap proteins, using gel isoelectric focusing technique

The objective in this study was the same as in the experiment No.2, i.e. we were trying to find any relative quantitative or qualitative changes in leaf sap proteins that could be related with flower development. The main difference in this study, however, was the use of gel isoelectric focusing technique, instead of electrophoresis, for protein separation.

Alaska peas were used in this experiment and were grown under the standard controlled conditions. At the time of flowering careful selection of the plants was carried out to obtain maximum uniformity among the experimental plants. Half of the selected plants were deflorated and the other half were left untreated. When there were three flowers on untreated controls, leaf samples were taken from each node of both flowering and deflowered plants. Three replicate plants were taken in each treatment and the experiment was repeated more than three times. The extraction of leaf saps was carried out at the same time, and the extracted saps of individual leaves were stored in deep freeze at $-20^{\circ}C$ for 2-3 days. The effect of freezing and thawing on sap proteins was tested by electrofocusing but no changes were observed. After isoelectric focusing protein bands were located by our own staining procedure.

In Fig. 5 a comparison is made between the patterns of leaf sap proteins (as separated by gel isoelectric focusing) of corresponding nodes in deflowered and control plants. It is evident from the figure that the patterns of sap proteins hardly differ at any corresponding nodes of deflowered and control plants. Prominent changes however can be seen with the physiological aging of leaves in each treatment, but no significance can be attributed to these changes in relation to any

LEAF PROTEINS

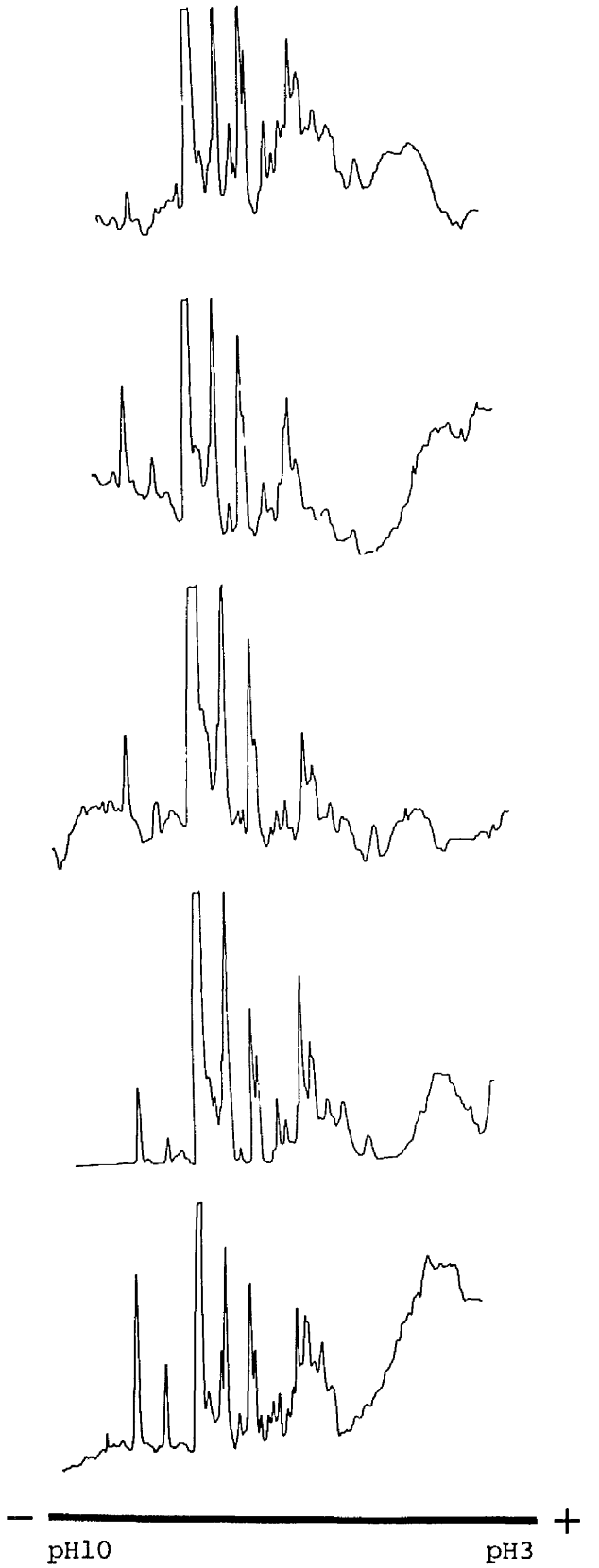


Fig. 6. Densitometer tracings of acrylamide gels showing the pattern of leaf sap proteins of Greenfeast peas after separation by isoelectric focusing and staining with Coomassie brilliant blue. Leaf node numbers are from base upward. There were eighteen nodes on the plant and the flower buds had just started appearing.

LEAF PROTEINS

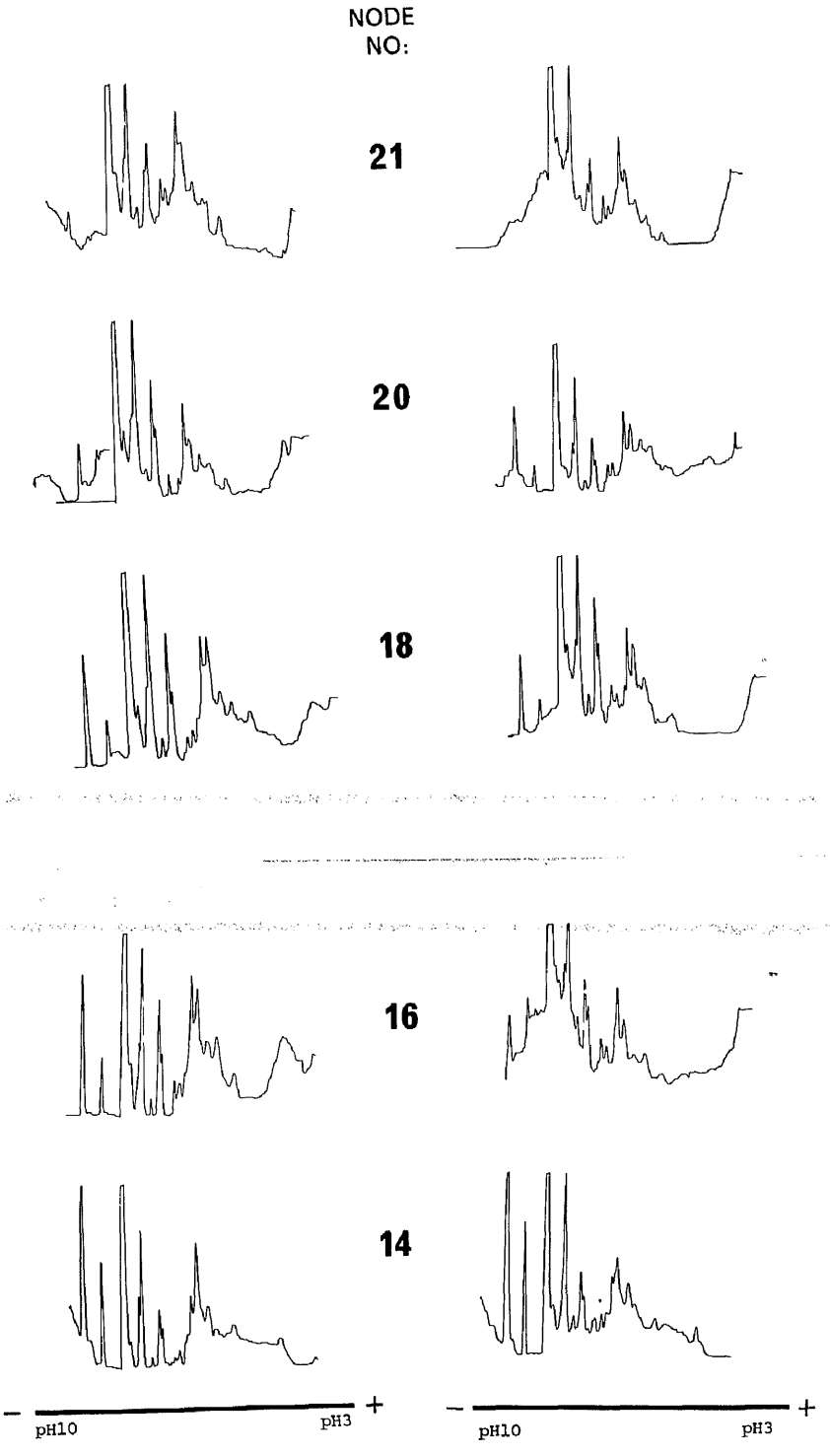


Fig. 7. Densitometer tracings of acrylamide gels showing the pattern of leaf sap proteins of Greenfeast peas after separation by isoelectric focusing and staining with Coomassie brilliant blue. Right hand series is from deflowered plants (total nodes 22) and left hand from untreated (total nodes 21) plants. The plants were of the same chronological age at the time of sampling. Leaf node numbers are from base upward.

senescence stimulus produced by developing flowers.

It therefore appears that although a far superior separation of sap protein is achieved by isoelectric focusing, it does not help to find any specific changes in sap proteins of leaves of any physiological age, young or old, that could be related to flower development. However, it provides good confirmation of our previous results.

The patterns of leaf sap proteins of cv. Greenfeast peas

Peas cv. Greenfeast used in this experiment were grown in the growth cabinet under the standard controlled conditions. At the time of flowering leaf samples were taken, and the leaf saps were extracted in the usual way. The separation of leaf sap proteins was carried out by our standardized procedure of gel isoelectric focusing.

Densitometer traces of acrylamide gels showing protein patterns of the leaves of different nodes are shown in Fig. 6. There seems to be quite a similarity in these scans and the ones of Alaska peas (shown in Fig. 4).

The effect of flower development on the leaf sap proteins of Greenfeast peas

The main objective of this study was to confirm the results obtained in previous studies in a different variety of plant material. Greenfeast peas were therefore grown under similar conditions as in previous studies. At the time of flowering uniform plants were selected and half of them were deflorated. After the formation of three flowering nodes on untreated plants, leaf samples were taken followed by the extractions of the saps. The sap proteins were then analysed by isoelectric focusing

LEAF PROTEINS

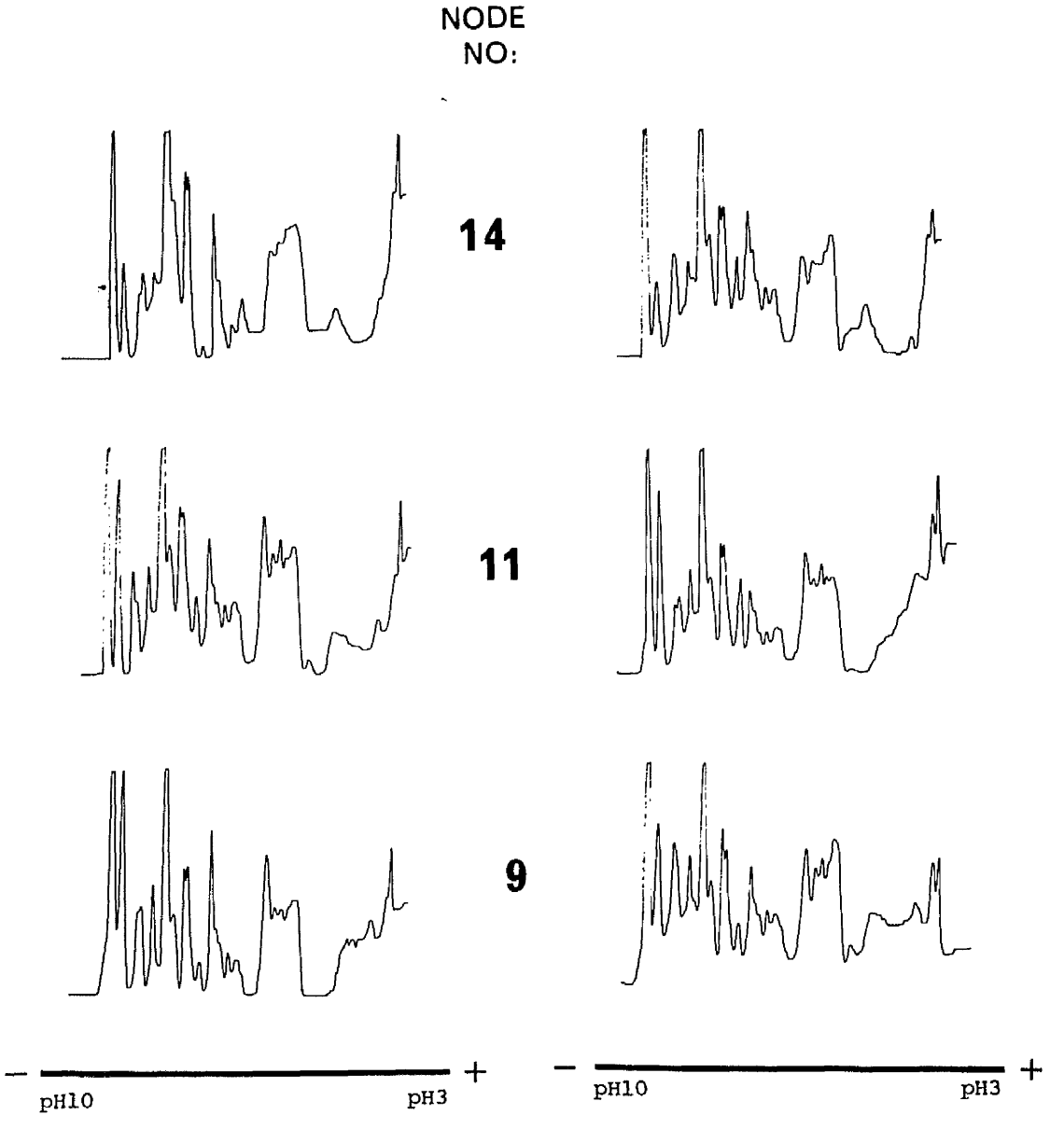


Fig. 8. Densitometer tracings of acrylamide gels showing the patterns of leaf sap proteins of Alaska peas after separation by isoelectric focusing and staining with Coomassie brilliant blue. Scans on the right-hand side are from deflowered (total nodes 20) and on left-hand side are from untreated plants (with 4 pods and total nodes 16). The plants were of the same chronological age at the time of sampling. Leaf node numbers are from base upward.

as described earlier.

The typical scans representing sap proteins of leaves of different nodes of flowering and deflowered plants are presented in Fig. 7 Again no difference can be observed in the proteins patterns of corresponding leaves in deflowered and control plants.

The results obtained in this study are quite in accordance with the results obtained in our previous studies. It therefore confirms that development of flower does not bring any observable changes in the sap proteins of the leaves of different physiological age, under our experimental conditions and analytical procedure.

The effect of fruit development on the leaf sap proteins of Alaska peas

After finding that the flower development, prior to fruiting, does not bring any specific changes in the leaf sap proteins, it was decided to see if any changes are brought about by the advance stages of floral structure. This study was therefore conducted to observe the effects of fruit development on the leaf sap proteins of Alaska peas.

Alaska peas were grown according to the standard procedure used in previous studies. Uniform plants were selected at the time of flowering and the treatment of defloration was started on half of them. The defloration was continued on the first treated plants while fruits were developing on untreated controls. Sampling of leaves from both deflowered and untreated plants was carried out when there were 4 pods (with at least one completely filled) on the untreated plants. In this experiment it was not possible to sample all the leaves at corresponding nodes as there were no corresponding nodes on untreated plants for the nodes above the 16th node of deflowered plants. Moreover leaves below the 8th node were mostly yellow in both treatments. The study of yellow leaves was

LEAF PROTEINS

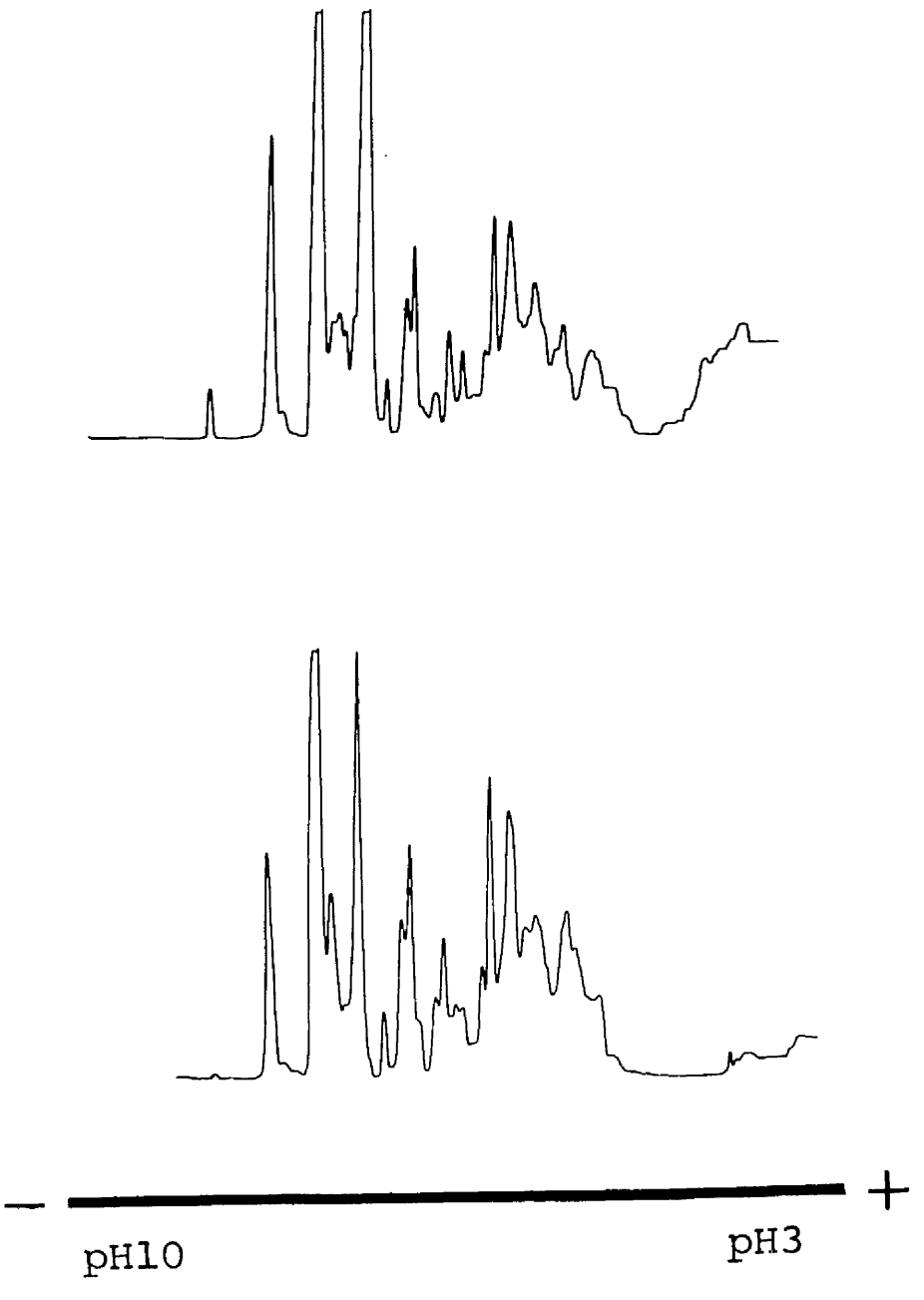


Fig. 9. Densitometer tracings of acrylamide gels showing protein pattern of young developing leaves at A, pre-flowering and B, flowering stages of the plants. The proteins were separated by isoelectric focusing and were stained with Coomassie brilliant blue.

avoided for the reason that we were interested to find the cause of senescence rather than the result of senescence. Therefore representative leaf samples were taken at the 9th, 11th and 14th node from both fruiting and deflowered plants. The experiment, however, was repeated three times with at least three replicate plants in each treatment. The extraction and analysis of leaf proteins (by electrofocusing) were carried out according to the standard procedure described in previous studies.

The separation of sap proteins of the leaves at corresponding nodes (9th, 11th and 14th) of deflowered and untreated plants are presented as densitometer traces of gels, after the isoelectric focusing and staining, in Fig. 8. Again no difference can be seen in the proteins patterns of the corresponding leaves in deflowered and control plants. These results, therefore lead to the conclusion that not only the flower development but also the fruit development brings very little change, if any, in the leaf sap proteins, as extracted and analysed by us.

The effect of flowering on the sap proteins of young developing leaves

In this study an attempt was made to see if at the early stages of flowering, any striking changes appear in the proteins patterns of young developing leaves. In other words, we thought that initiation of flowering might be producing some changes in the proteins patterns of new developing leaves thus triggering a stimulus for whole plant senescence.

In this study, therefore, a comparison is made (see Fig. 9) between the protein patterns of the young developing leaves of Alaska peas at the flowering and pre-flowering stages of the plants. However, looking at Fig. 9 there does not appear any observable difference in the protein patterns of the young leaves of flowering (B) and pre-flowering (A) plants. Thus even at the time of flower initiation there does not

LEAF PROTEINS

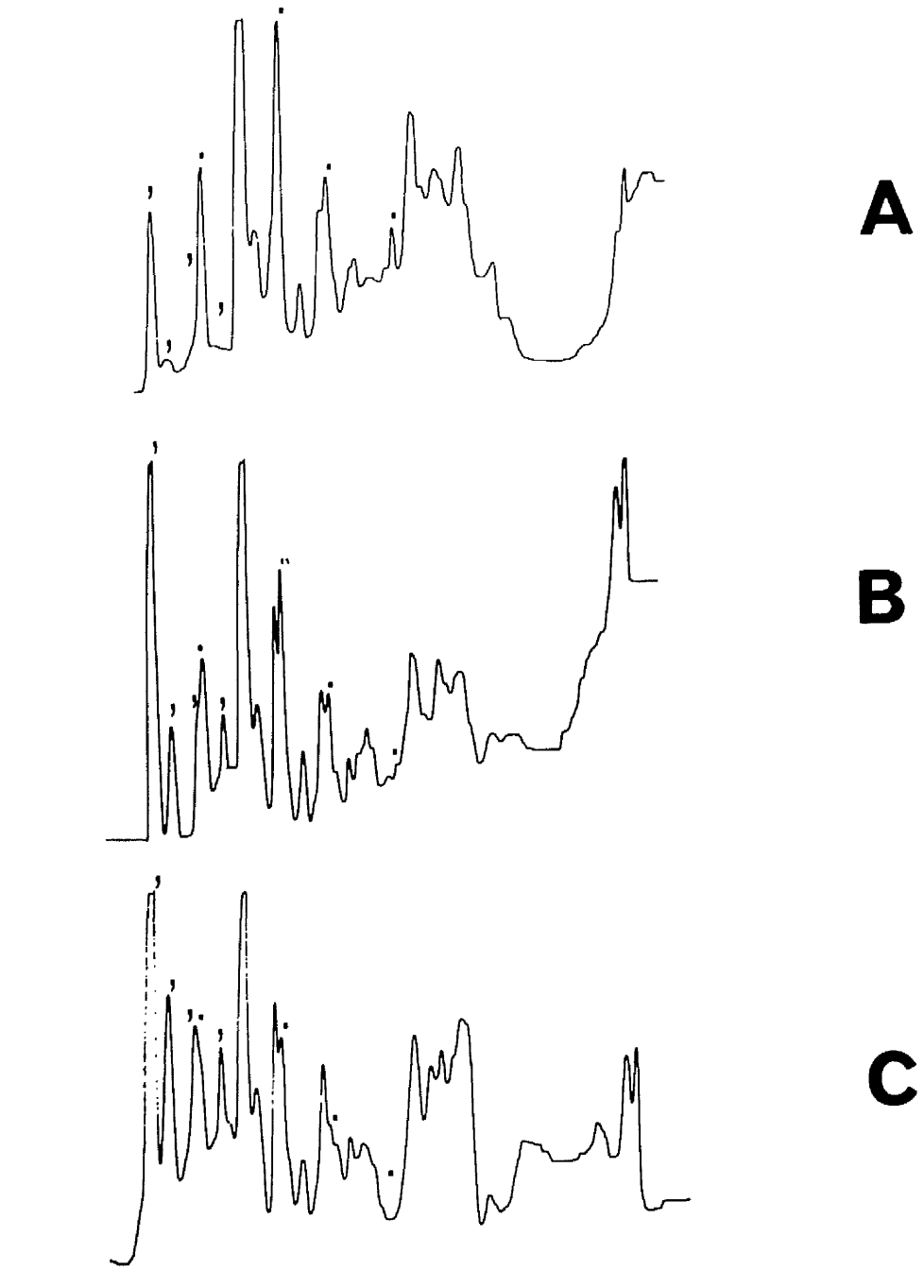


Fig. 10. Densitometer tracings of acrylamide gels showing pattern of sap proteins of 9th leaf of Alaska peas, A, just after complete development, B, after aging on test tube, C, after aging on intact plant. The proteins were separated by gel isoelectric focusing and were stained with Coomassie brilliant blue. (·) point to protein bands which decrease on leaf aging. (′) point to protein bands which increase on leaf aging.

seem to be any stimulus produced that could bring any observable changes in the sap proteins of young developing leaves.

