THE SENESCENCE OF THE CUT CARNATION (*Dianthus caryophyllus* L. CV. WHITE SIM) FLOWER

by

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Submitted in fulfilment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in the

Department of Botany, Faculty of Science,

University of Natal, Pietermaritzburg.

December, 1985.

PREFACE

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

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December, 1985

ACKNOWLEDGEMENTS

There have been many people who have aided me, directly or indirectly to produce this thesis and I am sincerely grateful to you all. I would like to especially thank the following people:

Professor J. van Staden for supervising this project. His dedication to this role is reflected in the fine research facilities he has organised together with the department.
The other two members of my supervisory committee, Professor R.N. Pienaar and Dr. N.A.C. Brown and the remaining staff members of the Botany Department.

Dr. Margaret Hutton (Furze) who has long been an inspiring influence.
Members of the Research Unit for Plant Growth and Development, past and present, for being a 'sounding-board' for many a theory and their forthcoming advice.

The technical staff, especially Mr.Tunnington, Mr. de Jong, Mr. Sarawan and Mr. Ndaba.
The staff of the Electron Microscope Units both in Pietermaritzburg, namely Mr. Bruton, Mr. Bandu and the late Mr. Kundalram, and in Durban, Mr. Evers.

The Oehley family who provided the opportunity to experience growing carnations and becoming aware of market demands. My special thanks to Sue for her impressive support in this venture and to Jean for the care showed in typing this thesis.

Floricadia (Heidelberg, South Africa) particularly Mr. Cunningham, Hazel and Rocky Naidoo. Their supply of flowers, even through the drought, was constantly appreciated and vital for the continuation of this research.

The C.S.I.R., their financial support for the last two years has been greatly appreciated.

Ian, my husband, for understanding my commitment this last year and help in proof reading.

And finally, my humblest gratitude to my parents for their unselfishness in the opportunities they have given me to attain such a goal.

CONTENTS

ABSTRACT

A review of the literature pertaining to cut carnation flower senescence and the regulatory role of plant hormones in this process revealed the value of this system in physiological studies. Carnation flower senescence is a good example of correlative senescence and therefore this final development stage involves an interaction between flower parts dying at the expense of the development of others. Due to the survival value of the seed, ovary growth occurs to the detriment of the surrounding flower parts especially the petals, the flower part that determines vaselife. This senescence strategy occurs, although at a later stage, even when pollination is unsuccessful. Additional ethylene applied using 2-chloroethyl phosphonic acid, which when incorporated into plant tissue produces ethylene, accelerated carnation flower senescence. If the carnation flowers are treated with silver thiosulphate, which prevents ethylene action, and ethanol, which inhibits ethylene biosynthesis, petal longevity is extended to the detriment of ovary growth. Correlating the physical appearance of the flowers in the presence and absence of ethylene with dry mass and labelled sucrose analyses, carbohydrate movement appeared to be a major event during the senescence of this cut flower. Such a conclusion could not be reached on dry mass analyses alone as the photosynthetic organs of the carnation flower contribute to the carbohydrate pool in the first days following harvest. Furthermore the respiratory pattern of the flower is not a steady decline. Concomitant with the natural ethylene emanation as the petals irreversibly wilt, so the respiratory rate

increases. On the other hand, the respiratory rate is greatly reduced with silver thiosulphate and ethanol treatment. In the presence of ethylene, together with the growth of the ovary there is an influx of carbohydrates from all the flower parts including the petals into the ovary. With silver thiosulphate and ethanol treatment the petals become the dominant carbohydrate sink. It thus appears that insufficient carbohydrates moving to the ovary may be the cause of the lack of ovary development. However, an experiment with isolated cultured ovaries on a modified MILLER'S (1965) medium lacking in plant hormones but with a range of sucrose concentrations showed that sucrose alone cannot stimulate ovary growth. The mechanism by which this source-sink relationship is determined appears to be controlled from the sink. The source organs contribute carbohydrates that are in excess of their metabolic needs. Acid invertase activity, maintaining the sucrose gradient into the sink, was considered as a mechanism by which sink strength could be controlled due to the parallel in other plant systems between the activity of this enzyme and sink strength. On investigation the levels of acid invertase activity are higher in the ovaries of senescing carnations than in the petals. This balance of invertase activity was reached mainly due to a decline in petal invertase activity. However, as silver thiosulphate treatment lowered the level of acid invertase activity in the ovary and this flower part was not the dominant sink with this treatment, acid invertase activity appears to contribute to sink activity in the senescing carnation flower. Nevertheless due to the immobility of sucrose through membranes, for the passive movement of sucrose down a concentration gradient, membrane permeability to sucrose would have to be altered. This is a possible

role of the plant hormones and specific ions. Furthermore, this ovary growth was correlated with chloroplast development in the ovary wall. In the presence of ethylene 'greening' or an increase in chlorophyll content during flower senescence was measured. This increase in the chlorophyll content did not occur in the silver thiosulphate and ethanol treated carnations. Relating this to chloroplast development, an electron microscope study showed that in the presence of ethylene the original amyloplast present at harvest developed into a chloroplast with thylakoids stacked into grana. With the ethylene inhibitory treatments, although thylakoids developed in the ovary wall chloroplasts, grana did not form. As chlorophyll is synthesised in the thylakoids, this chloroplast structure correlated with the chlorophyll measurements. The results of the parameters measured during the senescence of the cut carnation flower suggested that the other plant hormones besides ethylene were involved in this process. Endogenous cytokinin measurements showed that, overall, the level within the cut flower declined as the flower senesced. The ovary cytokinin levels did not steadily decline but increased as the petals irreversibly wilted. This peak of cytokinin activity was common to ovaries of flowers treated with 2-chloroethyl phosphonic acid and naphthalene acetic acid, treatments that accelerated senescence. Previous workers showed that a silver thiosulphate treatment prevented this increase in cytokinin activity in the ovary. This, together with the lack of ovary development, suggests that the ovary cytokinin activity may be a crucial event in the regulation of carnation flower senescence. To confirm such a hypothesis zeatin was injected into the ovary but was found ineffective in mobilising sucrose and accelerating petal senescence. It was only when both zeatin and indoleacetic acid

were applied to the ovary that sucrose mobilisation and accelerated petal senescence occurred. Thus auxins together with cytokinins appear important in ovary development. The importance of the presence of auxin in ovary development was further recognised by a naphthalene acetic acid treatment being far more effective in stimulating the growth of isolated cultured ovaries than kinetin. Auxin treatment increased the size of the cells within the ovary wall and the development of the chloroplasts within these cells to a greater extent compared to control and kinetin-treated ovaries. It was thus hypothesised that the auxin levels in the ovary were protected against conjugation by the presence of adequate levels of cytokinins. When the cytokinin levels dropped, as in the petals, ethylene could then accelerate auxin conjugation resulting in a retardation of growth. Sink tissues, such as the ovary, with a higher cytokinin and hence auxin content, may utilise mobilised assimilates from the petals thus contributing to petal senescence. To further prove this hypothesis an investigation into the site of ethylene action using the silver ion as a tool was initiated. A review of the histochemical and histological literature revealed that common silver binding sites in plants included sulphydryl groups, chloride ions, ascorbic acid and invertase. Each was considered as potential channels via which ethylene could effect its physiological response but no conclusion was reached. Because of this a decision on the importance of the translocatory path of a ten minute silver thiosulphate pulse within the flowerhead and its accumulation within the receptacle could not be reached.

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INTRODUCTION

With the world population developing into numbers that will soon outstrip the food supply, agricultural research has concentrated on increasing food availability. Initially emphasis focused on crop production but an alternative means to extend food availability exists. This simply involves preserving the harvested crop for human consumption (LIEBERMAN, 1983). An estimated twenty-five to eighty percent of all fresh fruit, vegetables and ornamental crops are never utilised because of problems arising after their harvest (STABY, ROBERTSON, KIPLINGER AND CONOVER, 1978). Thus the study of plant postharvest physiology is now recognised as a vital area of agricultural research. Postharvest studies begin when the whole or part of the plant is removed from its growing environment, which initiates the senescence of the plant parts, ultimately ending in their death. Once plant senescence is understood, this developmental process can then be manipulated for the preservation of the crop.

There were two main reasons for selecting the cut carnation (*Dianthus caryophyllus* L. cv. White Sim) flower as a system in which to study the senescence process. Firstly, the carnation flower is of great commercial importance. Carnations are a major cut flower crop throughout the western world. In South Africa carnations rate the second most popular cut flower to the *Chrysanthemum* (KOTZE, 1985, personal communication)*. In the United States of America, 613 million

* Multiflora Ltd., Marjorie Street, City Deep, Johannesburg, RSA.

carnation blooms were harvested and sold in 1969 alone (BALL, 1975). Thus the commercial importance of this flower can be appreciated. The grower's responsibility however, does not end at the harvest of his crop. For consumer satisfaction the blooms must be received in a condition capable of giving a good vaselife. Basic cultivation practices such as: a well selected cultivar, a controlled nutrient and disease-free environment, naturally contribute to the longevity of the flower. It has been realised that other factors which follow the harvest of the flower also contribute greatly to extend the life of the bloom.

Postharvest cut flower treatments originate from 'old wives' tales. It was recommended that by adding a spoonful of sugar and a copper coin to the water of a bowl of flowers, the blooms would last longer. It is now known that the sugar (sucrose) acts as an important photosynthetic carbohydrate supplement whilst the copper coin probably had bacteriocidal properties, thus preventing xylem blockage and maintaining a continued water uptake. On a large commercial scale, such a treatment did not appear practical. However, with more being understood about the postharvest period of the flower it was realised that by overcoming basic problems, such as poor handling and utilising refrigeration techniques, flower longevity could be greatly extended. So successful was the implementation of these methods that growers were able to extend their market range and still provide blooms with a good vaselife. Hence the less populated tropical countries now exploit the winter European and North American markets. Exemplary of this expanded market is the situation between Columbia and the United States of America. American growers were beginning to find it difficult to produce a profitable

crop as the average price per flower was slightly decreasing and production costs doubling (BALL, 1975). To overcome this many short cuts and innovations were adopted. One of these was to move and grow carnations in a more favourable climate. An ideal carnation cultivating climate was found in Columbia. In this environment the flowers can be grown with only the protection of a bamboo framework of shade cloth. This offsets the expense of greenhouse materials and heating, major production costs of the American floriculturalists. Utilising basic postharvest knowledge, that is overcoming poor handling and refrigerating the harvested flowers, Columbian-grown carnations, are now transported and sold in the United States of America dominating the market (GILLETTE, 1979). Thus the economic influence of this research can not be ignored and probably has played a major influence in this field.

The second reason for choosing the cut carnation flower is physiological. Early postharvest studies revealed that the cut carnation flower is an ideal system in which to study the events prior to the death of its tissues. This final stage of development is termed senescence and defined refers to the deteriorative changes which are natural causes of death (LEOPOLD, 1975). Within the flower, senescence occurs in each part at a different rate. The interaction of these senescing flower parts results in the overall wilted-petal appearance of the whole flower as it dies. The application of postharvest treatments extending or decreasing the longevity of the flower indicate that this senescence process can be manipulated. These treatments have been utilised by researchers to aid in analysing the physiology of the senescing carnation.

Indicative of the botanical value of this system is the vast literature output. The number of papers published using the cut carnation flower as the plant material are nearing the thousand mark (STABY AND CUNNINGHAM, 1985). It thus is necessary to generally review what is presently known, physiologically, of this flower in context with the research covered in this thesis.

CHAPTER ONE

LITERATURE REVIEW

Flower senescence

> *'What most people think of as flowers have little to do with the reproductive process of plants. They are simply flags that can be waved to attract the right insects for pollination to take place. Usually these brightly coloured organs are petals, and as such, they have evolved from leaves. It is the jewel displayed at the heart of the structure that is really interesting'.*
> (GROUNDS, 1980).

Flowers, like all other organs, exhibit an organised pattern of development. Floral induction of a vegetative shoot stimulates the apical meristem to form an assemblage of sterile and reproductive flower parts enclosed as a bud. It is from this calyx-protected bud that the petals burst attracting pollinators to their exposed stigma and stamens and then the senescence process begins.

Senescence can be studied at many levels within the plant from single cells to whole organisms. In angiosperms, flower senescence in monocarpic plants heralds the dramatic death of the whole plant, whilst in polycarpic plants, such as the carnation, it is restricted to

the flower itself. Harvesting this flower from its parent plant accelerates the overall senescence of the bloom. Because of this, senescence was formerly regarded as essentially starvation (MOLISCH, 1928). With the realisation that this process requires the metabolism of breakdown products, including the translocation of carbohydrate and nitrogenous compounds into other plant parts, it is now considered a stage of normal growth. Thus, with the flower being composed of anatomically and developmentally diverse flower parts, each senescing at a different rate, correlative senescence determines the overall appearance of the flower. Correlative senescence is when the death of one plant part is influenced by the growth of another. The term was coined when studying leaf development. In this organ, senescence may be accelerated by an actively growing region within the plant, such as a fruit. Removal of such a growing region delays the senescence of the neighbouring leaves (SETH AND WAREING, 1967). Biologically this competition for the plants resources between actively growing regions and mature plant parts may be extremely important in saving valuable plant metabolites for their reutilisation in younger or storage tissues. Additionally, the senescence phenomenon can be considered as a kind of communication system dictating the growth and form of the flower or plant. The physical impact of flower senescence is seen by the condition of the petals. Not all flower petals senesce in the same fashion; in one extreme the petals abscise from the remainder of the flower, whilst at the other extreme the first signs of petal senescence are determined by a change in pigmentation. In the carnation, petal senescence is determined by the petals wilting. Different degrees of wilting do exist. Reversible petal wilting is due to the loss of turgor water

whilst irreversible wilting terminates in the death of the petals (NICHOLS, 1977b). Concurrent with this petal wilting the ovary enlarges, 'greens' and, if pollinated, develops seeds. Clearly the cut carnation flower represents a closely integrated system of an actively growing region and maturing plant parts.

While attached to the parent plant, losses from the carnation flower due to respiration and transpiration are replaced from the assimilate pool and root uptake of water, respectively. Removal of the flower from its normal nutrient source makes this explant entirely dependent on its own food reserves and moisture content. If these requirements are not exogenously supplemented they could become limiting. The rate at which this limiting situation arises is to a large extent dependent upon the kind and intensity of the physiological activity of that detached flower.

Water deficit in cut flowers is considered a major cause of rapid senescence (AARTS, 1957; LEVITT, 1972; ROGERS, 1973). Immediately after picking, the carnation flower is exposed to water stress caused by increased water loss and limited water uptake (HALEVY AND MAYAK, 1981). With poor postharvest handling, even temporary water stress has been shown to accelerate senescence (MAYAK AND HALEVY, 1974; BOROCHOV, TIROSH AND HALEVY, 1976). Water stress also builds up with the ageing of the cut flower even when held in water. This was initially thought to be due to deposits plugging the xylem vessels and hence obstructing water uptake (DURKIN AND KUC, 1966; LARSEN AND FROLICH, 1969; RASMUSSEN AND CARPENTER, 1974). These xylem

obstructions can arise either from the development of microorganisms (LARSEN AND CROMARTY, 1967; LARSEN AND FROLICH, 1969; ROGERS, 1973; MAYAK, GARIBALDI AND KOFRANEK, 1977) or the release of products resulting from oxidative processes (DURKIN AND KUC, 1966; LINENBERGER AND STEPONKUS, 1976). However, CAMPRUBI AND FONTARNAU (1977) showed that no direct relationship exists between flower longevity and the flower preservative action on the level of xylem plugging. A silver nitrate treatment was initially used for its bacteriocidal property, but (as will be discussed) was later found to be an effective ethylene inhibitor, a mechanism by which senescence is delayed (MAYAK, GARIBALDI AND KOFRANEK, 1977).

Water loss through stomatal behaviour can also contribute to water stress. The mechanism of stomatal movement is a subject of intense discussion (LEVITT, 1974; RASCHKE, 1975). The early theory that starch breakdown contributes to the increase of osmotic pressure in the guard cells through sugar formation has been modified. During stomatal opening, starch disappears from the chloroplasts at the same time as potassium ions enter the guard cells, whilst during stomatal closure, the reappearance of starch parallels the loss of potassium ions. Starch hydrolysis may provide the organic anions with which potassium uptake is associated. The role of plant hormones in affecting this stomatal behaviour has been well recognised. Cytokinins and gibberellins (LIVINE AND VAADIA, 1965) have been found to induce stomata to open whilst auxins (FERRI AND LEX, 1948), abscisic acid (JONES AND MANSFIELD, 1970) and ethylene (MADHAVAN, CHROMINISKI AND SMITH, 1983) cause stomatal closure. VITAGLIAND AND HOAD (1978) suggested that

ethylene may exert its effect through abscisic acid as ethylene stimulates abscisic acid production in plants (GOLDSCHMIDT, GOREN, EVEN-CHEN AND BITTNER, 1973). Ethylene, abscisic acid and auxin promote flower senescence and stomatal closure. This indirectly suggests that the continued water flow and gaseous exchange through the cut carnation flower may be an impetus needed to maintain flower vitality.

Reduction in water flow results in weakened photosynthesis, assimilate transport being disrupted and the inhibition of growth processes, which also includes the accumulation of nutrients in storage organs (KURSANOV, 1984). The most affected of these is the inhibition of meristematic growth following loss of turgor. Assimilates are thus not required which logically leads to phloem transport inhibition. This view of KURSANOV (1984) is not universally agreed with. It appears that photosynthesis can be immediately reduced on water stress (HODDINOTT, EHRET AND GORHAM, 1979) but that translocation seems to be the plant function most resistant to water shortage (CANNY, 1984).

On the other hand, stomatal closure would reduce gaseous exchange. As the carnation flower remains a living entity when removed from its parent plant, this impeded gaseous exchange could affect two vital processes, photosynthesis and respiration. Carbon dioxide levels have been shown to decline after an application of abscisic acid to leaves (CUMMINGS, KENDE AND RASCHKE, 1971). This may be hormonally significant as carbon dioxide is an inhibitor of ethylene action (UOTA, 1969). However, these gaseous levels cannot be directly related to stomatal movement. Respiratory rates also greatly affect the carbon dioxide and oxygen levels.

The respiratory pattern of the carnation flower as it ages is not a steady decline as one would expect. A definite peak of activity occurs as it matures (COORTS, 1973). This is followed by a second short dramatic peak in the respiratory drift before a final decline. The second peak in the respiratory pattern probably indicates the final senescence stage as it is coincident with petal wilting. Analogies to the climacteric respiratory rise of many fruits have been drawn with this secondary respiratory rise in the carnation flower (LARSEN AND FROLICH, 1969).

The closeness between the water and carbohydrate status of the cut carnation flower may biochemically be related to the extent by which respiration is associated with oxidative phosphorylation. During the initial dehydration stage, respiration is more closely associated with energy storage, whereas further dehydration uncouples these processes (ZHOLKEVICH AND ROGACHEVA, 1963; ZHOLKEVICH, 1968). Therefore, the increasing content of ATP in plant tissues at the initial stage of dehydration may also be a factor responsible for the higher linear velocity of assimilate transport (ZHOLKEVICH, 1968) and the more intensive loading of labelled sucrose into leaf veins (PLAUT AND REINHOLD, 1965; 1967). Alternatively, the osmotic balance is affected by the level of soluble sugars. In carnation flowers, during senescence the level of soluble sugars gradually declines, the increased level of fructose in the ovary being the exception (DIMALLA AND VAN STADEN, 1980). However, if petal longevity is solely dependent on sugar content, as the sugar content depletes, so the capacity for the petal to retain water is reduced. It thus appears that during the initial petal wilting stages, energy storage into carbohydrates occurs which are then shunted

from the petals. This movement may promote irreversible wilting as the osmotic balance cannot be attained again with the chemical constituents remaining in the petals. Thus the movement and utilization of petal carbohydrates may well be the determining factor in petal wilting.

Sucrose is considered the predominant translocatory carbohydrate in plants. This is because firstly, it is the major carbohydrate in phloem tissue; secondly, it appears in the sieve elements ahead of other sugars when radioactive carbon dioxide is assimilated by photosynthesising leaves; and lastly, the sucrose content in conducting phloem elements can be correlated to the movement of assimilates in plants. For these reasons also, sucrose is recognised as the translocatory carbohydrate in carnation flowers (HARRIS AND JEFFCOAT, 1972; HO AND NICHOLS, 1975). The evolutionary advantages of selecting sucrose for this role are numerous. Being a stable non-reducing sugar it can travel in the phloem metabolically inert in the absence of invertase, whilst structurally it contains twice as much carbon per gram-mole as the hexoses. However, both the above advantages may be acquired with other metabolites. For example, mannitol and other non-reducing polyalcohols or tetra-oligosaccharides carry even more carbon in the molecule than sucrose. PONTIS (1977) and PAVLINOVA AND TURKINA (1978) recognised the real essence of sucrose as a translocatory carbohydrate being in the property of its covalent bond. Both apomeric carbons of sucrose are interlinked by a (1-2) bond in which the furanose configuration of the fructose residue seems to be responsible for the high negative value of free hydrolysis energy

of this bond (ΔF_o = 6600 cal $mole^{-1}$). This approaches the energy rich γ-phosphorous bond in ATP (ΔF_o = 7000 cal $mole^{-1}$) and exceeds the usual glycosyl bonds of di- and polysaccharides (ΔF_o = 3000 cal $mole^{-1}$) and sugar phosphates (ΔF_o = 3000 - 4500 cal $mole^{-1}$). Hence sucrose is transported not only as a carbon carrier but also as an energy source. On sucrose metabolism this energy may aid the hexose residues to be transferred to other acceptors in the presence of appropriate enzymes, entering the metabolism without additional activation.

To determine the direction of assimilate movement in correlative senescence, WARREN-WILSON (1972) proposed the source-sink theory. This theory names the region within a plant of high metabolic activity that will utilise assimilates as the 'sink'. Regions of assimilate production, whether mobilised from photosynthetic tissues or storage organs, were alternatively termed 'sources'. Agriculturally, much attention has been focused on manipulating this source-sink relationship in plants. Dry matter directed to the harvested plant part may prove one of the most promising and efficient means of increasing productivity (WAREING AND PATRICK, 1975).

For the source-sink theory to function, a source must produce an excess of assimilates which are then available to other plant parts. The source of these assimilates may be from photosynthetic tissues or storage organs whose storage compounds are being mobilised. Of the storage carbohydrates in plants, more is probably known of starch and sucrose than all the other storage carbohydrates grouped together, probably due to their common occurrence. DIMALLA AND VAN STADEN (1980)

detected the presence of sucrose, glucose, fructose, inositol and sorbitol, with xylose only being present in the ovary. The absence of starch was noted suggesting that sucrose is not only the principal translocatory carbohydrate, a major product of photosynthesis but also the main storage sugar. This makes sucrose a most vital chemical constituent in carnation flowers.

The ability to synthesise sucrose is a general characteristic of both the photosynthetic and non-photosynthetic cells in higher plants (AP REES, 1984). Evidence from both these systems reveals that plants produce sucrose via sucrose phosphate synthetase and sucrose phosphate phosphatase. With the knowledge that this disaccharide does not readily cross the chloroplast envelope and products of photosynthesis move from the chloroplast mainly as triose phosphates, sucrose phosphate synthetase has been located external to the chloroplast in the cytoplasm. As for the regulation of this sucrose synthesis, it has been suggested that when sucrose synthesis exceeds transport, the consequent accumulation of this sugar restricts further synthesis through enzyme inhibition (HEROLD, 1980). With no direct investigation yet been made, this hypothesis remains open for consideration. Sucrose levels, however, are not only dependent on sucrose synthesis and transport but also its metabolism.

Invertase (β-fructofuranosidase) was the first enzyme known to act on sucrose. In the presence of water this sugar is broken down into its constituents, glucose and fructose. This enzyme acts at two pH optima; acid invertase (pH 3,5 to 5,1) and neutral or alkaline

invertase (pH 7,0 to 7,8). Acid invertase is the dominant form, so much so that for many the term acid invertase is synonymous with plant invertase. Carnation petal invertase showed the most activity at about pH 5 although activity was detected from pH 3,4 through to 7,95 (HAWKER, WALKER AND RUFFNER, 1976). There appears to be a dual location of this enzyme in plant cells, in the vacuole and free space. The relative proportions between these two sites are thought to vary with tissue and its state of differentiation (AP REES, 1984). Considering the cellular location of acid invertase and its pH sensitivity, ESCHRICH (1980) hypothesised that this enzyme is controlled by adjusting the pH of the free space. This may be effected by the cellular release of acids or cations, particularly oxalic acid and potassium and calcium ions. In the carnation flower over eighty percent of the invertase activity remains within the petals throughout its postharvest life (DIMALLA AND VAN STADEN, 1980). This invertase activity is the greatest at harvest declining with age. Alkaline invertase is also ubiquitous in higher plants but is not as active as acid invertase. In the carnation flower, with melezitose and raffinose being metabolised which are acid invertase specific, alkaline invertase has not been considered important (HAWKER, WALKER AND RUFFNER, 1976).

Finally, the last enzyme known to be involved in sucrose synthesis is sucrose synthetase. The reaction catalysed by this enzyme is reversible. Sucrose synthetase has a broad pH optimum for sucrose synthesis (pH 7,5 to 8,5) but a sharp quite different optimum (pH 6,0 to 6,5) for sucrose cleavage. Measuring sucrose synthetase at pH 8,5, HAWKER, WALKER AND RUFFNER (1976) found that this enzyme was more

active in the carnation compared to other petal tissues but acid invertase activity is one and a half times greater than sucrose synthetase activity. This correlation is actually meaningless as at pH 8.5 sucrose synthetase is synthesising sucrose whilst acid invertase is cleaving the sucrose molecule. It does, however, indicate that both processes can occur simultaneously thus enforcing the concept that collectively, all four enzymes of sucrose metabolism adjust the sucrose concentration of a source tissue.

The vacuole has long been considered a repository for sucrose and other assimilates but it was only when methods for vacuole isolation became available, that this could be confirmed (LEIGH, AP REES, FULLER AND BANFIELD, 1979). In red beet, sucrose storage was confined entirely to the vacuole (LEIGH, AP REES, FULLER AND BANFIELD, 1979), but in other tissues sugars were also detected exterior to the tonoplast (NISHIMURA AND BEEVERS, 1979; WAGNER, 1979; THOM, MARETZKI AND KOMOR, 1982; WAGNER, KELLER AND WIEMKEN, 1983). The accumulation of sucrose in the vacuole may be an active process as it occurs against a concentration gradient (THOM, KOMOR AND MARETZKI, 1982). But once within the confines of the tonoplast it is stored for future use.

The size of the respirable pool in the carnation tissues is not only affected by sucrose metabolism and storage but also by the movement of carbohydrates between the flower parts. The first step in sucrose mobilisation involves sucrose hydrolysis within the vacuole (LEIGH, 1984). Currently little is known of the ensuing hexose transport from the vacuole and thus leaves the whole question of sucrose

mobilisation open. From this point though, the symplastic continuity between the various source cells and the uniformity of sucrose content of these tissues suggests that sucrose moves within the plasmodesmata connections to the sieve element and companion cell region of the phloem. However, prior to its active accumulation into the phloem, sucrose appears to enter the apoplast (GEIGER, 1975; GIAQUINTA, 1980). This is true in sugar beet and maize tissues (HEYSER, 1980) but the presence of the symplastic continuity from the mesophyll to the companion cells via the phloem parenchyma may indicate that in other species, some but certainly not all, loading could occur entirely within the symplasm.

Phloem loading is the process whereby the major translocated sugars are selectively and actively accumulated into the sieve tubes prior to their export from the source. It is generally accepted that this loading generates the driving force for the mass flow of assimilates from source to sink by decreasing the osmotic potential of the sieve tubes, which in turn generates a water potential gradient and water influx into the phloem (GIAQUINTA, 1980). Present lines of evidence support a sucrose-proton cotransport system for the loading of sucrose into the phloem tissue (BAKER, 1978; HEYSER, 1980; KOMOR, ROTTER, WALDHAUSER, MARTIN AND CHO, 1980). In such a system, the driving force for the loading of sucrose is an electrochemical potential gradient of protons across the phloem membranes, which is established by a proton-extruding mechanism, presumably a membrane ATPase (GIAQUINTA, 1979). Sucrose is thought to enter the apoplast near the phloem, interact with a sucrose-specific carrier on the phloem membrane

and cross the membrane in response to the proton motive force established by the higher proton concentration of the apoplast relative to the interior of the phloem. In this manner the energetically driven downhill movement of protons into the phloem is coupled to the secondary active loading of sucrose. Protons are continuously extruded to maintain the proton gradient by an active proton pump on the phloem plasma membrane.

In many source-sink systems, the unloading of this sucrose from the phloem may occur down a concentration gradient. Hence unloading and the transfer of sucrose to nearby consuming cells will occur without further expenditure of energy by sieve elements or their accessor cells (GEIGER AND FONDY, 1980). Consumer cells could regulate this passive unloading by maintaining a low concentration of sucrose or, in other terms, the driving force of sucrose unloading is via sucrose utilisation by consumer cells. Such a hypothesis was structured from the work of WALKER AND HO (1977 a,b) who demonstrated maximal import of photosynthate by small fruit whose rapid metabolism maintained a low internal sucrose concentration. However, with the phloem being highly pressurised and generally resistant to leakage along its entire length (WARDLAW, 1965), the companion cells and, in some cases, transfer cells may facilitate unloading, perhaps by providing a variably permeable plasmamembrane for efflux of solute. Possible effectors suggested include specific ions (FONDY AND GEIGER, 1980) and plant hormones (PATRICK, 1976; TANNER, 1980) which may also be the stimulus used by plant parasites for their attraction of assimilates (ELZEN, 1983).

As valid as this hypothesis appears, the term 'attraction' stimulated alternate concepts for the control of sink activity. The sink could control assimilate movement to it via a requirement message rather than the activity of its metabolic activities. ANTOSZEWSKI AND MIKA (1971) suggested that this message was carried by special metabolic substances or plant hormones transmitted by the growing cells, whilst KURSANOV (1984) proposed that a bioelectric pulse was the message. The concept of a requirement message would have little value if the unloading of sucrose occurred down a concentration gradient.

The only known example of the unloading of sucrose in the flower occurring against a concentration gradient is into the floral nectaries (THORNE AND GIAQUINTA, 1984). Extremely high rates of sugar movement across nectary membranes are a salient feature of their physiological activity. Because of this it is argued that the nectaries serve as 'gates' through which plants dispose of excess sugars if their influx into the phloem exceeds the possibility of assimilation by sinks (FREY-WYSSLING AND HÄUSERMANN, 1960). Nectar secretion is usually regarded as an ecological adaption intended to attract pollinating insects to the flower. This may well be a major function in most cases, nevertheless, in non-flowering plants nectaries exist in leaves suggesting they perform a broader range of functions. ZIEGLER (1965 a,b) proposed that an additional function of these nectar gates was to facilitate the transport of nitrogenous substances via the phloem tissue. A major difference between the nectar and phloem sap is the lower nitrogenous content of the former (LÜTTGE, 1961). Since sucrose is a major component of phloem sap, which moves via a concentration gradient,

the incomplete assimilation of sucrose by sinks would hinder the movement of other substances. This particularly includes the nitrogenous compounds that the tissue may require during this period. So the expulsion of unused excess sugars via the nectaries and the reabsorption of nitrogenous substances may be of major importance for the distribution of substances other than carbohydrates in plants. In this light, the energy cost of active transport to unload the sugars against a concentration gradient may be worth the maintenance of continued transport. Symplastic coupling within the system of sieve tubes, companion cells, parenchyma cells and secretory cells of the nectary glands allows many possible sites for active transport.

Thus far the transport of carbohydrates has been dealt with at the biochemical and cellular level and MURRAY, MAUK AND NOODEN (1982) rightly point out that more effort has been directed at analysing these components of the vascular system than understanding the functional and spatial connections between the groups of cells. The vascular system is generally envisaged as a circular flow of materials within the plant, primarily upward through the xylem and downward through the phloem, but the actual flow patterns are more complex and restricted due to the vascular connections between individual organs. For example, leaves along a stem axis are arranged in vertical ranks or rows which are termed orthostichies. Leaves in the same orthostichy tend to share a primary vascular pipeline which to some degree is separate from that of other orthostichies. Lateral connections and anastomoses between strands may provide some interconnection. The vascular patterns can be more complex with different organs, besides leaves, also connected

with separate vascular traces. This vascular division may have developed to restrict the spread of pathogens as well as conserve energy and resources by limiting the formation of unneeded inter-connections. Whatever the reasons, it is a facet to be considered when studying the long distance transport of compounds.

HARRIS AND JEFFCOAT (1972) were the first to study the translocation of assimilates within the carnation plant. By exposing leaves on the shoot to radioactive carbon dioxide, they found that labelled carbon assimilates moved preferably to the flower. Radioactivity detected within the flower did, however, decrease with distance of the labelled leaf from this organ. Photosynthate movement into the flower was at a maximum in the two weeks preceding flower opening with little labelled carbon being detected in the roots. It thus, appears that by the time the flower is harvested the majority of carbohydrates needed for its development have been provided by the photosynthesising leaves. Redirection of leaf assimilates away from the flower only occurs when the flower senesces or is removed from the plant.

As floral tissues represent an active sink region in the carnation plant, it is to be expected that they would possess well-developed mechanisms for offloading assimilates from the phloem (HARRIS AND JEFFCOAT, 1974). The accumulation of labelled assimilates in the flower was largely attributed to movement into the petals (HARRIS AND JEFFCOAT, 1972) and thus this flower part was considered to possess a major attracting force in flower sink activity. With the assimilates being metabolised into a variety of sugars besides sucrose, HARRIS AND

JEFFCOAT (1974) proposed that assimilate accumulation may involve the inversion of sucrose. Petal invertase activity was thus measured and found to be far greater than invertase activity in the stem (HARRIS AND JEFFCOAT, 1974) and also in the other remaining flower parts (DIMALLA AND VAN STADEN, 1980; HALABA AND RUDNICKI, 1981; 1983). Hence the idea of invertase activity influencing sink activity was initiated.

Once the flower is severed from the parent plant, a major source of carbohydrate is unavailable to the bloom. The impact of this deprivation can be exemplified by supplementing the sucrose levels of the cut flower with an exogenous source and witnessing the extension of flower longevity. So even in its isolated state, the flower remains a carbohydrate sink. Within the flower though, the petals do not remain the dominant sink. As the carnation flower senesces, the sink switches to the ovary. NICHOLS AND HO (1975a) demonstrated this by applying labelled sucrose via a stem feeding solution. Prior to the petals irreversibly wilting, labelled sucrose moved to the petals, yet following their senescence, the majority of the radioactive carbon was found within the ovary. To investigate if this source of ovary radioactivity came via the petals, the petals were fed labelled sucrose which was found to move to the ovary, nectaries and stem (NICHOLS AND HO, 1975b). It was thus concluded that during senescence the sink strengths of the ovary, nectar and stem became greater than the original petal sink. Additionally, these results implied that the petals became a source of translocated substrate on their senescence. This remains a controversial issue. MOR, REID AND KOFRANEK (1980) alternatively propose that the petals and ovary compete

for a common supply of carbohydrate.

Thus, while there is a general agreement that carbohydrates are vitally important for petal longevity and ovary development, the exact relationship and degree of transport between these flower parts remains unclear. However, before such relationships can be further investigated, the role of plant hormones in carnation flower senescence must also be reviewed. Plant hormones regulate the senescence process of the carnation flower. Whether this action is through their known influence on assimilate transport is a question in point.

Plant hormones

The genetic constitution of a plant determines the basic form and pattern of its development making it a recognisable member of its species. However, a control system must exist to ensure the integration and coordination of the orderly sequence of events during plant development. SACHS (1882) suggested that this coordination of growth was performed by chemical messages. Although much controversy surrounds this issue at the present time (see Chapter 5), positive evidence has accumulated to support the plant hormones as being the chemical messengers coordinating plant growth. These plant hormones can only regulate growth processes contained within the genetic material. Once a genetical process has been induced, the plant hormones may regulate the intensity and rate of the development but they may not completely prevent the process. The hormones capability to adjust the induced development will depend on the concentration of its active form as well as the

number of vacant sites to which this active hormone can attach. The sensitivity of the carnation flower to hormones does alter with its development as can be seen with the flowers response to ethylene during various growth stages (BARDEN AND HANNAN, 1972; CAMPRUBI AND NICHOLS, 1978). Furthermore, senescence has been shown to be regulated by a pattern of growth hormone levels (BRUINSMA, 1983).

A great deal of attention will be focused on ethylene, not only because biochemically the most is known of this plant hormone but also because the majority of the postharvest treatments to extend flower longevity are directly evaluated on their ability to inhibit ethylene production. Ethylene, structurally, is the simplest hormone known. Yet at each stage in crop production from germination to harvest and postharvest handling, there are plant processes subject to modification by this plant hormone. Of all the known plant growth hormones ethylene is the only one that, under normal physiological conditions, exists as a gas.

The ability of certain gaseous agents in the air to modify plant growth was known for many years before ethylene was identified as the active component. The ancient Chinese hastened fruit ripening by placing the unripened fruits in a chamber containing burning incense. Likewise, the flowering of pineapple plants was accelerated with fire smoke. The discovery of ethylene being the gaseous active component, however, was only made in 1901 by NELJUBOV. Observing germinating pea seeds he found that they grew in a horizontal direction if confined to the

laboratory atmosphere, yet when air was drawn from outside the laboratory, the seedlings resumed their normal growth. A series of experiments analysing illuminating gas isolated ethylene as the active component.

The first report of ethylene-induced floral senescence was made by CROCKER AND KNIGHT (1908). They found that carnations are extremely sensitive to ethylene. Often it is assumed that this ethylene-sensitivity holds true for all flowers. This may be true but their dose-response relationships are diverse. The carnation and orchid flowers are blooms that are very sensitive to ethylene (SMITH, PARKER AND FREEMAN, 1966).

Ethylene occurs as the result of industrial pollution, from the combustion of fossil fuels and is synthesised in plants. The initial suggestion that plants produce ethylene stemmed from a report by COUSINS (1910) that oranges produced a gas that promoted the ripening of bananas. GANE (1934) chemically proved this, thereby providing the necessary evidence that ethylene is a plant hormone.

Unfortunately interest in this plant growth regulator waned with the discovery that auxin promoted ethylene production. Many of the auxin effects are indirectly due to increased ethylene production. Furthermore, the difficulty of measuring ethylene production via bioassays or crude chemical techniques also hindered research. With the introduction of gas chromatography (HUELIN AND KENNETT,1959), one of the quickest and most accurate assays for ethylene

estimation was developed. Likewise, the increased use of 2-chloroethyl phosphonic acid, which when absorbed by living tissues produces ethylene (WARNER AND LEOPOLD, 1969), provided a convenient means of ethylene-treating field plants. Thus since 1960, there has been a vast increase in ethylene research with the results summarised in many excellent reviews (YANG, 1980; ADAMS AND YANG, 1981; SISLER AND GOREN, 1981; DAGANI, 1984; SMITH AND HALL, 1984; YANG AND HOFFMAN, 1984). The majority of this information is pertinent to this thesis as it provides a theoretical basis on which the role of ethylene in flower senescence may be understood and manipulated.

The initial precursor of ethylene was not easily isolated because the simple chemical structure of ethylene allowed many compounds, through various chemical reactions, to be possible candidates for this pathway. Consequently a number of compounds were proposed as ethylene precursors which included linolenic acid, propanol, β-alanine, ethanol, ethane, acetic acid, fumaric acid and methionine (YANG, 1974). Tissues in their homogenised state lose their ethylene-biosynthetic capacity. By adding methionine, copper II ions and abscorbic acid, such an *in vitro* system produces more ethylene than when methionine is substituted with any of the other proposed ethylene precursors (LIEBERMAN AND MAPSON, 1964). So effective is methionine as a precursor that plant tissues administered labelled methionine produce an equal proportion of radioactive ethylene (LIEBERMAN, 1979). These results suggest that methionine is the major, if not sole precursor of ethylene.

The conversion of methionine to ethylene differs in plants compared

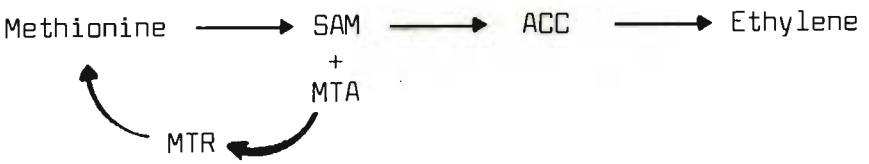
to chemical systems. In both instances the C-3 and C-4 of methionine are converted to ethylene and the C-1 to carbon dioxide. In the chemical system ethylene is formed from methionine via the intermediate methional ($CH_3S-CH_2-CH_2-CHO$) and the CH_3S-group of methionine yields a volatile dimethyl sulphide. In plant tissues, methionine but not methional, serves as the ethylene precursor. The CH_3S group is not converted into volatile products but is retained within the tissue (YANG AND HOFFMAN, 1984).

The concentration of methionine in plant tissue is very low so that if a normal rate of ethylene production was maintained and the sulphur not recycled, the sulphur would become limiting. So important is this sulphur recycling that the intermediate, S-adenosylmethionine (SAM) is thought to have evolved solely for this purpose. Experimental evidence for SAM being an intermediate was presented by ADAMS AND YANG (1977). The degradation products produced if ethylene is derived from SAM are 5'-methylthioadenosine (MTA) and a hydrolysis product, 5'-methylthioribose (MTR). This hydrolysis of MTA to MTR is the pathway via which the CH_3S group of methionine is conserved (MURR AND YANG, 1975). Both these compounds were isolated by ADAMS AND YANG (1977).

To detect the next intermediate in the ethylene biosynthetic pathway, the difference between aerobic and anaerobic conditions played a crucial role. A nitrogenous atmosphere causes the cessation of ethylene production but when re-exposed to air, a surge of ethylene emanation occurs. Interpretation of these events indicated that an intermediate accumulated during anaerobic incubation and is subsequently

converted to ethylene upon exposure to oxygen. This intermediate was later identified as 1-aminocyclopropane-1-carboxylic acid (ACC). This final step in ethylene biosynthesis is thus oxygen dependent (ADAMS AND YANG, 1979).

Hence, the ethylene biosynthetic pathway in plants appears to be:



The first enzyme involved in this pathway is methionine adenosyltransferase which catalyses the conversion of methionine to SAM (CHOU, COULTER, LOMBARDINI, SUFRIN AND TALALAY, 1977). The conversion of SAM TO ACC is controlled by ACC synthetase, an enzyme specific for SAM with a molecular weight of 55,000 (BOLLER, HERNER AND KENDE, 1979). From a series of experiments, ACC synthetase is believed to be a pyridoxal enzyme (YU, ADAMS AND YANG, 1979). This enzyme group facilitates the elimination of a proton from the α-carbon and an amino acid yielding a carbonium ion. In the case of SAM, the positive sulphonium ion destabilizes the molecule thermodynamically thereby causing an intramolecular nucleophilic displacement reaction by the carbonium yielding ACC and MTA (YANG AND HOFFMAN, 1984). As SAM provides a positive sulphonium ion to facilitate the γ-elimination reaction in the formation of ACC, the SAM step is again shown to be an important development in ethylene biosynthesis.

In the ACC to ethylene transformation, the mechanistic details are still unclarified. ADAMS AND YANG (1979) initially postulated that oxidase-derived hydrogen peroxide cleaves the cyclopropane ring of ACC. This releases a two carbon fragment, ethylene. The remainder of the ACC molecule forms carbon dioxide, formic acid and ammonia. PIRRUNG (1983) explored the feasibility of three alternate mechanisms for ACC to ethylene biosynthesis. These involve either a nitrene, nitrenium ion and radical cation intermediates. The lack of stereospecificity rejected the intermediacy of a nitrene or a nitrenium ion. Thus it appears that ACC is converted into ethylene through an amine radical cation via two one-electron oxidation steps. The loss of the stereochemistry is due to the free rotation of the carbon to carbon bond that evolves ethylene. Despite many uncertainties, PIRRUNG's (1983) model has gained support as it correctly predicts that cyanide, not formate, is a biosynthetic product. This cyanide is immediately incorporated into asparagine.

This conversion of ACC into ethylene is catalysed by a membrane-bound enzyme, which has been designated the name, ethylene-forming enzyme (EFE)(HOFFMAN, YANG, ICHIHARA AND SAKAMURA, 1982). It was proposed by JOHN (1983) that this enzyme acted by being asymmetrically organised in the plasma membrane of plant cells. Thus the generation of ethylene from ACC is coupled to an electrogenic flow of protons into the plant cell. This hypothesis was based on the dependency of this reaction on cell and membrane integrity (LIEBERMAN, 1979). Additionally a protonophore, 2,4 dinitrophenol, inhibits the reaction (YU, ADAMS AND YANG, 1980). However, there is much evidence which does not support

this hypothesis (BOROCHOV AND ADAM, 1984). The addition of ATP was found to be a potent inhibitor rather than a stimulator of EFE activity. EFE activity is preserved in a membrane fraction and the loss of membrane integrity as a result of detergent-induced solubilisation of the membrane had no effect on EFE activity. Thus the model proposed by JOHN (1983) cannot be accepted. The role of an *in vivo* transmembrane potential is now thought to be the transport mechanism of ACC into the cell compartment (BOROCHOV AND ADAM, 1984).

With the pathway and most of the mechanistic details of ethylene biosynthesis established, attention focused on the regulation of ethylene biosynthesis. Rates of ethylene production during the development of higher plants varies from organ to organ and time of development (ABELES, 1973). In the carnation cut flower, ethylene production occurs in three phases; the first being a low steady rate which accelerates into a second maximum emanation. This rise in ethylene production signals the terminal stage of flower senescence after which in the last stage, the level of this plant growth regulator declines. It thus appears that the ethylene content of the flower is precisely controlled. This is expected,especially as ethylene regulates such a variety of processes at different times in the life cycle of plants and the cut flower passes through many developmental phases whilst senescing.

To determine the control of ethylene biosynthesis from a cut carnation flower, changes in the levels of endogenous ethylene precursors were examined and their effect on ethylene production quantified. In carnation petals, at the onset of senescence, the autocatalytic ethylene

production was accompanied by a rapid increase in ACC content. The amount of ACC present is almost proportional to the amount of ethylene produced. Thus it is considered that ACC synthesis is the only limiting enzyme in the ethylene biosynthetic pathway (BUFLER, MOR, REID AND YANG, 1980). Many of these experiments were adapted from previous studies on ripening fruits and mung bean hypocotyl segments (ADAMS AND YANG, 1977; 1979; IMASEKI, 1983). Although this autocatalytic ethylene rise later decreased, the ACC content remained high. In this case it was presumed that the ethylene-forming enzyme (EFE) is inactivated due to the deterioration of cells whereas ACC synthetase remains active (BUFLER, MOR, REID AND YANG, 1980). Young carnation petals are incapable of converting ACC to ethylene (MAYAK, LEGGE AND THOMPSON, 1981), and as the methionine conversion to ACC does not appear rate limiting for ethylene production (BOLLER, HERNER AND KENDE, 1979), these observations suggest that these two ethylene biosynthetic enzyme systems are not active within young carnation petal tissue. This may be due to the enzyme not being synthesised or being inhibited. The evidence presented here suggests that ethylene biosynthesis is regulated by the induction and repression of ethylene biosynthetic enzymes, especially ACC synthetase. Thus senescence could be controlled by the induction of these enzymes producing ethylene.

The greatest stimuli of ethylene biosynthesis during the postharvest period of a cut flower are from mechanical injury (harvesting the flower), temperature extremes, insect infestation, disease and chemicals (ABELES, 1973). The stress created induces the synthesis of ACC synthase which, in turn, causes a rapid accumulation of ACC and a

marked increase in ethylene production (YU AND YANG, 1980; BOLLER AND KENDE, 1980; KONZE AND KWIATKOWSKI, 1981; WANG AND ADAMS, 1982). This 'stress ethylene' in the cut carnation may be the peak ethylene emanation prior to petal senescence. The time lapse from harvest to this ethylene emanation probably is the period needed for a stress situation to arise within the flower.

This time lapse could be the period taken for a regulatory substance to accumulate and thus stimulate ethylene biosynthesis. The exogenous application of auxins and cytokinins stimulates ethylene production (ABELES, 1972; LAU AND YANG, 1975; LIEBERMAN, 1979; IMASEKI, 1983). In auxin-treated tissues, auxin induces continuous *de novo* synthesis of ACC synthase and the increasing enzyme supplies an increasing amount of ACC, the immediate precursor of ethylene (KANG, NEWCOMB AND BURG, 1971; YOSHII AND IMASEKI, 1981). The auxin promotion of ethylene production thus explains the similarity of growth responses by these two plant hormones in plant systems (MORGAN AND HALL, 1964).

There are two schools of thought on the mechanism by which cytokinins modify ethylene production. LAU AND YANG (1973) propose that kinetin stimulates ethylene production by preventing indoleacetic acid conjugating mainly into indoleacetylaspartic acid, which is inactive in inducing ethylene production (ABELES AND RUBINSTEIN, 1964; BURG AND BURG, 1968; CHADWICK AND BURG, 1970). They showed that kinetin enhanced indoleacetic acid uptake and suppressed the conversion of indoleacetic acid to indoleacetic acid conjugates. Confirming this hypothesis, a close relationship was demonstrated between the rate of ethylene

production and the level of free indoleacetic acid, which was regulated by kinetin.

On the other hand, benzyladenine has been shown to increase ethylene production several fold without altering the ACC content (IMASEKI, 1983). Cytokinin action is thus thought to stimulate the formation of ethylene from ACC, a mechanism differing from auxin-ethylene evolution. The effectiveness of this cytokinin action on ethylene production, however, is greater with lower ACC concentrations supplied. Moreover, benzyladenine stimulates the ACC-forming activity so that, although ethylene production increases, the ACC content of the tissue remains constant (IMASEKI, 1983). In spite of the disagreement as to the regulatory role of cytokinin on ethylene biosynthesis, both schools of thought have observed an increase in ethylene evolution with cytokinin application.

Overall, the measurement of ethylene levels from a cut carnation flower suggests that ethylene biosynthesis is finely regulated to a definite pattern. Although ethylene is an extremely soluble gas it is not translocated around the plant in significant quantities (JERIE, ZERONI AND HALL, 1978). Yet application of ethylene to one plant part can influence ethylene production in another distant part. The mechanism by which this occurs is unknown but it seems likely that a compound is produced in response to ethylene treatment which is involved in ethylene production elsewhere and must, presumably, be translocated (SMITH AND HALL, 1984). ACC has been shown to be produced in one plant part and translocated to another where it leads to increased ethylene biosynthesis

(AMRHEIN, BRENING, EBERLE, SKORUPKA AND TOPHOF, 1982). NICHOLS (1977a) showed that the rate of ethylene biosynthesis differs with carnation flower part. The styles and the lower portion of the petals produce the most ethylene. The significance, however, of controlled ethylene biosynthesis in the cut carnation flower will remain unknown until the action of ethylene is clarified.

Whilst vast progress has been made in the area of ethylene biosynthesis, our understanding of ethylene action has remained obscure. The majority of evidence on ethylene action is of secondary events, the result of some primary mechanism(s). Prior to 1970, the work of BURG AND BURG (1965; 1967) represented the only significant attempt to probe ethylene action at the primary level. To mimic the biological action of ethylene, they found that structurally the compound must possess a terminal carbon atom adjacent to an unsaturated bond. This biological action is inversely related to molecular size and substituents which lower the electron density in the unsaturated position and reduce activity. Since these requirements for ethylene-like activity closely resemble those for metal-olefin binding, they postulated that *in vivo* ethylene binds to a metallic receptor site. As the silver ion has long been utilised to complex with olefins in gas chromatography (BEDNAS AND RUSSEL, 1958; ATKINSON, RUSSEL AND STUART, 1967), the ethylene-action site requirements were first thought to be similar to those governing silver-olefin complexes. On analysis the copper I ion, being a soft metal, has more evidence supporting it as the metal complex involved (BURG AND BURG, 1967; BEYER, 1979). Indeed, reagents known to inhibit the copper-containing enzymes prevented ethylene binding (SISLER, 1982).

This evidence is inconclusive as the softness of a metal is determined not only by its valence state but also by ligands previously bound. Presumably in a protein, soft ligands are available which could soften other metals besides copper.

Further information on this binding site was gained by making use of the gaseous property of ethylene. The binding of ethylene *in vivo* can be measured by initially exposing the tissue to labelled ethylene, allowing the unbound ethylene to diffuse away by flushing the tissue with unlabelled ethylene and then measuring the amount of labelled ethylene that remains bound within the tissue (SISLER AND GOREN, 1981). ABELES (1973) estimated that only 0,000001 percent of the total plant tissue biomass contained ethylene receptor sites. Accurate determination quantified the bound ethylene at 0,27 $\mu\ell\ l^{-1}$ in tobacco leaves; 0,03 $\mu\ell\ l^{-1}$ in tomato fruits; 0,20 $\mu\ell\ l^{-1}$ in bean leaves and 0,15 $\mu\ell\ l^{-1}$ in citrus leaves (SISLER AND GOREN, 1981). As yet no biological proof exists that this binding of the labelled ethylene in the above measurements is to a physiologically active receptor. Sites of this ethylene-binding in the cell have been located in the extracted membrane fraction (SISLER, 1980). HALL, EVANS, SMITH, TAYLOR AND AL-MUTAWA (1982) suggested that the endoplasmic reticulum is the locus of ethylene-binding. Wherever the locus of ethylene-binding, SISLER, REID AND FUJINO (1983), proposed that once the plant growth regulator binds to this site, this reaction releases a 'second-messenger' molecule. This results in the transcription of new mRNA material from the genome. The proteins coded by this RNA are the enzymes which cause the symptoms of ethylene treatment. The ethylene

diffuses from the tissue or is destroyed (SISLER AND GOREN, 1981).

Ethylene was originally considered inert (McGLASSON, 1970; ABELES, 1972; 1973; VARNER AND HO, 1976) due to inadequate precautions taken with regard to the radiochemical purity of ethylene and microbial contamination. Once these interferences were eradicated, BEYER (1975b) demonstrated that ethylene metabolism is an integral part of ethylene biochemistry. Hence the significance of this ethylene metabolism in ethylene action is a major question to be considered.

In *Pisum sativum*, ethylene is metabolised by two apparently separate systems, one leading to the formation of carbon dioxide whilst the other leads to the incorporation of ethylene into unknown components in the tissue (BEYER, 1975b). As BEYER (1977) considered ethylene to be a natural senescence accelerator in cut carnation flowers, he further investigated ethylene metabolism in relation to ethylene action in this system. Ethylene oxidation was coupled with the later stages of senescence, following the natural surge of ethylene. Tissue incorporation, however, occurred at an earlier stage when ethylene oxidation maintained a low level. Of the different flower parts, the reproductive and receptacle tissues showed the greatest ethylene metabolite incorporation. Accounting for this difference in ethylene metabolism, ethylene oxidation and ethylene incorporation may well have independent physiological roles that are interrelated but not necessarily coupled.

There are three possible roles of ethylene metabolism in plants. Firstly, ethylene metabolism may remove ethylene from the system;

secondly, the oxidation of the hormone at the binding site may be the requirement for ethylene action and lastly, the product(s) of ethylene may serve to alter tissue sensitivity to ethylene.

Regulating endogenous ethylene levels by metabolism does not appear to be the purpose of this process. Indeed, only a small percentage of ethylene normally synthesised by plant tissue is metabolically degraded (BEYER, 1981) and when this degradation occurs it does not correlate with periods of peak ethylene production, a time when ethylene removal or detoxification would seem needed (BEYER AND SUNDIN,1978). Even the fact that ethylene rapidly diffuses from the tissue would make such an inactivation system superfluous.