Changes in leaf sap proteins during the course of aging of detached leaves

In this experiment an attempt was made to study the changes appearing in leaf sap proteins during the senescence of detached leaves, in order to see if they differ from the changes that appear with the physiological aging of intact leaves. The point was to find the influence of whole plant, if any, on the aging of intact leaves.

Alaska peas were grown for this experiment under the controlled condition. Fully expanded leaves at 9th node of flowering plants (with 5 flowers) were detached along with the internode between 9th and 8th node. The axillary buds at the 9th node were carefully crushed with a fine forcep. The leaves were then placed in the test tubes containing water in such a way that the internodes were well immersed in water while the leaves stayed above the water surface. First leaf samples were taken at this stage and only the leaf saps were extracted, excluding the internode, and stored in the deep freeze at -20°C. Other leaves, in the test tubes, were then left for ten days to age in the growth cabinet under the same environmental conditions as used for growing plants in previous studies. After 10 days a second sampling of the leaves was done and the leaf sap was extracted as described before. The experiment was repeated three times with at least 3 replicates at each stage. Proteins separation was carried out by isoelectric focusing as described before.

The typical proteins patterns of the leaves at the time of first sampling are shown in Fig. 10A. The patterns of proteins after 10 days aging in test tubes are shown in Fig. 10B. The protein peaks that increase with the aging of leaves have been marked with (,) and the ones that decrease during aging are marked with (.).

APICAL PROTEINS

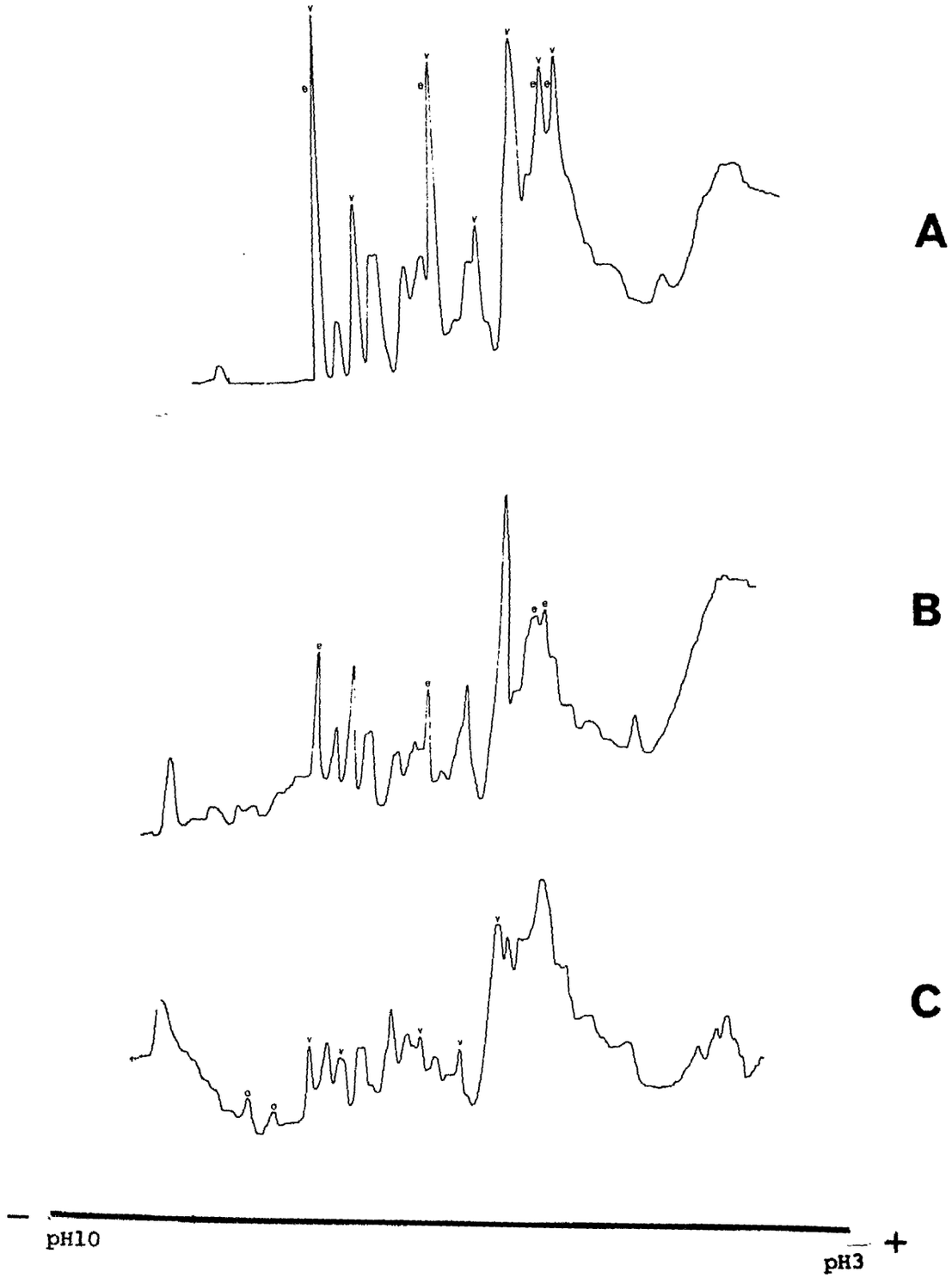


Fig. 11. Densitometer tracings of acrylamide gels showing pattern of apical proteins after separation by gel isoelectric focusing and staining with Coomassie brilliant blue. (A) seedling stage, (B) flowering stage, (C) final stage of differentiation into flower.

Fig. 10C shows the pattern of sap proteins of a leaf at 9th node on its aging on intact plant (there were 4 pods and 16 nodes on the plant). Comparing Fig. 10B with 10C it appears that similar changes appear in the patterns of sap proteins during the aging of an intact or detached leaf.

Protein changes during different stages of apical growth and differentiation

Apical growth in peas ceases soon after flowering by differentiating into a final flower. This cessation can be delayed by defloration but only to a limited extent and again terminating into a final flower. This study was therefore conducted as an initial investigation to study changes in apical proteins during different stages of apical growth and differentiation.

Alaska peas were grown under the standard controlled conditions. Protein analysis (by gel isoelectric focusing) of apical portions were carried out at three stages; first at seedling stage just at time of first leaf expansion, second just before flowering, and third just before differentiation into final flower (when there were 5 flowers on the plant). Apices (1 mm to 1.5 mm apical portion) were removed from plants using fine forceps and needle. Ten apices were then crushed in the glass tubes (containing .2 ml of 4% sucrose solution in water), above the polymerized gel column, with the fine forceps, to extract water soluble proteins. Two such replicate samples were analysed by isoelectric focusing at all the three stages, and the experiment was repeated only 2 times.

Fig. 11 a,b,c, show proteins patterns of apical proteins at seedling, flowering and near final cessation stages respectively. It is evident from the figure that some peaks (marked 'V') show a quantitative depression from seedling to final stage. It is also evident that even at flowering

stage a quantitative depression appears in some peaks marked 'e'. It, therefore can be concluded that proteins patterns of apical meristems of peas differ at the three different stages studied, but it is difficult to say whether or not the changes observed are effected by flower development as no such studies were conducted where corresponding plants were kept vegetative.

DISCUSSION

The ease with which defloration can effectively delay senescence in annuals has now made it generally accepted fact that flower development is the main controlling agent of whole plant senescence in annuals. The mechanism of its control, however, has been differently advocated, i.e. either through its nutrient mobilization effect (Molisch 1928) or because of some special senescence stimulus (Leopold et al 1959). If the effect is only of nutrient mobilizing nature then there should only be a general hydrolysis with increased translocation. But if the effect is through some special senescence stimulus then one would anticipate some dramatic changes in the plant specific to flower development. The studies described in this section were therefore conducted as a first step to confirm whether or not flower development brings specific changes in plant metabolism, as reflected by protein composition, related to whole plant senescence.

Leopold et al (1959) suggested that first a stimulus is produced at the stage of flower development (in soyabean and male spinach plants) and the second and more drastic effect appears at the time of the ripening of the seeds (soyabean). We therefore started to find out specific metabolic changes (in leaf sap proteins) that floral structure may bring at different stages of its development.

As proteins play vital role in plant metabolism it was reasonable to conceive the possibility that flower could mediate its effect through some proteins or enzymes. Moreover a frequent correlation has been made by various workers (Osborne 1962, Shaw et al 1965, Anderson & Rowan 1965, 1966, Mettale & Dove 1968, Atkin & Sirvastava 1969, 1970, Patricia & Titus 1972, Martin & Thimann 1972) between protein and/or enzymes and the senescence of the particular organ. It was therefore decided to see if such correlations are also possible in whole plant senescence. In

other words we were trying to find specific changes in proteins (of leaf sap) related to flower and then to the whole plant senescence. It was also in view, however, that if such correlation were possible then we might be able to get an objective method of measuring senescence in plants.

The reason for choosing leaf as the site for the effect of flower development was simply because early works (Molisch 1928, Leopold et al 1959) considered the aging of whole plant on the basis of its senescing leaves.

Results presented in Figs. 3, 5 and 7 show that the patterns of leaf sap proteins, as separated by disc electrophoresis or isoelectric focusing, do not differ at corresponding nodes of flowering or deflowered plant (cvs. Alaska and Greenfeast). It therefore suggests that development of flower prior to fruiting does not bring any observable changes on the patterns of leaf sap proteins, as extracted and analysed by us. The advanced development stage of floral structure, i.e. fruit filling, was therefore next studied. The results of this study are shown in Fig. 8 and again no changes specific to the effect of fruit filling can be observed in the leaf sap protein patterns. It was then decided to compare protein patterns of youngest leaves of the plants at flowering and pre-flowering stages in order to see if any changes occur at early stages of flower initiation. However, it is evident from Fig. 9 that protein banding of the youngest leaves from the plants of different stages of development are very similar.

In these experiments although we are unable to find any specific changes in leaf sap proteins in relation to various developmental stages of floral structure, but changes in the patterns of sap proteins with the physiological aging of the leaves were quite prominently noticed (compare patterns of proteins of leaves at higher node number with the

lower ones in Fig. 3, 5 and 7). It was therefore thought to look if the changes that appear with the aging of intact leaves are influenced by the whole plant metabolism or are due to the metabolism of the leaf itself. For this purpose changes in the patterns of proteins were then studied during the aging of detached leaves under the same environmental conditions as for the intact leaves. However, as seen in Fig. 10, it appears that changes in the protein patterns of leaf sap follow a similar trend during the aging of detached leaves just as in intact leaves, under our experimental conditions.

In the study of apical proteins some peaks (marked 'e' in Fig. 11) were observed to decrease at the time of flowering and these, along with other peaks (marked 'v' in Fig. 11) showed a further relatively quantitiative depression near the final stage of apical differentiation (Fig. 11). It is, however, difficult to say whether the changes observed were specifically due to flower development or because of an independent progressive decline in the vigour of apical meristems themselves, as pointed out by Lockhart & Gottschall (1961). To explain it further, it would be necessary to conduct more experiments in order to study proteins patterns of apical meristems at stage in plants corresponding to flowering plants but kept vegetative by some environmental conditions, as described by Lockhart & Gottschall (1961). But in the absence of such experiments here we can only point out that quantitative depression appears in certain protein peaks at different stages of apical growth and differentiation.

The main conclusions that can be drawn from these studies are:

(1) The development of flower and fruit on the plant produce very little effect, if any, on the leaf sap proteins according to our experimental procedures. But we cannot emphasise here that flower and fruit development does not produce any senescence

stimulus, because it is quite possible that stimulus produced is of a nature other than proteins; may be of a hormone or growth regulating nature.

(2) Changes in proteins patterns during the aging of intact leaves appear to follow an independent route which remains the same even if the leaves were detached.

(3) The methods employed for the analysis of leaf proteins cannot be used for measuring whole plant senescence.

Section B HORMONE STUDIES

Introduction

The effects of microgram quantities of plant hormones on various plant growth processes are so profound that they have become a leading topic of research in all fields of plant physiology. Their effects on senescence are also quite striking; the way different plant hormones can retard or enhance senescence in various plant organs has been discussed in the introduction. Although it is difficult to describe an exact mechanism as to how they operate in plant metabolism, a general understanding from the current literature suggests that they are involved in the control of nucleic acid and protein or enzyme synthesis (Van Overbeek 1966, Black & Edelman 1970). Both of these groups of compounds have been closely related to senescence and thus suggests that plant hormones are important in the control of senescence. Moreover, besides their effects on the senescence of detached or intact plant organs, various workers have also described their close relationship with the mechanism of whole plant senescence. Loomis (1953) suggested that high concentration of auxins in the fruit could be responsible for the nutrient mobilization effect that the fruit seems to possess. Wareing & Seth (1967) confirmed the nutrient mobilization effect of high concentration of plant hormones, but they suggested another possibility of plant senescence control, where they proposed that a senescence delaying factor (cytokinin) synthesised by roots is diverted by developing fruits and therefore a decreased supply of this factor to other plant parts could be the cause of whole plant senescence. A similar suggestion was also made by Sitton *et al* (1967) who found decreased quantities of cytokinin in root exudate of sunflower after its complete development and therefore suggested that lower supplies

of cytokinin from the root might be the cause of shoot senescence.

The effect of plant hormones in retarding or enhancing senescence of explants, their involvement in nucleic acid and protein synthesis and their possible role in the mechanism of whole plant senescence therefore, constitute the bases for these studies. The main objective, however, was to explain and confirm whether or not the cause of whole plant senescence in annuals is due to change in endogenous levels of different growth regulators.

The construction of experiments in these studies was very much the same as in protein studies, i.e. we were trying to see if flower and fruit development bring any changes in endogenous levels of plant hormones that could be held responsible for a major role in whole plant senescence.

Ideally one would like to study changes in all of the known and unknown specific or non-specific growth promoters or inhibitors in order to see if they are correlated with senescence. But with the available techniques of separation and identification of different growth regulators (especially the unknown) it was extremely difficult, if not impossible, to conduct a study of such a magnitude. Therefore, although we were quite interested in some inhibitory zones on TLC of our leaf extracts, we decided to concentrate our attention only on the known plant hormones. Because there was only a limited time available for these studies, it was not even possible to study all of the known growth regulators, and therefore we had to further restrict our plan of work only to GAs, ABA and cytokinin.

The choice of extraction solvent for endogenous GAs, ABA and cytokinins varies from author to author but basically there are three solvent systems, i.e. (1) a polar organic solvent, (2) aqueous organic solvent, and (3) aqueous buffer solvent. In the initial trials there-

fore, it was attempted to see the extraction capacity and feasibility of different solvent systems for our own work. First, comparison was made between the organic (absolute Methanol) and aqueous (buffer) solvents, in which organic solvent (Methanol) proved to be a better system as it was simple and more convenient to handle and with equally good extraction capacity (Badr et al 1971). With aqueous extraction another difficulty was that during acid hydrolysis of proteins there can be a danger of dehydration and isomerization of ABA (Lenton et al 1970). Organic solvent system (Methanol) was further compared with aqueous organic solvent (80% Methanol) and was found to extract less dry weight (the weight of the residue left after the evaporation of solvent from the extract) and therefore had less difficulties of purification. Extraction of phytohormones with the organic solvent (Methanol) was therefore adopted as the standard procedure as it proved to be the most satisfactory procedure for our comparative work. Although further trials were made to test different organic solvents no other solvent proved to possess any extra advantage. Further purification and separation procedures have been discussed in Materials and Methods.

Various trials were also made for the selection of different bioassays and the basis of choice were mainly the speed, sensitivity, convenience and suitability to our conditions. However the selection of dwarf pea bioassay for Gibberellins was mainly because we were extracting our GAs from the peas and so we were adopting a highly desirable practice of bioassaying back on to the same species as that for which the material was extracted.

The space in our growth cabinets was not enough to grow sufficient numbers of plants for hormone extraction studies, therefore the experiment had to be conducted in the field. Unfortunately the seedling

from the first sowing caught disease and so the experiment was restarted after a month by planting new seedlings on a different site. It took about four months to complete the experiment in the field, and as it was conducted in the second year of the course so the repetition of field experiment was not possible. However extractions of hormones in each treatment were repeated from the same sample.

Leaf samples were taken at the seedling stage (when there were 4-5 total nodes), flowering stage (at the time of flower initiation), a fortnight after first defloration (when there were 3-5 complete flowers on the untreated plants but pod formation had not started), at fruiting stage (when there were 4-6 pods on untreated plants with at least one completely filled) and finally at the stage of fruit maturity (when at least three pods had gone yellow).

The leaf samples (collected in polythene bags) were frozen, freeze-dried and powdered as described in Materials and Methods. 30 g of powdered material were used in each replicate extraction unless otherwise stated. The purification and separation of different hormones were carried out according to the standard procedure described earlier. However, the final sequence of separation on TLC and bioassay was as follows:

GAs and ABA separation

The fraction 'G' containing GAs and ABA from the sephadex column was reduced and was applied to a TLC plate (0.75 mm thick) which was then developed in solvent system 1 consisting of chloroform - ethyl acetate - acetic acid (60:40:5 v/v). Two zones, the first corresponding to GA_3 and GA_1 (Rf 0.25) 'A' and second zone corresponding to $GA_{5,4,7}$ and ABA (Rf 0.45) 'B' were removed and eluted separately. The eluate of the zone 'A' was bioassayed directly at this stage. The eluate of the zone 'B' was then applied on another TLC plate which was then

developed in solvent system II (details of the preparation of this solvent system and its use has been described in Materials and Methods). Zones corresponding to GA_5 (Rf 0.4), ABA (Rf 0.5), GA_7 (Rf 0.6) and GA_4 (Rf 0.65) on the plate developed in solvent system II were removed and eluted separately and then bioassayed.

Separation of Cytokinins

The material from the acid extract of silver nitrate precipitates (for details see Materials and Methods) was applied on a preparative plate (Silica gel HF 254) which was then developed in solvent system III consisting of n-butanol and ammonia sp.g. 0.88 (4:1 v/v). Two zones, first corresponding to Zeatin (Rf 0.5) 'Z' and second zone (Rf 0.62) were removed and eluted separately and then bioassayed. In earlier trials other zones did not show any activity and therefore were not assayed later in routine work.

To make sure that the biological activities of extracted materials corresponding to different growth regulators (in chromatographic behaviour) are not, completely or partially, from the substances in solvents or silica gel, blanks were also run corresponding to natural samples. However, as shown in Table 10 the bioassays of different blanks showed no biological activities .

A recovery reconstruction experiment was also carried out in which a known quantity of GA_3 was run through the whole extraction procedure along with the plant material. The bioassay results of the final eluate showed 50% recovery of the added hormone.

The effect of flower development on the endogenous levels of plant hormones (ABA, cytokinins and GAs) in the leaves (at flowering node, a node above and a node below flowering node) of Greenfeast variety of garden peas.

Leaves from the above mentioned three nodes were collected separately from both untreated and deflowered plants a fortnight after the first defloration treatment. The leaves of the same node number were bulked in each treatment and analysed as described before.

Table 1-3A show the bioassay results of ABA like activity of the extracted material (with similar chormatographic properties as ABA) from the leaves at flowering node, a node above and a node below flowering node respectively of both treatments. There was persistently no significant difference in the bioassay results of replicate extractions, therefore the observation of both replicates were pooled together in each treatment. The results presented for comparative treatments are therefore the mean of their respective pooled observations.

It is evident from the data presented in Tables 1-3A that there is no significant difference between the endogenous levels of ABA like substances extracted from the leaves at corresponding nodes (at all of the three nodes studied) of untreated and deflorated plants.

In the bioassay for cytokinin like activity all the cotyledons (5) in each dish (the test solution in each dish was either of the two zones, i.e. 'Z' or 'ZR' of an individual extraction) were weighed collectively. Because accurate weighing of individual cotyledons was so time consuming that with the available time it was not a practical way of working and therefore collective weights had to be taken. In the absence of individual observations we cannot present mean weights of cotyledons in each treatment along with the standard error of mean, therefore total weights of cotyledons obtained in the bioassay of

replicate extractions in each treatment are presented separately as A and B (on all tables from 1-7B).

The data presented in tables 1-3B shows no real difference in the cytokinin like activity of the leaves at the corresponding nodes (at all of the three nodes studied) of untreated and deflowered plants. (14 days after first defloration).

The bioassay results of Gibberellin like activity (of the zones corresponding to GA_3 on TLC. Zones corresponding to $GA_{5, 4}$ and 7 showed no activity and therefore need not be mentioned here) are presented in tables 1-7C as mean internode (third) length of peas (var. Meteor) from the pooled observations obtained in the separate bioassays of replicate extractions.

The data presented in tables 1-3C shows no significant differences in the endogenous level of gibberellin like substances of the leaves at corresponding nodes (at all of the three nodes studied) of untreated and deflowered plants.

It therefore appears from the above results (shown in tables 1-3) that flower development prior to pod formation does not bring any observable changes in the endogenous levels of ABA, Cytokinin and Gibberellin like substance of the leaves as compared with deflorated plants under our experimental conditions.