Conflicting evidence, however, surrounds the hypothesis that ethylene metabolism is required for the physiological responses of ethylene. Evidence against this hypothesis includes the unrelated dose-response curve of ethylene action and metabolism above an ethylene concentration of 10 $\mu\ell\ l^{-1}$ (BEYER, 1984). Additionally propylene, although structurally similar to ethylene, is metabolised far more rapidly than ethylene and yet is about a hundred times less effective physiologically than ethylene (BEYER, 1978). These latter observations cast doubts on the metabolism-action ethylene relationship in plant tissue (DODDS AND HALL, 1982; SISLER AND YANG, 1983). Counteracting this negative evidence are a number of correlations between changes in tissue sensitivity and ethylene metabolism (BEYER, 1978; 1979). For example, ethylene oxidation increases when *Ipomaea* petals become responsive to ethylene action (BEYER AND SUNDIN,1978). A quantitative

relationship also exists between an ethylene action antagonist, the silver ion, and ethylene action and metabolism (BEYER, 1979; EVANS, SMITH, TAYLOR AND HALL, 1985). In peas, silver ion application reduced the growth inhibition response and ethylene metabolism in a parallel fashion. Another antagonist of ethylene action, carbon dioxide, also reduces ethylene metabolism.

A final role of ethylene metabolism to be considered in plants concerns the products of this biochemical process altering tissue sensitivity to ethylene. Ethylene oxide has recently been shown to increase tissue sensitivity to ethylene (BEYER, 1984). Ethylene oxide will not mimic ethylene action when applied alone but the synergistic effect of ethylene and ethylene oxide has been positively demonstrated in the 'triple response' in peas, leaf abscission in cotton and growth stimulation in rice. This raises the question as to whether or not products of ethylene metabolism, especially ethylene oxide, may regulate tissue sensitivity to ethylene.

BURG AND BURG (1965) originally proposed that ethylene action involves the binding of ethylene and oxygen to the metal of a metalloenzyme, ethylene being a dissociable activator molecule. From the evidence presented above, however, ethylene binding and metabolism appear closely related. This was further substantiated by the recent work of GOREN, MATTOO AND ANDERSON (1984) who again using the silver ion, a potent inhibitor of ethylene, found that ethylene binding in tobacco leaves was prevented as was chlorophyll degradation. The silver ion, as previously discussed, also prevents ethylene metabolism in

peas (BEYER, 1979; EVANS, SMITH, TAYLOR AND HALL, 1985). Thus to incorporate ethylene metabolism into the hypothesis of BURG AND BURG (1965), BEYER (1984) modified their hypothesis so that initially ethylene oxide was formed which then initiated the ethylene oxidation and action sequence. In this manner the evidence for ethylene action from binding sites and ethylene metabolism can be reconciled.

With due respect, although much progress has been made to identify the primary site of ethylene action and its mechanism, this is an area of much needed research. Likewise, the detailed events by which an ethylene-induced growth response occurs also remains unknown. The difficulty with analysing ethylene action in a senescence system is that this developmental process entwines the simultaneous degradation and synthesis of vital cellular constituents. This metabolic conflict between the degradative hydrolysis of cell wall and storage polysaccharides and large increases in cell permeability (SACHER, 1966) with the increase in RNA and protein synthesis (RICHMOND AND BIALE, 1966; FRENKEL, KLEIN AND DILLEY, 1968; HULME, RHODES, GALLIARD AND WOOLTORTON, 1968; HULME, RHODES AND WOOLTORTON, 1971) are features in common with stress phenomena. Plants respond to stress conditions by the initiation of repair processes, which occur simultaneously with the degradative reactions. Thus increases in RNA and protein synthesis, beginning shortly after ethylene treatment, may be the result of ethylene triggering a degradative response with the ensuing RNA and protein synthesis merely representing a secondary repair mechanism (McGLASSON, WADE AND ADATO, 1978). It is with this concept in mind that the primary action of ethylene in plant tissues will be considered.

A possible avenue of plant hormone action could be via enzyme induction. Using cycloheximide, an inhibitor of protein synthesis, enzyme synthesis as well as the action of ethylene was prevented (RHODES, WOOLTORTON, GALLIARD AND HULME, 1968). This confirms that although ethylene accelerates the loss of protein in senescing tissues, certain enzymes are synthesised. Supporting this hypothesis, BUFLER, ROMANI AND REID (1983) found that the protein synthetic capacity of all the carnation flower parts, as determined by their polysomal state, initially decreased when the flower was cut. On the ethylene surge, however, the polysomal population increased. This confirms that increased protein synthesis is coincident with ethylene production and the senescence of cut carnation flowers.

In 1973, ABELES reviewed the literature pertaining to ethylene stimulation of enzyme induction. Comparing ethylene-treated tissues with controls, greater quantities of the following enzymes have been found: acid phosphatase, ATPase, α-amylase, catalase, cellulase, chitinase, chlorophyllase, cinnamic acid 4-hydroxylase, cytochrome c reductase, diaphorase, β-1,3-glucanase, invertase, malic enzyme, pectin esterase, peroxidase, phenylalanine, ammonia lyase, polygalacturonase, polyphenol oxidase, protease and pyruvic carboxylase. This enzyme induction is not always a direct result of ethylene action as was shown with malic enzyme, β-1,3-glucanase, phenylalanine ammonia-lyase and peroxidase. Unfortunately, as promising as this information appears, the direct biochemical involvement of ethylene in enzyme induction has not been elucidated and hence remains open for confirmation. Concerning enzyme activity, as yet no direct ethylene effect has been detected.

Invertase, α-amylase (ENGLES AND ZANNIS, 1930), peroxidase (RIDGE AND OSBORNE, 1970) and adenosine triphosphatase (OLSON AND SPENCER, 1968) are a few of the enzymes that were unaffected by ethylene.

Indirect evidence for greater enzymatic activity in the presence of ethylene has been implicated in the regulation of several physiological processes that, for their initiation, required cell wall dissolution and cell lysis through an increased production or release of hydrolytic enzymes (MATILE, 1975). These processes include abscission of leaves (JACKSON AND OSBORNE, 1970; SAGEE, GOREN AND RIOV, 1980) and flower buds (WONG AND OSBORNE, 1978; CAMERON AND REID, 1983); abscission and dehiscence of fruits (JACKSON, MORROW AND OSBORNE, 1972; LIPE AND MORGAN, 1972), the softening and maturation of fruits (PRATT AND GOESCHL, 1969; LIEBERMAN, 1975); and the development of gas-filled cavities in the stems of herbaceous dicotyledons (KAWASE, 1974; 1978). This property of ethylene was also seen in the formation of aerenchyma in *Zea* roots (DREW, JACKSON, GIFFARD AND CAMPBELL, 1981). Thus it appears that ethylene stimulates the activity or production of these hydrolytic enzymes but this is only a supposition until further investigation.

An alternate course of ethylene action could be via adjusting membrane permeability. As senescence includes processes leading to cell disorganization, ethylene could initiate these senescence processes. Cell order is generally maintained by spatial separation of reactants by partially permeable protoplasmic membranes. With ethylene being lipid-soluble and a large portion of membranes consisting of lipid,

ethylene could directly effect membrane permeability. The most obvious change in membrane permeability occurs during fruit ripening when high levels of ethylene are emitted. Yet evidence suggests that membrane permeability is a result of ripening and not the cause (BRADY, O'CONNELL, PALMER AND SMILLIE, 1970). Augmenting this conclusion is the fact that carbon monoxide, an ethylene analog, does not share lipid solubility characteristics but has the same physiological activity. In carnation petals the ethylene rise occurs before the onset of tonoplast permeability changes (MAYAK, VAADIA AND DILLEY, 1977). This increase in petal tonoplast permeability is accompanied by a massive loss of phospholipids (BOROCHOV, HALEVY, BOROCHOV AND SHINITZKY, 1978; SUTTLE AND KENDE, 1978). It has thus been concluded that ethylene does not directly affect membrane integrity, but rather indirectly via its effect on cellular metabolism and phospholipid loss (HALEVY AND MAYAK, 1981).

The question of the primary action of ethylene in the senescence of the cut carnation remains unknown. The marked senescence-accelerating effect exogenous ethylene has on the carnation flower indicates the major role this plant hormone occupies in carnation flower postharvest physiology. Evidence suggests that the primary action of ethylene is via inducing enzyme synthesis. The generalisation in such a statement, however, bringsone no closer to the secret of ethylene's biochemical action. In fact at this point, if it were not for the noteworthy response of the carnation flower to ethylene, it would be tempting to suggest that ethylene's autocatalytic surge is a result of the senescence

process, the signal of the final disintegration of cellular organisation. Indeed, the mere sight of the carnation flower, on the one hand, possessing petals that are irreversibly wilting towards their death whilst on the other hand, the ovary is developing and forming seeds, forces one to question ethylene as the sole regulator of this process. It has been previously noted that the auxins and cytokinins stimulate ethylene biosynthesis. Even so, such a hormonal interaction does not explain the physical developmental conflict apparent within the carnation flower.

Cytokinins

Severing the flower from the parent plant removes it from a constant source of compounds that may play a major role in the control of senescence. One chemical constituent thought to be greatly limited by the isolation of the flower are the cytokinins. Much controversy surrounds the sites of cytokinin biosynthesis but present evidence has only conclusively indicated that the root systems of plants produce cytokinins (BURROWS AND CARR, 1970; WHEELER, 1971; ENGELBRECHT, 1972; KRIESEL, 1976; WANG, THOMPSON AND HORGAN, 1977; VAN STADEN AND DAVEY, 1979). If this organ were the sole source of cytokinins, only those cytokinins within the flower explant at harvest would be available to influence growth responses under cytokinin regulation. Yet genetically speaking, it must be realised that all living plant material has the capacity to synthesise this hormone. Buds, seeds and fruits, because of their high cytokinin content, have also been proposed as sites of cytokinin synthesis at some stage in their development (VAN STADEN AND DAVEY, 1979). To date this hypothesis has not been accepted one way or another. Failure to trace the incorporation

of labelled adenine, the cytokinin precursor, into cytokinins in young developing fruits of *Lupinus albus* (VAN STADEN AND CHOVEAUX, 1981), *Phaseolus vulgaris* leaf explants (VAN STADEN AND FORSYTH, 1985) and even excised maize roots (VAN STADEN AND FORSYTH, 1984a) on the one hand, whilst being successful with tobacco cell cultures (NISHINARI AND SYÔNO, 1980); crown-gall tissue from *Vinca rosea* (STUCHBURY, PALNI, HORGAN AND WAREING, 1979; PALNI, HORGAN, DARREL, STUCHBURY AND WAREING, 1983) and in excised tomato roots (VAN STADEN AND FORSYTH, 1984b), on the other hand, has created a dilemma resulting in an inconclusive situation. Nevertheless, as the endogenous cytokinin levels decrease from the time the carnation flower is harvested (VAN STADEN AND DIMALLA, 1980) this would suggest that flower removal dissociates it from any cytokinin input.

Supplements of kinetin (MAYAK AND DILLEY, 1976; MAYAK AND KOFRANEK, 1976), benzyladenine (MACLEAN AND DEDOLPH, 1962; HEIDE AND OYDVIN, 1969) or isopentenyl adenine (MAYAK AND KOFRANEK, 1976) in the holding solution of the cut carnation flower extend their longevity. The effectiveness of these treatments, however, depends on the concentration applied and environmental conditions. High concentrations or a prolonged weak cytokinin pulse are detrimental to longevity (WATERS, 1964; HALEVY AND WITTWER, 1965; HEIDE AND OYDVIN, 1969). Environmentally, MAYAK AND DILLEY (1976) found that the extension of flower longevity by kinetin appears to depend on the light energy prevailing during anthesis. Overcoming these problems, cytokinin-treated carnations have a reduced sensitivity to exogenous ethylene (MAYAK AND DILLEY, 1976; EISINGER, 1977) and have a delayed and

reduced peak of ethylene production (EISINGER, 1977). It is via this action on ethylene biochemistry that cytokinins are considered to extend carnation flower longevity (MACLEAN AND DEDOLPH, 1962; HEIDE AND OYDVIN, 1969; MAYAK AND DILLEY, 1976; MAYAK AND KOFRANEK, 1976; EISINGER, 1977; COOK, RASCHE AND EISINGER, 1985).

COOK, RASCHE AND EISINGER (1985) showed that the decrease in ethylene biosynthesis,when carnation flowers are treated with benzyladenine,is due to a decrease in the endogenous ACC level and a reduced capacity of the tissues to convert ACC to ethylene. Similar results have been reported by MOR, SPIEGELSTEIN AND HALEVY (1983) based on their work with isolated petals. These results, however, conflict with previously discussed work on the regulation of ethylene biosynthesis by cytokinins in mung bean hypocotyls (LAU AND YANG, 1973; IMASEKI, 1983). In this case ethylene biosynthesis was enhanced by cytokinin application. In terms of benzyladenine movement within the cut carnation flower, ethylene emanation may be determined by the flower part to which this cytokinin is translocated. Basally applied labelled cytokinin mainly remains within the stem with a small portion flushing in and out of the petals and a slight accumulation occurring in the ovary (KELLY, 1982). This stem accumulation of cytokinins is interesting in that EISINGER (1977) found that carnation flowers with shorter stem lengths senesced faster than those with longer stems. Exogenously supplementing the cytokinins of the short stemmed flower extended their longevity so that they senesced simultaneously with the long stemmed flowers. Root originating cytokinins were thus concluded to regulate senescence because shorter stems would have less cytokinins.

This stem accumulation of cytokinins may provide a constant source of cytokinins for the flower.

The potential of cytokinins in maintaining flower or petal longevity was recognised in the work of MAYAK AND HALEVY (1970). Using rose petals they demonstrated a higher endogenous cytokinin activity in young petals than in older ones. Also a short-lived rose variety had lower levels of cytokinins than a longer lasting variety, and adding cytokinin delayed senescence of the short lived variety. Additionally, VAN STADEN AND DIMALLA (1980), determining endogenous cytokinin levels of ageing carnation flowers, found that levels dropped during petal senescence. Thus in the carnation flower, the cytokinins appear to play a 'protective' role against senescence rather than being solely responsible for the stimulation of ethylene emanation. The concept that cytokinins are involved in delaying senescence is well known. Application of a droplet of cytokinin to a shaded leaf prevented yellowing in the region of the droplet, producing, what has been termed, the 'green island effect' (RICHMOND AND LANG, 1957). During leaf senescence this loss of chlorophyll is a secondary step. The cytokinins stop the degradation of chlorophyll by maintaining the RNA and soluble protein levels, stabilising the polysome aggregates and suppressing the changes in respiratory rate and mitochondrial coupling normally associated with senescence (TETLEY AND THIMANN, 1974). The role of cytokinins is thus generally seen as a sustaining factor for cellular protein synthesis rather than a triggering molecule especially as cytokinins raise amino acid incorporation.

By definition, cytokinins are also known as promotory factors of cell division in tissue culture. SKOOG AND MILLER (1957) found that the addition of auxin and nutrients to undifferentiated tobacco pith callus stimulated few cell divisions and no cellular differentiation. The addition of the purine base, adenine, along with auxins resulted in the cells dividing and a large cellular mass being created.

Furthermore, entwined with the stimulation of cell division and the senescence-delaying action of cytokinins, this group of plant hormones is known to be involved in nutrient mobilisation. In fact, after detecting that the endogenous cytokinin levels within the carnation ovary actively increased during natural senescence, VAN STADEN AND DIMALLA (1980) theorised that the increased localisation of cytokinin in the ovary created a preferential sink for nutrients and carbohydrates. This would result in the characteristic ovary dry mass increase that occurs during carnation flower senescence (NICHOLS, 1976; DIMALLA AND VAN STADEN, 1980).

The line between the stimulation of growth and the mobilisation of nutrients needed for this growth is very fine. Cytokinin application to a detached leaf creates the 'green island' effect as well as attracting amino acids, namely glycine, via intracellular transport from the surrounding untreated tissue (MOTHES, ENGELBRECHT AND KULAJEVA, 1959). A long distance action of cytokinin, mobilising glycine from one primary bean leaf to another (LEOPOLD AND KAWASE, 1964) through the phloem (MULLER AND LEOPOLD, 1966) has also been demonstrated. Whether this amino acid movement, however, is a result of direct

cytokinin control or due to the retardation of the senescence process within the sink tissue is questionable. Kinetin mobilises non-proteinogenic amino acids (MOTHES, ENGELBRECHT AND SCHÜTTE, 1961) inferring that an amino acid concentration gradient is not necessary for their continued translocation. On the other hand, OSBORNE (1962) showed that senescence can be retarded in a system where amino acid directed transport is prevented. Therefore, although cytokinins effectively mobilise amino acids, especially in isolated organs, the mechanism of action is unknown.

NICHOLS (1976) showed that ovary size increased during flower senescence due to cell enlargement with no further cell division. In the light of this information, the increase in endogenous cytokinins in the ovary is not to stimulate cell division. With the most prevalent feature of senescing plant organs being gradual loss of RNA and, as a consequence of this, reduced protein synthesis, cytokinin action in the ovary could be involved in sustaining cellular protein synthesis (OSBORNE, 1962) and mobilising the amino acids needed for this cellular enlargement. Cytokinins have also been suggested to be active in carbohydrate mobilisation. PATRICK, JOHNSTONE AND WAREING (1979) showed that kinetin placed on the decapitated stem of *Phaseolus vulgaris*, mobilised sucrose towards it. In tomato plants, adverse light conditions cause the cytokinin levels to drop, which in parallel caused a decrease in assimilate import (LEONARD AND KINET, 1982). The limiting step in assimilate import in cytokinin deficient tissues appears to be the hydrolysis of sucrose (HO, 1984). As yet, a relationship between a hydrolysing enzyme, such as invertase, and

cytokinin action has not uniformly emerged from different plant tissues (MORRIS AND ARTHUR, 1984). Further work is needed here before any conclusion can be drawn.

On the other hand, cytokinins and carbohydrates are closely related during translocation. The cytokinin riboside form is more soluble than the adenine derivative and the storage form is a cytokinin-o-glucoside. Moreover, the movement of excess cytokinins from the point of application appears to imitate that of assimilate translocation. Organs that contain high levels of cytokinin activity, such as fruits and their seeds (LETHAM, 1973; 1974; KOSHIMIZU, KUSAKI, MITSUI AND MATSUBARA, 1967; VAN STADEN, DAVEY AND BROWN, 1982) are also strong assimilate sinks during their development (WARDLAW, 1968). Buds, roots and the cambium show a similar high cytokinin, high assimilate utilisation relationship (VAN STADEN AND DAVEY, 1979). HUTTON (1982) found that radioactive zeatin moved passively with the assimilate stream in the phloem. Reinforcement of this theory was demonstrated by foliar application of labelled benzyladenine and monitoring its translocation. Radioactivity was detected in the actively growing regions, the highest being in young leaves which may act as a sink or be 'waiting' to pass the cytokinins into the apical bud (ABO-HAMED, COLLIN AND HARDWICK, 1984) resembling assimilate movement. It may thus be this close cytokinin-carbohydrate relationship that causes the influx of cytokinins into the ovary during ovary enlargement instead of the alternative 'attraction' of carbohydrates by cytokinins. Interesting is the observation that for a kinetin treatment to extend the longevity of a carnation flower, after being exposed to periods of

low light intensity during winter, it must be supplemented with sucrose (MAYAK AND DILLEY, 1976).

Thus far the cytokinins have been considered as a group of the plant hormones. Extraction and identification by a soybean callus bioassay, however, has shown that the carnation flower contains cytokinin compounds that coelute with zeatin, zeatin riboside, zeatin glucoside, isopentyladenine and isopentyl adenosine (VAN STADEN AND DIMALLA, 1980). Each of these compounds probably differs in their activity within the carnation flower as a selection of cytokinin metabolites have shown varied responses on soybean callus tissue (PALNI, PALMER AND LETHAM, 1984). The activity sequence found in soybean callus tissue was: zeatin riboside > zeatin > o-glucosides of zeatin and zeatin riboside and their dihydro derivatives > lupinic acid (an alanine conjugate of zeatin) > 7- and 9-glucosides of zeatin which were almost inactive. Additionally, their metabolic rate may also be important for their activity. The cleavage of the N^6 side chain of cytokinins by oxidation is the dominant form of cytokinin metabolism observed in soybean callus. KELLY (1982) analysed labelled benzyladenine metabolism in the carnation and found that after the flower aged a day, forty percent of this compound had metabolised into two unidentified products. This capacity for benzyladenine metabolism was universal within the flower tissues. To date, this is the sole cytokinin metabolism investigation in the cut carnation flower accentuating the need for further knowledge in this area of cytokinin biochemistry.

KELLY (1982) proposed that the role of cytokinins accumulating in

the ovary may be to induce ethylene production (LAU AND YANG, 1975). This ethylene emanation would cause the ovary to swell since this physiological response can be induced by exogenous ethylene (NICHOLS AND HO, 1975a). He argued that inhibitors of ethylene production, such as silver thiosulphate, inhibit the swelling of the ovary (MOR, REID AND KOFRANEK, 1980). This silver thiosulphate treatment also prevents the natural rise of cytokinins within the ovary (VAN STADEN AND DIMALLA, 1980). KELLY (1982), furthermore, has not accounted for the varied physiological response between the different flower parts, especially the petals and ovary, during flower senescence. MAYAK AND HALEVY (1970) considered the cytokinins to have a 'protective' role in the flower against senescence. The high cytokinin levels in the ovary could sustain cellular protein synthesis, mobilise the needed amino acids and carbohydrates, as well as blocking ethylene action. The decreasing cytokinin levels in the petals would not sustain these metabolic activities and hence allow irreversible petal wilting to occur. As cytokinin-treated carnations have been shown to have a reduced sensitivity to exogenous ethylene (MAYAK AND DILLEY, 1976; EISINGER, 1977), this 'protective' role of cytokinins may be against ethylene action. This hypothesis, however, does not account for the cytokinin-ethylene production relationship outlined by KELLY (1982).

Auxin

Previously it was noted that auxin promotes ethylene production (MORGAN AND HALL, 1964; BURG AND BURG, 1966; LIEBERMAN, 1979; YOSHI AND IMASEKI, 1981). Likewise, kinetin was also shown to increase ethylene production (LAU AND YANG, 1973; IMASEKI, 1983). This kinetin action occurs indirectly via protecting the metabolism of indoleacetic

acid, the levels of which determine ethylene emanation (ABELES AND RUBINSTEIN, 1964; BURG AND BURG, 1968; CHADWICK AND BURG, 1970; KANG, NEWCOMB AND BURG, 1971). Exogenously applied indoleacetic acid rapidly conjugates to form indoleacetylaspartic acid, a compound inactive in inducing ethylene production. Kinetin markedly suppresses this indoleacetic acid conjugation resulting in a higher level of free indoleacetic acid and a lower level of indoleacetic acid conjugates (LAU AND YANG, 1973). Additionally SCHNEIDER AND WIGHTMAN (1978) stated that kinetin also repressed the formation of the isoenzymes of indoleacetic acid oxidase and peroxidase. On further investigation (GASPAR AND XHAUFFLAIRE, 1967; JAIN, KADKADE AND HUYSSE, 1969; LEE, 1971; 1974), however, little correlation between the capacity of kinetin to increase endogenous indoleacetic acid levels and to inhibit indoleacetic acid-oxidase has been found (NOOR SALEH, 1981). Alternatively, the movement of indoleacetic acid to the site of kinetin application could also be contributing to the increased levels of this hormone (LAU AND YANG, 1973). Nevertheless, despite the needed confirmation of the biochemical relationship between the auxins and cytokinins, it is well recognised that high levels of indoleacetic acid are maintained with kinetin treatment (FUCHS AND LIEBERMAN, 1968; NOOR SALEH AND HEMBERG, 1980). Relating this to the cut carnation flower, the increased cytokinin activity within the ovary may well be maintaining high auxin levels; whilst in the petals, the reduced cytokinin levels would allow for auxin metabolism.

Ethylene reduces the endogenous indoleacetic acid levels in different plant tissues (ERNEST AND VALDOVINUS, 1971; BEYER, 1975a;

LIEBERMAN AND KNEGT, 1977). This reduction may result from increased conjugation and decarboxylation (BEYER AND MORGAN, 1969; ERNEST AND VALDOVINUS, 1971; MINATO AND OKAZAWA, 1979; RIOV AND GOREN, 1979), decreased synthesis (ERNEST AND VALDOVINUS, 1971) and reduced transport from the site of synthesis (BEYER, 1973). The majority of evidence supports ethylene increasing the conjugation of indoleacetic acid (RIOV, DROR AND GOREN, 1982), yet this may not be a direct effect of ethylene but rather a result of greater indoleacetic acid accumulation in ethylene-pretreated tissues (BEYER AND MORGAN, 1969). Thus the ethylene emanation that occurs immediately prior to carnation petal senescence probably reduces the levels of indoleacetic acid save where the endogenous cytokinin levels are high. This hypothesis is suggesting that auxin levels in different flower parts, protected from metabolism by a high cytokinin content, may be important in carnation flower senescence. It would therefore appear that revealing the auxin action within these carnation tissues is crucial to understanding the senescence phenomenon in this system.

What has been revealed about auxin in relation to the carnation flower is extremely scant. In 1972, JEFFCOAT AND HARRIS, questioning the hormonal regulation of the distribution of carbon-labelled assimilates in this flower, extracted and, using the wheat coleoptile straight growth test, quantified the endogenous auxins. Even though crude in method, auxin-like substances were detected in the ovary. The presence of inhibitors were blamed for masking any auxin activity in the petals which showed positive signs for indoles by an alternate colorimetric test. Furthermore, indoleacetic acid in place

of the flower was active in increasing the proportions of labelled assimilates moving upwards from the source leaf.

Although there is much evidence to indicate that indoleacetic acid plays a regulatory role in assimilate partitioning, much debate revolves around the mechanism of this auxin action (WAREING AND PATRICK, 1975; MORRIS, 1982). This may well be due to the lack of correlating observations obtained from artificial systems of decapitated plants back to the whole plant situation (WAREING AND PATRICK, 1975). Nevertheless two views on this auxin-carbohydrate regulated transport have emerged. On the one hand, indoleacetic acid appears to act via invertase (SACHER AND GLASZIOU, 1962; SACHER, HATCH AND GLASZIOU, 1963; GLASZIOU, WALDRON AND BULL, 1966; GAYLER AND GLASZIOU, 1969; MORRIS, 1982). Sink tissues frequently contain high specific activities of invertase which has been suggested to be responsible for maintaining a sucrose gradient (MANNING AND MAW, 1975; BAKER, 1978; WALKER, HO AND BAKER, 1978; KEENER, DE MICHELLE AND SHARPES, 1979). High auxin levels have also been detected at sites of high sink activity. For example, in maize plants infested with *Ustilago maydis*, the stimulation of carbohydrate accumulation in infected tissues was correlated with both increased invertase activity (BILLETT, BILLETT AND BURNETT, 1977; CALLOW, LONG AND LITHGOW, 1980), and with a large rise in the indoleacetic acid level. Of importance is that high invertase levels bear closely with expansion growth (GIAQUINTA, 1979; MORRIS AND ARTHUR, 1984) and high auxin levels (PRESSEY AND AVANTS, 1980). Cellular expansion is the means by which the carnation ovary enlarges (NICHOLS,

1976). This, however, indicates that the problem that where hormones are effective in stimulating both growth and invertase activity, it is clearly important to determine which response occurs first. If growth precedes the observed changes in invertase then it is unlikely that the effect of the hormones on assimilate mobilisation could be mediated by a direct action of the hormone on tissue invertase levels (MORRIS, 1982). The high levels of auxin activity in the carnation ovary (JEFFCOAT AND HARRIS, 1972) may thus primarily be involved in cellular expansion with the energy requirement for this process being the cause of the increased invertase activity. VEIERSKOV AND SKYTT ANDERSEN (1982) found indoleacetic acid kept the carbohydrates available as high concentrations of low molecular weight sugars. However, using sugar cane internodal tissues, GAYLER AND GLASZIOU (1969) produced evidence that auxin application increased the enzyme-forming capacity of invertase by stabilising the mRNA for invertase.