<u>The effect of fruit development on the endogenous levels of plant hormones (ABA, Cytokinin and GAs) in the leaves of Greenfeast variety of garden peas</u>

The sampling of leaves at fruiting stage was carried out from all the nodes above first flowering node in both treatments i.e. untreated and deflorated plants. Leaves of different nodes of different plants were bulked together in each treatment.

The bioassay results of endogenous levels of ABA, Cytokinins and Gibberellin like substances (extracted materials of similar chromatographic and biological properties as their respective authentic samples) of both untreated and deflorated plants are presented in Table 4 (A, B and C respectively). It is evident from the data shown in Table 4 (A, B and C) that ABA, Cytokinin and GA like activities measured from the leaf extracts of both treatments (untreated and deflorated plants) do not show any significant difference. It therefore suggests that flower development (as mentioned earlier) and fruit development (prior to maturity) do not produce any observable effects on the endogenous levels of plant hormones (ABA, Cytokinin and GAs) in the leaves of Greenfeast variety of peas, as extracted by us.

The effect of fruit maturity on the endogenous levels of plant hormones ABA, Cytokinins and Gibberellins) in the leaves of Greenfeast variety of garden peas

As in the fruiting stage the sampling of leaves was carried out from all the leaves above flowering node which were separately bulked in each treatment. The quantity of powdered leaf material used in each replicate extraction of both treatments was 40 gms.

The bioassay results of endogenous ABA, Cytokinin and Gibberellin like substance in both treatments (untreated and deflorated plants) are shown in Table 5 (A, B and C respectively).

It is evident from the Table 5A that ABA like activity measured in the leaves of untreated plants is significantly higher than the leaves of deflorated plants. In Tables 5B and 5C it can be seen that no Cytokinin or Gibberellin like activities were measured in the leaf extracts of untreated plants whereas leaf extract of deflorated plants

show significant activities for both Cytokinin and Gibberellin like substances over the controls.

These results, therefore, suggest that (under our experimental conditions and extraction procedures) after the maturity of fruits there appear significant changes in the endogenous level plant hormones of the leaves with an increase in ABA like activity and decrease in Cytokinin and Gibberellin like activities as compared with the levels in the leaves of deflorated plants.

The effect of flower initiation on the endogenous levels of plant hormones (ABA, Cytokinin and GAs) in the leaves of Greenfeast variety of garden peas

The effect of flower initiation was studied by comparing the levels of hormones in the leaves at the time of flower initiation with the levels at the seedling stage of plants. Leaf samples at seedling stage were collected from three week old plants which had 4-5 total number of nodes with no visible flower buds. Leaf samples at flowering stage were taken from the plants that had just started flowering, having 2-3 mm buds with no petal colour formation. The seedling plants were grown near the end of the main experiment (from which leaves sample at all other stages were collected), because initially the studies on endogenous levels of plant hormones were started mainly to look at the effect of defloration. But during the course of this experiment it was also decided to compare hormone levels of leaves in plants at flowering and pre-flowering stage for an additional information to see if any interesting changes appear in the endogenous hormone levels of leaves at the early stages of flower initiation. Therefore, as we had not taken leaf samples at seedling stage, new seedlings were raised near the end of the first main experiment.

The bioassay results of ABA, Cytokinin and Gibberellin like substances (of both untreated and deflorated plants) are shown in Table 6, A, B and C respectively. It is evident from the data presented in Table 6 (A, B and C) that endogenous levels of ABA, Cytokinin and Gibberellins (in leaves) do not differ significantly between the two stages of plants studied.

Three gibberellin fractions corresponding to $GA_{5, 4 \text{ and } 7}$ on TLC (developed in solvent system II) that showed no biological activity with previous extract in dwarf pea bioassay were tested using lettuce hypocotyl bioassay. And a zone corresponding to GA_5 (Rf 0.4 in solvent system II) was found active in the sample of both treatments. The other zones, however, showed no GA like activity. The bioassay results of the zones corresponding to GA_5 (of untreated and deflorated plants) are shown in Table 8. The data presented in Table 8 show that the leaves of the plants at flowering and pre-flowering stages do not significantly differ in endogenous levels of GA like substance.

The results obtained in this study, therefore, lead to the conclusion that under our experimental conditions and extraction procedures there does not appear any change in the levels of hormones (ABA, Cytokinin and GA) of the leaves at the onset of flowering in the Greenfeast variety of garden peas.

<u>The effect of flower initiation on the endogenous levels of plant hormones (ABA, Cytokinin and GAs) in the apices of Greenfeast variety of garden peas</u>

The finally powdered materials of apical portions collected from the plants at flowering and seedling stages were not enough to use 30 gms of the plant material in the replicate extractions, therefore only 15 gms of the powdered material were used in each replicate

extraction of both treatments (seedling and flowering stages). The bioassay results of ABA, Cytokinin and Gibberellin like substances (of both flowering and seedling stages) are shown in Tables 7 (A, B and C respectively) and 9 (bioassay results of GAs like substances).

The data presented in Table 7A show a significant increase in the levels of ABA like activity (of the apices) at the time of flowering; a slight decrease in the levels of cytokinin fraction 'ZR' can also be seen. The levels o fCytokinin fraction 'Z' and Gibberellin (both GA_3 as shown in Table 7C, and GA_5 as shown in Table 9) like substance however show no significant difference between the two stages studied.

Table 1. Endogenous levels of plant hormones in the leaves at flowering node of untreated and deflowered plants

A Bioassay results of ABA like activity expressed as mean coleoptile length

	Extracted material: Untreated plants	Extracted material: Deflowered plants	Control	1.0 µg ABA	10.0 µg ABA	100.0 µg ABA
Mean coleoptile length in mm	12.8† ± 0.25	12.8† ± 0.29	18.0 ± 0.24	15.6 ± 0.24	13.0 ± 0.24	10.2 ± 0.38

† Zone corresponding to ABA on TLC (in two solvent systems) Rf in solvent system II = 0.5

B Bioassay results of Cytokinin like activity expressed as total weight of radish cotyledons (5)

	Extracted material: Untreated plants Z†	Untreated plants ZR*	Deflowered plants Z†	Deflowered plants ZR*	Control	0.1 µg Zeatin	1.0 µg Zeatin	10.0 µg Zeatin
Total wt. of cotyledons (5) in gms	A- 0.1651	A- 0.1209	A- 0.1683	A- 0.1192	1,000	0.1304	0.1669	0.1842
	B- 0.1601	B- 0.1287	B- 0.1621	B- 0.1308				

† Zone corresponding to Zeatin on TLC in solvent system III (Rf 0.5)
* 1.5 cm zone above the Zeatin zone on TLC (Rf 0.62)
- A,B correspond to replicate extraction

C Bioassay results of Gibberellin like activity expressed as mean internode length

	Extracted material: Untreated plants	Extracted material: Deflowered plants	Control	0.01 µg GA_3	0.1 µg GA_3
Mean internode length in mm	10.7† ± 0.56	10.2† ± 0.25	4.4 ± 0.40	9.6 ± 0.51	16.0 ± 0.71

† Zone corresponding to GA_3 on TLC in solvent system I (Rf 0.25)

Table 2. Endogenous levels of plant hormones in the leaves at one node above flowering node of untreated and deflowered plant at flowering stage

A Bioassay results of ABA like activity expressed as mean coleoptile length

	Extracted material		Control	1.0 µg	10.0 µg	100.0 µg
	Untreated plants	Deflowered plants				
Mean coleoptile length in mm	12.7† ± 0.216	12.7† ± 0.177	17.1 ± 0.35	14.8 ± 0.43	12.1 ± 0.24	10.4 ± 0.17

† Zone corresponding to ABA on TLC (in two solvent systems) Rf in solvent system II = 0.5

B Bioassay results of cytokinin like activity expressed as total weight of radish cotyledons (5)

	Extracted material				Control	0.1 µg Zeatin	1.0 µg Zeatin	10.0 µg Zeatin
	Untreated plants		Deflowered plants					
	Z†	ZR*	Z†	ZR*				
Total wt. of cotyledons (5) in gms	A- 0.1491	A- 0.1163	A- 0.1548	A- 0.1232	0.1070	0.1227	0.1576	0.1653
	B- 0.1568	B- 0.1209	B- 0.1523	B- 0.1200				

† Zone corresponding to Zeatin on TLC in solvent system III (Rf 0.55)
* 1.5 cm zone above the Zeatin zone on TLC (Rf 0.62)
- A,B correspond to replicate extractions

C Bioassay results of Gibberellin like activity expressed as mean internode length

	Extracted material		Control	0.01 µg GA_3	0.1 µg GA_3
	Untreated plants	Deflowered plants			
Mean internode length in mm	10.0† ± 0.45	9.7† ± 0.21	4.4 ± 0.40	9.6 ± 0.51	16.0 ± 0.71

† Zone corresponding to GA_3 on TLC in solvent system I (Rf 0.25)

Table 3. Endogenous levels of plant hormones in the leaves at one node below flowering node of untreated and deflowered plants at flowering stage

A Bioassay results of ABA like activity expressed as mean coleoptile length

	Extracted material		Control	1.0 µg ABA	10.0 µg ABA	100.0 µg ABA
	Untreated plants	Deflowered plants				
Mean coleoptile length in mm	13.2† ± 0.22	13.3† ± 0.21	18.0 ± 0.24	15.5 ± 0.24	13.0 ± 0.24	10.2 ± 0.38

† Zone corresponding to ABA on TLC (in two solvent systems) Rf in solvent system II = 0.5

B Bioassay results of Cytokinin like activity expressed as total weight of radish cotyledons (5)

	Extracted material				Control	0.1 µg Zeatin	1.0 µg Zeatin	10.0 µg Zeatin
	Untreated plants		Deflowered plants					
	Z†	ZR*	Z†	ZR*				
Total wt. of cotyledons (5) in gms	A- 0.1912	A- 0.1398	A- 0.1793	A- 0.1449	0.0782	0.1002	0.1281	0.1363
	B- 0.1868	B- 0.1479	B- 0.1832	B- 0.1523				

† Zone corresponding to Zeatin on TLC (Rf 0.55)
* 1.5 cm zone above the Zeatin zone on TLC (Rf 0.62)
- A,B corresponds to replicate extractions

C Bioassay results of Gibberellin like activity expressed as mean internode length

	Extracted material		Control	0.01 µg GA_3	0.1 µg GA_3
	Untreated plants	Deflowered plants			
Mean internode length in mm	10.6† ± 0.52	11.0† ± 0.55	4.4 ± 0.40	9.6 ± 0.51	16.0 ± 0.70

† Zone corresponding to GA_3 on TLC in solvent system I (Rf 0.25)

Table 4. Endogenous levels of plant hormones in leaves at all nodes above flowering node in untreated and deflowered plants at fruiting stage

A Bioassay results of ABA like activity expressed as mean coleoptile length

	Extracted material		Control	1.0 µg ABA	10.0 µg ABA	100.0 µg ABA
	Untreated plants	Deflowered plants				
Mean coleoptile length in mm	12.7† ± 0.23	12.5† ± 0.18	0.171 ± 0.40	15.0 ± 0.47	12.8 ± 0.32	10.6 ± 0.15

† Zone corresponding to ABA on TLC (in two solvent systems) Rf in solvent system II = 0.5

B Bioassay results of cytokinin like activity expressed as total weight of radish cotyledons (5)

	Extracted material				Control	0.1 µg Zeatin	1.0 µg Zeatin	10.0 µg Zeatin
	Untreated plants		Deflowered plants					
	Z†	ZR*	Z†	ZR*				
Total wt. of cotyledons (5) in gms	A- 0.1560	A- 0.1214	A- 0.1560	A- 0.1229	0.1070	0.1227	0.1576	0.1653
	B- 0.1580	B- 0.1203	B- 0.1614	B- 0.1304				

† Zone corresponding to Zeatin on TLC in solvent system III (Rf 0.5)
* 1.5 cm zone above the Zeatin zone on TLC (Rf 0.62)
- A,B correspond to replicate extraction

C Bioassay results of Gibberellin like activity expressed as mean internode length

	Extracted material		Control	0.01 µg GA_3	0.1 µg GA_3
	Untreated plants	Deflowered plants			
Mean internode length in mm	13.7† ± 0.57	13.9† ± 0.58	6.8 ± 0.20	15.6 ± 1.33	17.0 ± 0.55

Zone corresponding to GA_3 on TLC in solvent system I (Rf 0.25)

Table 5. Endogenous levels of plant hormones in the leaves at all nodes above flowering node of untreated and deflowered plants at the stage of fruit maturity

A Bioassay results of ABA like activity expressed as mean coleoptile length

	Extracted material		Control	1.0 µg ABA	10.0 µg ABA	100.0 µg ABA
	Untreated plants	Deflowered plants				
Mean coleoptile length in mm	10.8† ± 0.21	12.7† ± 0.22	17.1 ± 0.40	15.0 ± 0.47	12.8 ± 0.32	10.6 ± 0.15

† Zone corresponding to ABA on TLC (in two solvent systems) Rf in solvent system II = 0.5

B Bioassay results of Cytokinin like activity expressed as total weight of radish cotyledons (5)

	Extracted material				Control	0.1 µg Zeatin	1.0 µg Zeatin	10.0 µg Zeatin
	Untreated plants		Deflowered plants					
	Z†	ZR*	Z	ZR*				
Total wt. of cotyledons (5) in gms	A- 0.0998 B- 0.0879	A- 0.1003 B- 0.0890	A- 0.1361 B- 0.1299	A- 0.1132 B- 0.1218	0.1000	0.1304	0.1669	0.1842

† Zone corresponding to Zeatin on TLC in solvent system III (Rf 0.5)
* 1.5 cm zone above the Zeatin zone on TLC (Rf 0.62)
- A,B correspond to replicate extractions

C Bioassay results of Gibberellin like activity expressed as mean internode length

	Extracted material		Control	0.01 µg GA_3	0.1 µg GA_3
	Untreated plants	Deflowered plants			
Mean internode length in mm	6.4† ± 0.27	12.9† ± 0.58	6.8 ± 0.20	15.6 ± 1.33	17.0 ± 0.55

† Zone corresponding to GA_3 in solvent system I (Rf 0.25)

Table 6. Endogenous levels of plant hormones in the leaves at flowering and seedling stages of plants

A Bioassay results of ABA like activity expressed as mean coleoptile length

	Extracted material		Control	1.0 µg ABA	10. µg ABA	100.0 µg ABA
	Flowering stage	Seedling stage				
Mean coleoptile length in mm	13.7† ± 0.26	14.4† ± 0.22	18.2 ± 0.28	15.6 ± 0.29	13.2 ± 0.36	10.6 ± 0.18

† Zone corresponding to ABA on TLC (in two solvent systems) Rf in solvent system II = 0.5

B Bioassay results of Cytokinin like activity expressed as total weight of radish cotyledons (5)

	Extracted material				Control	0.1 µg Zeatin	1.0 µg Zeatin	10.0 µg Zeatin
	Flowering stage		Seedling stage					
	Z†	ZR*	Z†	ZR*				
Mean wt. of cotyledons (5) in gms	A- 0.1270	A- 0.0993	A- 0.1163	A- 0.0938	0.0782	0.1002	0.1281	0.1363
	B- 0.1201	B- 0.0981	B- 0.1265	B- 0.1023				

† Zone corresponding to Zeatin on TLC in solvent system III (Rf 0.5)
* 1.5 cm zone above the Zeatin zone on TLC (Rf 0.62)
- A,B correspond to replicate extractions

C Bioassay results of Gibberellin like activity expressed as mean internode length

	Extracted material		Control	0.01 µg GA_3	0.1 µg GA_3
	Flowering stage	Seedling stage			
Mean internode length in mm	10.40† ± 0.40	9.60† ± 0.42	5.00 ± 0.32	2.80 ± 1.02	16.8 ± 1.07

† Zone corresponding to GA_3 on TLC in solvent system I (Rf 0.25)

Table 7. Endogenous levels of plant hormones in the apices at flowering and seedling stages of plants

A Bioassay results of ABA like activity expressed as mean coleoptile length

	Extracted material		Control	1.0 µg ABA	10.0 µg ABA	100.0 µg ABA
	Flowering stage	Seedling stage				
Mean coleoptile length in mm	15.3† ± 0.20	16.7† ± 0.28	18.2 ± 0.28	15.6 ± 0.29	13.2 ± 0.36	10.6 ± 0.18

† Zone corresponding to ABA on TLC (in two solvent systems) Rf in solvent system II = 0.5

B Bioassay results of Cytokinin like activity expressed as total weight of radish cotyledons (5)

	Extracted material				Control	0.1 µg Zeatin	1.0 µg Zeatin	10.0 µg Zeatin
	Flowering stage		Seedling stage					
	Z†	ZR*	Z†	ZR*				
Total wt. of cotyledons (5) in gms	A- 0.1232	A- 0.0880	A- 0.1280	A- 0.1000	0.0671	0.0946	0.1252	0.1308
	B- 0.1260	B- 0.0918	B- 0.1196	B- 0.0979				

†Zone corresponding to Zeatin on TLC in solvent system III (Rf 0.5)
* 1.5 cm zone above the Zeatin zone on TLC (Rf 0.62)
- A,B corresponds to replicate extraction

C Bioassay results of Gibberellin like activity expressed as mean internode length

	Extracted material		Control	0.01 µg GA_3	0.1 µg GA_3
	Flowering stage	Seedling stage			
Mean internode length in mm	9.90† ± 0.35	10.60† ± 0.54	5.00 ± 0.31	8.8 ± 1.02	16.8 ± 1.07

† Zone corresponding to GA_3 on TLC in solvent system I Rf (0.25)

Table 8. Bioassay results of Gibberellin like activity expressed as mean hypocotyl length

	Extracted material		Control	0.01 µg GA_3	0.1 µg GA_3
	Flowering stage	Seedling stage			
Mean hypocotyl length in mm	3.9† ± 0.22	4.6† ± 0.24	1.8 ± 0.44	4.1 ± 0.24	9.5 ± 0.33

† Zone corresponding to GA_3 or 2O on TLC in solvent system II Rf 0.4

Table 9. Bioassay results of Gibberellin like activity expressed as mean hypocotyl length

	Extracted material		Control	0.01 µg GA_3	0.1 µg GA_3
	Flowering stage	Seedling stage			
Mean hypocotyl length in mm	4.1† ± 0.16	3.8† ± 0.19	1.8 ± 0.44	4.1 ± 0.24	9.5 ± 0.33

† Zone corresponding to GA_5 or 2O on TLC in solvent system II Rf 0.4

Table 10. Bioassay results of blanks for ABA, GAs and cytokinins

ABA like activity in wheat coleoptile bioassay (mean coleoptile length in mm)		GAs like activity in dwarf pea bioassay (mean internode length in mm)		Cytokinin like activity in radish cotyledon bioassay (total wts. of cotyledons (5) in gms)		
Control	Blank	Control	Blank	Control	Blank	
					Z	ZR
18.2 ± 0.28	17.4 ± 0.18	5.0 ± 0.32	4.6 ± 0.25	0.0782	0.0721	0.0801
17.1 ± 0.40	16.8 ± 0.26	4.4 ± 0.40	4.6 ± 0.24	0.1000	0.0938	0.0898
17.1 ± 0.35	16.6 ± 0.28	5.0 ± 0.32	4.2 ± 0.38	0.0671	0.0711	0.0691
		† 1.8 ± 0.19	† 1.77 ± 0.21			

† Lettuce hypocotyl bioassay

The effect of exogenous applications of GA_3, Kinetin and IAA on the general pattern of senescence in Greenfeast variety of garden peas

The views of different workers regarding the role of plant hormones in the control mechanism of whole plant senescence have been discussed in the beginning of this section. In short it has been suggested that senescence in plants can be effected by phytohormones in two possible ways i.e., (i) either by a decline in the endogenous quantities of hormones in various parts of the shoot (either by diversion into fruit or due to lower synthesis) or (ii) by the nutrient mobilization effect of their high concentration in the fruits.

In previous studies, described in this section, we were trying to confirm the first possibility by measuring the endogenous levels of hormones in the leaves in the presence and absence of fruits to see if any quantitative depression occurs due to intact fruits. But with the results obtained we were unable to confirm this hypothesis, as no significant differences were observed in the endogenous levels of phytohormones (we looked at ABA, Cytokinin and GAs) in untreated and deflorated plants unless leaves of untreated plants had already gone yellow. So it was decided to see if the role of hormones could be explained in terms of second hypothesis (nutrient mobilization effect) along with confirmation of our previous results, if possible. This study was, therefore, designed on the basis of two main assumptions, i.e. (i) if we assume that hormones effect senescence by mobilizing nutrients due to their high concentrations in the fruits then this effect should be accelerated if their concentration in the fruits are raised by the exogenous application; (ii) alternatively if plant senescence is effected by their diversion into fruits then the exogenous application of hormones into fruit should retard this effect. In other words we were trying to accelerate the effect of nutrient mobilization, if it is

so, or otherwise retarding the effect of decrease levels of hormones (due to their diversion into fruits), if that is so, so that a difference could be observed when compared with controls.

GA_3, Kinetin and IAA were used for the exogenous application of hormones in this study. Three concentrations of each compound (0 ppm, 30 ppm, 300 ppm) were used and each concentration of every hormone was applied in conjunction with all the three concentrations of the two other hormones in separate treatments. A total of 27 combinations ($(GA_3)3x(K)3x(IAA)3 = 27$) were therefore tested with two replicate plants per treatment.

Greenfeast variety of peas was used in this experiment and the plants were grown under the standard controlled conditions described earlier. The different treatments of hormones were prepared in lanoline, which were then applied at the peduncle of completely developed flowers prior to pod formation. The treatment was applied by giving a small cut in the peduncle into and over which lanolin containing a hormone treatment was applied. The second application of treatments was given after the complete pod formation, just at the time when seeds started developing.

Leaf samples for chlorophyll extraction were taken from the first flowering node when the pods were nearly filled. The freshly cut leaves were weighed and their boundaries were drawn on the papers to calculate their areas. The individual leaf samples (kept in tinfoil bags) were then stored in deep freeze.

The experiment was continued until the pods started yellowing, but no striking differences were observed among the different treatments at any stage of flower and fruit development. To further explore the effects of different treatments, young scions (two week old seedlings) were grafted on the old plants of the experiment (after removing their

fruits). The idea was that we might be able to observe some effects of the treatments from the differential growth of the scion. However there was no success in grafts union and therefore the experiment had to be terminated.

The chlorophyll extraction from different leaf samples (stored in deep freeze) was carried out by first keeping the leaf in boiling water for 3 minutes, after which the leaf was transferred to hot acetone. The acetone extraction of chlorophyll from the leaf was continued until the leaf was clearly bleached. Each chlorophyll extract (in acetone) was diluted to 50 ml with acetone and then the absorbance was taken at 665 mμ. However no significant difference was observed (see Appendix III) in the absorbance (calculated on the basis of per unit leaf area) of different treatments.

From the results obtained in this experiment it appears that the exogenous application of hormones (GA_3, K and IAA) in different concentrations (0, 30, 300 ppm) as used by us do not produce any observable effects on the chlorophyll contents of leaves (at first flowering node) nor on the general pattern of senescence in the plants.

The fact that in this study the application of exogenous hormones (GA_3, K and IAA) did not have any senescence retarding effect, can be considered as a further support to our previous findings where no quantitative change was observed in the levels of endogenous hormones (GAs, ABA and Cytokinins) due to developing flowers and fruits. But it is hard to draw any definite conclusion from this study unless a more detailed work is carried out using higher concentrations, more numbers of applications, different methods of application, with detailed quantitative analysis of different organic and inorganic nutrients of the leaves and a larger number of replicates.

DISCUSSION

After the original observation of Richmond and Lang (1957) that Kinetin can retard senescence in Xanthium leaves, various other workers (as mentioned in the Introduction) have also shown the effect of different growth regulators in retarding or enhancing the senescence. To confirm that growth regulators are really involved in the regulation of senescence, it is necessary to show that correlative changes also appear in their endogenous levels during senescence. Attempts were therefore made by different workers to study the levels of endogenous hormones during senescence, and it was successfully shown that during senescence there appears a progressive decline in GAs and Cytokinin like substances and an increase in ABA like substances (Fletcher et al 1969, Mayek & Halvey 1970, Chin & Beevers 1970).

Thus, considering that changes in growth regulator do appear during senescence and also that defloration can delay senescence in intact plants (Molisch 1928, Leopold et al 1959, Lockhart and Gottschall 1961) then the question arises that whether the senescence delaying effect of defloration is to maintain the balance in endogenous levels of hormones, otherwise disturbed by developing flowers and fruits. Although suggestions have been made that senescence in intact plant could be due to decline in some growth regulators (as mentioned earlier) and also that exogenous application of hormones can delay senescence of intact plants (Engelbrecht 1964, Fletcher 1969), but no attempt has been made to study the changes in the endogenous levels of growth regulators due to flower and fruit development as compared to deflorated plants of same chronological age. In this study therefore an attempt was made to see if the flower and fruit development is associated with changes in the endogenous levels of hormones in the leaves (thus enhancing their senescence) in

contrast to deflorated plants where senescence is delayed.

The results obtained in the experiment showed no significant difference in the endogenous levels of growth regulators (GA, ABA and Cytokinin only were studied) in untreated and deflorated plants during the flower and fruit development on untreated plants (see Tables 1-4). A significant decline in the levels of Gibberellins and Cytokinins like substance with increase in ABA like substances was observed in untreated plants compared to deflorated plants (see Table 5) after the fruit maturity. But it cannot be concluded from these results that senescence of untreated plants occurs due to changes in their endogenous levels of growth regulators at the time of fruit maturity, Because the leaves on untreated plants after the maturity of their fruits were nearly completely yellow compared to deflorated plants that had a fair number of green leaves (this was because node formation in deflorated plants was continued and so new leaves were produced which were green whereas in untreated plants further growth of nodes was checked after fruiting and therefore older leaves were yellow. The leaves on deflorated plants at corresponding nodes to untreated plants were also yellow). Thus an objection can be raised that the changes observed in untreated plants after the maturity of fruits are the result of senescence rather than the cause. Because if changes in hormone levels would have been the cause of leaf senescence then one would expect them to appear before the visible occurrence of senescence. Thus unless changes in hormone level are not shown prior to visible senescence of leaves at any stage of floral development, it would be hard to say that flower and fruit development (at a certain stage) enhance senescence through changes in endogenous levels of hormones. Therefore in contrast to the speculative suggestion of Sitton et al (1967) and Wareing & Seth (1967) our results show that flower and fruit development does not

produce any observable changes in the levels of endogenous growth regulators that could be considered as the cause of senescence. But for more definite conclusions, time course studies after the complete development of fruit to fruit maturity will be necessary.

The results presented in Tables 6-9 show the endogenous levels of growth regulators in the leaves of peas at flowering and seedling stages, but no emphasis can be given to these results as seedling plants were grown under shorter days and lower temperatures than the plants at flowering stage. And as endogenous hormone levels have been shown to vary under different photoperiods (Zeevart 1969, Heid & Skoog 1967, Vanstaden & Wareing 1972) therefore it is difficult to isolate the effects of flower emergence from the effects of photoperiod. The main conclusions that can be drawn from this study are the following:

(1) The endogenous levels of ABA, Cytokinins and Gibberellins like substances in the leaves of Greenfeast variety of peas do not differ in the presence or absence of developing flowers and fruits (before fruit maturity).

(2) The senescence of leaves on intact plants is associated with a decline in Cytokinin and gibberellin like substances and in increase in ABA like substances.

Section B SURGICAL STUDIES

Introduction

It is noticeable in the earlier works relating to whole plant senescence that surgical experiments have been of great help in investigating the aging process in annuals (Molisch 1928, Leopold et al 1959, and Lockhart & Gotschall 1961). In fact surgical experiments are commonly used to elucidate various physiological phenomena of plants (Murfet 1971, Phillips 1969, Scott & Berrie 1973).

Thus considering the significance of surgical studies in earlier work, and specially when we had to repeat some of the previous work for the confirmation and then for its extension, it was therefore decided that this approach of research should also be included in our studies on the control mechanism of plant senescence.

In the initial experiments described in this section a general survey of whole plant senescence was carried out in order to provide confirmation of the previous work and also to observe other responses resulting from the accepted methods of surgery and novel combinations of them. In later experiments, however, our main objective was to investigate in detail the role of fruit development and ripening in whole plant senescence.

General Survey of Senescence in Garden Peas

The mid-season variety of garden peas var. Greenfeast was used in these studies, and the plants (6 plants/treatment) were grown under the standard controlled conditions, excepting where cold treatment was given at $8^{\circ} \pm 2^{\circ}C$.

In this survey various attempts were made to observe the effects of defloration, defoliation, decapitation (individually and in various combinations), cold treatment and light on the general pattern of senescence in peas. In all cases, lateral buds were allowed to grow, if growing, with a view that whole plant senescence may be delayed if the growth of laterals continued. In other words the idea was that it may be that even if all the leaves on the main stem have gone yellow, the plant may still be viable and capable of instituting activity in axillary buds.

Due to some electrical faults in the growth cabinet, the experiment had to be scrapped three times before observations could be begun and therefore results were delayed for nearly a year.

The effect of defloration

The reason for conducting this study was mainly to repeat the previous work under our own conditions, and therefore to have a practical observation of the known fact - "Removal of flowers delays senescence".

The flower buds were removed along with peduncle prior to petal expansion. Removal of flower buds, and so the prevention of fruit set, was continued on the first treated plant throughout the experiment. The experiment was terminated when fruits on untreated plants were fully matured and dried.

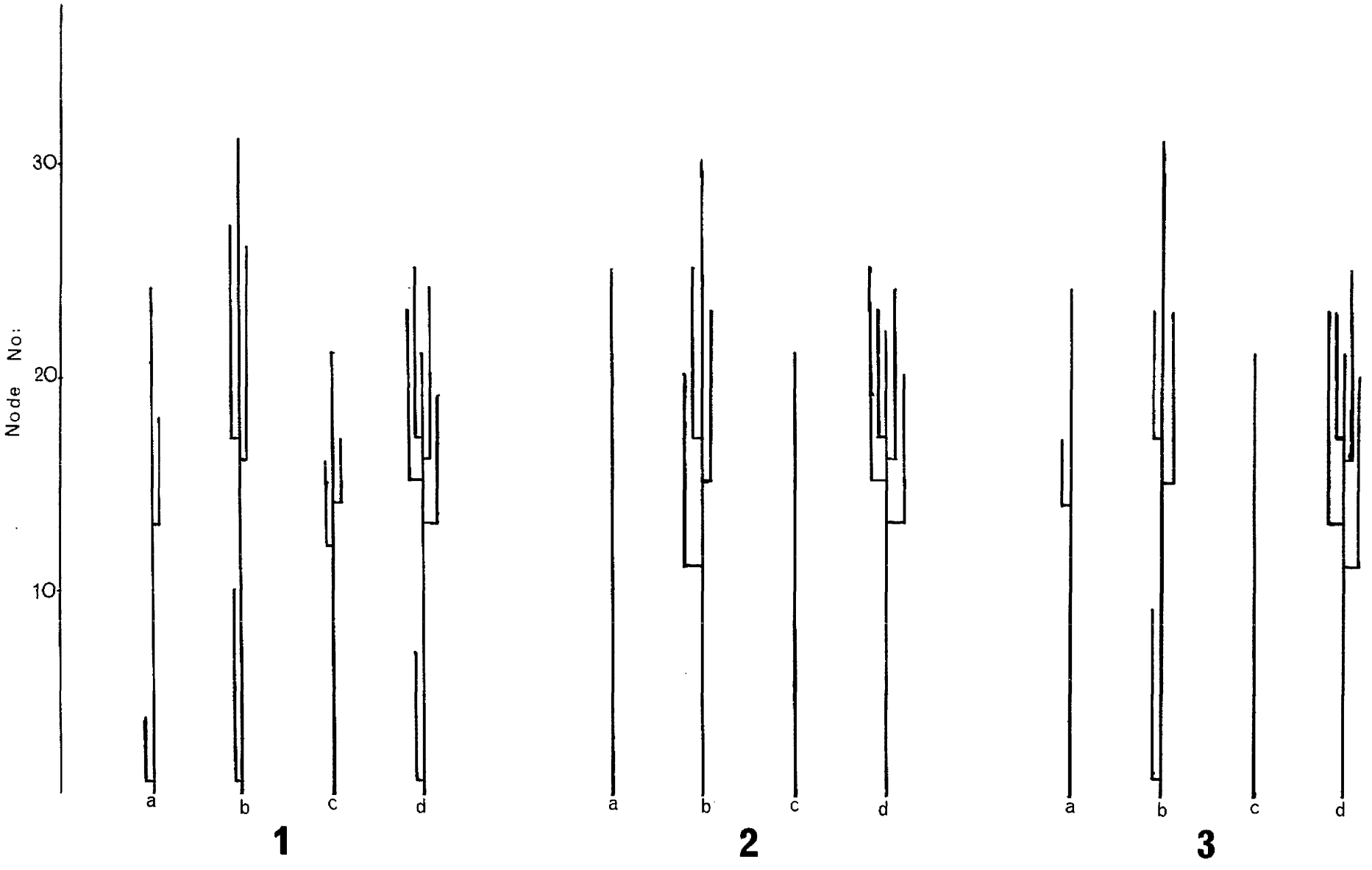

Node No:
30
20
10
a
b
c
d
1
a
b
c
d
2
a
b
c
d
3

Fig. 12. A model nodal situation of laterals in Greenfeast peas with different treatments of defoliation, defloration and decapitation is shown to scale. The length of individual branch is drawn on average basis.

1 = No defoliation.

2 = Defoliation keeping 4 leaves.

3 = Defoliation keeping 6 leaves.

(a) = No defloration, no decapitation.

(b) = Defloration.

(c) = Decapitation.

(d) = Defloration + decapitation.

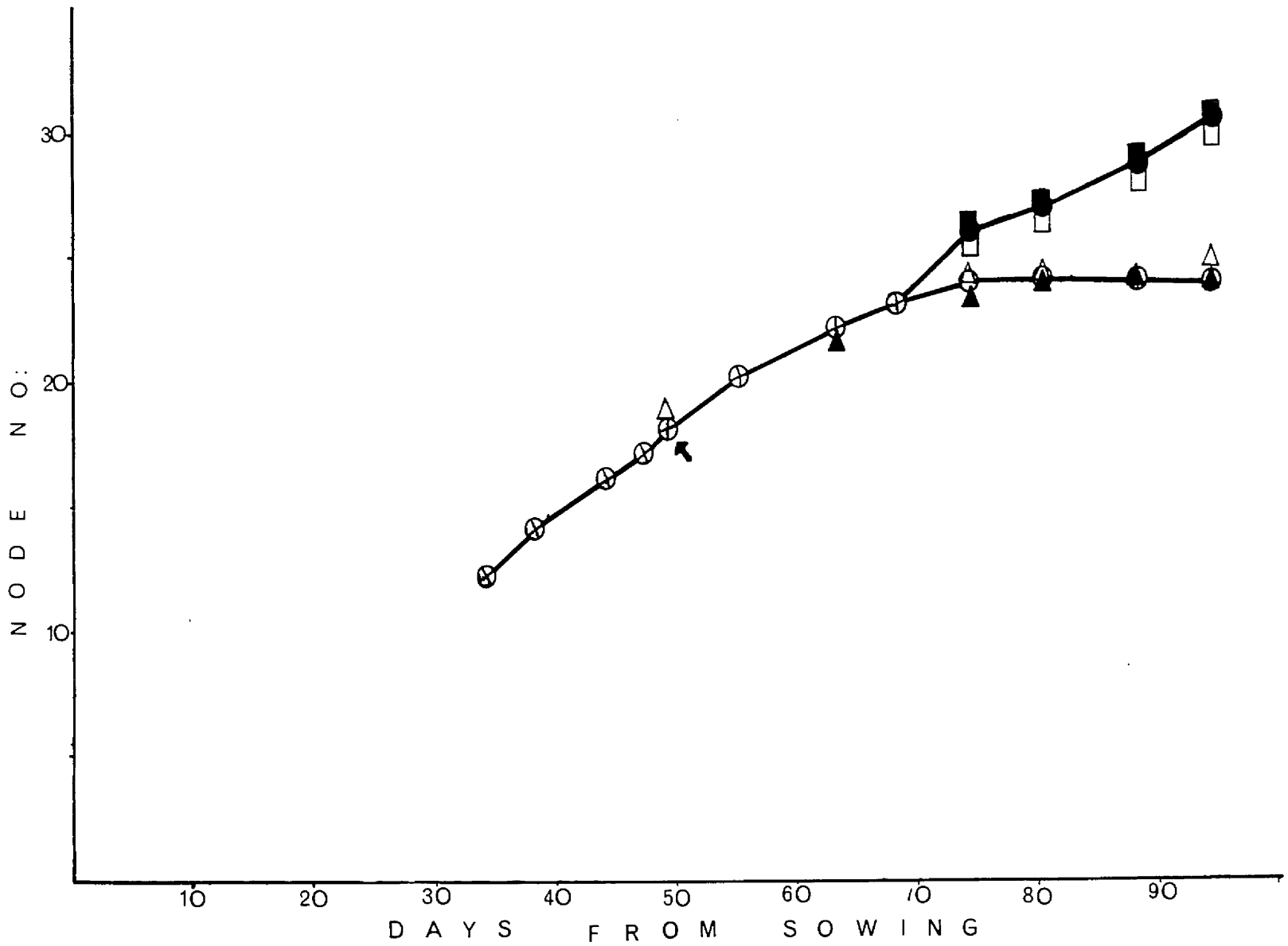

NODE NO:
30
20
10
10
20
30
40
50
60
70
80
90
DAYS FROM SOWING

Fig. 13. Effect of defoliation and defloration on node formation in Greenfeast peas.


When more than three treatments appear at the same spot.


Flowering node.


Control (no defoliation, no defloration).


Deflorated (no defoliation).


Deflorated and defoliated keeping 4 leaves.


Deflorated and defoliated keeping 6 leaves.


Defoliated keeping 4 leaves (no defloration).


Defoliated keeping 6 leaves (no defloration).

The most striking effect of defloration was manifest in the vigorous growth of axillary buds, whereas in untreated plants the growth of axillary buds occurred rarely (see Fig.12 also see Appendix V). The removal of flower buds from the axillary branches induced the production of tertiary axes but these branches rarely produced any appreciable number of nodes, and were thin with very short internodes and small leaves.

The growth of the main stem was also continued, with the formation of new nodes and leaves, if flower and fruit development were prevented by defloration. The growth of the main stem however soon started declining (when fruits on the untreated plants were nearly filled) by progressively forming shorter internodes and smaller leaves, even when flower buds were continually removed from all parts of the plant. The internode length of the new nodes after 28 nodes was extremely reduced (a fourth of the length of internodes at the time of flowering) by the time fruits on untreated plants were completely matured. The apical growth of the untreated plants, however, ceased as soon as their fruits started filling. The sequence of node formation of the main stem in untreated and deflorated plants is shown in Fig. 13 Although from the Fig. 13 there does not appear any depression in the rate of node formation in the deflorated plant, however, the vigour of apical growth was markedly reduced as appeared from the size of the internodes, leaves and the thickness of the stem.

The senescence of leaves on the main stem of the deflorated and untreated plants showed a similar pattern of yellowing nearly at the same time on the corresponding nodes (see Table 11). However, deflorated plants remained green, when untreated plants were completely yellow after the maturity of their fruits, mainly because of the young leaves, either on the axillary branches or on the newly formed extra

Table 11. Effects of various treatments of defoliation, defloration and decapitation on general appearance of Greenfeast peas, as compared with controls at the time of harvest

	Treatments	Stem	Leaves	Branching	Pods	Flower Node	Total Nodes	Remarks
Defoliation keeping four leaves	No defloration No decapitation	Yellow	Mostly dried but a few greenish yellow	Absent	3 Wrinkled	18	25	Dying
	Defloration	Green	Green. Basal leaves of branches slightly yellow	Vigorous		17	30	Growing
	Decapitation	Greenish yellow	Mostly dried but a few green with strong yellow spots	Absent	2 Inflated	18	21	Dying
	Defloration Decapitation	Green	Yellow on main stem; green on branches	Vigorous		18	22	Growing
Defoliation keeping six leaves	No defloration No decapitation	Yellow	Dried	Rare	3 Wrinkled	18	24	Dead
	Defloration	Green	Green. Basal leaves of branches yellow	Vigorous		19	31	Growing
	Decapitation	Greenish yellow	Dried	Absent	4 Wrinkled	18	21	Dying
	Defloration Decapitation	Green	Yellow on main stem; green on branches	Vigorous		18	21	Growing
No defoliation	No defloration No decapitation	Dried	Dried	Slightly	4 Wrinkled	18	24	Dead
	Defloration	Green	Top leaves (9) green, rest dried. Branch green with basal leaves yellow	Vigorous		18	31	Growing
	Decapitation	Dried	Dried	Slightly	4 Wrinkled	18	21	Dead
	Defloration Decapitation	Green	Dried on main stem. Green on branches except basal	Vigorous		18	21	Growing

nodes on the terminal portion of the main stem corresponding to which there were no nodes on the untreated plants (because apical growth on untreated plants ceases by the time their fruits are half filled, while it continues on deflorated plants), in other words on plant parts which did not exist on untreated plants.

Leaves on the lateral branches appeared to have a slow progression of senescence compared to those on the main stem. Even when the leaf, at the node of which the lateral branch originated, was completely dried, the lateral still had most of its leaves green. The vigour of lateral bud growth, however, declined nearly parallel to the main stem.

It, therefore, can be concluded from this study that under our experimental condition, removal of flower buds can delay senescence in peas, this delay is due to the inception of axillary branching and also to the continued production of new leaves by the main axis.

The effect of defoliation

Osborne (1955) reported that in maturing leaves a senescence factor is produced which increases as the leaf ages and then on its translocation into the petiole causes leaf abscission. Chin & Beever (1970) showed that with aging of leaves, the endogenous levels of ABA increase. Thus if maturation and senescence of leaves is associated with ABA and/or a senescence factor, and considering that old leaves of peas do not abscis, then a possibility arises that in peas, mature and old leaves might have a significant influence in accelerating whole plant senescence by translocating an unknown kind of senescence factor and/or ABA into the other parts of the plant. If this is so, then one would expect that removal of mature and old leaves would have a senescence retarding effect. But Hopkinson (1966) suggested that old leaves perform a beneficial function in plant growth by supplying their

breakdown products to young growing regions. This means, contrary to the first hypothesis, that the removal of old leaves might have an adverse effect on growing regions and so the growth of apices and fruits may be decreased.

Still different is the view of Molisch (1928) and others (as mentioned earlier) who think that development of fruits mobilizes nutrients from the leaves, and then the leaf senesces due to exhaustion. Simon (1967) explained it, that fruit can exhaust the leaves by mobilizing amino acids from the leaves which are produced during protein turnover. Then considering that protein turnover is a normal process in the leaves (as mentioned earlier), one would expect that if old leaves are removed then fruit will mobilize nutrients from young leaves thus causing their premature death. But if fruits are capable of photosynthesis (because developing fruits are always green) and also for the synthesis of proteins from inorganic nitrogen (because excised pods have been shown to develop on agar containing inorganic nutrients (N) (Baldev et al 1965) and so act just as sinks where breakdown products could be deposited (Peterie et al 1939), then by removing old leaves one may not find any difference in the plant growth and senescence.

The possibilities that old leaves might have a beneficial effect of supplying nutrients and at the same time that it could also have an adverse effect by producing senescence factor or ABA make the situation complicated for experimentation. However, if ABA in the plant causes bud dormancy (Wareing & Saunders 1971), and if we remove fruit and so reduce the demand for nutrients, then it could be expected that removal of old leaves might enhance axillary bud growth. But if by the removal of old leaves nutrient supply is also checked, which may be necessary for branch development, then it would mean that by the removal of old leaves there would be a greater number of branches

produced but the growth of each branch would be very poor. I have used the words very poor because increasing the number of branches itself decreases the growth per branch (Lockhart & Gottshall 1961), and if this is because the competition for available nutrients is increased then under lower supply of nutrients the growth should be markedly poor. Apical meristems as the vigorous sinks for carbohydrates have been shown by Ryle (1970). As apical dominance of the main stem is known to suppress the growth of axillary buds (see Phillips 1969) then it can be suggested that defloration along with decapitation will eliminate this effect. Although our knowledge regarding senescence factor is only slight, however, as it is known to accelerate abscission, so one can assume that in other plant parts too, it would have some sort of killing action.

In general we assumed that the effect of ABA and/or senescence factor would be more qualitative (like dormancy of buds or killing or death of apical meristems) rather than quantitative such as decline in the vigour of growth that we would expect from lower nutrient supply.

Thus by the removal of old leaves we could expect the following possibilities:

(1) A vigorous axillary growth (in the absence of fruits and apices), because of lack of ABA or senescence factor. But poor development of the branches if old leaves had a simultaneously beneficial function by providing catabolism materials to be used anabolically by the younger parts of the plants.

(2) Premature death of young leaves due to nutrient mobilizing effect of fruit development.

(3) Poor fruit growth due to lack of nutrient supply from old leaves.

(4) No effect if fruits were capable of synthesising their storage

Table 12. Effect of defoliation and decapitation on dry weight and moisture percentage of fruits/plant of Greenfeast peas at the time of harvest

Treatment	Dry wt. of fruits per plant in gms	Moisture percentage in fruits per plant
Defoliation keeping 4 leaves	3.17 ± 0.37	41.18 ± 4.81
Defoliation and decapitation keeping 4 leaves	2.72 ± 0.25	55.23 ± 4.29
Defoliation keeping 6 leaves	4.04 ± 0.41	33.73 ± 3.46
Defoliation and decapitation keeping 6 leaves	4.21 ± 0.29	37.80 ± 4.61
Decapitation	6.09 ± 0.24	26.73 ± 6.02
Control	4.67 ± 0.41	32.38 ± 10.67

materials themselves.