On the other hand, a number of studies showed increased assimilate movement with indoleacetic acid application occurring in non-growing tissues. Nutrients move within six hours of indoleacetic acid treatment, a period considered too brief for the involvement of sink activity (BOOTH, MOORBY, DAVIES, JONES AND WAREING, 1962; DAVIES AND WAREING, 1965). In agreement, PATRICK AND WAREING (1973) found that respiration, protein synthesis and sucrose metabolism were unaffected when indoleacetic acid was applied to internodes of decapitated bean seedlings. They concluded that this hormone may be acting more directly on the transport process than via invertase activity. The feasibility that auxin could act on longitudinal transport processes in the

phloem (DAVIES AND WAREING, 1965; HEW, NELSON AND KROTKOV, 1967) is questionable considering auxins, being transported basipetally, must act along the whole pathway between shoot apex and source (MORRIS, 1982). Therefore although it is clear that auxins stimulate assimilate movement, the precise mechanism of its action remains obscure.

Considering auxin action in the carnation flower from an opposing angle, SACALIS AND NICHOLS (1980) found that the application of moderate levels of the synthetic auxin, 2,4-dichlorophenoxyacetic acid enhanced carnation flower senescence. As indoleacetic acid stimulates ethylene production (YOSHII AND IMASEKI, 1981) endogenous auxins were thought to naturally regulate the pattern of ethylene emanation from the carnation flower (NICHOLS, 1966). This ethylene production is directly related to the levels of precursor, ACC (HOFFMAN AND YANG, 1980). WULSTER, SACALIS AND JANES (1982) proposed that either these ACC levels fluctuated with ACC transport to the site of ethylene synthesis or from increased ACC synthesis in response to auxin movement into the petals from other areas. In an isolated petal system, indoleacetic acid treatment enhanced their senescence and increased the duration and amount of ethylene production, hence supporting the latter proposal.

Thus far, the connection between these conflicting roles of auxin action, assimilate mobilisation and the acceleration of cellular enlargement with the stimulation of ethylene and its cellular degradative action, is not forthcoming. Through fruit postharvest studies, yet another facet of auxin action and its relationship with ethylene is demonstrated which may solve this dilemma. In fruit development, the

auxins have long been recognised as senescence retardants (SACHER, 1973) as exogenous auxin applications on different fruits, with effective auxin penetration, delay the onset of ripening. This conservation of longevity occurs even with a marked ethylene production and respiratory increase (VENDRELL, 1969; FRENKEL AND DYCK, 1973). As endogenous auxin levels decline with the maturity of the fruit (COOMBE, 1960; IWAHORI, 1967; ABEL-RAHMAN, THOMAS, DOSS AND HOWELL, 1975) and ripening can be delayed with infiltrating auxin treatments, fruit ripening may then be related to a deficiency in auxin (FRENKEL, 1972). Thus in fruit systems the endogenous auxin content appears to 'protect' the tissues against ethylene action. The carnation ovary, being an immature fruit, may indeed be physiologically similar. Thus as auxin levels rise in the ovary ethylene production is stimulated which acts on the auxin-deprived petals whilst being ineffective on the ovary itself due to auxins protective role. This ovary auxin content could also be effective in mobilising assimilates and stimulating cellular expansion. However, as attractive as this hypothesis appears, petals supplemented with auxins senesced at a greater rate than untreated petals. If auxins do maintain the carbohydrates as low weight molecular sugars (VEIERSKOV AND SKYTT ANDERSEN, 1982) respiration could render the carbohydrates limiting. Whatever the mechanism of action of auxins it is apparent that the difference between petal and ovary development does not lie in a single plant hormone but the interaction of many.

The role of the remaining known hormones, although unfortunately not further investigated in this thesis, cannot be overlooked.

Exogenous application of gibberellins to the flower bud caused an increased accumulation of carbon-labelled assimilates in the bud (HARRIS, JEFFCOAT AND GARROD, 1969), a property of gibberellins that was maintained even when the flower was replaced by a drop of this hormone on the flower stem (JEFFCOAT AND HARRIS, 1972). Although difficult to quantify gibberellins, two gibberellin-like substances were extracted from the flower, substantiating their role in assimilate mobilisation (JEFFCOAT, SCOTT AND HARRIS, 1969; JEFFCOAT AND HARRIS, 1972). Indirectly related to this, gibberellins stimulate petal enlargement when the petals are isolated (GARROD AND HARRIS, 1978) and within the whole plant (NICHOLS, 1968). Even with these growth promotory properties though, exogenous gibberellin treatments have little effect on longevity (NICHOLS, 1968).

Abscisic acid, like ethylene or due to its stimulated accumulation by ethylene, antagonises the effect of the gibberellins, cytokinins and auxins (LEOPOLD AND KRIEDEMAN, 1975). Thus, it is not surprising that abscisic acid treatment accelerates carnation flower senescence (MAYAK AND DILLEY, 1976). It also may be due to its accumulation within the petals that the rose and carnation flower senesces (MAYAK AND HALEVY, 1972; RONEN AND MAYAK, 1981).

With the flower consisting of many physiological and anatomical diverse parts, indeed for this organ to function as a unit, it is essential that the growth and development of its tissues be coordinated. From this discussion, plant hormones definitely appear to be the regulators of carnation flower senescence. Nevertheless, even

with the endogenous plant hormone levels quantified, their metabolites identified and the effect of exogenous plant growth regulators applications observed, many facets of this growth coordination remain open for investigation.

Manipulating flower senescence

Another means of investigating the regulation of flower senescence is by applying a variety of synthetic chemicals and relating their known biochemical properties with the response of the flower. Such substances can range from enzyme inhibitors, uncouplers of phosphorylation, free radical scavengers to the relatively simple preservative solutions which have been revealing by themselves.

Originally the majority of preservative solutions were composed mainly of a sugar to improve water relations within the cut flower. As sucrose is the translocatory carbohydrate, it is preferably applied over glucose and fructose. For a short period after treatment, a high respiratory rate prevents sugar accumulation but from then on the sugars are stored within the flower. This collection of sugars maintains or increases the osmotic pressure. However, this osmotic property of the sugars is not maintained towards the end of corolla life. At this stage, osmotic energy fell more rapidly than could be accounted for by loss of sugars and potassium, indicating massive cell disruption (NICHOLS, 1977 b). Indeed, sucrose treatment underlines the importance of maintaining carbohydrate levels but it appears that this is not the determining component controlling flower longevity.

Vascular blockage by bacteria was thought to accelerate cut carnation flower longevity. The most commonly used antimicrobial compounds used in floral preservative solutions are the salts of 8-hydroxyquinoline, aluminium and silver. It was through investigation of silver as a bacteriocide that its anti-ethylene properties in the cut carnation flower were realised.

AARTS (1957) found that of the silver ions, silver nitrate and acetate, were the most effective bacteriocides. Their main disadvantage being that they photo-oxidised and hence could not be stored or used for any length of time. Their effectiveness in delaying senescence was, however, later found to be due to ethylene action and biosynthesis inhibition. HALEVY AND KOFRANEK (1977) found that silver nitrate pretreatment was far more effective if applied to the petals when compared to a basal uptake. This signified that the bacteriocide property of the silver ion was not the only factor extending flower longevity. Beyer (1976a) realised the potential of this ion as an anti-ethylene agent, and correlated it to the action of silver nitrate treatment on the petals of the carnation flower. Basally-applied silver nitrate was considered ineffective as an ethylene-inhibitor due to its poor translocation to the flowerhead (KOFRANEK AND PAUL, 1972). Silver nitrate moves upwards in the stem via the xylem (VEEN AND VAN DE GEIJN, 1978) participating in a chromatographic type of transport (BELL AND BIDDULPH, 1963). The exceptionally high affinity for silver by the negatively charged sites of the xylem is exemplified by the fact that the presence of other cations usually promotes such movement but is ineffective in this case retarding further

translocation (VEEN AND VAN DE GEIJN, 1978). So for silver nitrate movement all available sites have to be saturated.

To overcome the relative immobility of the silver ion, the negatively charged thiosulphate complex was used. As negatively charged ionic complexes are not, or to a much smaller extent, subject to absorption and exchange processes within the xylem, the silver thiosulphate complex moved at a far greater rate (2 ms^{-1}) through the xylem (VEEN AND VAN DE GEIJN, 1978). Important is that even in this form, the physiological action of this ion is still effective in that the senescence of the cut carnation is delayed.

Since this discovery, the silver thiosulphate complex has been used as an inhibitor of ethylene action and ethylene biosynthesis in the cut carnation flower. In research it has proved a most useful tool to determine the effect of ethylene action. Nevertheless, all silver-ethylene related results should be treated with caution until the site(s) of silver attachment or the inhibited biochemical processes are identified (see Chapter 5). Furthermore, the action of silver thiosulphate is only effective in ethylene-sensitive flowers. In cut flowers relatively insensitive to ethylene, such as the rose, a silver thiosulphate treatment is ineffective (DE STIGTER, 1981). The challenge thus arises to discover what action of the silver thiosulphate complex makes it so valuable in delaying carnation flower senescence.

Other inhibitors of ethylene action include carbon dioxide, ethylene oxide and substituted benzothiadiazoles. The former two

treatments have not been popular among floriculturalists due to the need for controlled or modified atmosphere storage. Ethylene oxide is also explosive and toxic. The substituted benzothiadiazoles have been shown to antagonise the action of ethylene in carnation flowers (CARPENTER AND DILLEY, 1973) but it has not been widely explored in other plant systems.

The elucidation of the ethylene biosynthetic pathway led to many other alternative enzyme inhibiting treatments preventing the production of this hormone. Two examples of such ethylene biosynthetic inhibitors are, firstly, rhizobitoxine and its analogs which act by preventing the conversion of SAM to ACC by ACC synthase (BAKER, WANG, LIEBERMAN AND HARDENBURG, 1977) and secondly aminooxyacetic acid which is an inhibitor of ACC synthase (FUJINO, REID AND YANG, 1981). With the sites of action of these compounds in the ethylene biosynthetic pathway known, treatments with these synthetic compounds are physiologically extremely valuable. Economically, however, an ethylene action inhibitor would be far more protective against accelerated flower senescence considering the pollutant presence of ethylene (ABELES, 1973).

Free radical scavengers, antioxidants and oxidant protectants are also considered capable of retarding flower senescence. Ethanol is a free radical scavenger (BEAUCHAMP AND FRIDOVITCH, 1970). A basal treatment of a four percent ethanolic solution extends carnation flower longevity up to fourteen days (HEINS, 1980). Ethanol with its chemical structure was initially considered an ethylene precursor but this was found to be incorrect (BIALE AND YOUNG, 1962). The prevention

of ethylene biosynthesis by ethanol suggests that a natural anaerobic accumulation of ethanol (THOMAS, 1925; THOMAS AND FIDLER, 1932) may prevent ethylene biosynthesis (HEINS, 1980). In an unrelated system, isolated wheat roots, GUDJNSOTTIR AND BURSTRÖM (1962) found that non-toxic ethanol levels increased cellular enlargement that was inhibited by light. However, ethanol action within the flower is considered to be via scavenging free radicals (BAKER, LIEBERMAN AND ANDERSON, 1978). Other free radical scavengers such as benzoate, n-propyl gallate, inhibit ethylene production of fruit tissue slices suggesting that ethylene biosynthesis involves a free radical step. Interesting is that the cytokinins are also considered to be free radical scavengers (LESHEM, WURZBURGER, GROSSMAN AND FRIMER, 1981).

A final group of chemicals that are capable of delaying carnation flower senescence are the plant growth retardants and inhibitors. How these substances elicit their senescing delaying response is not understood. Suggestions include interactions with plant hormones, adjusting metabolic processes that increase the tolerance of plant tissues to stress, and bacteriocide activity.

The subject of this review was to outline the valuable progress made in the investigations on the senescence of the cut carnation flower. An off-shoot of the success of this research is seen with floriculturalists now being able to extend carnation flower longevity up to two weeks.

However, the endogenous regulation of this flower senescence event remains unsolved. The tissue variety, both anatomically and physiologically, forces senescence not only to involve the death of floral tissues but the continued development of others. As a result this study on the senescence of the cut carnation flower is not confined to degradative processes but concentrates on the influence the growth processes may have in this final stage of development. Thus such processes as cell expansion, chloroplast development, photosynthesis, respiration, assimilate transport and vascular development are all part and parcel of this investigation.

CHAPTER TWO

MANIPULATING FLOWER SENESCENCE

Introduction

Basically three postharvest treatments were used in this thesis. They were chosen for their marked effect on the senescence phenomenon occurring in the cut carnation flower. The first treatment enhanced the senescence of the bloom by supplementing the natural levels of ethylene. This was controlled by basally applying a solution of 2-chloroethyl phosphonic acid (commercially termed Ethrel or Ethephon) which, when absorbed by living tissues, is metabolised to form ethylene (WARNER AND LEOPOLD, 1969). Agriculturally this compound made a vast impact as it provided a convenient means of applying ethylene to plants.

The remaining two treatments delay the senescence process in the cut carnation flower and thus are of potential economic importance. These treatments are a silver thiosulphate basal pulse directly after harvest (VEEN AND VAN DE GEIJN, 1978) and a continuous basal application of an ethanolic solution (HEINS, 1980). In contrast to the ethylene treatment, it is well documented that these two latter treatments delay senescence by preventing ethylene production, which is associated with irreversible petal wilting. Silver thiosulphate treatment also appears to prevent ethylene action (BEYER, 1976a,b; HALEVY AND KOFRANEK, 1977; VEEN AND VAN DE GEIJN, 1978; DIMALLA AND VAN STADEN, 1980; VEEN, 1983). At the outset preliminary experiments were conducted to

observe and quantify the physical effect of these postharvest treatments on cut carnation flower senescence.

Materials and Methods

Plant material

Flowers of *Dianthus caryophyllus* L. (cv. White Sim), grown by Floricadia, Heidelberg, Transvaal, were harvested at the fully open stage, cooled to 4°C and then transported overnight to Pietermaritzburg. Prior to experimentation the stems were recut and the flowers immediately subjected to the following treatments:

1. Immersion in distilled water; termed control.

2. Immersion into a 20 mg l^{-1} 2-chloroethyl phosphonic acid solution (WARNER AND LEOPOLD, 1969); termed ethrel.

3. Pulsed for ten minutes with a silver thiosulphate solution [4mMol silver nitrate ($AgNO_3$) with 16mMol sodium thiosulphate ($Na_2S_2O_3$)] and then immersed into distilled water (REID, FARHOOMAND, PAUL AND KOFRANEK, 1979), termed STS.

4. Immersion into a four percent ethanolic solution (HEINS, 1980), termed ethanol.

The treated flowers were maintained under continuous light at 23°C and were harvested as they aged. Depending on the experiments

conducted the flowers were divided into petals, ovary, receptacle, calyx, bracts and stem (Fig. 2.1).

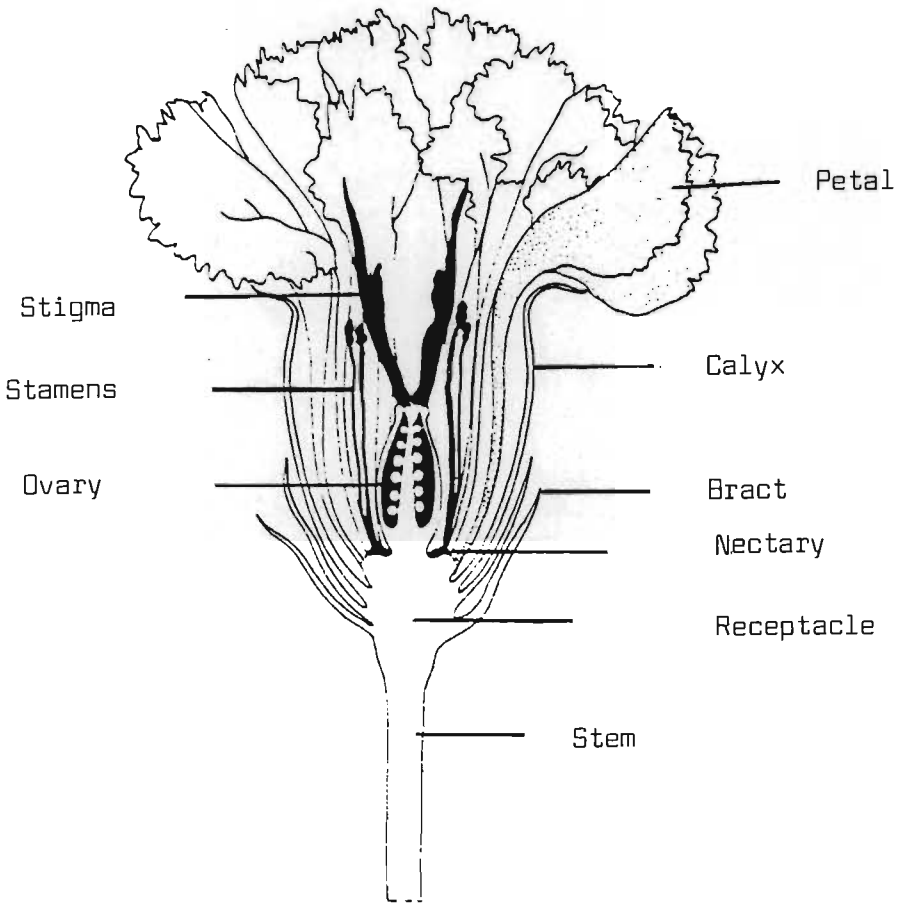


Figure 2.1 : Diagram of the carnation (*Dianthus caryophyllus* L. cv. White Sim) flower.

Chlorophyll determination

The intact ovary was placed into a glass vial and immersed in 7 mℓ of dimethyl sulphoxide. The chlorophyll was extracted (without grinding)

into the fluid by incubating at 65°C for two hours. The extractant was then transferred to a graduated tube and made up to a total volume of 10 mℓ with dimethyl sulphoxide. This extract could be assayed immediately or transferred to vials and stored between 0 - 4°C until required for analysis (HISCOX AND ISRAELSTAM, 1979).

In initial experiments, an alternate method for chlorophyll determination was used. This method required homogenisation of the ovary with 80 percent acetone. After centrifugation the chlorophyll content of the supernatant was immediately determined.

The chlorophyll extracted by either method was measured in a Varian DMS 90 double beam spectrophotometer at 440 nm and 665 nm, respectively. These wavelengths were chosen as these were the regions where chlorophyll extracted from carnation ovaries showed the greatest peaks of absorption (Fig. 2.2).

Electron microscopy technique

One millimetre square sections of the carnation flower tissue were fixed at 4°C in six percent glutaraldehyde buffered at pH 7,2 with a 0,05 Molar sodium cacodylate buffer. After twenty-four hours the glutaraldehyde was removed and after being washed twice for thirty minutes with the same buffer, the tissue was post-fixed with two percent osmium tetroxide buffered as above. This was followed by a further three thirty minute washes with the buffer. The material was then dehydrated in an alcohol series followed by a double wash in propylene

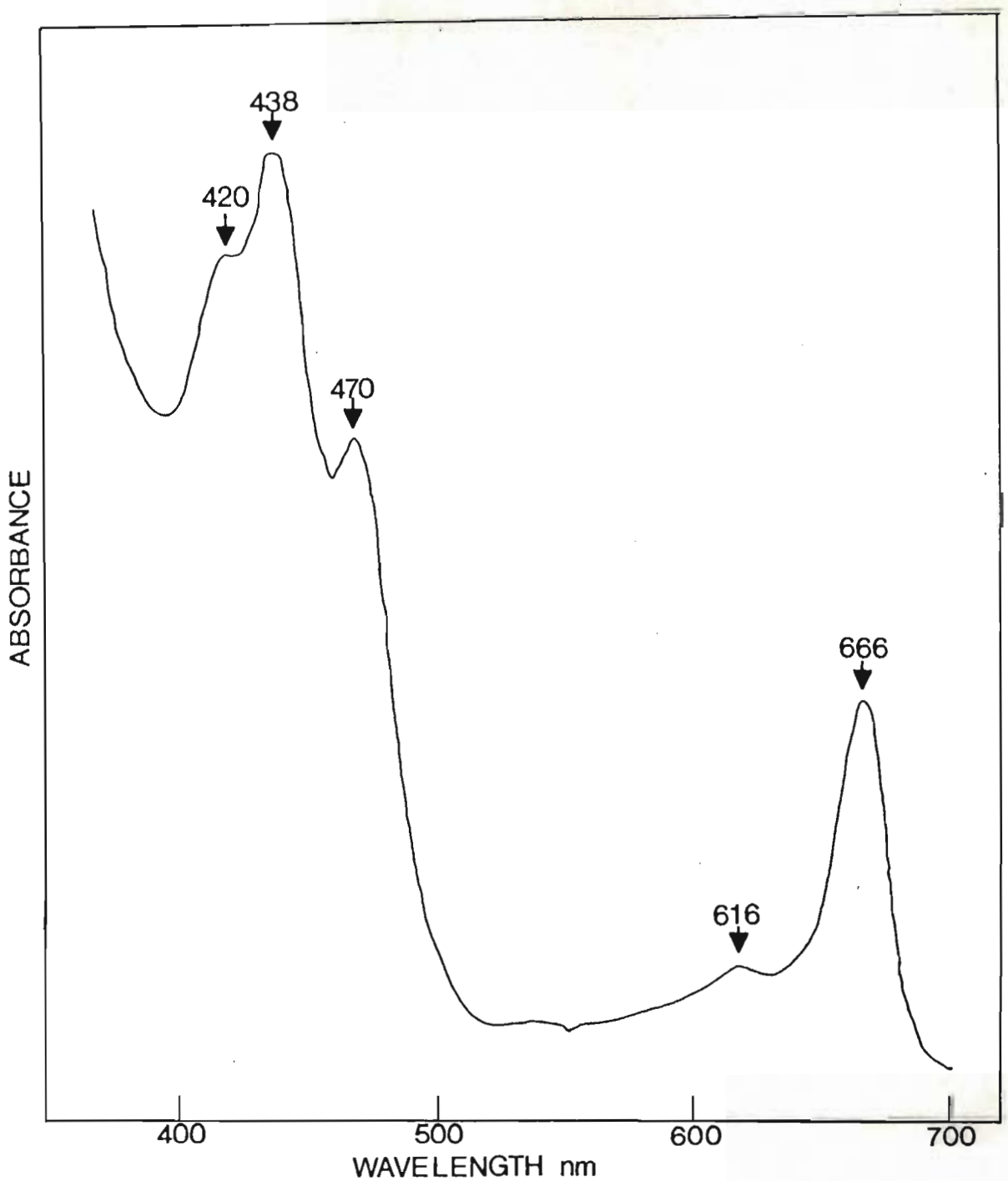


Figure 2.2 : Absorbance spectrum of a chlorophyll extract from the carnation ovary.

oxide. Impregnation of the tissue with Epon araldite was achieved by adding increasing amounts of resin to subsequent propylene oxide washes. For final impregnation the tissues were placed in pure resin. Polymerisation lasted forty-eight hours at 70°C. Sections *ca* 700 Å were cut with a glass knife on a LKB microtome and stained with uranyl acetate and lead citrate (REYNOLDS, 1963). The sections were viewed with a transmission electron microscope (JEOL-100 CX) at an accelerating voltage of 80 kV and photographed.

Results and Discussion

A preliminary experiment showed that increased stem length extended flower longevity and maintained the greatest fresh and dry mass within the flowerhead (Fig. 2.3). As a sucrose staying solution delays flower senescence longer than the control and increases fresh and dry mass (Table 2.1), stem originating carbohydrates appear important in determining flower longevity.

Table 2.1 : Effect of an eight percent staying solution on the longevity, fresh and dry mass of the cut carnation flowerhead. Standard error is indicated.

Treatment	Longevity (days)	Fresh Mass (g)	Dry Mass (g)
Control	7 ± 1	3,45 ± 0,22	0,91 ± 0,10
Sucrose	10 ± 1	7,45 ± 0,53	1,71 ± 0,31

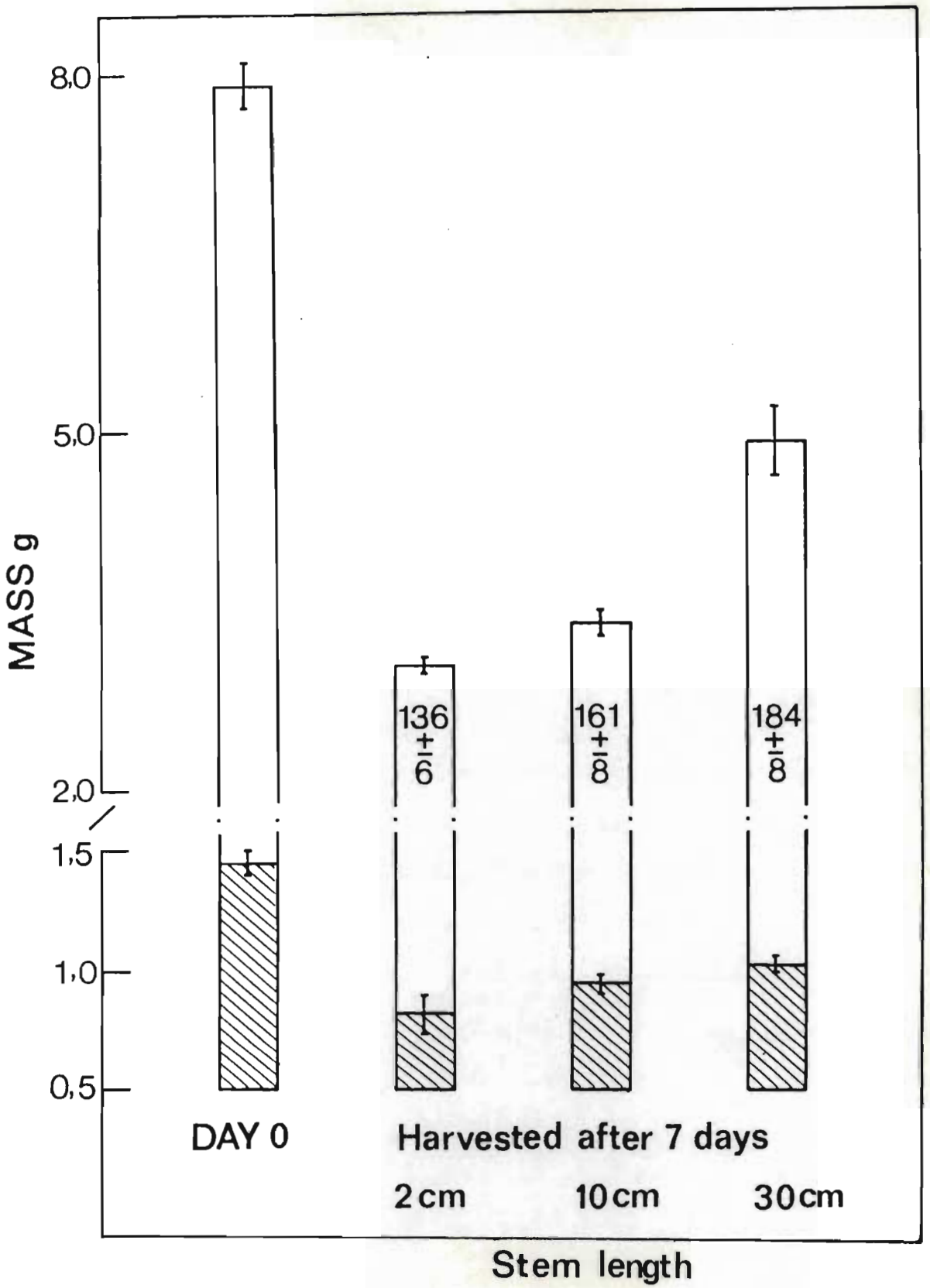


Figure 2.3 : The effect of stem length on the fresh (□) and dry (▨) mass of the cut carnation flowerhead seven days after harvest. $^{136}_{+}$ indicates the longevity, in hours, of the flower. Vertical bars indicate the standard error of the mean.