Two levels of defoliation, i.e. keeping 4 or 6 leaves on the plant, were investigated with a view to studying the most effective stage of defoliation. While removing leaves, stipules were also removed because in peas stipules are modified to provide a major photosynthesising organ. In different treatments both levels of defoliation were studied in conjunction with defloration, decapitation and defloration along with decapitation.

Defoliation (at both levels) had very little effect on the growth rate of main stem (Fig. 13). There was also no or a slightly depressing effect (with and without the treatments of defloration, decapitation and defloration + decapitation) on the branching pattern of plants (Fig. 12). The yield of fruits per plant on dry weight basis was, however, decreased due to defoliation (Table 12). The senescence of leaves and stem (senescence being estimated by visual observation of yellowing in the green tissue) was slightly delayed by defoliation (Table 11).

Thus, under our experimental conditions, as the senescence of upper leaves in the presence of fruits was unaffected or slightly retarded due to defoliation, then it can be suggested that fruit development may not have a nutrient mobilizing effect unless nutrients are available for transport. The observation that fruit development was decreased, but not checked, with the removal of old leaves suggests that old leaves have some beneficial function by supplying nutrients for fruit development, but at the same time fruit seem to be capable of synthesising most of their requirements.

In general it appears that under our experimental conditions senescing old leaves of peas have little observable influence on the acceleration of whole plant senescence.

The effect of decapitation

It is well known that decapitation results in the growth of axillary branches (see Phillips 1969). As pea plants under our growth cabinet conditions do not normally produce branches, and also that on an average they do not bear more than 4 pods per plant, it was decided to decapitate plants after the formation of 4 flowering nodes. The objective was to see if the initiation of axillary growth has any influence on plant senescence. It was envisaged that development of branches may compete with fruits for nutrient thus causing reduced growth of fruits but by producing new nodes and leaves they might be able to prolong the life of the plant. In another treatment, flowers were also removed along with decapitation in order to see the extent of competition of fruit for nutrient on the growth of laterals. Another thought in the mind was that by creating two nutrient mobilizing centres, if they were, then the leaves on the main stem may show accelerated effects of senescence.

The effect of decapitation was, however, different from expected, as in the presence of developing fruit, axillary growth did not show any enhancement (Fig. $_{12}$). The development of fruit was, however, enhanced, and, as shown in table $_{12}$, gave highest yield/plant on dry weight basis. The maturation and drying of the pods was also earlier than controls which can be seen from the percentage moisture content of pods at the time of harvest in different treatments. The general senescence (as marked by the yellowing and drying of the tissue) in decapitated plants was increased and on an average they died 5 days earlier than controls.

When decapitation was carried out in conjunction with defloration the axillary bud growth was increased as compared to the plants which were only deflorated (Fig. 12). The senescence of leaves on the branches of deflorated + decapitated plants was, however, very much similar to the ones which were only deflorated.

From these observations it appears that under our experimental conditions the axillary bud growth (in peas) after flowering is under the control of developing flower rather than apex. However in the absence of flowers the apex appears to have a weak depressing effect on axillary bud growth. The observation that decapitation can enhance fruit development, and in the absence of fruits it enhances axillary growth, leads to suggest that development of apical meristems involves the consumption of some growth factors from other parts of the plant, which in the decapitated plants are diverted into fruits or in the absence of fruits into the axillary branches thus enhancing their growth. In general it can be concluded from above observations that under our experimental conditions fruits induced senescence in peas cannot be delayed by decapitation.

The effect of cold treatment

It has been described that peas grow better at lower temperatures (lower than 25°C) while at 30°C or above their growth is adversely affected and hence their senescence is enhanced (Went 1957, Lockhart & Gottschall 1961). It was also observed previously in this laboratory (unpublished observations) that senescence of peas can be d elayed by growing them at very low temperatures of 6° to 8°C. Thus as we know that senescence in peas is promoted by fruit development which is prevented by defloration, and also considering that fruit induced senescence might be due to some special stimulus , it was decided to

see if fruit induced senescence could be overcome by the cold treatment of the plants. Considering a possibility that cold treatment may delay senescence by adversely affecting the fruit development (as reported by earlier workers of this department), though it may not have any control over the special senescence stimulus, if any, of the maturing fruits. Therefore it was decided to make observations on the effect of cold treatment on plants at various stages of their growth, especially at pre- and post-developmental stages of fruits.

Two varieties of garden peas, i.e., Greenfeast and Alaska were used in this experiment. The seedlings were raised under the standard controlled conditions ($18^{\circ} \pm 2^{\circ}C$ and 18 hr photoperiod). For cold treatment the plants were transferred into a growth cabinet maintained at $8^{\circ} \pm 2^{\circ}C$ with 18 hr photoperiod, at seedling, flowering and fruiting (nearly filled) stages. In actual practice plants were sown at different times in the growth cabinet maintained at $18^{\circ} \pm 2^{\circ}C$, so that all the three stages of plants were transferred at the same time into the cold growth cabinet.

Under the cold growth cabinet condition, i.e. at $8^{\circ} \pm 2^{\circ}C$, the development of plants was slowed down, so that even after 4 weeks there was hardly any node formation on the plants at any of the three stages. The development of flowers and fruits was also checked. The leaves on the plant, of any stage, however, showed no signs of further yellowing.

As the growth of the plants was extremely slow and it was apparent that at this rate of growth the experiment will need longer time than that available for recording any observation,

, this

experiment therefore had to be terminated after four weeks. The plants were however transferred back to the growth cabinet maintained at $18^{\circ} \pm 2^{\circ}C$, where they resumed their normal growth and after the

ripening of their fruits died as usual indicating that exposure to cold treatment for four weeks had no effect on senescence at 18°C.

It is difficult to conclude anything important from this experiment, unless more variations in the temperature (8° - 20°C) are tested. We were unable to test due to frequent electrical faults in the cabinets and shortage of time.

The effect of light on apical growth

Lockhart & Gottschall (1961) showed that apical senescence in peas is independent of flowering, and is due to some degenerative changes taking place within the apex itself. Looking at general pattern of growth in peas, it can be seen that the apex in the seedling stage, is contained within the unexpanded leaves, but as the plant grows and especially after flowering the younger leaves are no longer tightly held but are open and at the time of final cessation of growth the apex is completely exposed. As radiation effect has said to accelerate senescence by increasing the degeneration processes of cells (though in animal cells) (Varner 1961, Curtis 1967), it was decided to see if light has any effect on the senescence of apical meristems. Thus if light has any effect on the acceleration of senescence of apices, then it can be expected that by keeping apices in the dark their senescence might be delayed.

The apices were kept in the dark by masking them with small vials wrapped in tin foil. These vials were supported on canes (which were stuck in the pots) and by using special clips it was possible to ensure that the apex to be kept covered as the internodes expanded. Sufficient foil was left extending over the top of the vial, which was gently rolled around the apical portion (topmost portion of the plant above the uppermost fully expanded leaf) after it has been inserted in the vial to prevent

diffusion of reflected light into the vial.

The effect of masking was tested (using 8 replicate plants) at $18^{\circ} \pm 2^{\circ}C$ and $8^{\circ} \pm 2^{\circ}C$, but in both cases the same length of photoperiod was used, i.e. 18 hr photoperiod.

At $18^{\circ} \pm 2^{\circ}C$ the masked internodes started elongating, thickening and turning white. At the end of 2nd week (from the time of masking) axillary buds of the plants started growing. At that time apices were uncovered under the dim light to see their condition, and it was observed that stems around the apices were extremely swollen while their apices were drying and abscissing. The uncovered plants were then placed back into the growth cabinet. However during the next week, apices of the main stem did not resume growth, while axillary branches continued their growth.

At $8^{\circ} \pm 2^{\circ}C$, masking had no effect on the plants (these plants were grown under the standard growth cabinet conditions, and at the time of masking were transferred to another cabinet maintained at $8^{\circ} \pm 2^{\circ}$ C). Three weeks after masking, apices were uncovered under dim light, and it was noticed that covered apices were exactly the same as their comparative controls (at $8^{\circ} \pm 2^{\circ}C$), and, unlike the plants at $18^{\circ} \pm 2^{\circ}C$, had no swellings, discoloration or shrivelling. However, even the control plants at this temperature showed no growth, and so it is possible that the reason for masking having no effect may be because of the low level of active growth of the plants at that temperature.

It therefore looks from this experiment that our treatments of masking and temperature were very severe, as either the tissues were killed (as in case of masking at $18^{\circ} \pm 2^{\circ}$ C) or their growth was nearly checked ($8^{\circ} \pm 2^{\circ}C$ temperature). Therefore further studies with dim lights, different qualities of lights and at different temperatures

would be necessary for any conclusions to be drawn from this study.

DISCUSSION

In his classic book, Molisch (1928) described that development of fruits, in annual plants, exhaust the organic reserves of the leaves due to which, leaves senesce and so the plant dies after the ripening of its fruits. Later Leopold et al (1959) attempted to quantify the effects of flower and fruit development. In their experiment they decapitated the plants (soyabean) and then prevented axillary growth by pricking out axillary buds (that started growing), and then measured the effects of various stages of fruit development by counting the number of leaves that remained green during the course of time in various treatments. Lockhart & Gottschall (1961) repeated Leopold et al's work using peas and these also followed a similar pattern. These workers concluded that flower and fruit development has a major influence on plant senescence. However they all used the senescence of leaves as the main indicator of whole plant senescence. These organs do not constitute the whole plant and while they may have a profound role in whole plant senescence they are not the only parts of plant body involved. It seems that they considered it questionable, that with the senescence of leaves an annual plant would be able to survive. And so fruit development which can induce leaf senescence can be thought of as the main cause of whole plant senescence. Probably for this reason most research on whole plant senescence consists of studies of leaf senescence.

Certainly the leaf performs the most important function of photosynthesis, without which a plant, unless parasitic, can hardly show any appreciable growth. So does it mean that senescence of such an important organ is directly related to the senescence of the whole plant? Hopkinson (1966), however, said that senescence of old leaves

is in fact beneficial for plant growth, as their breakdown products are used by growing parts of the plant.

In an accident in our growth cabinet, where due to some electrical faults, the temperature in the cabinet was raised between 37° - 45°C for 5 hours, we observed that all the leaves of the plants (plants with 5-6 nodes) in that cabinet shrivelled and senesced. However, when the normal temperature (18°C) in the cabinet was resumed, the plants again started growing with the formation of new nodes and new leaves. Similarly in another instance when all of the leaves from some plants (5-6 nodes) were removed, the growth of the plants still continued and new leaves were formed on the plants. Therefore a question arises that if the removal of all leaves from the plant or the sudden death of all leaves on the plant due to high temperature does not stop the growth of the plant then how can a fruit induced senescence of leaves be considered as the sole cause of plant senescence. Of course, a point can be raised here that fruit induced senescence is a slow process and therefore during this period, there is a possibility that some senescence accelerating compounds are produced in the leaf causing plant death.

Therefore, in this general survey, besides looking at the general aspects of plant senescence, we placed special emphasis on the role of leaves in the senescence of the whole plant. In this connection we looked at various possibilities (as mentioned earlier) concerning how fruit can induce leaf senescence and how leaf senescence can effect plant death. In all our experiments, we allowed any natural bud growth to develop, since we considered that suppression of growth would itself mean induction of senescence (see Leopold 1964).

Our main observations in this study were -

(i) Removal of flowers delays plant senescence.

(ii) In the absence of fruits the growth of apical buds continue,

though later on it slowly declines. The axillary buds also start growing, and this also declines after some time.

(iii) The senescence of leaves at corresponding nodes of deflorated and fruiting plants follows a similar progression of senescence in chronological time.

(iv) The removal of old leaves results in lower yield of fruits.

(v) The senescence of young leaves in the absence of old leaves is hardly enhanced by flower and fruit development.

(vi) Removal of old leaves does not affect the growth rate of the main stem nor the total number of nodes per plant.

(vii) In the absence of flower and fruits, removal of old leaves produces no or a slightly depressing effect on axillary growth.

(viii) Removal of old leaves in the absence of flowers, does not affect the growth of the main stem or axillary branches, as compared with deflorated controls.

(ix) Decapitation (after flowering) has no effect on axillary growth but it increases fruit development.

(x) In the absence of flower and fruits, however, decapitation slightly enhances axillary growth.

These observations can be explained -

(i) As in the presence of fruits, axillary and apical growth is checked (which is prominent in deflorated plants) then it suggests that fruit development and maturity has an important influence on the senescence of apical meristem.

(ii) As the senescence of leaves of the same physiological age on the main stem progresses similarly in normal and deflorated plants then it can be argued that fruit has little or no effect on leaf senescence. This further implies that the role of the fruit is in controlling apical senescence.

(iii) As the removal of old leaves results in lower yield of fruit, but the development of fruit is not completely abandoned, nor the senescence of young leaves adversely affected; then it suggests (as Petrie et al (1939) described) that fruits do not cause senescence of leaves by diverting nutrients, but in fact act as a sink for the breakdown products from the senescing leaves.

(iv) As the removal of old leaves does not have any senescence retarding effect on the apical growth, in the presence or absence of fruit, then it seems that old leaves have little effect by producing senescence accelerating compounds.

(v) As the growth of apical meristems declines even in the absence of flowers, then it suggests that senescence of apical meristems is an independent process but can be enhanced by fruit development.

(vi) With the cessation of apical growth, the formation of new leaves stops, while the senescence of old leaves continues; this then suggests that the cessation of apical growth is the most decisive feature in whole plant senescence.

Therefore, under our experimental conditions, the main conclusions that can be drawn from this study are: Removal of flowers delays senescence in peas, by allowing the development of axillary bud and apical growth to continue for a short time. Fruit development does not cause leaf senescence but in fact is only benefited by leaf senescence. The senescence of whole plant occurs due to cessation of apical growth. Therefore it suggests that correct indication of whole plant senescence is the cessation of the growth of its apical meristems, rather than the senescence of its leaves, because as long as apical growth continues new leaves are produced and plant continues to survive. Thus in studies of whole plant senescence the effect of fruit development should be looked at in relation to apical growth rather than the senescence of its leaves.

Studies on correlation phenomenon in senescence in peas

In peas the death of the plant ensues after its apical meristems have lost their capacity for further growth and its leaves have gone yellow or dried. Many workers advocate that senescence in annuals, marked by the yellowing of their leaves, is accelerated by the development and maturity of their fruits. In our previous studies, though our results did not prove that leaf senescence could be used to measure plant senescence, however, it was clearly observed that in peas, fruits have a profound effect on plant senescence by accelerating the senescence of apical meristems. Therefore it appears that among different plant structures a correlative phenomenon may exist during the senescence of the whole plant.

From the literature we know that fruit could effect senescence in plants either by mobilizing various organic materials from other parts of the plant or by producing a special senescence stimulus or by both. However, in the studies of other workers it is difficult to distinguish which of these is the predominant factor or if both are of equal importance. For example, Leopold et al (1959) found that removal of developing fruit delays senescence in soyabean plants. However, their observation that even the removal of complete fruits can also delay plant senescence (though for a short period) led them to suggest that with the ripening of fruits a special stimulus is produced that accelerates plant senescence. Lockhart & Gotschall (1961) repeated this work on peas and were of the same view that ripening of fruits is associated with the production of a special senescence accelerating stimulus. Thus, considering that in those studies the effect of senescence stimulus were observed on those plants which had already been, allegedly, exhausted of their nutrients because of the previous fruit development, then the question arises - is the senescence stimulus also effective

in accelerating senescence in healthy leaves which have not been exhausted? Because there can be a possibility that stimulus produced at the time of fruit ripening may be of a weak nature that could only accelerate senescence in weak and exhausted leaves but may not have any effect on young and healthy leaves. So if the senescence stimulus has no effect on the leaves that have not been exhausted, then obviously it would mean that this stimulus of ripening fruits is only of secondary importance. Probably for this reason the role of senescence stimulus has not been investigated in detail by other workers. In this study, therefore, our objective was to differentiate more clearly between the effects of nutrient exhaustion and senescence stimulus and also, if possible, to ascertain which one of these effects has a main or decisive influence on the senescence of the whole plant.

Assuming that senescence stimulus would have a sharp qualitative effect while the effect of exhaustion would be slow and quantitative, it was decided to quantify the effect of fruits (by allowing different numbers of fruit to develop in different plants of the same chronological age) so that a clearer recognition of the effect of senescence stimulus could be observed. In other words the idea was that if in one treatment of an experiment, we permit a single pod to develop on each plant and in other treatment if we allow all the pods to develop, then the effect of exhaustion on the plants of first treatment will be markedly less compared to the plants of second treatment, but if ripening of fruit produces a really intense effect of senescence, then even from one fruit we might be able to observe a marked senescence accelerating effect as compared with completely deflorated plants.

By defloration we prolong the growth of the main axes and induce branching. With the objective of observing the effect of fruit development and ripening on the deflorated main axes, it was decided to

deflorate the plants in order to induce brancing so that the effects of fruit (the effect of development and ripening) present on a branch could be observed on a continuously deflorated main stem. At the same time we were also interested to see if the effects of fruit development and ripening are localised or general. This particular aspect was also investigated by allowing different numbers of fruits (one or all) to develop on main axes (at node No. 23 et seq) after the induction of branching (by the removal of first formed flower) and then deflorating the other plant parts.

Greenfeast variety of garden peas was used in this experiment, and the plants were grown under the standard controlled conditions. At the time of flowering, flower buds were removed with a pair of fine forceps from all plants of every treatment including controls. After 19 days continuous defloration, when the axillary branches were well established and there were 23 nodes on the main axes, different numbers of flowers on a branch or main stem (one or all) on plants of different treatments were labelled to permit the study of their development and maturity while the remainder of plant was continuously deflorated. Six replicate plants were used in each treatment and there were two control treatments i.e. (1) completely deflorated plants, and (2) plants which were first deflorated but then fruits were allowed to develop at all sites of the plants at the same chronological step as in other treatments. The quantitative effects of the development of different number of fruits on a plant were measured by counting the number of nodes formed on the main stem. The measurement of the effects of senescence stimulus was, however, quite difficult, because we knew from our previous experiment that by the time fruit matures on untreated plants, the apical growth on deflorated plants of same chronological age also declines. Moreover, assuming that the stimulus

Table 13. Total number of nodes on the main stem of Greenfeast peas, as affected by different number of fruits at different sites of the plants

	Fruits only at the main stem		Fruits only at an axillary branch		Completely deflorated plants	Controls (with fruits at all sites)
	One fruit	All fruits (Average 3)	One fruit	All fruits (Average 2)		(Average 5)
Total No. of Nodes	33.0 ± 1.05	28.0 ± 0.51	34.0 ± 1.25	34.0 ± 0.56	37.0 ± 0.44	28.0 ± 0.48

Table 14. The effect of fruit development on total number of nodes formed on fruit bearing and deflorated stems of fasciated plants

	Plants with two fruits				Plants with one fruit			
	Deflowered stem		Stem bearing fruits		Deflowered stem		Stem bearing fruits	
Number of nodes	a	30	a	21	a	33	a	31
	b	31	b	21	b	32	b	29

a, b represent replicates

would effect some sudden changes it was decided that the recording of the effects of fruit ripening (senescence stimulus) should be done by visual observation. The experiment was terminated when fruits in different treatments were completely yellow and dried.

During the selection of seedlings for this experiment a number of plants exhibiting basal fasciation, with two equal and identical stems above 6th node were observed. This serendipity offered an opportunity for complimentary study on nutrient exhaustion and senescence stimulus. The interesting point was that in these plants above the point of fasciation two stems arose with nearly the same vigour for apical growth, and therefore, by allowing fruit development and maturity on one stem while continuously deflorating the other it was possible to see if their effects are localised or general. There were four fasciated plants and it was decided to study the effect of development and maturity of one or two fruits (using 2 replicate plants in each case) present on any one of the two fasicated stems while continuously deflorating the other. These plants were kept in the same growth cabinet where the main experiment was being carried out and were similarly looked after.

The table (13) gives the data of total number of nodes formed on the main axes of peas (var. Greenfeast) in the presence of different number of fruits, either on main stem or on a branch compared with controls. It can be seen from these data that the development of a single fruit on a main stem or on a branch is significantly effective in suppressing node formation on main stem as compared with completely deflorated controls. The development of different number of fruits (one or all) show a pronounced quantitative effect on apical growth of main axes if the fruit were present on the main stem, but the presence of fruit on a branch does not show such a quantitative effect on node formation of main stem. Similarly table 14 indicate that in

A

B

Plate 2. The condition of Greenfeast peas before (A) and after (B) the maturity of different number of fruits at different sites of the plants. There was a fifteen days' time period between the two photographs.

fasciated plants the development of different number of fruits (one or two) have a prominent quantitative effect on the apical growth of fruit bearing stem but their corresponding deflorated stems do not exhibit such a quantitative effect. These results therefore suggest that under our conditions, though the development of fruits can have a general effect on the supression of apical growth, their effect on localised regions is exhibited more strongly.

Ripening of fruit was quite effective in accelerating the yellowing of green tissue. Plate 2A shows the general appearance of representative plants from different treatments at the time of complete fruit development prior to maturity. Fifteen days after taking first photographs (presented in plate 2A), when the fruits became matured and yellow, then randomly selected plants from different treatments were again photographed; these photographs are presented in plate 2B It is evident from plate 2A that at the time of complete development of different number of fruits on main stem or on a branch, most of the leaves on main stem above first flowering node remained green. And it can be seen in plate 2B that after the ripening of fruits, within 15 days after the complete development of fruits, all the leaves on the main stem show intense effects of senescence marked by yellowing and drying. It is also clear in plate 2B that maturity of a single fruit on the main is equally effective in accelerating leaf senescence (including young leaves) as in controls that had on an average 5 fruits. The presence of fruits on a branch, however, seem to be less effective in accelerating the senescence of leaves on the main stem compared with the case when fruit were present on main stem (plate 2B). In fasciated plants the ripening of fruit was similarly effective in enhancing leaf senescence of its own stem but had no observable effects on the deflorated stem.

Plate 3. Deflorated and fruit bearing stems of fasciated plant. Typical yellowing of apices due to fruit maturity can be seen on fruit bearing stem. Photographs taken after the yellowing of fruits.

Another striking effect of fruit ripening was exhibited in the yellowing and withering of apices of main stem and branches. Interesting to note was the observation that yellowing of apices took place even if leaves surrounding them were still green. The yellowing of apices on main stem, however, did not take place when the fruits were present on a branch, though the laterals other than fruit bearing branch showed similar effects of apex yellowing as the branch bearing fruits. In fasciated plants the apices of deflorated stems remained unaffected by the ripening of fruits on the corresponding other stems of the plants . Plate 3 show a typical example of apex yellowing in a fasciated plant where the apex was naturally open.

The dramatic yellowing of leaves and apices with maturity of a single fruit on the plant, provides a positive evidence that under our conditions, the ripening of fruits in peas is associated with the production of a special senescence stimulus.