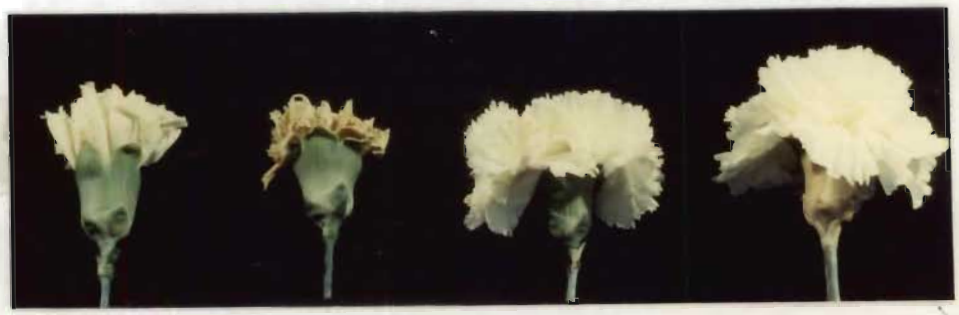
In any investigation considering the relationship between the flower parts, it thus appears necessary to eliminate or minimise the stem effect on the flowerhead as far as possible. To achieve this in all subsequent experiments, unless otherwise stated, the stems were trimmed routinely to two centimetres.

With the experimental system established, the carnation cut flowers were exposed to the three postharvest treatments. As expected the silver thiosulphate and ethanol treatments increased carnation flower longevity over a hundred percent whilst the 2-chloroethyl phosphonic acid staying solution accelerated flower senescence so that their vaselife was reduced to three days (Table 2.2).

The appearance of the petals from these postharvest treatments differed as they senesced. Following the ethylene increase, the petals irreversibly wilted becoming flaccid before finally 'browning'. With silver thiosulphate and ethanol treatments, the first sign of petal senescence occurred when individual petals appeared 'burnt' (Fig. 2.4). This suggests that, in the latter case, a water deficiency was not the cause of petal senescence.

Irrespective of petal appearance during senescence, this process was accompanied by a decrease in petal fresh mass (Fig. 2.5). The rate of this fresh mass loss was greater with control and 2-chloroethyl phosphonic acid-treated flowers than with the ethylene-inhibitory treatments. Thus, although the cut flower had a continuous supply of

Table 2.2 : The effect of different postharvest treatments on the longevity and appearance of cut carnation flowers. Standard error is indicated.

	CONTROL	ETHREL	STS	ETHANOL
APPEARANCE (7 days after harvest)				
Longevity (days)	7 ± 1	3 ± 1	11 ± 1	11 ± 1

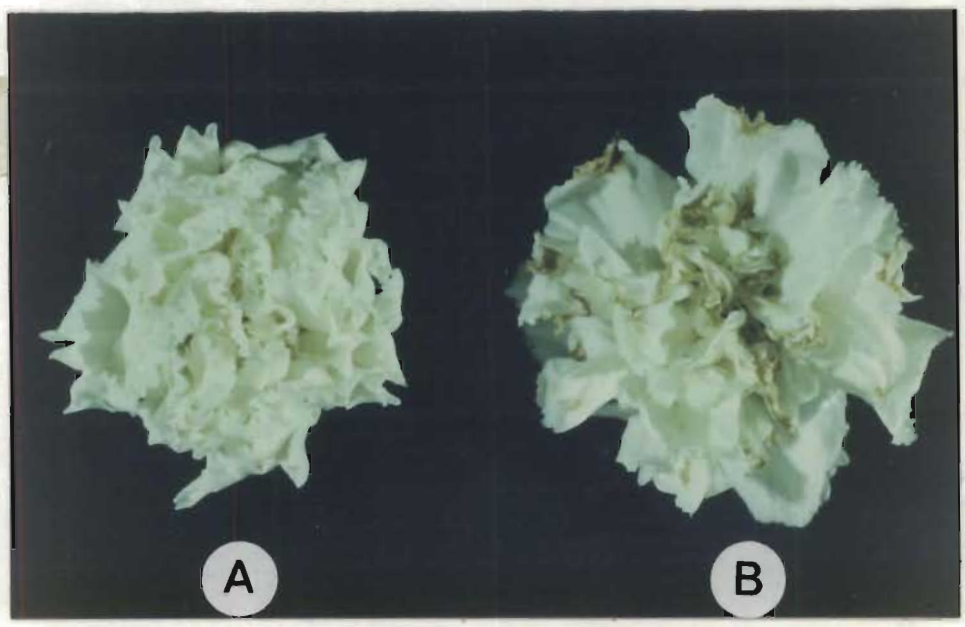


Figure 2.4 : Appearance of senescing cut carnations. The control (A) petals wilted irreversibly whilst the petals from silver thiosulphate pulsed flowers (B) appeared to be 'burnt'. The control flower and silver thiosulphate flower , from harvest, have aged seven and twelve days respectively.

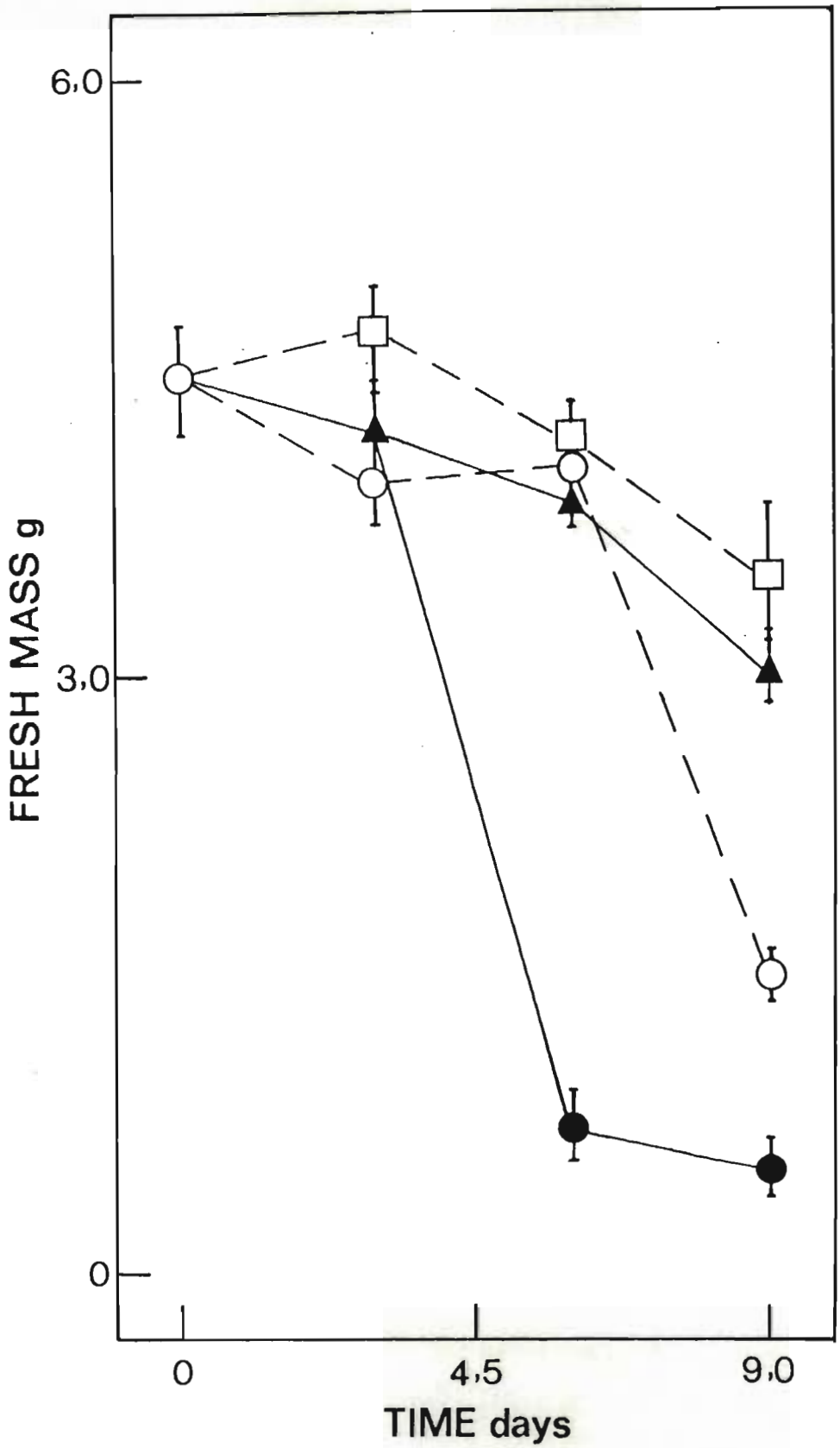


Figure 2.5 : Petal fresh mass of control (-○-), 2-chloroethyl phosphonic acid (-●-), silver thiosulphate (-▲-) and ethanol (-□-) treated cut carnation flowers. Vertical bars indicate standard error of the mean.

water available, on petal senescence, the capacity to utilise this water source was impaired. The control and 2-chloroethyl phosphonic acid treatment lost this water retaining capacity at a greater rate than the silver thiosulphate and ethanol treatments. On the other hand, the cellular expansion required for ovary growth needs a high water potential in the control and 2-chloroethyl phosphonic acid-treated flowers. The question thus arises as to the biochemical or structural imbalance that occurs to cause this change in the water status of the cut flower petals.

Dividing the flower into its separate parts, the increase in ovary size in the presence of above basal levels of ethylene was most noticeable, especially when contrasted to the retarded ovary growth of the silver thiosulphate and ethanol-treated flowers (Table 2.3). Additionally, the large 2-chloroethyl phosphonic acid-treated ovaries had 'greened' considerably in comparison to the ovaries from the senescence-delaying treatments.

Quantification of the chlorophyll content of the ovaries showed that from harvest there was little change with silver thiosulphate and ethanol treatments. On the other hand, control and 2-chloroethyl phosphonic acid-treated ovaries steadily synthesised more chlorophyll (Fig. 2.6). An ultrastructural investigation was undertaken to determine the cause of these chlorophyll differences between ethylene-treated ovaries and ovaries in the absence of ethylene.

Table 2.3 : The effect of the postharvest treatments on the growth of the ovary in the cut carnation flower nine days after harvest. Standard error is indicated.

	DAY 0	CONTROL	ETHREL	STS	ETHANOL
APPEARANCE (9 days after harvest)					
Width (mm)	7,3±0,15	9,6±0,23	11,0±0,20	8,1±0,26	8,0±0,26
Height (mm)	11,8±0,36	12,5±0,25	13,4±0,15	12,0±0,25	11,5±0,16

At harvest, the plastids in the ovary wall resembled amyloplasts. These are non-pigmented organelles located in tissues removed from the light which store starch (ESAU, 1977) (Plate 2.11). Considering the physical enclosure or shading effect of the petals around the ovary, the presence of amyloplasts in the ovary wall would be expected. Within three days from harvest, these amyloplasts lost their starch and began to change into chloroplasts (Plate 2.2). The plastids in this control ovary increased in size and, although haphazard in arrangement, thylakoids appeared and began to stack into grana. It is within these thylakoids that the chlorophyll is localised and thus thylakoid development accounts for the increased chlorophyll measurements. However, compared with the chloroplast from stem tissue (Plate 2.12)

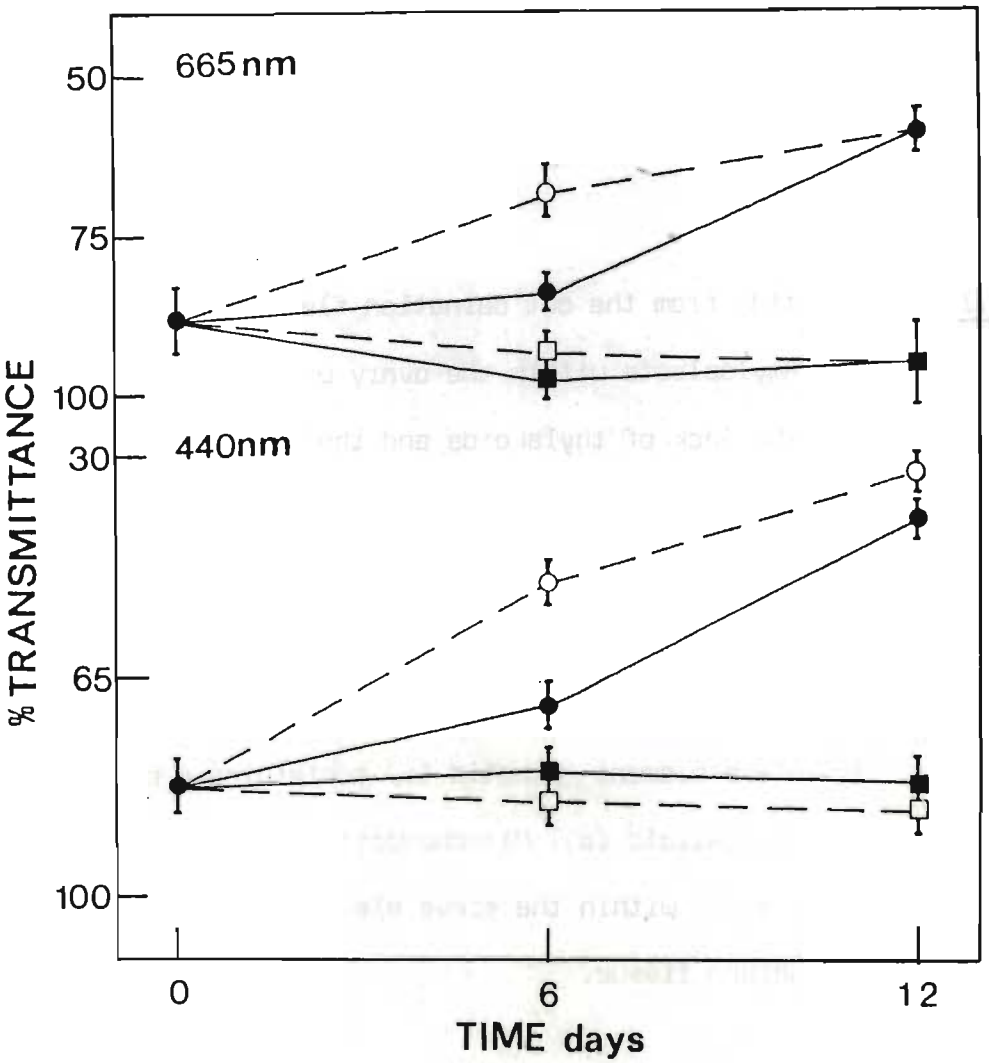
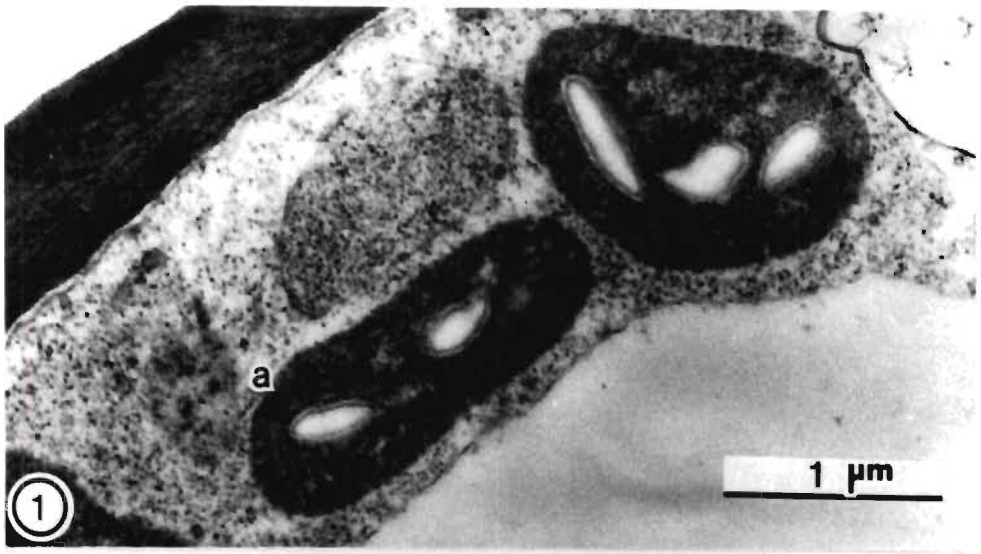
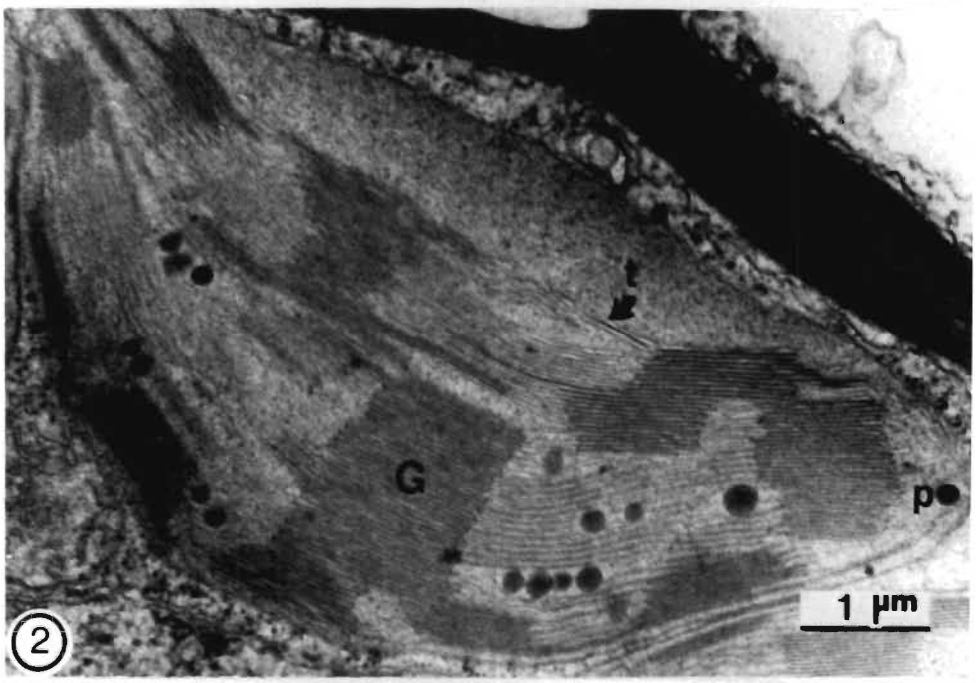


Figure 2.6 : The chlorophyll content of ovaries from control (-●-), 2-chloroethyl phosphonic acid (-○-), silver thiosulphate (-□-) and ethanol (-■-) treated cut carnation flowers over a twelve day period from harvest. Transmittance was measured at 665 nm and 440 nm. Vertical bars indicate the standard error of the mean.

Plate 2.1 : Plastids from the cut carnation flower.

1. Amyloplasts within the ovary wall at harvest. Note the lack of thylakoids and the presence of starch (a).
2. A chloroplast from stem tissue. Thylakoids (t) are stacked into grana (G) and plastoglobuli (p) are present within the chloroplast.
3. Sieve element plastid (e) containing a protein crystalloid (c). Mitochondria (m) are also present within the sieve elements of the phloem tissue.


a
1 µm
1


G
p
1 µm
2

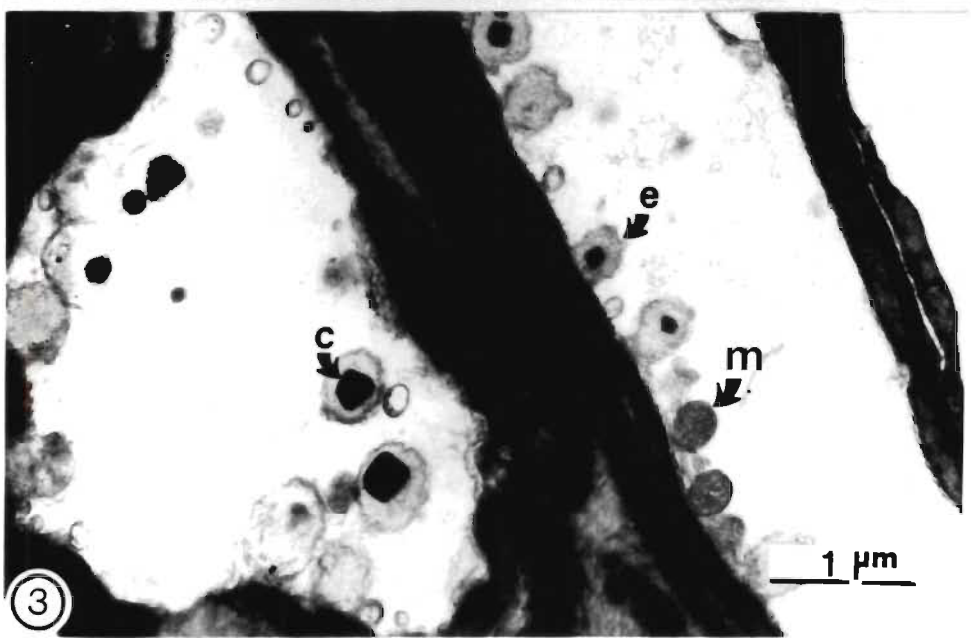
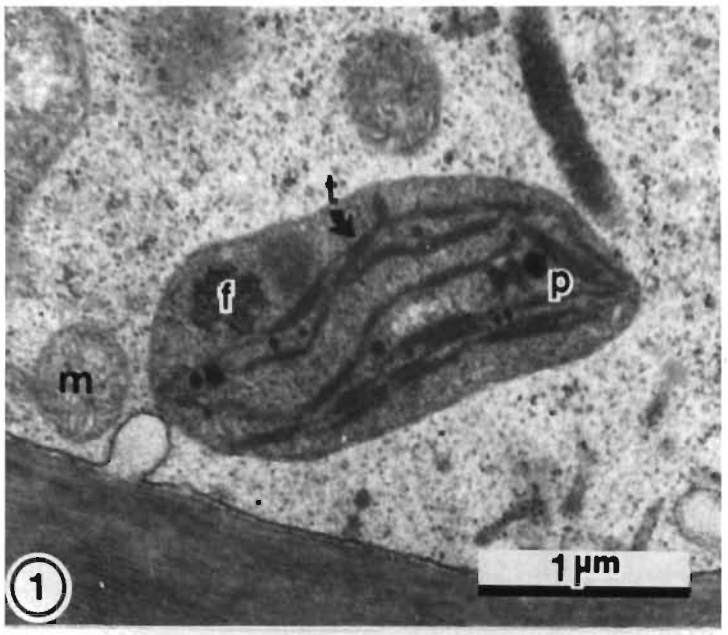
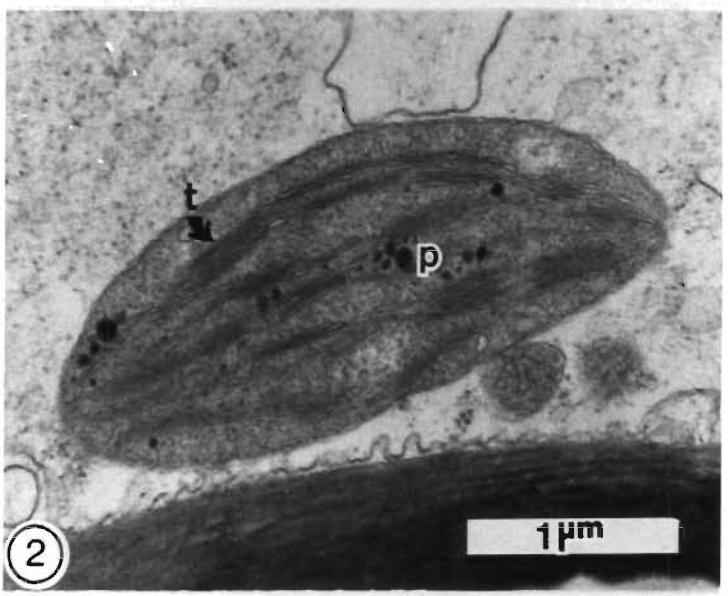

e
c
m
1 µm
3

Plate 2.2 : Chloroplasts from the ovary wall of a control carnation flower.

1. Three days after harvest. The thylakoids (t) have begun to stack into grana. Plastoglobuli (p) and a ferrotin body (f) are also present within the chloroplast. Mitochondria (m) are present.
2. Six days after harvest. Further stacking of the thylakoids (t) into grana had occurred with no obvious increase in the number of plastoglobuli (p) within the chloroplast.
3. Nine days after harvest. The number of thylakoids (t) stacked into grana had increased further. Fewer plastoglobuli (p) were present.


t
f
p
m
1 µm
1


t
p
1 µm
2

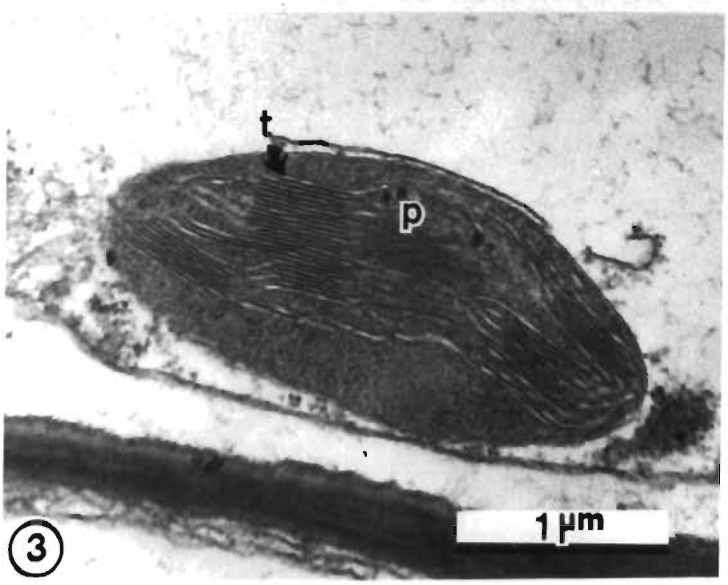
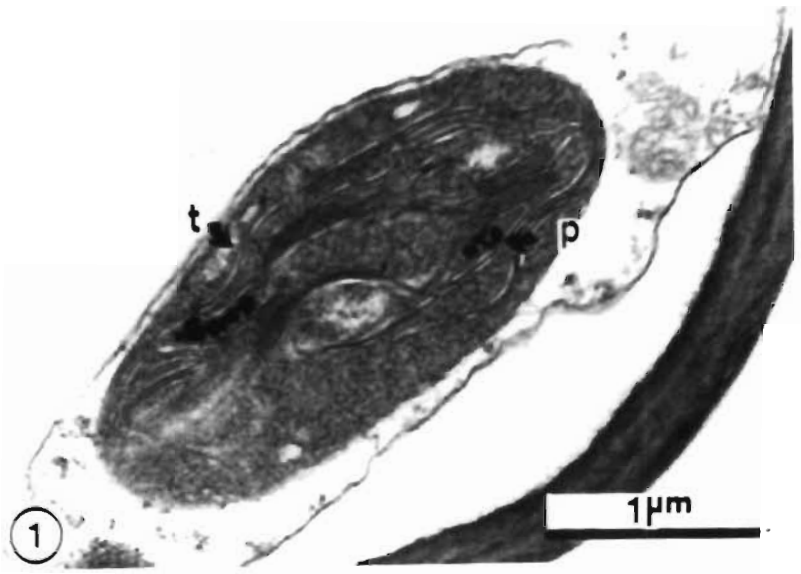
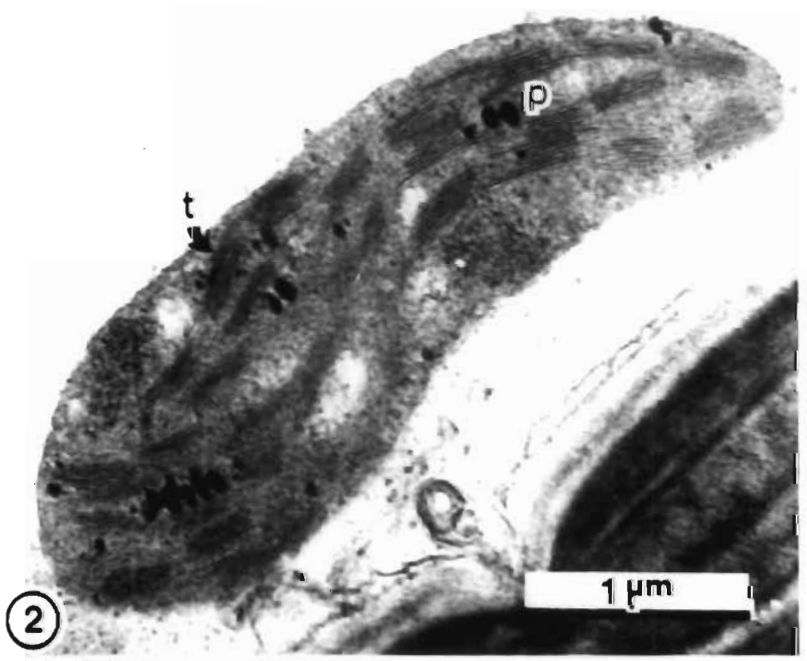
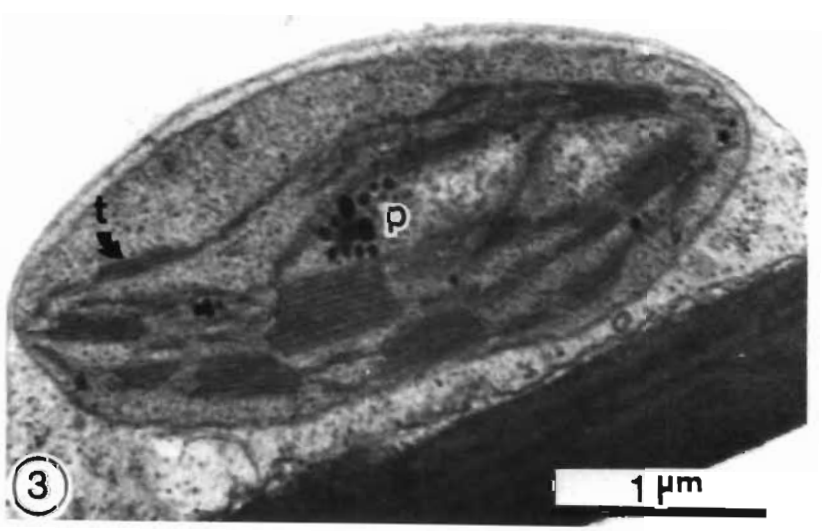

t
p
1 µm
3

Plate 2.3 : Chloroplasts from the ovary wall of a 2-chloroethyl phosphonic acid treated carnation flower.