Further investigations on the senescence stimulus of ripening fruits

In the preceding study a convincing evidence of senescence stimulus produced by the ripening fruits was observed. The yellowing of apices after fruit maturity appeared to be the most interesting aspect of senescence stimulus, because we were of the view that the cessation of apical growth is of prime importance in the control mechanism of whole plant senescence. However, as this effect of fruit ripening was observed on fairly old apices, so it was not clear that if the previously observed yellowing of apices was due to a strongly effective senescence stimulus or it was just a side effect of a weak stimulus on the independently senescing tips. Therefore, it was decided to conduct further investigations in order to have more confirmative evidence on the effect

of senescence stimulus on plant apices. In the first place we were interested to study the effect of fruit ripening independent of nutrient exhaustion effect of fruit development, on young and vigorously growing apices. Thus, considering that senescence stimulus is produced at the time of fruit ripening, whereas nutrient exhaustion effect, if any, appears during the development of fruits (Leopold et al 1959), it was decided to graft scions bearing complete fruits prior to their maturity, on to the young root stocks at vegetative stage.

Greenfeast variety of garden peas was used in this experiment. The scions bearing at least 3 complete fruits (incomplete fruits were removed) were side grafted on young root stocks having 9 visible nodes. In the other treatment fruits were removed from the scion just before grafting. Six replicate grafts were carried out for each treatment. The grafts were kept in moisture saturated atmosphere (by covering with clear polythene bags) at the standard temperature and photoperiodic conditions. However the grafts union did not take place excepting in a single case where fruits were removed prior to grafting. The experiment was repeated for two more times but besides any exceptional plants (3 out of 16, in plants where fruits were removed) there was hardly any success in graft union.

In order to find an alternate way of testing the effect of fruit ripening on young apices, it was decided to graft scion from young and vegetative plants (seedlings) on the root stocks bearing complete fruit that had not started yellowing. The idea was that a pea seedling var. Alaska) that has 4-5 nodes will produce another 10-12 nodes, but if this shoot is grafted on a fruit bearing root stock just before the ripening of fruits, and if ripening of fruits produces a senescence stimulus that kills apices, then these grafted shoots (of seedlings) will not grow; but if the fruits were removed prior to ripening and

so the production of senescence stimulus is prevented then the grafted shoots will grow normally and will produce further 10-12 nodes. However, if plant senescence occurs due to its exhaustion by developing fruit, then even if the fruit were removed from root stock prior to maturity, the growth of the grafted scion will either be very poor or it will not grow at all. Simply the result anticipated from this experiment were:

(1) A vigorous growth of young grafted scions on root stocks where fruits were removed prior to maturity, but no growth of the grafts if the fruits were allowed to mature on root stocks. Thus providing a convincing evidence that fruit maturity produces a senescence stimulus that kills apices, but fruit development does not exhaust the plant to allow further growth.

Or -

(2) No or similarly poor growth of the grafts on both defruited and fruit bearing (ripening fruits) root stocks. Thus providing a conclusive evidence that fruit development exhausts the plant and prevent it to support further growth.

Or -

(3) A vigorous growth of the grafted scions on defruited and fruit bearing root stock. Thus providing a conclusive evidence that fruit development and maturity has neither any effect on young apices nor it has any effect on the capacity of plant to permit further growth, and so indicating that independent nature of apical senescence in peas is the main cause of plant death after fruiting.

Alaska variety of garden peas was used in this experiment. Young scions from seedling peas (having 4-5 nodes) were side grafted on fruiting root stocks (with fruits nearly filled) below the 1st fruiting node. The grafts were covered with clear polythene bags and were kept under the standard controlled conditions. Graft union (marked by

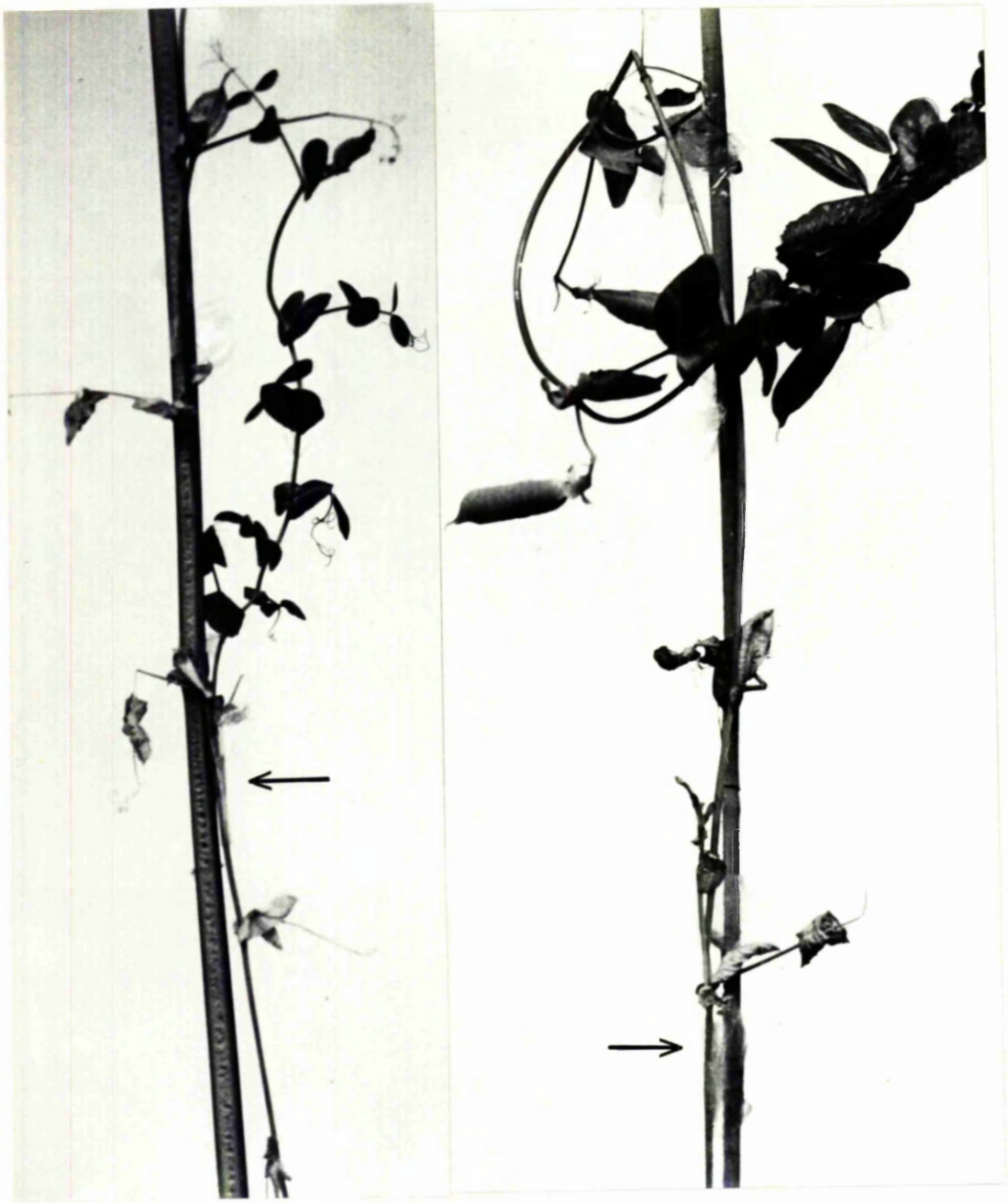


Plate 4. The condition of young scions grafted onto fruiting root stocks below first flowering node. Scion started growing on defruited stocks (left), but did not grow on untreated stock (right). Arrows indicate graft union. Photographs 20 days after grafting.

Plate 5. The fruiting of scion on defruited root stock. The young scion was grafted below the first flower node of fruiting stock. Arrow indicates graft union.

callus formation at the site of joint) was 100% successful in this experiment. Polythene bags were removed from the plants soon after the graft union has taken place (within a week). These grafted plants were then divided into two groups each having 10 replicate plants. In one group all the root stocks were completely defruited while in the other group fruits on the root stock were allowed to mature.

The results obtained in this experiment were quite striking as all scions on defruited root stocks started growing while the scion on root stocks with intact ripening fruit showed no growth in any plant. The condition of grafted scions on defruited and fruit bearing root stocks 20 days after grafting is shown in plate 4 . The growth of young scions on defruited root stocks was quite vigorous and they formed flowers and fruited just as normal plants (with 10 total nodes on an average) (see plate 5), while the apices of young scions on fruit bearing root stock started yellowing and therefore never showed any growth.

As in the presence of ripening fruits, none of the grafted scion showed any growth but instead its apices started yellowing, whereas all the scions on defruited root stocks showed normal growth and development, therefore, it can be concluded, that under our experimental conditions the ripening of fruits in peas, produces a fairly strong senescence stimulus that can kill even the young and vigorous apical meristem; and that the development of fruits does not exhaust the plant to allow further growth. Since in this experiment the grafting was carried out after the root stock had produced nearly fully developed fruits we can conclude the effect we observed is due to maturity, but it may be that development of fruits also have effect on young apices, which could only be tested by grafting young scion to root stock with their pods at early stage of development.

Plate 6. The condition of young scions grafted on to fruiting root stock above the flowering nodes. Scion started growing on defruited stock (left), but did not grow on untreated stock (right). Arrows indicate graft union. Photographs 20 days after grafting.

A study on the movement of senescence stimulus within the plants

In the first experiment of this series it was observed that ripening of fruits on a branch is less effective in accelerating leaf and apical senescence of main stem than if the fruit were present on main axes. In fasciated plants also, the deflorated stem remained unaffected by the ripening of fruits on corresponding fruit bearing stem. These observations prompted a study of the movement of senescence stimulus within the plant. In this study, therefore, our objective was to investigate if the senescence stimulus is capable of moving in a non-polar fashion or its movement is confined to a specific direction.

The movement of senescence stimulus in the downward direction was clearly seen in the previous experiment where young scions, grafted below the first flowering node of the root stock, were killed by the ripening of fruits above the graft joint. To find if the stimulus is also capable of moving in the upward direction, it was decided to graft young scions above the fruiting nodes of the root stock.

Alaska variety of garden peas was used in this experiment and the young scions were side grafted near the apex of the fruiting plants so that all the fruits on the root stocks were below the graft joints. Twenty such grafts were carried out using the same method of grafting as in the previous experiment. With the exception of one, all the grafts showed successful union. The successfully grafted plants were divided into two lots, with 9 replicates in one group and 10 in the other. In the group having 9 replicates, all the fruits from the root stocks were removed. The experiment was carried out under the standard controlled conditions. The results obtained in this experiment were very similar to the ones obtained in the previous experiment, i.e. all the scions on defruited root stocks started growing while on the root stock bearing fruits they died within a fortnight (plate 6). As

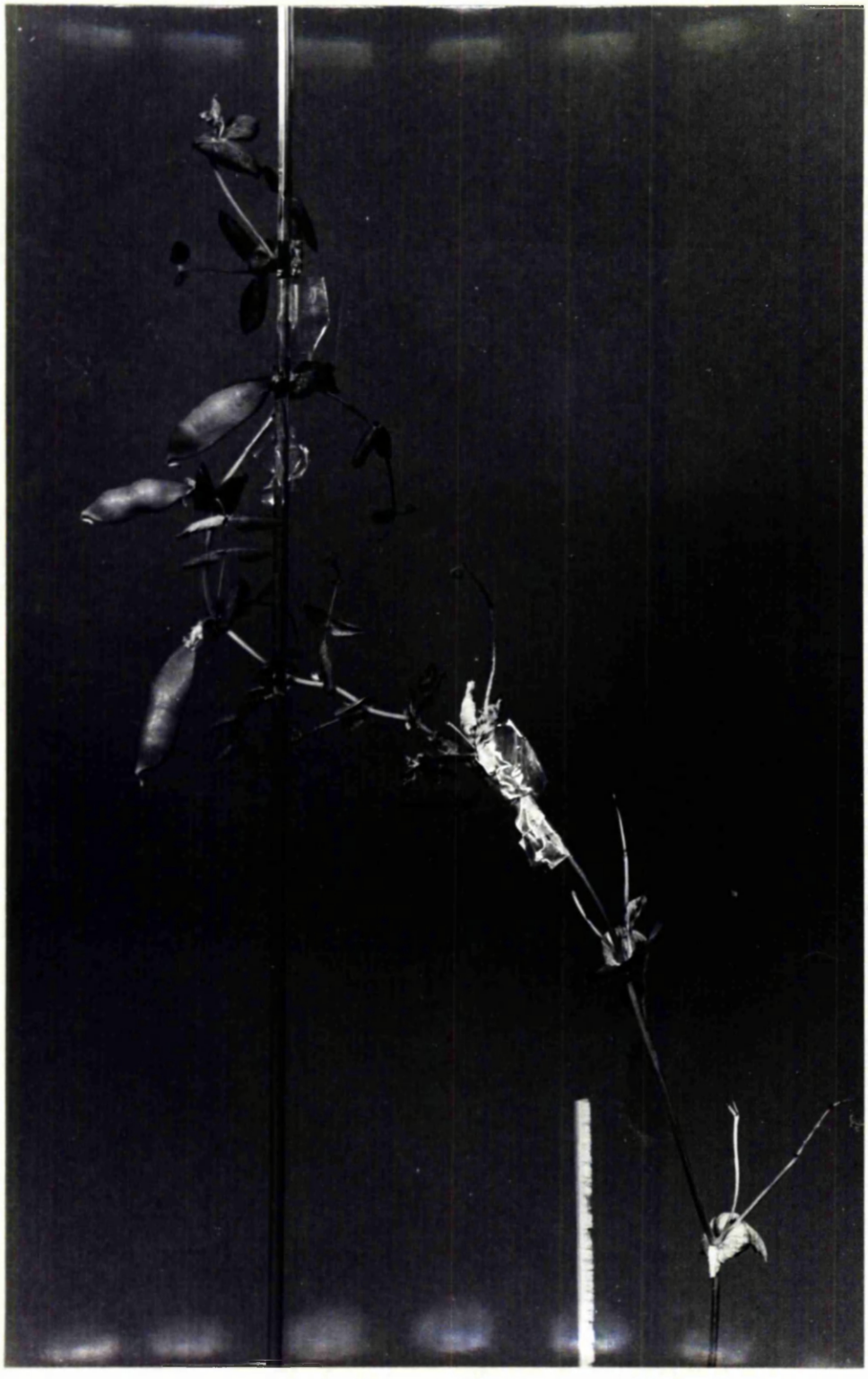


Plate 7. The fruiting of scion on defruited root stock. The young scion was grafted above the fruiting nodes of stocks. Arrow indicates the graft union.

shown in plate 7 the scions on defruited root stocks flowered and fruited as normal plants.

It can therefore be concluded that under our conditions, the senescence stimulus produced by the ripening pea fruits, is capable of moving in both upward and downward directions from its site of production, towards the growing apical meristem. However it is possible that vigorously growing apical meristems themselves mobilize this stimulus from the fruits, and so the difference in the results of earlier experiments, may be due to old apices of those plants with poor mobilization effect. However, irrespective of whether the stimulus moves passively towards the apical meristems or the apical meristems constitute an active sink for the senescence stimulus from the ripening fruits, it is evident from these results that it has a decisive role in causing the death of apical meristem.

An extension of the above experiment

It was concluded in the last experiment that fruit development prior to maturity does not exhaust the plant to support further growth, and that the maturity of fruit produces a stimulus which is strongly effective in killing apical meristem. To confirm it further, it was decided that the above experiment should be further extended by grafting young scions on the defruited root stocks of that experiment when fruits on their first scions were nearly filled. The objective was to test the effect of two times fruit development on the capacity of root stock to permit further growth, and also to see the effect of fruit ripening from the first grafted shoot upon a freshly grafted young scion on the same root stock. Thus it was decided that the growth of 2nd graft should be observed both in the presence of ripening fruits on first scion, and also by defruiting completely developed fruits

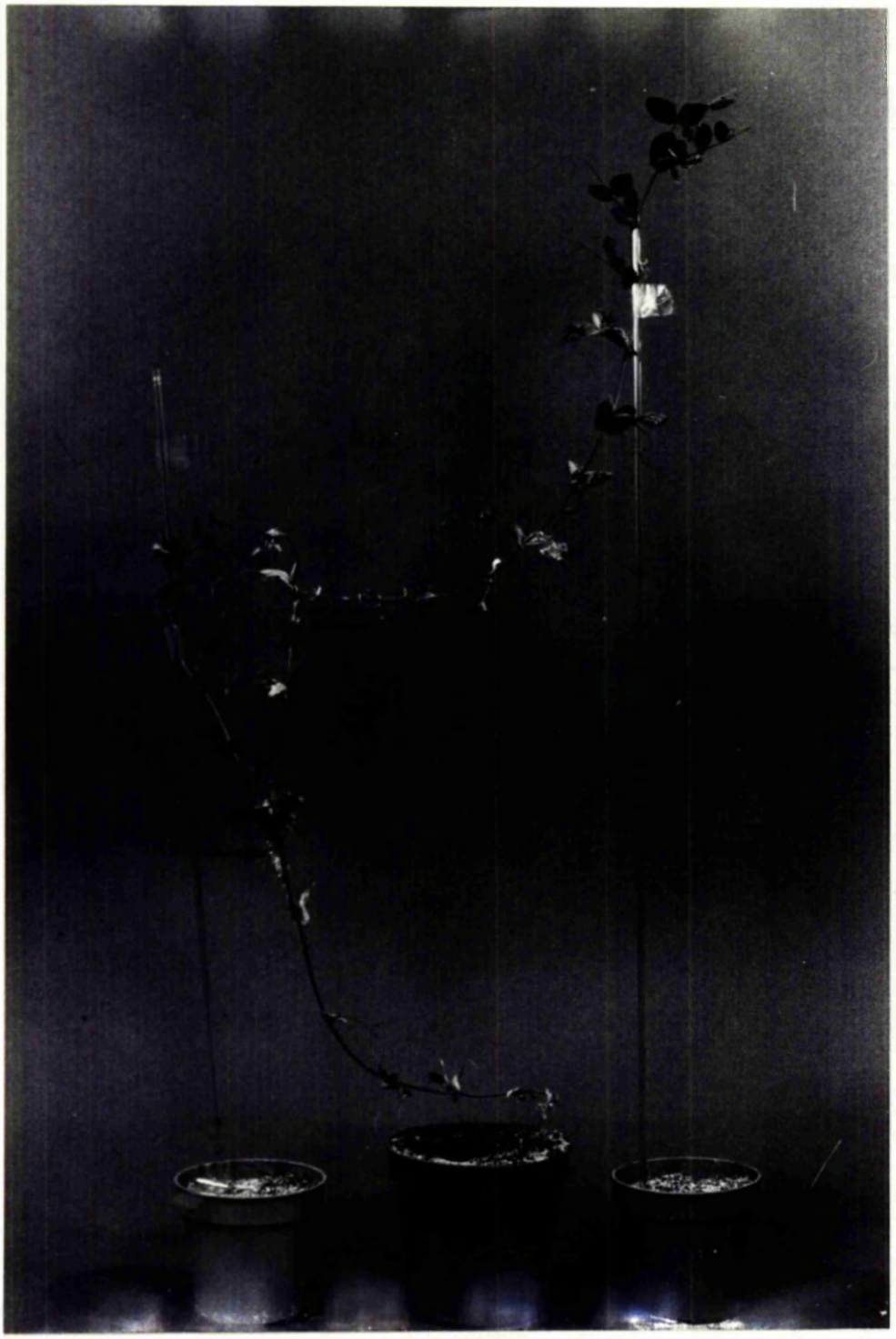


Plate 8. The growing second young scion grafted onto defruited root stock when the fruits from the first grafted scion of the stock were removed after complete development but prior to maturity. Single arrow (→) indicates the graft union of second graft and double arrow (⇉) indicates the union of the first graft. Photographs 18 days after second grafting.

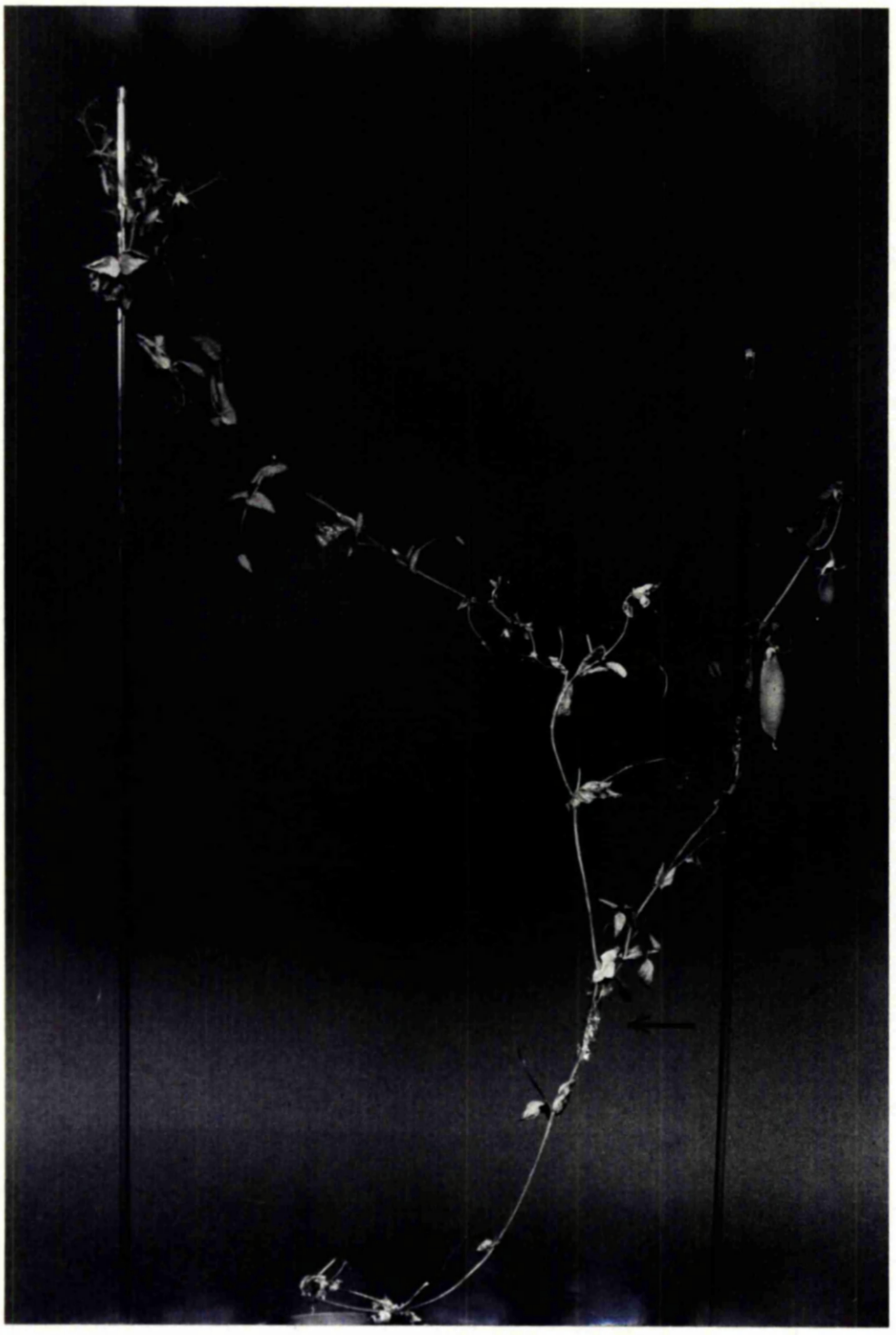


Plate 9. The fruiting of second young scion grafted on old defruited root stock when the fruits from it first grafted scion were removed prior to maturity. Single arrow (→) indicates the graft union of second graft and double arrow (⇶) indicates the graft union of the first graft.

prior to maturity.