1. Three days after harvest. Thylakoids (t) stacked into grana and plastoglobuli (p) are present within the chloroplast.
2. Six days after harvest. Thylakoids (t) stacked into grana and plastoglobuli (p) are present within the chloroplast.
3. Nine days after harvest. Thylakoids (t) stacked into grana and plastoglobuli (p) are present within the chloroplast.


t
p
1μm
1


p
t
1 μm
2

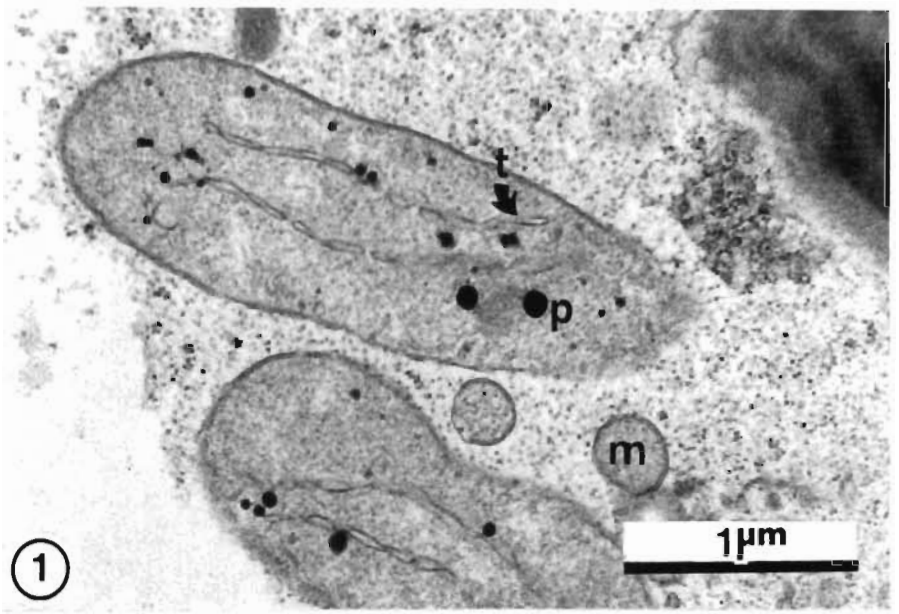

t
p
1 μm
3

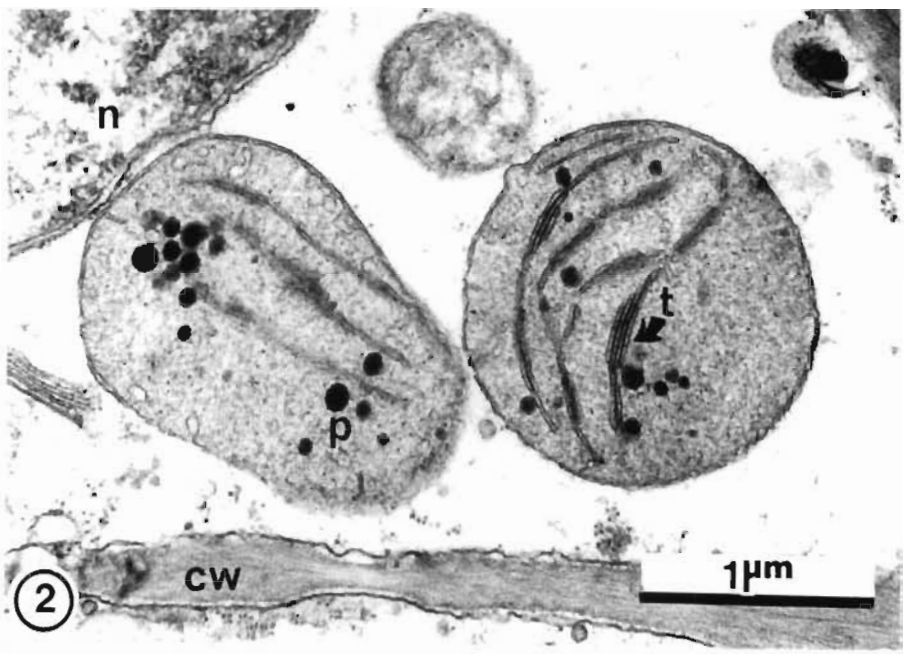
little further structural changes within the chloroplast occurred with time. The steady increase in chlorophyll content (Fig. 2.6) was probably due to the synthesis of chlorophyll in the existing thylakoids. Plastoglobuli were also observed suggesting that some membrane degradation occurred during amyloplast development into chloroplasts. There was little structural difference between the chloroplasts described above from the control ovary wall and those from an ovary wall in a 2-chloroethyl phosphonic acid-treated flowers. There was a slight increase in the thylakoids being stacked into grana and plastoglobuli were present. On the third day a ferrotin body could be seen (Plate 2.3).

Ethylene is generally known to disrupt the internal membranes of the chloroplast and induce an increase in chlorophyllase activity during fruit ripening (PURVIS, 1980; PUROHIT, 1982) and leaf senescence (SABATER AND RODRIGUEZ, 1978; SHIMOKAWA, 1979). This chloroplast degrading action of ethylene may be confined to mature chloroplasts. In the ovary wall, the chloroplasts were not mature and did not attain this status as can be seen when compared with a chloroplast from stem tissue (Plate 2.18). Ethylene thus appears to stimulate chloroplast development as well as the degradation of mature chloroplasts. The stimulation of chloroplast development in the ovary wall may be a direct action of ethylene, the interaction of ethylene with other plant growth regulators, or a result of the senescent process, for example, assimilate transport. This whole question of chloroplast development in the ovary wall will be discussed further in Chapter 4. However, pertinent at this stage, is that in silver thiosulphate (Plate 2.4)

Plate 2.4 : Chloroplasts from the ovary wall of silver thiosulphate pulsed carnation flower.

1. Three days after harvest. Single thylakoids (t) and a small number of plastoglolobuli (p) have developed within the chloroplast. Note the close association of the chloroplast with mitochondria (m).
2. Six days after harvest. The thylakoids (t) are beginning to stack into grana and a greater number of plastoglobuli (p) are present within the chloroplasts. The nucleus (n) and cell wall (cw) are indicated.
3. Nine days after harvest. The thylakoids appear to be disintegrating, an observation supported by the increase in plastoglobuli (p) in the chloroplasts. Mitochondria (m) have generally increased in size.


t
p
m
1μm
1


n
t
p
cw
1μm
2

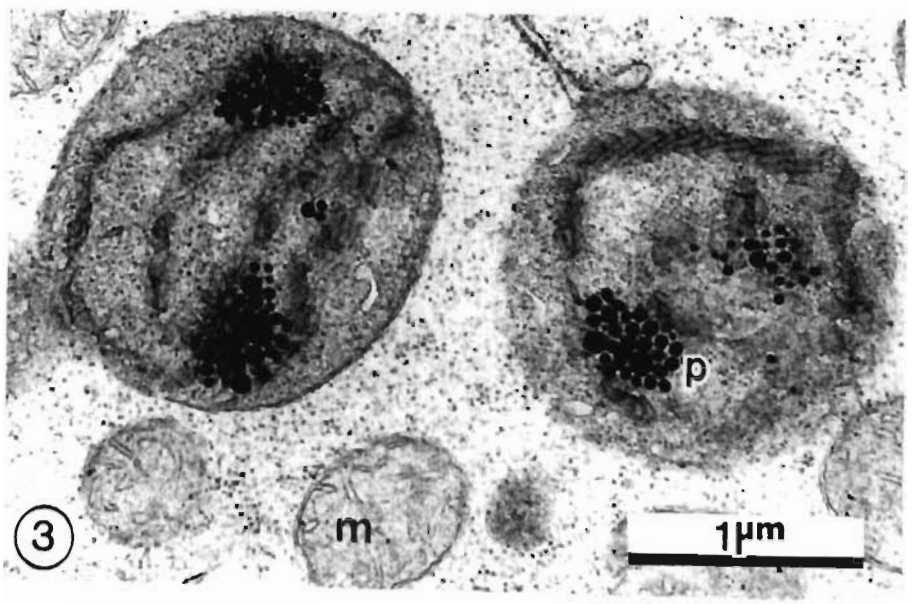
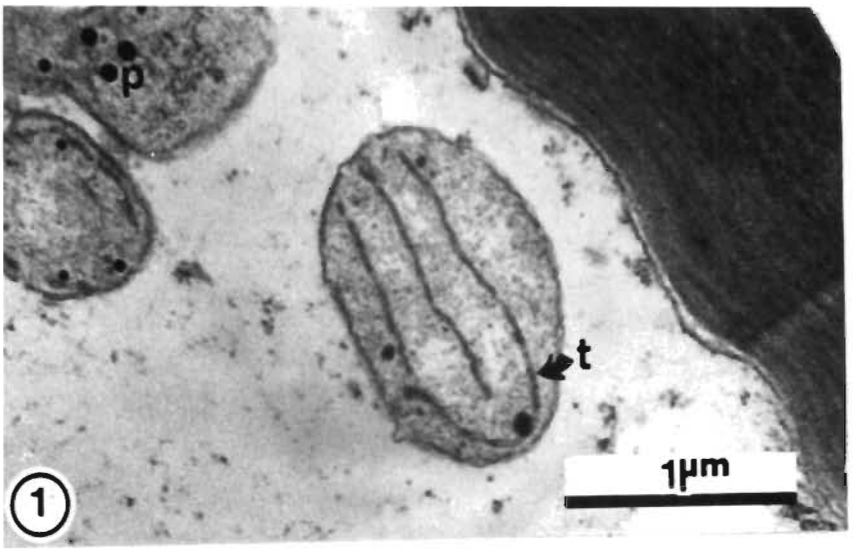
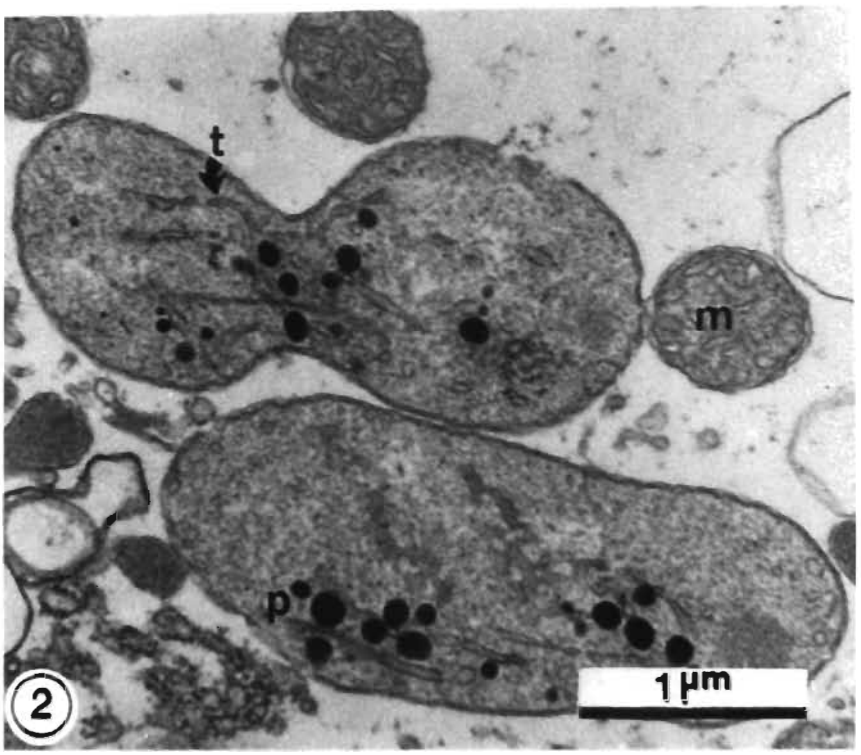

p
m
1μm
3

Plate 2.5: Chloroplasts from the ovary wall of an ethanol-treated carnation flower.

1. Three days after harvest. Single thylakoids (t) and a small number of plastoglobuli (p) are present within the chloroplast.
2. Six days after harvest. The chloroplasts have generally increased in size but the thylakoids (t) remain single and the number of plastoglobuli (p) have increased. Mitochondria (m) are indicated.
3. Nine days after harvest. The chloroplasts still contain single thylakoids (t) and there is no increase in the number of plastoglobuli (p).


p
t
1μm
1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t
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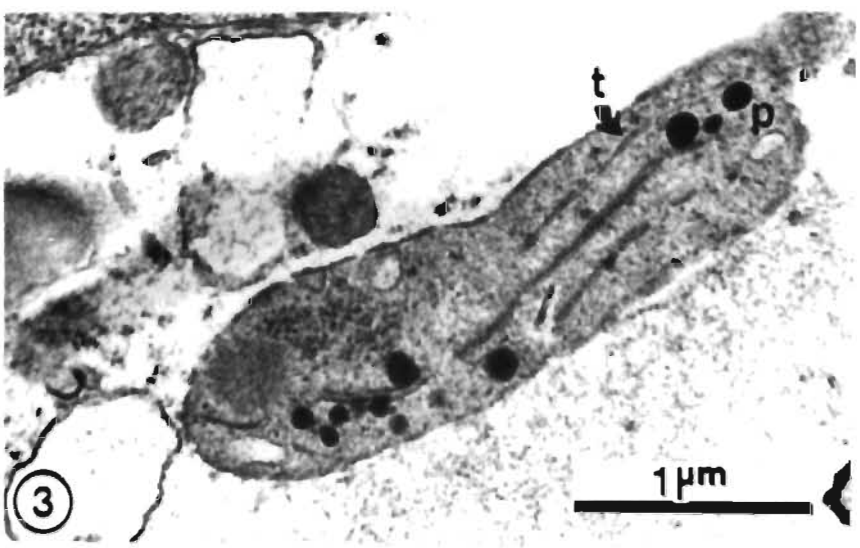
p
1 μm
2


t
p
1 μm
3

and ethanol-treated flowers (Plate 2.5) the ovary walls contained chloroplasts that developed little in comparison with the control and 2-chloroethyl phosphonic acid treatments. In both of the former treatments, the starch present in the plastids at harvest was also absent by the third day. Single thylakoids appeared in the chloroplast with a number of plastoglobuli which increased with time. The sparse thylakoid development in these chloroplasts is in agreement with the low chlorophyll content measured from these ovaries (Fig. 2.6).

During the chloroplast studies another organelle was noticed. The phloem sieve elements in the carnation flower often contained crystals surrounded by a double unit membrane (Plate 2.13). At first these crystal bodies were thought to be associated with a silver thiosulphate treatment but on a thorough investigation were detected in phloem tissue regardless of treatment. The presence of a double unit membrane suggests that this organelle could be either a plastid, mitochondria or nucleus. In the literature, NEUBERGER AND EVERT (1974) recognised that one of the early structural changes in the sieve elements was the formation of a protein crystalloid within the plastid. Thus this organelle is another modification of a plastid.

The anatomical difference between the ovary wall chloroplasts from control and 2-chloroethyl phosphonic acid-treated flowers compared to silver thiosulphate and ethanol treatments may well be due to assimilate availability for their development. Irrespective of postharvest treatment, from harvest the whole cut carnation flower decreased in dry mass but the distribution of this dry mass between

the flower parts alters with postharvest treatment. Considering the ovary, when senescence is accelerated with 2-chloroethyl phosphonic acid treatment, its dry mass increased compared to the control (Fig. 2.7). On the other hand the ovary from the ethylene-inhibitory treatments, silver thiosulphate and ethanol, did not alter in dry mass measurement from harvest. This indicates a lack of ovary growth with these treatments and probably also accounts for the lack of chloroplast development. The petal dry mass also differed between the ethylene producing-treatments and ethylene-inhibitory treatments. The petal dry mass declined rapidly with 2-chloroethyl phosphonic acid-treatment whilst with the ethylene-inhibitory treatments petal dry mass also decreased but at a much slower rate (Fig. 2.7). Although the rates of metabolism would be expected to differ between tissues, these dry mass results suggest a movement of assimilates from the petals to the ovary during ethylene-accelerated senescence (NICHOLS, 1968; MAYAK AND DILLEY, 1976; NICHOLS, 1976; CAMPRUBI AND NICHOLS, 1978; DIMALLA AND VAN STADEN, 1980). Thus it appears that ethylene may regulate the redirection of the assimilates within the flower to the ovary. If this is proved correct, this action of ethylene may be economically detrimental to the floriculturalist but considering agriculture crops it could prove a most valuable tool. Often the harvested plant parts from a crop are the fruits or seeds. If more assimilates were directed to these organs immediately prior to their harvest, the efficiency of crop growth to harvest would greatly increase. So while it is important to understand the regulation of assimilate partitioning within the flower to delay carnation flower senescence, on the other hand, this information could also be used to

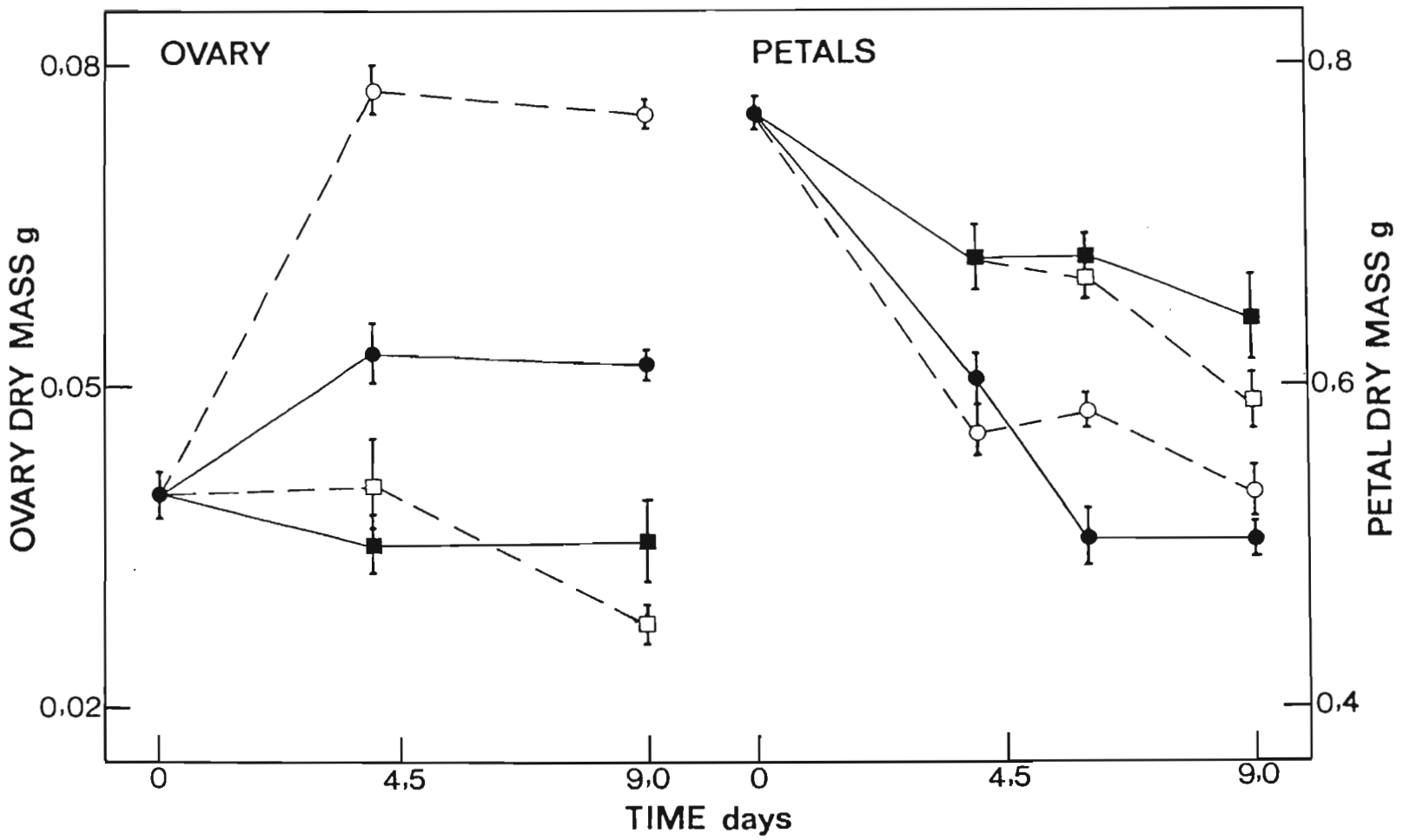


Figure 2.7 : The change in dry mass of the ovary and petals from control (-●-), 2-chloroethyl phosphonic acid (- ○-), silver thiosulphate (- □ -) and ethanol (-■-) treated cut carnation flowers over a nine day period from harvest. The vertical bars indicate standard error of the mean.

alter assimilate partitioning in the crop plant and hence increase crop yield.

Alternatively, the economically important treatments to the floriculturalist, silver thiosulphate and ethanol, appear to extend flower longevity by maintaining a threshold level of assimilates within the petals whilst depriving the ovary of necessary assimilates for growth. The similarity of the physiological response between these two treatments suggests that they act via the same mechanism. But silver thiosulphate treatment blocks ethylene action as well as preventing ethylene biosyntheis (VEEN, 1979b) whilst ethanol treatment only prevents ethylene emanation (HEINS, 1980). Furthermore, the silver effect on ethylene action is considered direct whilst ethanol scavengers free radicles which prevents ethylene biosynthesis. Thus the biochemical action of these two postharvest treatments in delaying carnation flower senescence differed but the physiological result was similar. This suggests that these two treatments, through different means, affect development through the same mechanism.

CHAPTER THREE

ESTABLISHMENT OF ASSIMILATE MOVEMENT

Introduction

NICHOLS AND HO (1975a) advanced a hypothesis that .... 'a change in the source-sink relationships of the flower parts contribute to the factors that determine the rate of flower senescence'. From their investigations with labelled sucrose they concluded that the petals were an important source of carbohydrate for ovary growth. Using longer stemmed cut carnations together with the techniques of removing specific flower parts, MOR, REID AND KOFRANEK (1980), questioned this petal contribution of carbohydrates to ovary growth. They found that ovary dry mass increased a full day before petal wilting symptoms became visible. Additionally, the removal of the ovary did not affect petal senescence. As for the increase in ovary dry mass, this still occurred even in the absence of the petals. Thus these workers suggested that ..... 'the petals and ovaries of cut carnations are competing for a common supply of carbohydrate, that the relative strength of these two sinks governs the partitioning of dry matter between them, and the silver thiosulphate strongly promotes the sink strength of the petals'. It is with great caution that conclusions on assimilate movement can be drawn solely from quantifying dry mass as these measurements are a result of metabolic activities that affect the carbohydrate pool, as well as the movement of sugars between the flower parts. Thus while

there is general agreement that carbohydrates are necessary for petal longevity and ovary development, the exact relationship and degree of transport between these flower parts remains unclear. It is therefore of great importance to clarify the movement of assimilates within the cut carnation flower during senescence.

Materials and Methods

Determination of labelled amino acid translocation

For analysis treated flowers were injected with 1 µℓ of L-/1-^{14}C/glutamic acid (specific activity 1,85 MBq mmol^{-1}) into the stem just below the bracts. After a twenty-four hour period these flowers were divided into petals, ovary, receptacle and calyx plus stem and then analysed for radioactivity. Analyses were made after harvest, and again three, six and nine days later.

The radioactivity in the flower parts was measured by oven-drying the material at 90°C for forty-eight hours. After weighing, each flower part was then ground in a separate mortar and pestle and three 10 mg subsamples weighed into scintillation vials. To these vials 0,5 mℓ of a digesting mixture of hydrogen peroxide (H_2O_2) and perchloric acid ($HClO_4$) 1:1 (v:v) was added. After sealing the scintillation vials they were placed in an oven at 60°C for three hours. Once cooled, 7 mℓ of Ready Solv EP scintillation fluor (Beckman) was added to each vial. The samples were then placed in the dark for a twenty-four

hour period prior to counting to eliminate chemoluminescence. The radioactivity was measured with a Beckman LS 3800 Scintillation counter and results expressed as disintegrations per minute (dpm).

Determination of labelled carbon dioxide fixation

To determine the extent to which various flower parts could assimilate carbon dioxide, whole flowers and flowers from which the petals were removed were placed in airtight containers. These flowers were then exposed to labelled carbon dioxide (about 100 000 dpm) produced by reacting barium carbonate ($Ba\,^{14}CO_3$ specific activity 2,1 MBq $mmol^{-1}$) with concentrated sulphuric acid (H_2SO_4). Twenty-four hours after the labelled carbon dioxide application, the flowers were removed and divided into their component parts.

The respective flower parts were extracted for sugars. This was done by immediately boiling the flower parts in 50 mℓ of eighty percent ethanol for fifteen minutes, filtering out the debris and acidifying the effluent to pH 2.5 with hydrochloric acid (HCℓ). This extract was then passed through Amberlite cation exchange resin (IR -120, H^+ form, BDH chemicals Ltd) at a rate of 15 mℓ h^{-1}. After washing the columns with a further 50 mℓ of eighty percent ethanol, the ethanolic eluants were combined and reduced almost to dryness. This was resuspended with ten percent *iso*-propanol to a final volume of 3 mℓ. Total radioactivity associated with each extract was determined by taking the average counts of three 1 mℓ aliquots. This was done by airdrying each sample, redissolving the sugars in

1 mℓ of hundred percent methanol and then adding 10 mℓ of a scintillation cocktail (toluene containing 4,0 gl^{-1} PPO (2,5 - Diphenyloxazol)($C_{15}H_{11}NO$) and 0,20 gl^{-1} 2,2 -p-Phenylen-bis-(4-methyl-5-phenyloxazol)(Dimethyl-POPOP) and counting in a Beckman LS 3800 Scintillation counter.