The 2nd grafting of young scion (with 4-5 nodes) was carried out on the defruited root stocks of last experiment, below the first graft joint, when fruits on the first scion were nearly filled. Six grafts out of nine were successful. The root stock with successful union to 2nd scions were divided in two groups each having 3 replicates. In one group the fruits of the first scion were allowed to mature while in the other these were removed prior to maturity. The root stocks of both groups were transferred into bigger pots (6 x 6 inches) to prevent restricted root growth.

Similar to the previous results, it was observed that the new scions on defruited plants start growing while they die on the plants bearing ripening fruits (see Plate 8).

Due to faults appearing in the temperature control of growth cabinets, the plants were transferred to another cabinet (maintained at the same temperature and photoperiodic condition) where they were attacked by aphids. The spray of commercially available greenfly killers did not prove effective, therefore physical removal of aphids with a brush was regularly practised. However the new scions continued their growth on defruited root stocks, and then flowered and fruited, but their fruiting vigour marked by number and size of the pods was comparatively low (see plate 9).

The results of this experiment confirm that under our conditions, fruit development in peas does not prevent the root stock for supporting further growth of a non senescent apex, but ripening of fruits produces a distinct senescence stimulus that kills apical meristem and thus leads the plants to final death.

Plate 10. The condition of young scion of Greenfeast peas grafted on to root stock (var. Greenfeast) that had its fruit completely matured. Arrows indicate graft union. Photographs 20 days after grafting.

The effect of senescence stimulus on plant parts other than leaves and apices

After demonstrating the effect of senescence stimulus on apices and leaves, this study was conducted to see if it also affects plant roots thus causing as such the senescence of whole plant. Thus, if senescence stimulus does affect root or/and translocation from roots, then a graft would be unlikely to succeed in case of root which has its fruits ripened. To test this hypothes's it was decided to graft young scions on to the root stock that had their fruits completely matured and yellow.

Alaska and Greenfeast varieties of garden peas were used in this experiment and the plants were grown under the standard controlled conditions. The fruits from the root stock plants were removed after they had become completely yellow, and then the young scions (from vegetative plants) were grafted at lower nodes of the stem where it was still succulent. Six replicate plants were used of each variety. In Alaska peas only one graft was successful and in Greenfeast peas four plants had the successful union with the grafts.

As shown in plate 10 the young grafted scions did not show any growth for three weeks after which they senesced and died.

As after the ripening of intact fruits on the root s tock the young scions do not grow, whereas they grow vigorously if the fruits had been removed from root stock just prior to maturity, it therefore suggests that under our condition the senescence stimulus produced by ripening fruits not only affects leaves and apices but also the roots, and therefore the plant can no longer sup-port further growth.

The effect of senescence stimulus on axillary bud growth

While in peas, the apex of main stem appear to senesce even without

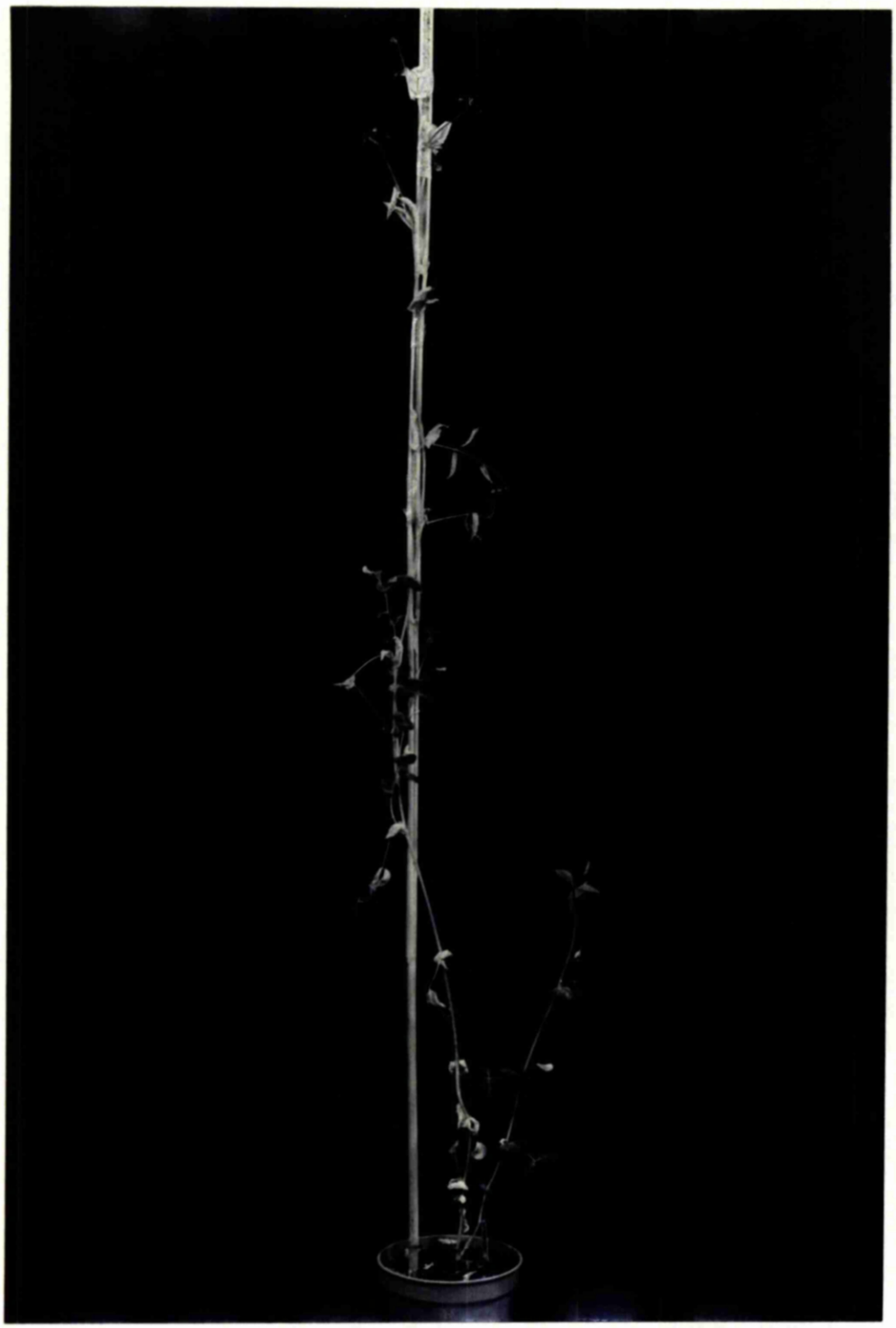


Plate 11. Delayed senescence in Alaska peas by removing complete fruits prior to maturity is manifest in axillary bud growth.

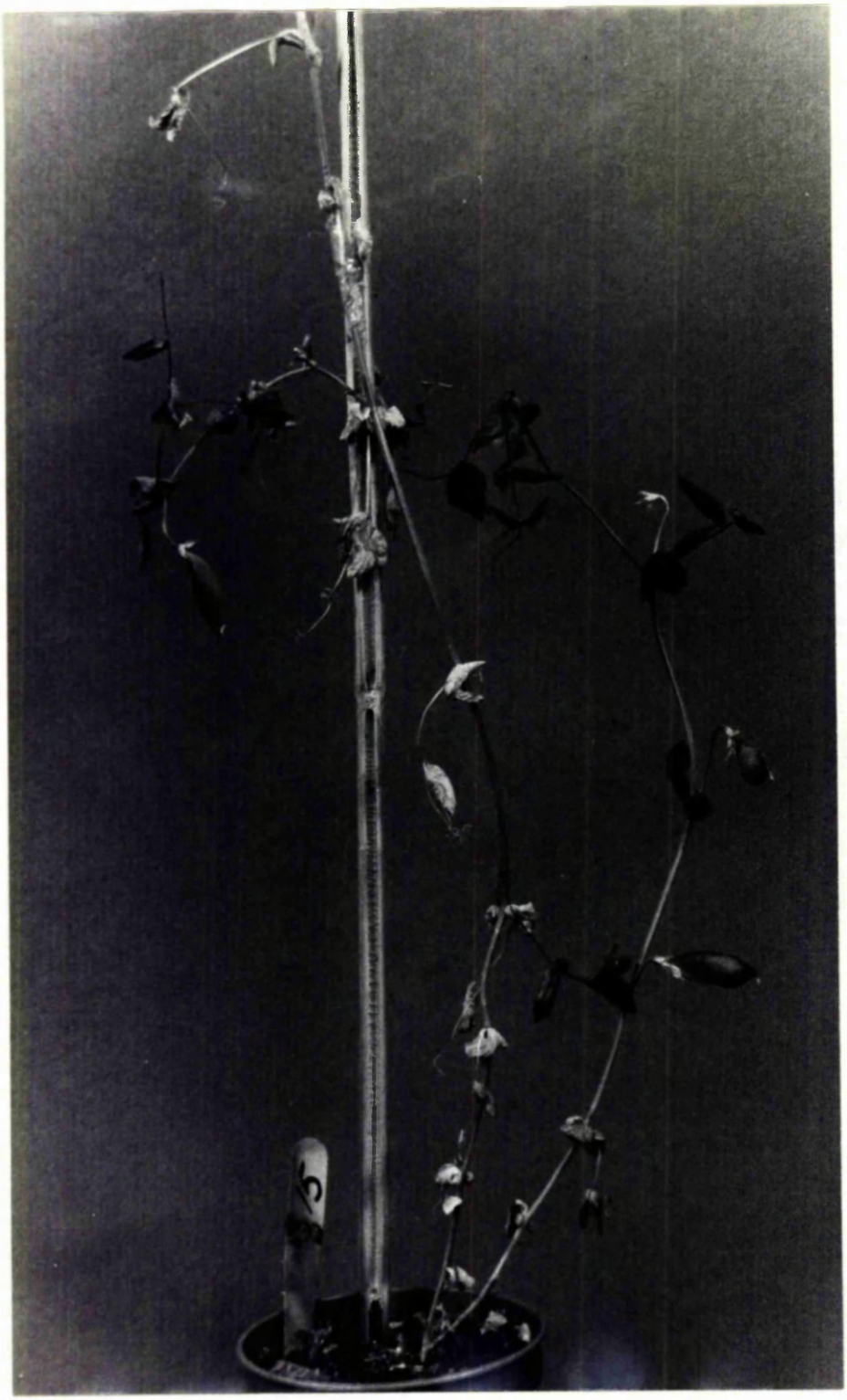


Plate 12. Fruit development on axillary branch of Alaska pea plant. The axillary branch was produced by the removal of complete fruits prior to maturity.

the influence of senescence stimulus, it may seem that there is no need for the production of a specific senescence stimulus, but in terms of correlations the axillary bud will either develop so making the plant potentially perennial or they could be suppressed. This would imply that main function of senescence stimulus is to control axillary bud development in the absence of any apical dominance which would result from the presence of unhealthy activity of the apex of main stem. To test this hypothesis it was decided to defruit plants after their complete development but prior to maturity. The experiment was repeated once.

Alaska variety of garden peas was used in this experiment, and plants (6 replicates) were grown under the standard controlled conditions. The fruits were removed after their complete development but prior to hardening and yellowing.

As shown in plate 11 the removal of complete fruits resulted in the growth of axillary buds. Though the growth of axillary buds was not as vigorous as the grafted scion from a seedling plant, and also that there was hardly any appreciable development of fruits (plate 12), but the life of plant was prolonged for at least four weeks.

These results, therefore, support our hypothesis that senescence stimulus performs an important function in the control mechanism of whole plant senescence by killing the potentially active meristem of axillary buds.

GENERAL DISCUSSION

After flowering and fruiting an annual plant dies - but how? In the introduction we suggested several possible answers to this question and then discussed many of them in the light of previous work. However it was realised that there is only insufficient work to support any particular hypothesis that could explain the control mechanism of whole plant senescence. Therefore it was decided to elaborate some of the previous work to attempt to obtain a clear understanding. It is, however, conceivable that detailed studies on each of the various hypotheses are beyond the scope of this thesis. Therefore we had to be selective in choosing any one of the hypotheses for detailed investigation. Our choice for the theory of senescence stimulus was mainly because it has been least investigated.

As discussed in the introduction there is a possibility that senescence stimulus might be one, or a group of compounds produced by flowers and/or fruits that causes or accelerates leaf senescence, or it may be a sudden change in endogenous leaf metabolites in response to photoperiod, or through some other mechanism. However it looked fairly evident that the leaf is the main site either for the production or for the response of senescence stimulus. But considering that in annuals flowers and fruits have the most striking effect on plant senescence and that their removal can defer senescence, we therefore decided to concentrate our attention towards the senescence stimulus produced by flowers and fruits.

In the beginning of our studies we thought that if flower and fruit produce any stimulus that affects leaf senescence by initiating some changes in the leaves, then in comparative studies on flowering and deflowered plants we might be able to observe some specific effects of senescence stimulus produced by flowers and fruits. However in various comparative studies on leaf proteins (described in Section $_{A}$)

we were unable to pick any quantitative or qualitative changes that could be related to developing flowers and/or fruits and so to any effect of senescence stimulus in proteins. In fact changes did appear in proteins during the aging of intact leaves, but it was not possible to relate these changes with the aging of the whole plant.

Similarly in plant hormone studies (Section B) there were no observable changes in the levels of ABA, GAs and cytokinin that could be related specifically to flower and fruit development. Our hormone studies however, showed that after the maturity of fruits we did observe some difference in hormone levels between deflorated and untreated plants, but it was difficult to associate the importance of our results in relation to plant aging, because at that stage the leaves on untreated plants were senescent while deflorated plants had a fair number of green leaves on the nodes that did not exist on untreated plants (due to the continuation of apical activity produces new nodes in the deflorated plants). Thus on deflorated plants the green leaves were on the newly formed nodes to which there were no corresponding nodes on the untreated plants, and therefore it was difficult to say if the changes observed were the cause or the result of leaf senescence. In those studies (both protein and hormones) however, it was noticed consistently that changes (both visible and endogenous) in leaves at corresponding nodes of deflowered and untreated plants appear parallel to each other at nearly the same chronological time. These observations were quite surprising, because even if there were no stimulus produced by the flower and fruit to cause any specific sudden changes, then at least any other alternative effect of fruit development should have accelerated the senescence of leaves as compared to the leaves of deflorated plants. Thus although it was quite evident from these studies that development of flowers and fruits does not appear to

produce any specific stimulus to cause observable changes in the leaves, at the same time it was difficult to conceive of an alternative mechanism of senescence control based solely on those results.

The surgical studies however were not begun initially to investigate the nature of senescence stimulus, but in fact to look at the importance of various plant organs in the control of whole plant senescence. In this survey it was observed that removal of old leaves neither increases nor decreases whole plant senescence; and also that development of flowers and fruits hardly affects leaf senescence, because neither was there any difference in the senescence of leaves at corresponding nodes of deflowered and untreated plants, nor was there any acceleration of senescence of young leaves in defoliated but fruiting plants. Thus, these observations coupled with the results obtained in protein and hormone studies (Section A and B respectively) provided fairly convincing evidence that senescence of leaves, which has always been given great importance in senescence studies, in fact is of less significance in the control of whole plant senescence. These results were later substantiated in other studies. For example in an experiment where fruits were removed when they were completely filled but prior to ripening, it was found that senescence in those plants was delayed by the growth of axillary buds, even when all the leaves on the main stem were completely dried.

Thus if leaf senescence is of a minor importance in controlling the aging of peas, and considering that in previous works the effects of flower and fruit (mobilization or senescence stimulus) have always been studied in relation to leaf senescence, then a question can be asked - do flowers and fruits have any role in the control of whole plant senescence? However the results of our experiment suggested that fruit development and maturity do have a significant influence

on the senescence of pea plants, because in the absence of fruits the growth of axillary and apical meristems extends the life of the plant. Thus after the completion of our general survey on the senescence of peas we were quite convinced that the senescence of the apical meristem is the true reflection of whole plant senescence and upon which fruits do have a significant influence. Therefore it initiated our interest to investigate further the effect of fruit development and maturity in relation to apical growth.

In the next experiment after the general survey (in Section C) an attempt was made to quantify the effects of fruit development and ripening on whole plant senescence with special interest on the apical growth. In this experiment for the first time quite striking effects of fruit ripening were observed on leaves and apices.

Then various grafting experiments were conducted to investigate the nature of senescence stimulus produced by the ripening of fruits. In these studies, first, it was shown that senescence stimulus is strong enough to kill even the vigorous apices of young seedlings, and also that it can move both acropetally and basipetally towards the growing apices. Later, it was revealed that the stimulus not only kills leaves and apices of all ages, but in fact it also affects other plant organs, so that after its production the death of the plant becomes inevitable. Finally it was also shown that in normal plants senescence can be delayed if the production of senescence stimulus is prevented. The delay in plant senescence, due to the prevention of fruit ripening, was manifest in the initiation of axillary growth, thus providing a support to our previous conclusions that cessation of apical growth has a more decisive role in the control of whole plant aging than the senescence of leaves.

In grafting experiments, while investigating the effects of

senescence stimulus, it was interesting to observe that young scions (shoots of seedlings) grafted on old root stock (whose fruits were removed after complete development) grow, flower and then fruit quite normally as the shoots of a normal plant; and also that if fruit were removed from the grafted scion (after complete development) and another young scion is grafted on the old root stock for the second time, then again it starts growing. The interesting point about these observations is that it looks quite clear that development of fruits (prior to maturity) does not exhaust the plant for further growth (in contrast to the conclusions of Molisch 1928), because as long as apical meristems remain capable of further growth the other plant organs (including the senescence of leaves) do not cause their senescence, even if fruit development (prior to maturity) had already been achieved twice on them. These results also provide convincing evidence against the suggestion of Sitton et al (1967) that shoot senescence may be due to decreased synthesis of cytokinin in the roots after flowering. Because, if shoot senescence occurs due to changes in root metabolism after flowering then in our experiment, grafts of young scions on old root stocks (that had been fruited before) should have grown either very poorly or not at all, whereas our results showed that root metabolism does not hinder shoot growth even after complete fruit development (prior to maturity) consecutively for two times. Thus if fruit development does not exhaust and so cause the senescence of leaves (as seen in first surgical experiment), roots (as mentioned above) and axillary buds (as seen in the last experiment), and also if fruits are capable of synthesising proteins and carbohydrate themselves (because explant fruit development has been shown on agar culture medium containing inorganic nutrients, Baldev et al 1965), then it appears that fruit development (prior to maturity) would have

only a minor role in the control of whole plant aging. However for definite conclusions it is necessary to verify the effect of fruit development on apical growth by grafting young scions on root stock that had just started flowering and then removing the fruits after their complete development but before ripening.

Lockhart & Gotschall (1961) reported that apical senescence in peas is an independent phenomenon and it occurs even if plants were kept vegetative throughout. In our experiments we also observed that apical growth loses its vigour, even if defloration is continuously practised (though no specific experiment was conducted for this purpose) and also that axillary buds do not grow as vigorously as the grafted young scions. Thus, although our results provide fairly conclusive evidence that senescence stimulus produced by ripening fruits has a profound effect of accelerating plant senescence, but considering that apical growth ceases even in the absence of fruits, it appears that cause of senescence in peas and their death at the close of the year is not only because of a senescence stimulus emanating from ripening fruits but also because they have only a limited capacity of apical growth. Thus in order to prolong the life of pea plants it would be necessary to control the effect of senescence stimulus as well as to enhance the vigour of apical growth.

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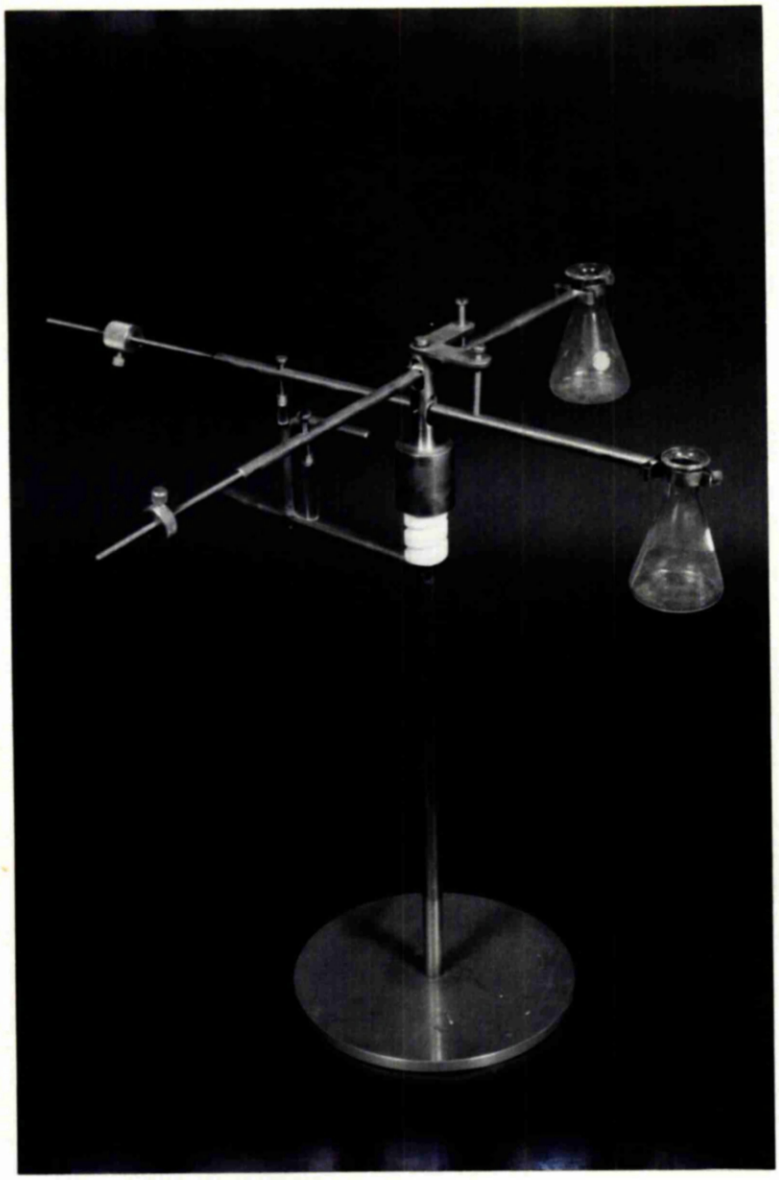
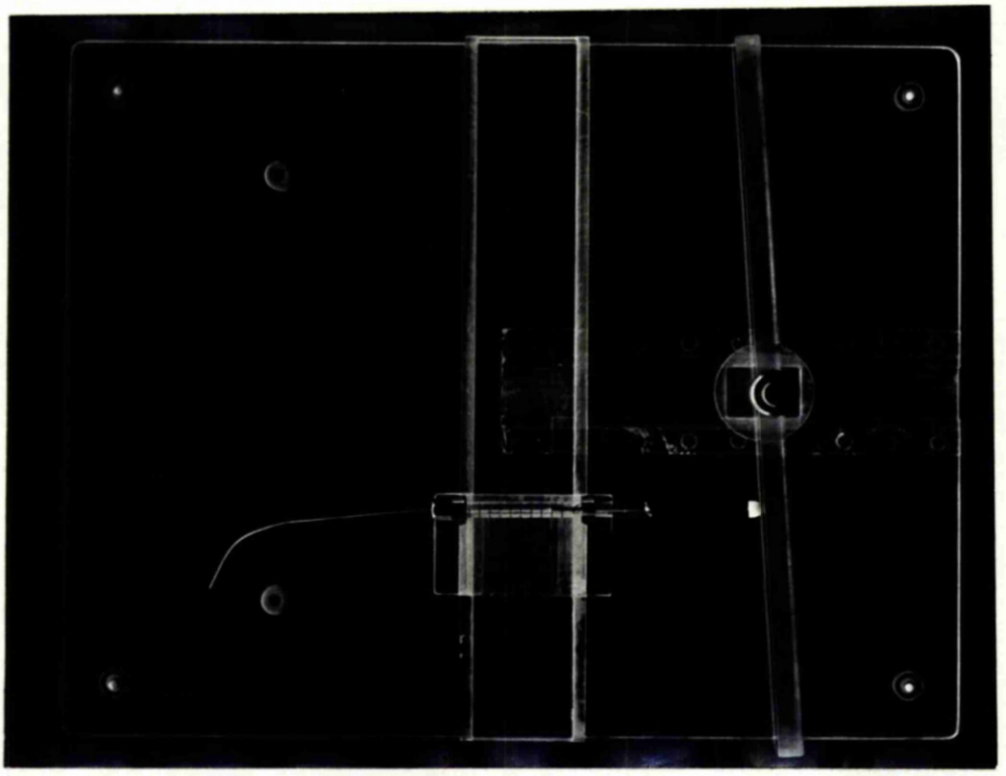
Abbreviations

ABA	Abscisic acid
GA	Gibberellin
GCMS	Gas chromatography coupled with mass spectrometry
GLC	Gas liquid chromatography
hr	Hour
IAA	Indole acetic acid
K	Kinetin
No. or no.	Number
pet. ether	Petroleum ether (redistilled at 40° - 50°C)
Rf	Retardation factor based on relative distance travelled $= \frac{\text{distance substance travels from origin}}{\text{distance solvent front travels from origin}}$
TCA	Tri-chlor-acetic acid
TLC	Thin layer chromatography
UV	Ultra violet
W/v	Weight/volume



APPENDIX I

Fraction collector for preparative work on column chromatography.