Determination of labelled sucrose translocation

To estimate the degree of sucrose movement from the stem, petals or ovary, 0,5 µℓ of ^{14}C-sucrose (specific activity 1,85 MBq $mmol^{-1}$) (about 345 000 dpm) was injected into the stem, two marked petals or the ovary. Twenty-four hours later the carnations were divided into the petals (labelled and unlabelled where necessary) the ovary and calyx plus stem. Radioactivity was measured by the method previously described (when amino acid translocation was determined). This procedure was repeated three, six and nine days after harvest.

For experiments using tritiated sucrose, 1 µℓ of ^{3}H-sucrose (specific activity 9.25 MBq $mmol^{-1}$)(about 310 000 dpm) was used to label the petals.

The efficiency of ^{14}C-sucrose recovery (61,4±15,4 percent) was greater than that attained with ^{3}H-sucrose (10,8±4,8 percent). This increased, however, when all the petals were labelled (50,3±15,2 percent).

Sugar separation by thin layer chromatography

Following the determination of gross radioactivity the remaining sugar extracts were refrigerated or aliquots streaked on thin layer chromatography plates (silica gel $60F_{254}$, Merck) and developed with n-butanol : acetic acid : diethyl ether : water 9:6:3:1 (HARBORNE, 1973). Sugars were tentatively identified using authentic markers with each extract and their chromatographic positions identified by spraying the plate with aniline hydrogen phthalate made up by dissolving aniline (9,2 mℓ) and phthalic acid (16 g) in n-butanol (490 mℓ), ethanol (490 mℓ) and distilled water (20 mℓ) (HARBORNE, 1973). The sugars that co-chromatographed with known standards were scraped into vials and the silica gel eluted with 1 mℓ of 100 percent methanol. The radioactivity associated with these eluants was determined as described before.

Sugar separation by gas liquid chromatography

A 500 µℓ aliquot of nectar was placed into a pill vial and reduced to dryness at 40°C. To this residue 0,5 mℓ of a solution of 25 mg $mℓ^{-1}$ hydroxylamine hydrochloride dissolved in pyridine was added, the vial stoppered and heated at 40°C for twenty minutes. This converted the sugars to their oximes. To silylise these oximes, once the solution had cooled, a 100 µℓ aliquot was carefully placed in a reactivial and taken to dryness under nitrogen at 40°C. The residue was reacted with 50 µℓ of Sil-A TMS derivative at room temperature for fifteen minutes. To separate these sugars by gas liquid chromatography, a Chromasorb

HP 80-100 glass column (approximately 2 m in length and with a 6 mm internal diameter) was used. The programme started at 125°C and was held for three minutes, whereafter the temperature was steadily increased at 2°C a minute to 260°C. A 0,5 µℓ sample of the silylised sugar was injected into the column for analysis and the retention times of the constituent sugars compared against authentic markers.

Determination of acid invertase activity

After homogenising 0,5 g fresh mass of the flower part in 20 mℓ ice-cold phosphate citrate buffer (pH 5,2) for five minutes, the homogenate was filtered under vacuum through Whatman No. 41 filter paper. The filtrate was then incubated with 20 mℓ of ten percent sucrose solution in a stoppered flask on a shaker at 25°C. One millilitre samples were withdrawn for analysis and the enzyme activity of these subsamples stoppered by keeping the samples at 80°C for two minutes. Small aliquots of the inactivated extracts were added to 1 mℓ 3,5-dinitrosalicylic acid reagent and boiled for five minutes. This reagent was prepared by dissolving 1 g of 3,5-dinitrosalicylic acid in 20 mℓ of 2N sodium hydroxide. On diluting to 70 mℓ with water, 30 g of potassium sodium tartrate was added. The mixture was diluted to 100 mℓ. After cooling the reaction mixture, 10 mℓ of water was added and the optical density read at 540 nm (DIMALLA AND VAN STADEN, 1980).

Results and Discussion

With respect to assimilate transport within the carnation flower, little work has been done on the amino acids even though they are a vital component of the assimilate pool. Difficulty in studying these compounds is due to their presence in low quantities; the total content of nitrogenous substances not exceeding more than a half a percent in the phloem sap of most plants. Usually about half this amount is represented by proteins whose involvement in long-distance transport is yet to be established. The other half includes amino acids, amides and other simple nitrogenous compounds that are believed to take part in phloem transport. Choosing a translocatory amino acid in the carnation flower was a problem. Phloem may be penetrated by virtually all the amino acids detected in leaf mesophyll tissue (KURSANOV, 1984) but different amino acids are translocated at dissimilar speeds (NELSON AND GORHAM, 1959). Of the commonly transported amino acids within the phloem, glutamic acid was chosen. The radioactive form of this amino acid was injected into the stem which, unfortunately, did not overcome the possibility that some label may be mechanically 'sucked' into the xylem vessels where the pressure is negative. Glutamic acid moved within the carnation flower regardless of postharvest treatment with the majority of radioactivity generally found within the stem, calyx and receptacle (Fig. 3.1). On the third day after harvest, relatively high levels of the label were detected in the ovary of all the treatments. As this occurred even in treatments delaying senescence, namely silver thiosulphate and ethanol, no correlation between the partitioning of dry matter and the translocation of the selected amino

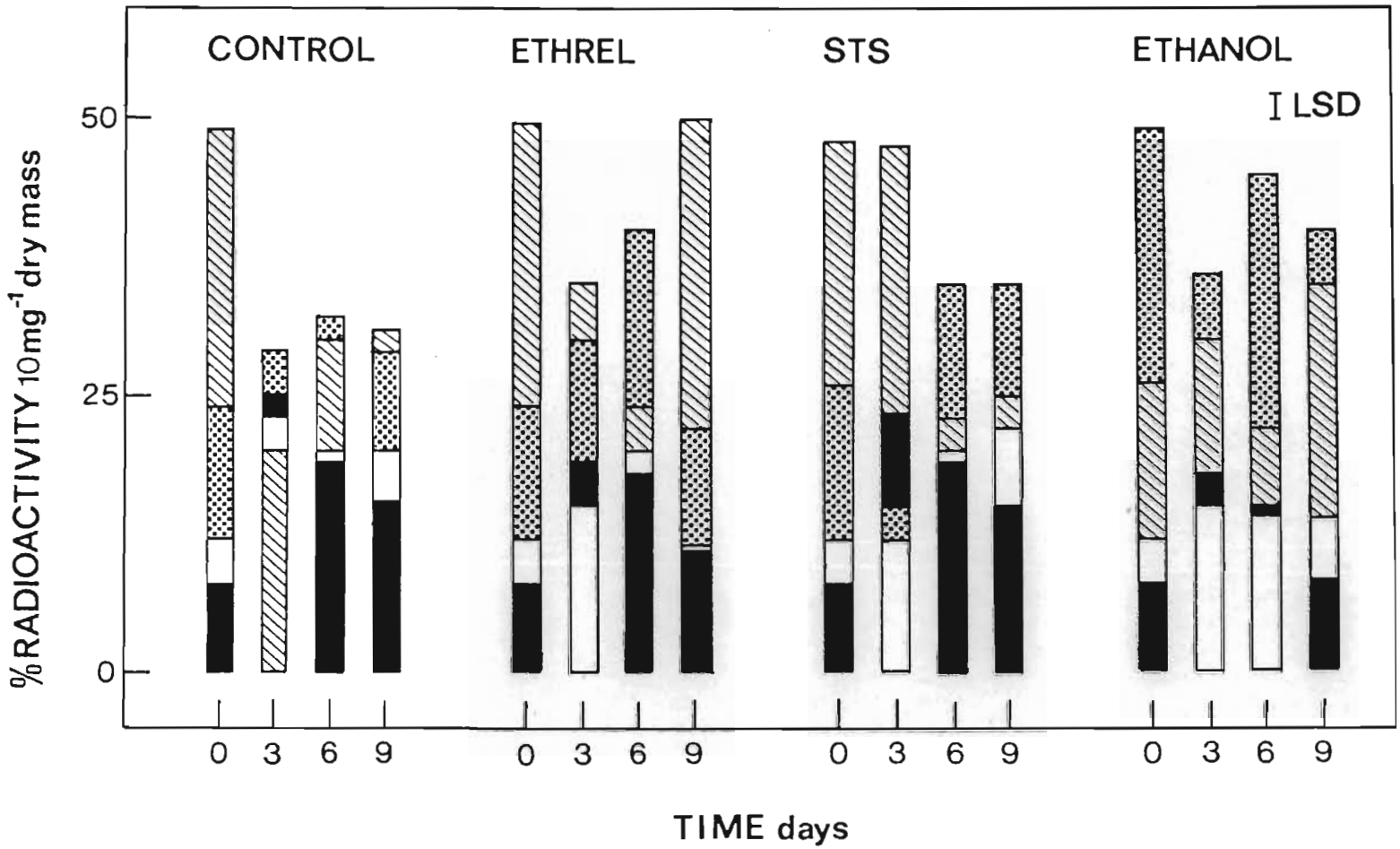


Figure 3.1 : The movement of labelled (^{14}C) glutamic acid within the carnation flower applied via the stem. Radioactivity (^{14}C) was measured from the petals (□), ovary (■), the receptacle (▩) and calyx plus stem (▨). The dpm's of each flower part are expressed as a percentage of the recovered ^{14}C within each treated flower. LSD (P=0,05) shows the least significant difference between the means.

acid during carnation flower senescence could be drawn. Interest thus focused solely on carbohydrate movement within the carnation flower.

To determine the movement of carbohydrates within the carnation flower, initially the influence of the petals in carbohydrate partitioning was gauged. This was investigated by removing the petals from the treated carnation flowers and quantifying ovary dry mass (Fig. 3.2). Treating a petalless flower with 2-chloroethyl phosphonic acid resulted in a significant decrease in ovary dry mass compared to the control. This suggests that in the presence of ethylene the petals of the whole flower are a weaker sink than the ovary and consequently the carbohydrates are transported to the ovary from the petals, stem, calyx and bracts. So in the petalless flowers, the lack of petals in the presence of ethylene may account for the decrease in ovary dry mass. This is in agreement with NICHOLS AND HO (1975 a,b) whom hypothesised that the petals are a source of carbohydrates for ovary growth. In the silver thiosulphate and ethanol-treated flowers ovary dry mass did not increase but removal of the petals from these flowers resulted in increased ovary growth. This implies that the ovaries could then utilise stem-derived carbohydrates more effectively. These assumptions were, however, taken on dry mass measurements without taking other carbohydrate pool modifying metabolic events into account.

An obvious event that could have been stimulated with ovary exposure, particularly in petalless flowers, was photosynthesis. Previous results (Fig. 1.6) have shown that in the control and 2-chloroethyl phosphonic acid treated flowers, the ovary chlorophyll levels increased as the

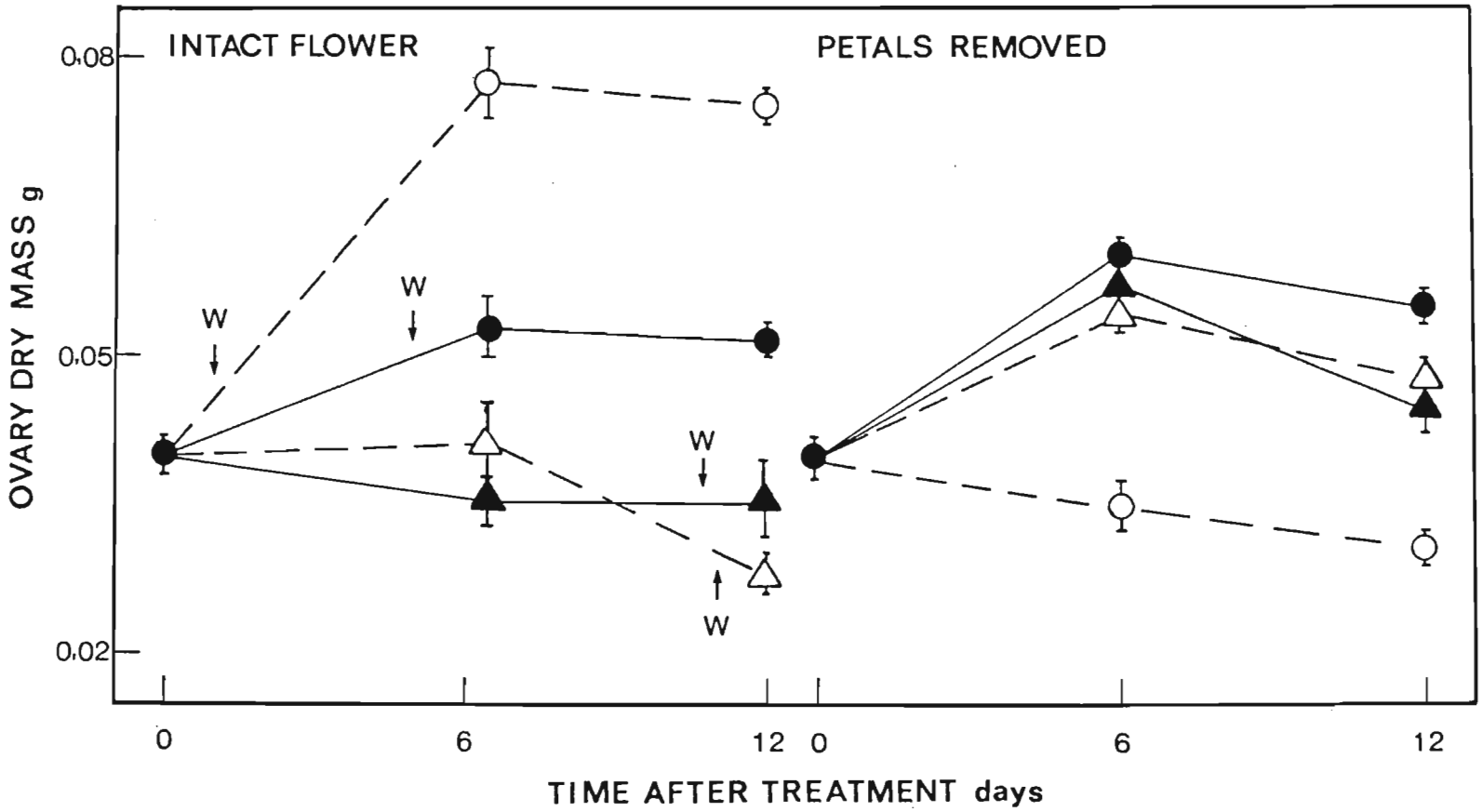


Figure 3.2 : The change in dry mass of the ovary over a period of twelve days from harvest from control (-●-), 2-chloroethyl phosphonic acid (-○-), silver thiosulphate (-△-) and ethanol (-▲-) treated cut carnation flowers. W signifies when petal wilting occurred. Vertical bars indicate standard error of the mean.

flowers aged whilst with silver thiosulphate and ethanol-treatments the ovary chlorophyll levels altered little from harvest. Petal removal resulted in a slight increase in the ovary chlorophyll content with silver thiosulphate and ethanol-treatments. In the case of petalless control and 2-chloroethyl phosphonic acid-treated flowers, the ovary chlorophyll content decreased or remained unchanged compared to that of the whole flower (Fig. 3.3). This suggests that additional photosynthates may be available for ovary growth in silver thiosulphate and ethanol treated petalless carnation flowers.

The ability of intact and petalless treated cut carnation flowers to fix carbon dioxide was thus compared directly after harvest, and again three and six days later. The amount of radioactivity detected in the sugar extracts from each system was then determined (Fig. 3.4). Labelled carbon dioxide was incorporated most efficiently by the flowers immediately after harvest. This level of carbon dioxide incorporation, however, decreased so that finally differences between treatments could not be detected. Thus the ability of the carnation flower to add to its carbohydrate pool via photosynthesis can occur during the first few days following harvest; the petalless flower being more efficient than the intact flower.

To establish the flower components responsible for carbon dioxide fixation, intact and petalless carnation flowers were exposed to labelled carbon dioxide for twenty-four hours after which they were divided into petals (where present), ovary and calyx plus stem. The soluble sugars were extracted from each flower part and the radioactivity associated with the sugar extract determined (Fig. 3.5).

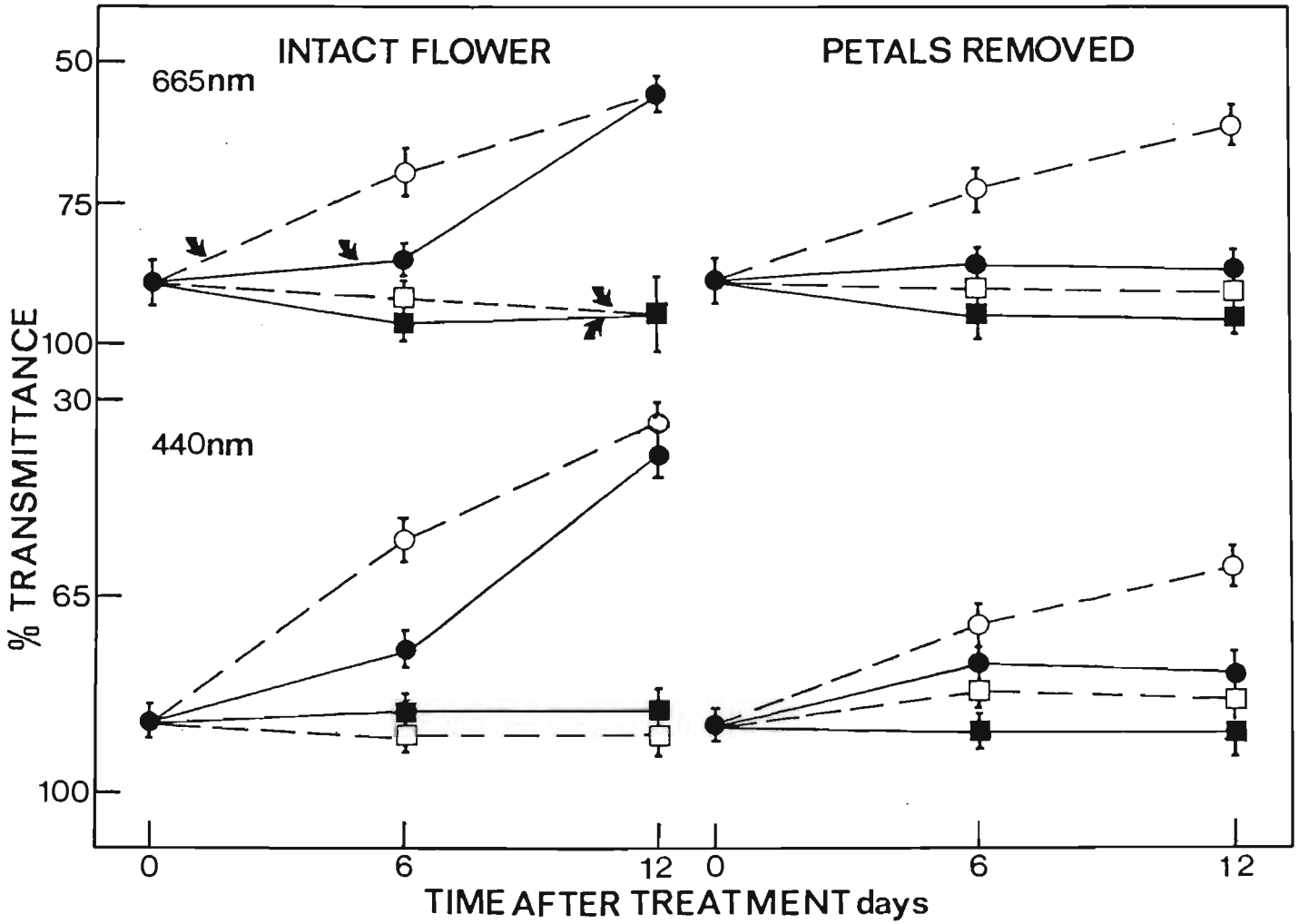


Figure 3.3 : The change in the chlorophyll content of the ovary from intact and petalless flowers from control (-●-), 2-chloroethyl phosphonic acid (-○-), silver thiosulphate (-□-) and ethanol (■) treated flowers. ↘ Indicates when petal wilting occurred. Transmittance was measured at 665 nm and 440 nm. Vertical bars indicate the standard error of the mean.

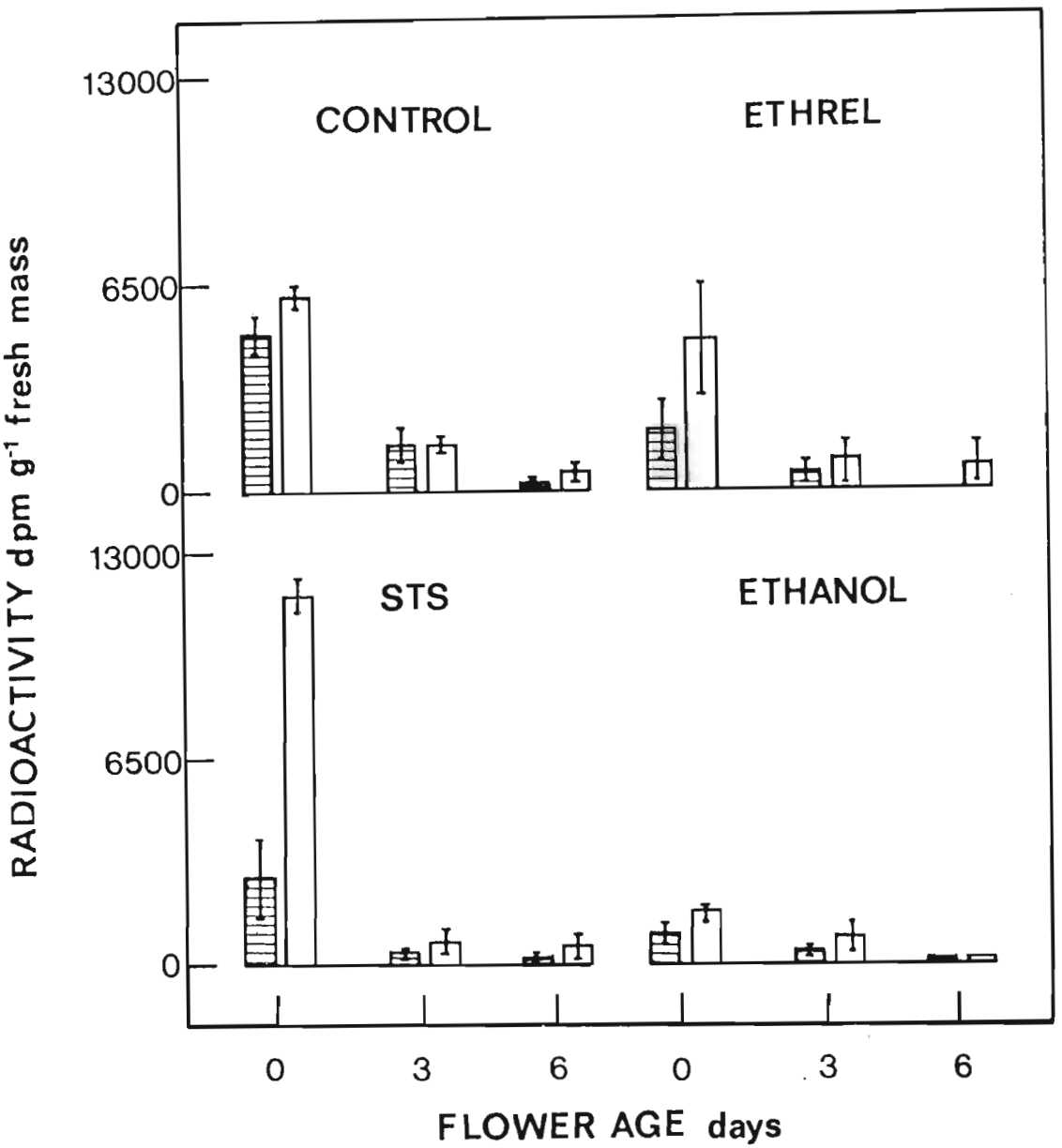


Figure 3.4 : The radioactivity (^{14}C) detected in the sugar extracts from intact (▤) and petalless (□) flowers from control, 2-chloroethyl phosphonic acid (ETHREL), silver thiosulphate (STS) and ethanol treated flowers. Vertical bars indicate the standard error of the mean.

In the control, 2-chloroethyl phosphonic acid and ethanol-treated intact flowers the sugar extracts from the calyx plus stem contained the highest amount of label. This was followed by the ovaries with the petals yielding the lowest level of radioactivity. In the silver thiosulphate-treated flowers the trend differed. In this instance a higher level of label was detected in the ovaries than in the calyx plus stem. Where the petals were removed the ovaries and calyx plus stem did not contain significantly different levels of label. Whereas it would appear that the ovaries were capable of incorporating carbon dioxide, the possibility of labelled carbon transport between the calyx and stem and the ovaries could not be disregarded since applied labelled sucrose can be transported from one part of the flower to another within twenty-four hours (HARRIS AND JEFFCOAT, 1972; NICHOLS AND HO, 1975 a,b). Furthermore, analysis of the carbon labelled sugar extracts of the ovaries showed that a considerable amount of radioactivity co-chromatographed with sucrose (Table 3.1).