APPENDIX II

Sample applicator designed for preparative work on TLC.

APPENDIX III

Analysis of variance table for experiment on page 68.

Source	df	Sum of squares	Mean square	F
Replicates	1	88.17	88.17	1
GA	2	1604.33	802.17	1.096
Kinetin	2	1158.33	579.17	0.79
IAA	2	2734.33	1367.17	1.82
GA x Kinetin	4	2051.01	512.75	0.70
GA x IAA	4	4738.01	1184.50	1.62
IAA x Kinetin	4	5674.01	1418.50	1.94
GA x IAA & Kinetin	8	13340.31	1667.54	2.28
Residual	26	19024.33	731.70	
Total	53	50412.83	-	-

Correction factor = 731504.16

APPENDIX IV

APPENDIX V

The effect of different treatments of defoliation, defloration and decapitation on the growth and senescence of Greenfeast peas

Tray No.	Treatment
(1)	Defoliation keeping 4 leaves
(2)	" " 6 "
(3)	Defloration + defoliation keeping 4 leaves
(4)	" " " 6 "
(5)	Decapitation + defoliation " 4 "
(6)	" " " 6 "
(7)	Defloration + decapitation + defoliation keeping 4 leaves
(8)	" " " " 6 "
(9)	Decapitation without defoliation
(10)	Defloration " "
(11)	Decapitation + defloration without defoliation
(12)	Control = without defoliation, decapitation and defloration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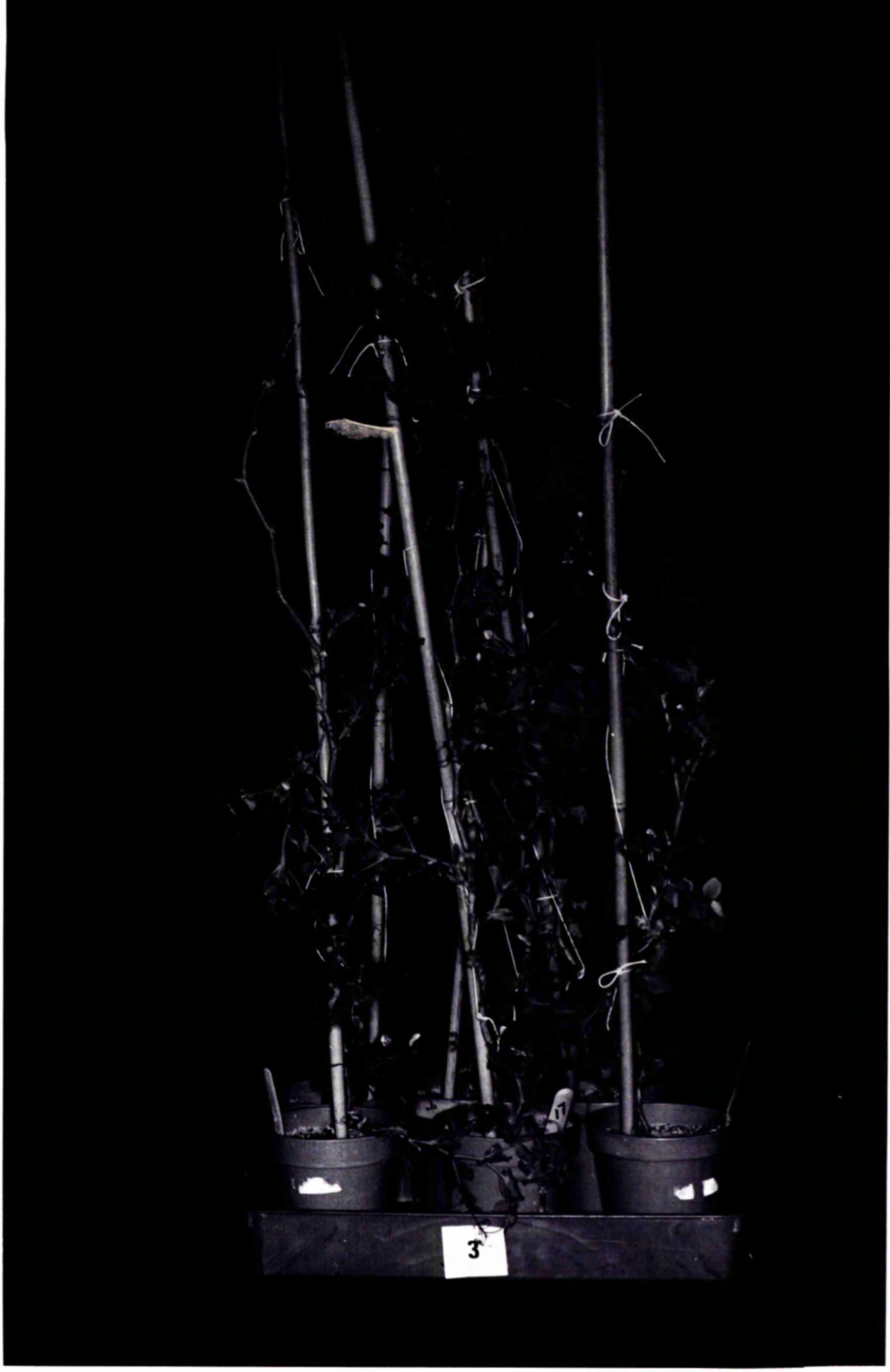
1


2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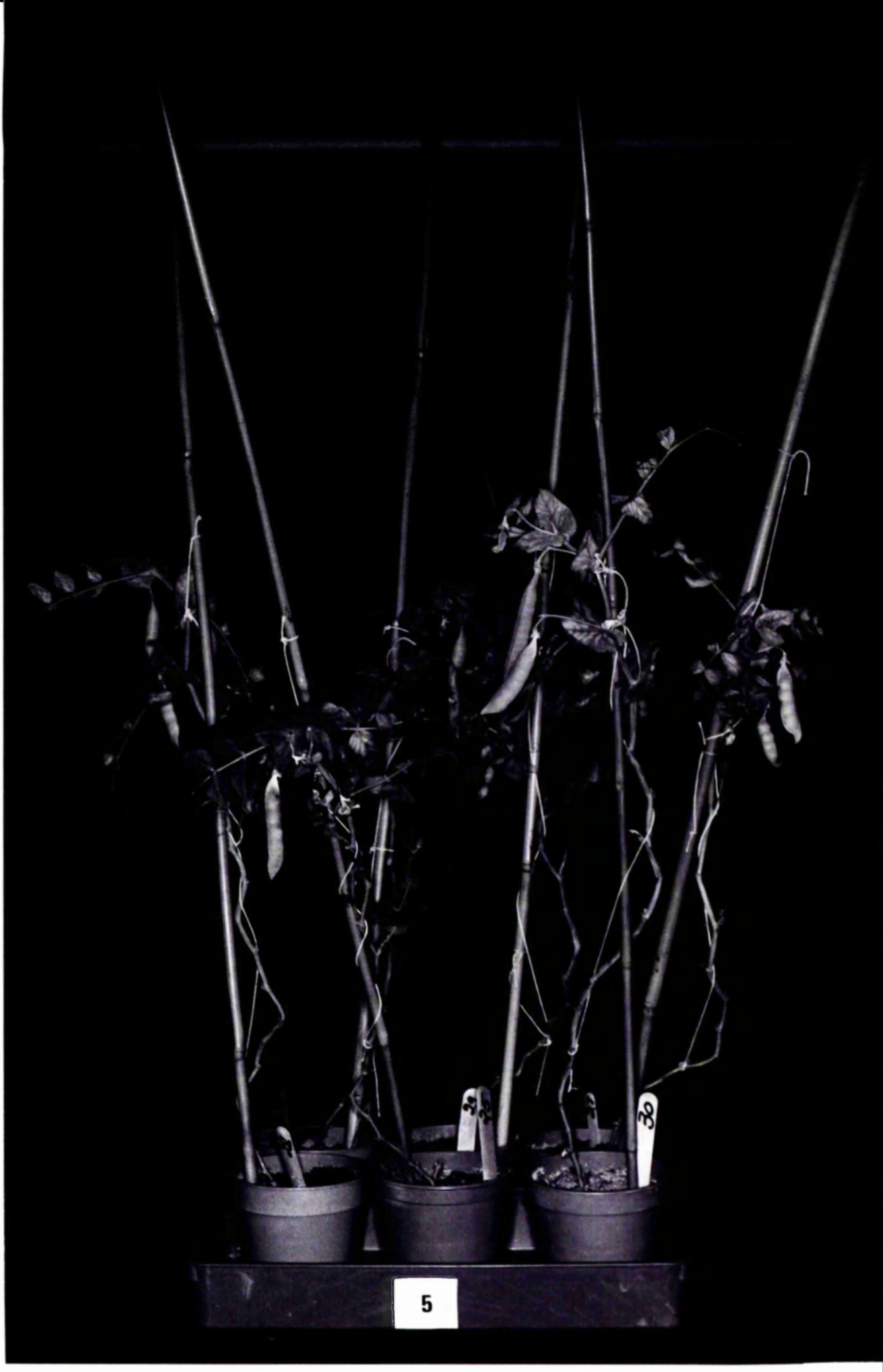
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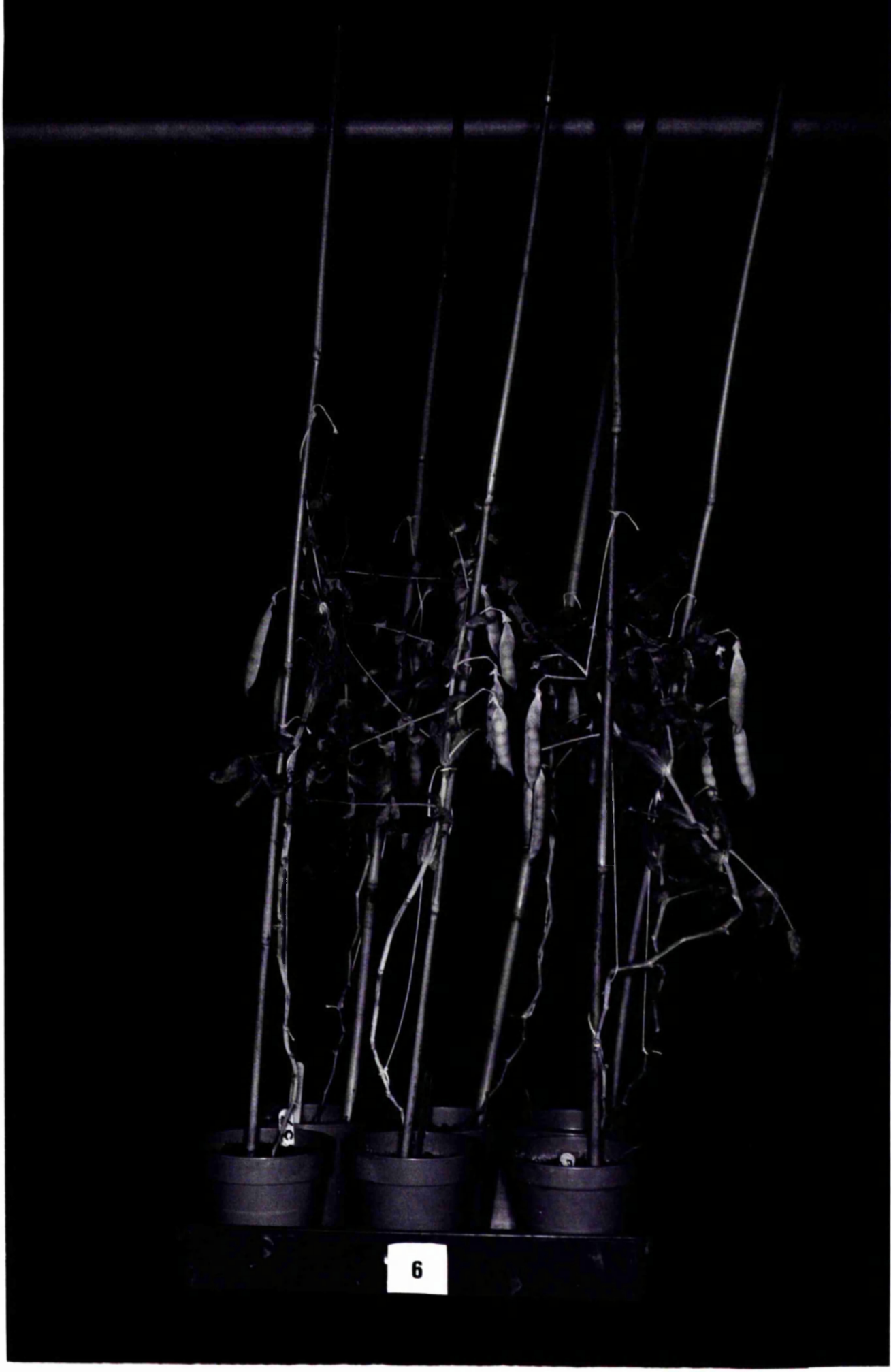
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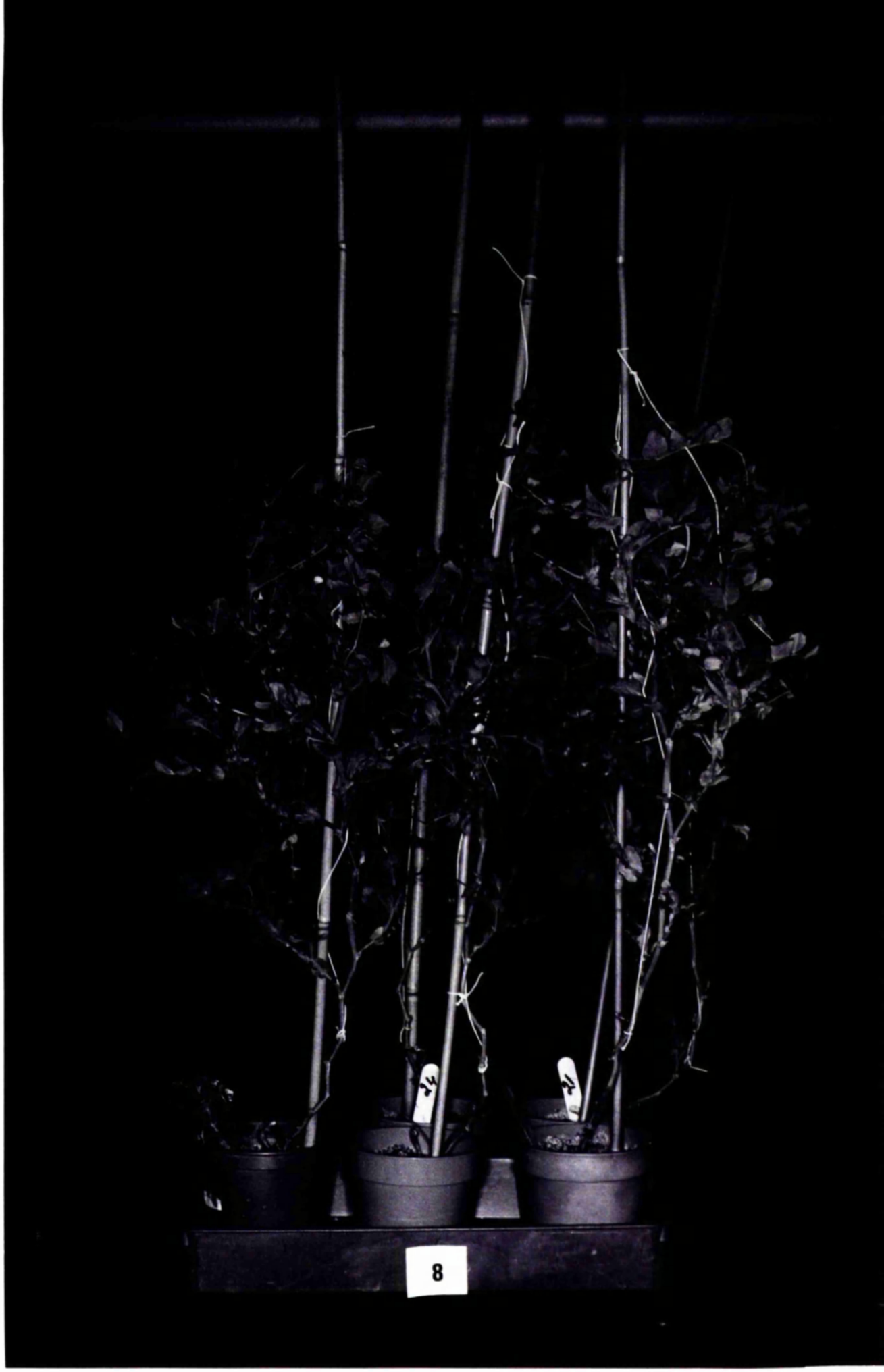
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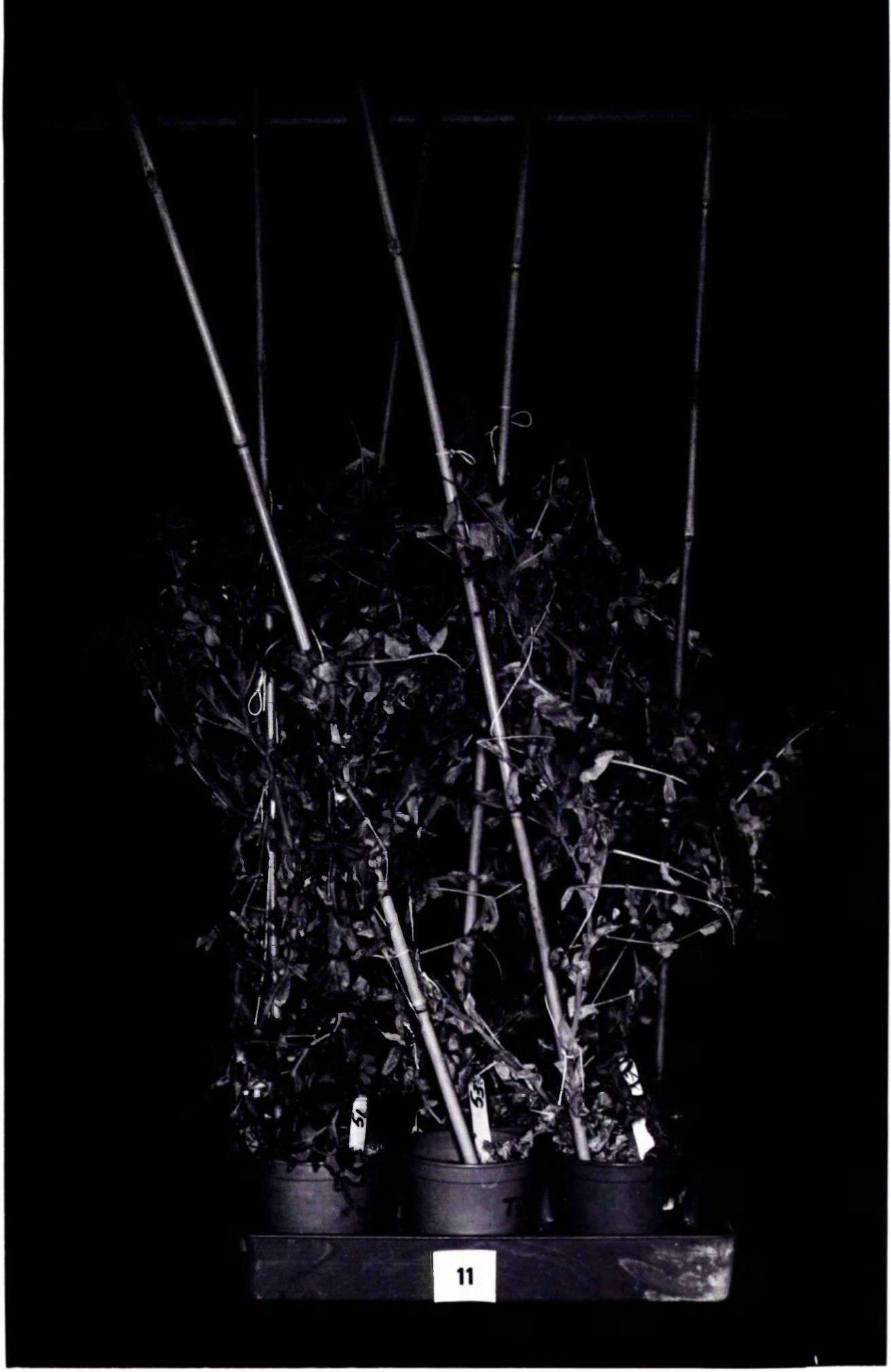
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