It was thus necessary to establish whether isolated ovaries could fix labelled carbon dioxide. When the ovaries were excised from the treated flowers and exposed to labelled carbon dioxide, labelled carbon incorporation into the sugars did not occur within the first twenty-four hours after harvest, irrespective of treatment (Table 3.2). Five days later, labelled carbon incorporation had taken place. By seven days the control and 2-chloroethyl phosphonic acid, which contained more chlorophyll, were still able to fix labelled carbon dioxide whereas this ability was much reduced or lost in the silver thiosulphate and ethanol treatments. These results suggest that the stem and calyx

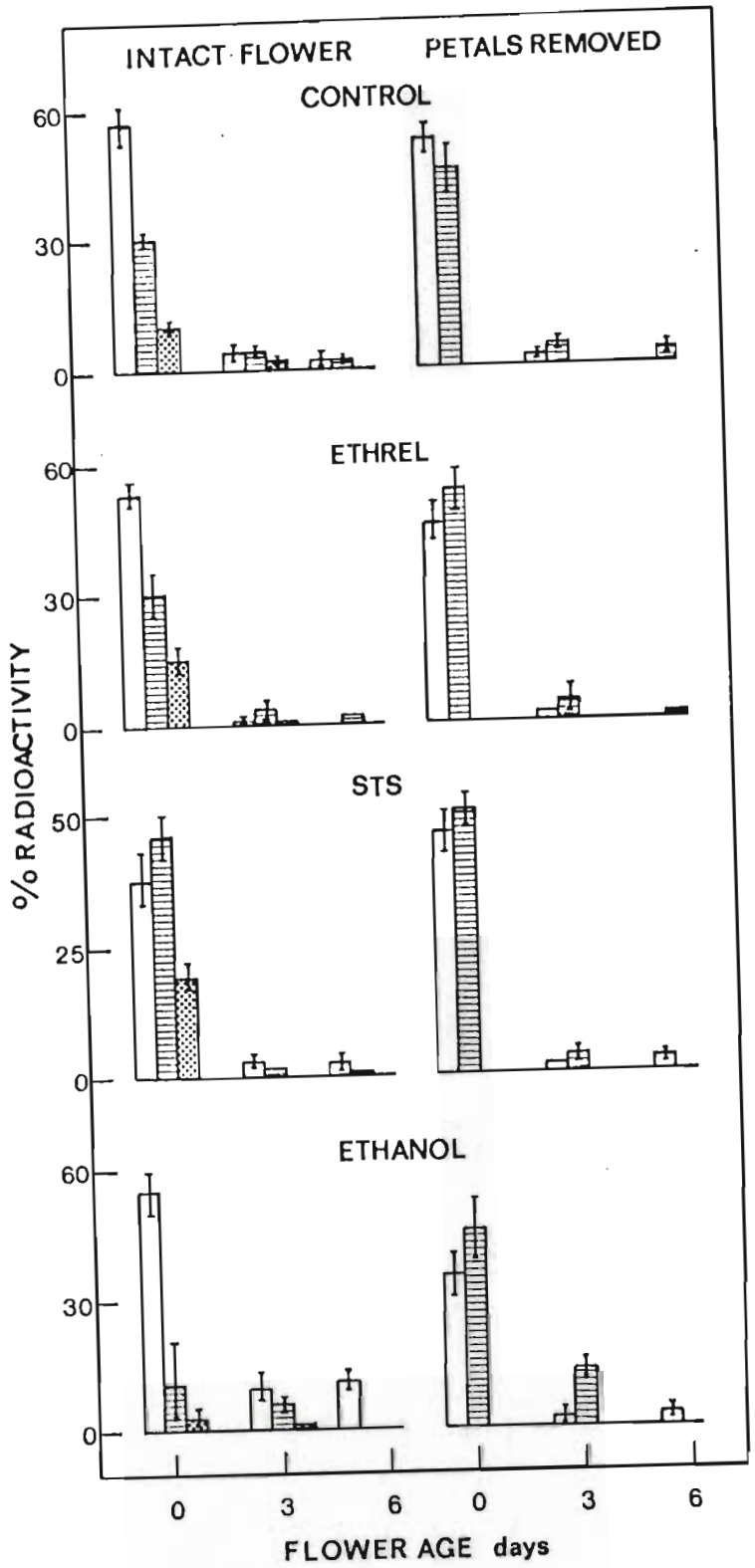


Figure 3.5 : The radioactivity (^{14}C) in sugars extracted from the calyx plus stem ( ), the ovary ( ) and the petals, when present ( ) from intact and petalless control, 2-chloroethyl phosphonic acid (ETHREL), silver thiosulphate (STS) and ethanol treated flowers after being exposed for twenty-four hours to a 0,03 percent $^{14}CO_2$ atmosphere. The dpm's measured from each flower part is expressed as a percentage within each flower. Vertical bars indicate the standard error of the mean.

Table 3.1 : Radioactivity (^{14}C) that co-chromatographed with sucrose (Rf = 0,20 on TLC plates developed with n-butanol : acetic acid : diethylether : water 9:6:3:1) twenty-four hours after freshly harvested flowers were exposed to a 0,03 percent $^{14}CO_2$ atmosphere. The radioactivity is expressed as a percentage of the total ^{14}C in the extracted sugars of each sample. Standard error is indicated.

Treatment	Radioactivity associated with sucrose (%)
Control	15,2 ± 2,3
Ethrel	18,3 ± 3,0
STS	10,7 ± 5,0
Ethanol	1,3 ± 0,4

Table 3.2 : Radioactivity (^{14}C) detected in the extracted sugars from ovaries excised from ageing treated cut carnation flowers after being exposed for twenty-four hours to a 0,03 percent $^{14}CO_2$ atmosphere. Standard error is indicated.

Treatments	Time after harvest (days)		
	1	5	7
Control	0	10261 ± 216	299 ± 6
Ethrel	0	10687 ± 58	120 ± 9
STS	0	1832 ± 1503	55 ± 18
Ethanol	0	7354 ± 2455	0

were responsible for most of the labelled carbon dioxide incorporation of the intact cut flower, and that the radioactivity which was detected in the ovaries after twenty-four hours was transported towards them from this source.

A thorough investigation of the movement of sucrose within the cut carnation flower was then undertaken. Firstly, labelled sucrose (^{14}C-sucrose) was injected into the stem of the treated carnations and the distribution of radioactivity within the flower monitored (Fig. 3.6). Initially, with the control and 2-chloroethyl phosphonic acid treatment, most of the label was detected in the petals. Following petal wilting, which occurred on the fifth day in the control and on the second day when ethylene levels were supplemented, the sink strength appeared to move from the petals to the ovary where the majority of the label accumulated. Later the calyx plus stem either became the stronger sink or transport of the applied labelled sucrose became hindered probably due to vascular blockage. With silver thiosulphate and ethanol treatments, the calyx plus stem retained the majority of label; yet after ageing three days the petals in both these treatments became the dominant sink. The petals with the silver thiosulphate treatment displayed stronger sink properties than the petals on ethanol-treated flowers. Thus, carbohydrates supplied to the flower from the stem were competed for by the petals and ovaries, the relative sink strength of these two organs depending on the time lapsed after harvest (Fig. 3.6) (MOR, REID AND KOFRANEK, 1980). The presence of ethylene also appears to change the source-sink relationship of the cut carnation in such a way that the ovary becomes the major

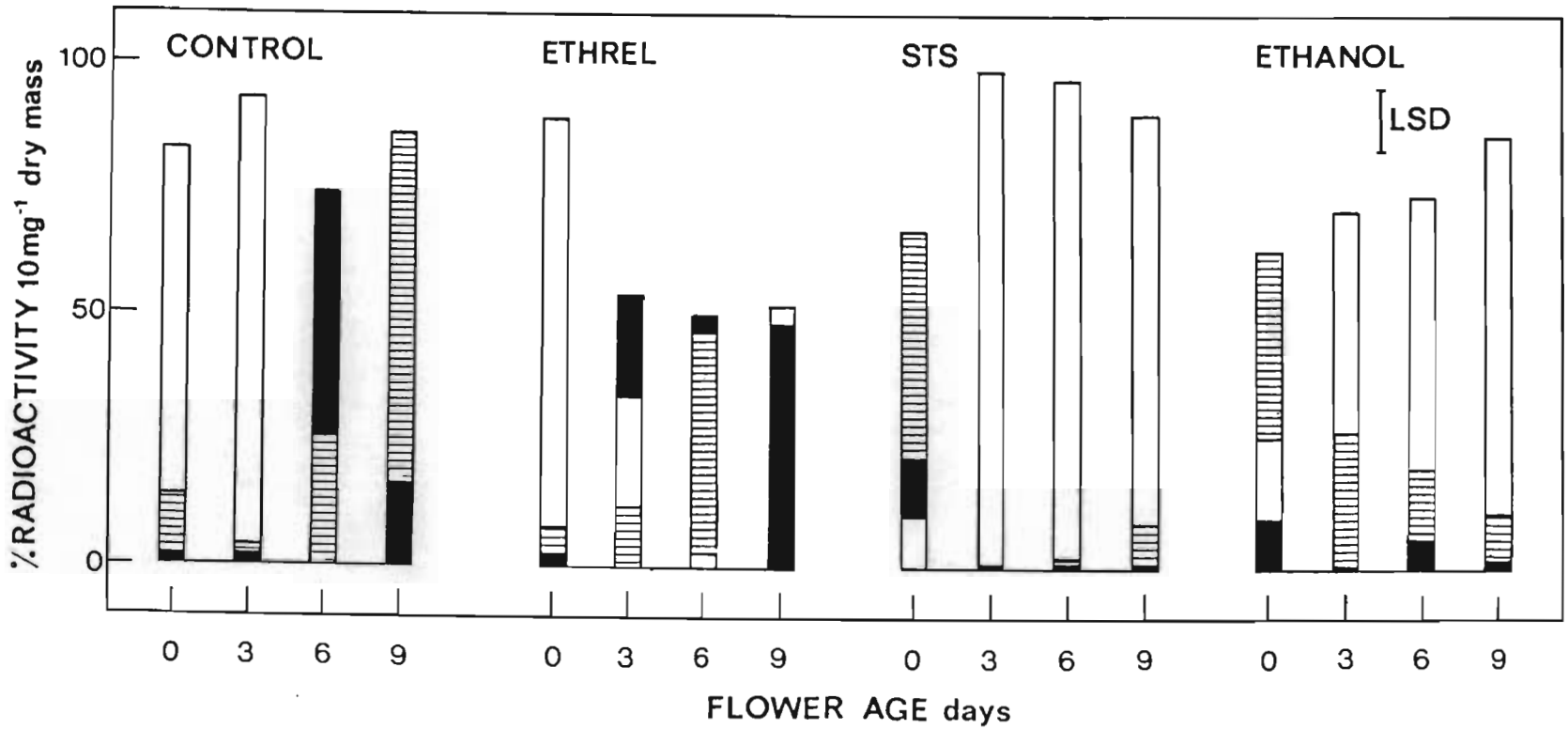


Figure 3.6 : The radioactivity (^{14}C) detected in the petals (□), ovary (■) and calyx plus stem (▤) of control, 2-chloroethyl phpsphonic acid (ETHREL), silver thiosulphate (STS) and ethanol treated flowers twenty-four hours after injection of labelled sucrose into the stem. LSD (P=0,05) shows the least significant difference between the means.

sink (NICHOLS, 1976; DUCASSE AND VAN STADEN, 1981).

To establish the validity of these conclusions, the petals and ovary, respectively, were also treated with labelled sucrose (^{14}C-sucrose) twenty-four hours prior to analysis. When the petals were labelled (Fig 3.8) on the control flowers, little sucrose movement into the ovary was detected. Furthermore, injecting sucrose into the ovary (Fig. 3.7), radioactivity was detected in the petals prior to petal wilting but following petal wilting remained within the ovary. In contrast, 2-chloroethyl phosphonic acid-treatment stimulated the movement of sucrose from the petals to the calyx plus stem and ovary during petal irreversible wilting (Fig. 3.7). The sucrose injected into the ovary did not move from this flower part until the petals were necrotic. Radioactivity was then detected in the calyx plus stem (Fig. 3.8). The differences in the pattern of movement between these two treatments that senesced in the presence of ethylene may be due to the intensity of sink activity. In the control flower the sink activity of the ovary does not become dominant until three days after harvest whilst with 2-chloroethyl phosphonic acid, dominant ovary sink activity is immediately apparent. The lack of movement of sucrose from the petals during the latter part of the experimental period is due to petal death, and hence cellular disruption, confining the sucrose within the petals. At this time there would be little available carbohydrate in the petals for redistribution.

With the ethylene inhibitory treatments, silver thiosulphate and ethanol, labelled sucrose applied to the petals generally remained

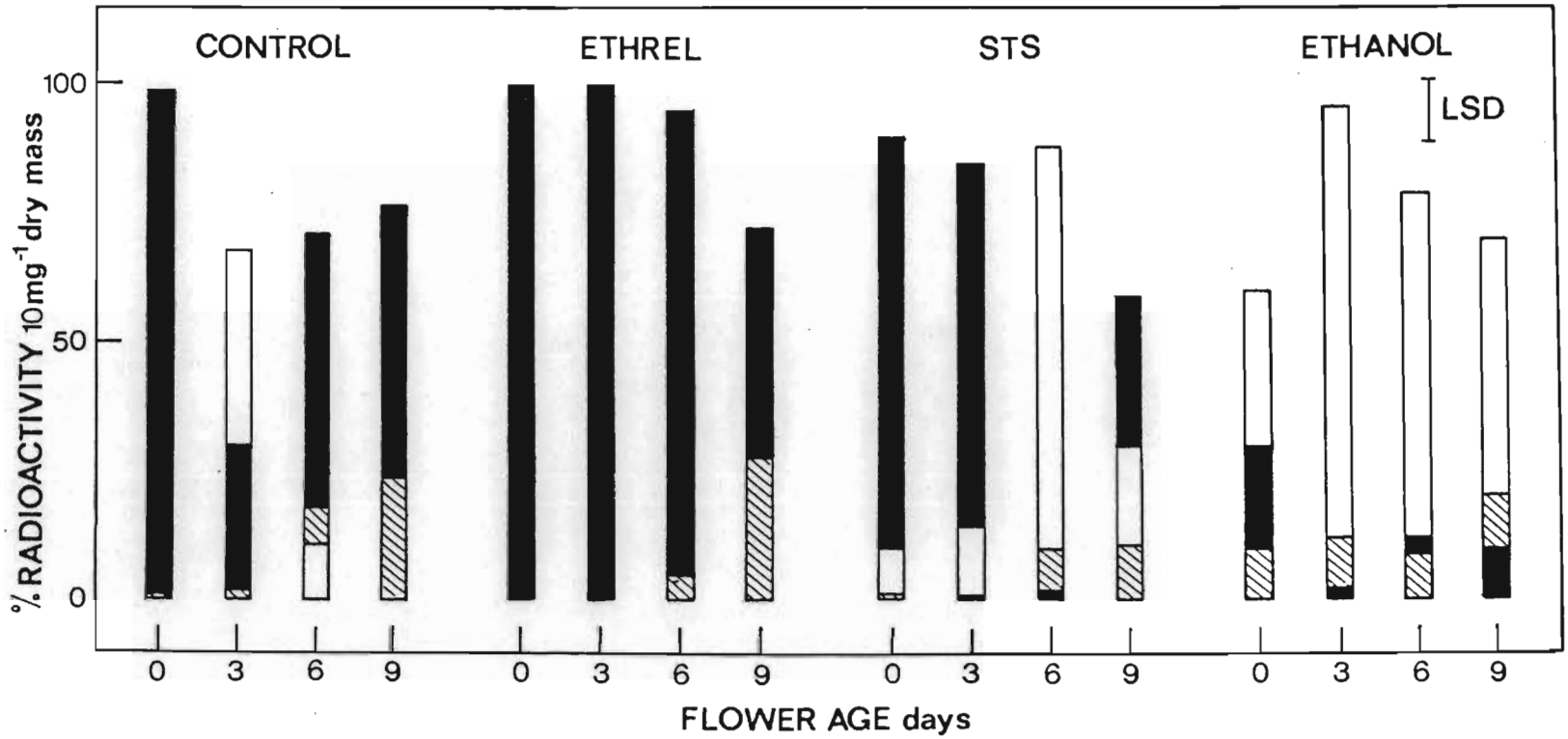


Figure 3.7 : The radioactivity (^{14}C) detected in the petals (□), ovary (■) and calyx plus stem (▨) of control, 2-chloroethyl phosphonic acid (ETHREL), silver thiosulphate (STS) and ethanol treated flowers twenty-four hours after injection of labelled sucrose into the ovary. LSD (P=0,05) shows the least significant difference between the means.

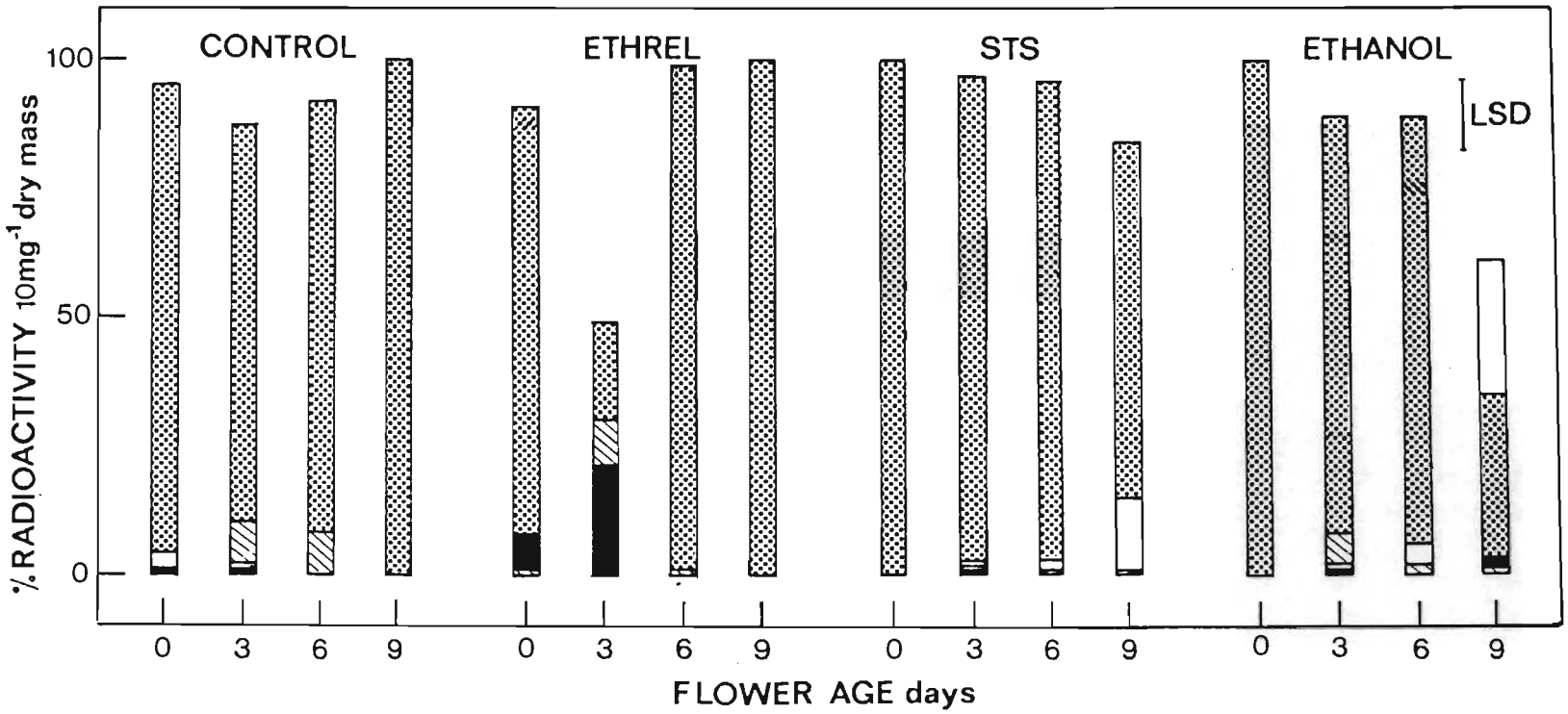


Figure 3.8 : The radioactivity (^{14}C) detected in the petals (□), ovary (■), calyx stem (▨) and labelled petals (▩) of control, 2-chloroethyl phosphonic acid (ETHREL), silver thiosulphate (STS) and ethanol treated flowers twenty-four hours after injection of labelled sucrose into the petals. LSD (P=0,05) shows the least significant difference between the means.

within the labelled petals. However,in the first days of the experiment some movement to the other flower parts was detected, the majority of the label being detected in the unlabelled petals. The dominant sink activity of the petals was further substantiated when the ovary was injected with labelled sucrose. Carbohydrate movement from this flower part was mainly to the petals. With silver thiosulphate-treatment, the majority of radioactivity moved to the petals on the sixth day after harvest whilst with ethanol treatment, movement of sucrose to the petals was apparent from the onset of the experiment. Hence it appears that the silver thiosulphate and ethanol-treated flowers were unable to assimilate the applied labelled sucrose in the ovary and thus exported it to other flower parts. This may account for the lack of ovary development in flowers exposed to these postharvest treatments.

The sucrose used in the above experiments was labelled equally on all carbons. Hence the question of labelled carbon dioxide evolution as a possible source of contamination had to be considered. NICHOLS (1973) and DUCASSE AND VAN STADEN (1981) realised this problem. Sucrose metabolises rapidly, that is within four hours of application, to produce labelled glucose, fructose and respiratory carbon dioxide. The measurement of this carbon dioxide production has been used to indicate the respiratory rate of carnation flowers at different senescent stages (COORTS, 1973). This is particularly relevant as it has been shown that the carnation flower is capable of carbon dioxide fixation (Fig. 3.4). Hence two four-day-old cut carnation flowers were placed together within a sealed container. One flower was labelled with sucrose (^{14}C-sucrose) whilst the other remained unlabelled. They were at the

period of their greatest respiratory activity. After a twenty-four hour exposure to labelled carbon dioxide the flowers were analysed separately (Table 3.3).

Table 3.3: Fixation of labelled carbon dioxide emanating from a sucrose labelled flower by an unlabelled flower. The sugars were extracted separately and the radioactivity determined (dpm g^{-1} fresh mass). Standard error is indicated.

Day labelled	Flower part analysed	Labelled flower (dpm)	Unlabelled flower (dpm)
4	Petal	2210 ± 243	0
	Ovary	130 ± 11	0
	Calyx	1216 ± 59	0
5	Petal	602 ± 175	0
	Ovary	1341 ± 770	0
	Calyx	0	0

No radioactivity was detected within the unlabelled flower confirming that there is no radioactive cross contamination between the flowers. Nevertheless, with carbon dioxide fixation occurring differentially between flower parts (Fig. 3.5), there remains the possibility of radioactive contamination between flower parts. In view of this, the use of tritiated sucrose (^{3}H-sucrose) was compared against carbon labelled sucrose (^{14}C-sucrose).

Two facets of sucrose labelling were tested. Firstly, the sucrose labelled on different atoms (^{14}C, ^{3}H) was compared (Fig. 3.9). When carbon-labelled sucrose was applied to only two petals, radioactivity was detected within the ovary. However, using the tritiated sucrose, no radioactivity was detected in the ovary. Tritiated sucrose does have a low recovery efficiency (10,8 $\pm$ 4,8 percent). Because of this, the second facet of sucrose labelling tested was the effect of labelling all the petals compared to only two petals. The recovery of labelled tritiated sucrose increased to 50,3 $\pm$ 15,2 percent. Furthermore, the greatest percentage of radioactivity was detected in the ovary (Fig. 3.9). Labelling all the petals with carbon-labelled sucrose showed a vast increase in radioactivity in the calyx plus stem and receptacle. Considering the possibility of labelled carbon (^{14}C) contamination and the photosynthetic potential of this tissue, using tritiated sucrose suggests that the majority of the sucrose movement from the petals is to the ovary suggesting that the calyx plus stem and receptacle accumulate radioactivity not solely due to translocation.

In summary, initially all the treated cut carnation flowers were capable of contributing to their soluble carbohydrate pool via photosynthesis (Fig. 3.4). Although the presence of ethylene stimulated the development of ovary wall chloroplasts (Plate 2.3) so that by the fifth day they themselves fixed labelled carbon dioxide (Table 3.2), it appears that the calyx plus stem have a far greater photosynthetic ability (Fig. 3.5) and transport a major portion of their fixed labelled carbon to the ovary. This indicates that ovary growth, which

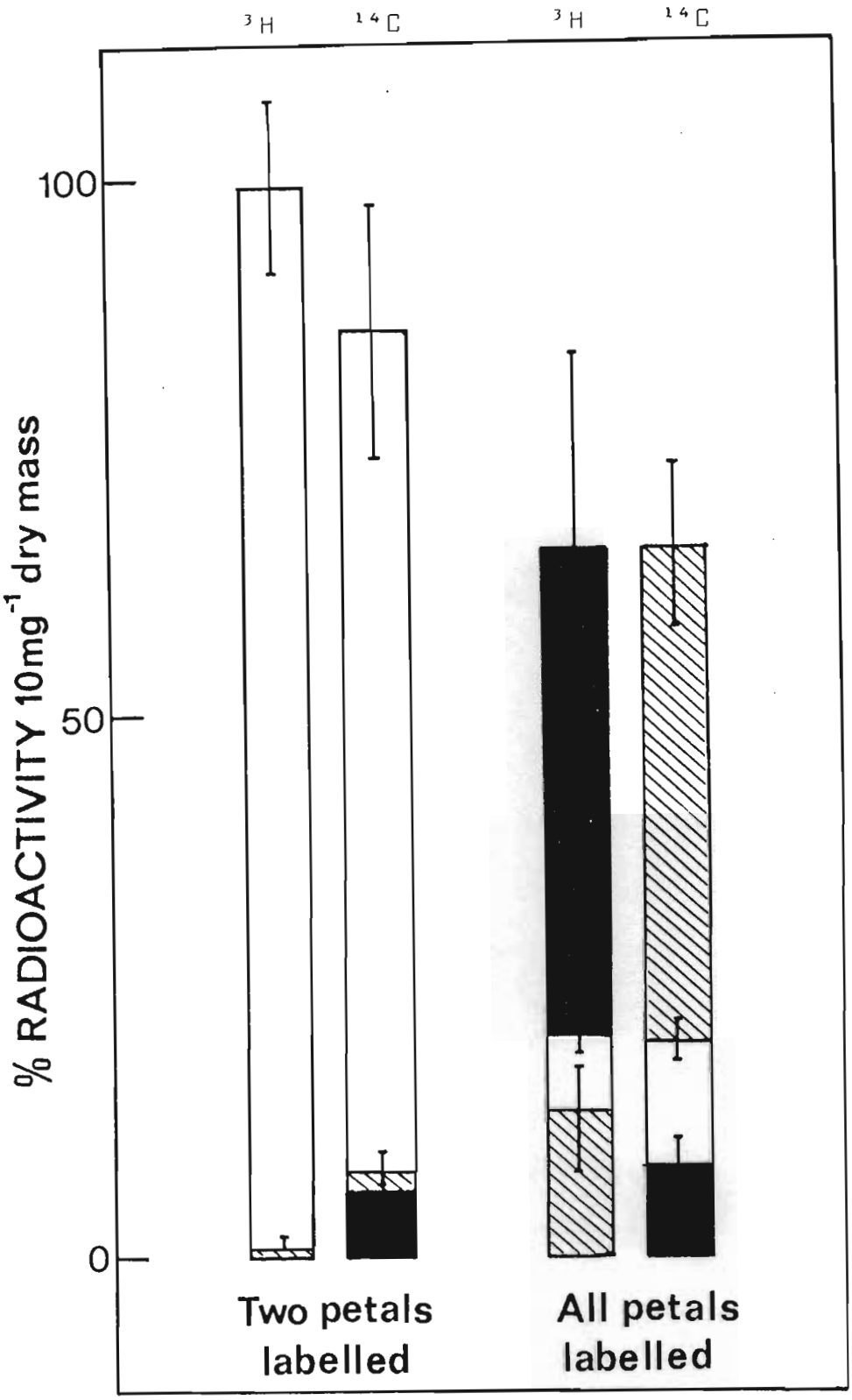


Figure 3.9 : The distribution of labelled ^{3}H-sucrose and ^{14}C-sucrose when all the petals or two of the petals were labelled four days after harvest. Radioactivity was measured from the petals (□), ovary (■) and calyx plus stem (▨). Vertical bars indicate the standard error of the mean.

is correlated with the presence of ethylene (NICHOLS, 1968; 1976; CAMPRUBI AND NICHOLS, 1979; DUCASSE AND VAN STADEN, 1981) occurred primarily in response to carbohydrates from external sources. These external sources of carbohydrate have been shown to be the stem (HARRIS AND JEFFCOAT, 1972; NICHOLS AND HO, 1975a; MOR, REID AND KOFRANEK, 1980) (Fig. 3.6) and the petals (NICHOLS AND HO, 1975b; DUCASSE AND VAN STADEN, 1981) (Figs. 3.7, 3.9). When supplementing the ethylene levels the ovary is the dominant carbohydrate sink in the carnation flower. With silver thiosulphate and ethanol treatment, which prevent ethylene action and biosynthesis respectively, the petals become the dominating sink and remain so,utilising the carbohydrates within the cut flower (DIMALLA AND VAN STADEN, 1980; HEINS AND BLAKELY, 1980; MOR, REID AND KOFRANEK, 1980; DUCASSE AND VAN STADEN, 1981). Because of this the ovary appears 'starved' which could account for the underdeveloped chloroplasts (Plates 2.4, 2.5).

Relating this information to the source-sink theory (WARREN-WILSON, 1972) the petals, prior to irreversible wilting, must be the region within the flowerhead with the highest metabolic activity. Whether the ovary, by becoming a centre of greater metabolic activity, causes the irreversible wilting of the petals is a point in question. However, following the senescence of the petals, the ovary is the flower part that shows the most development and hence utilises the most carbohydrates. The source of the carbohydrates appears to be any flower part that contains an excess of carbohydrates over and above its metabolic requirement. The movement of the sugars from a flower part will depend primarily on the position and intensity of sink activity as

well as the sugar load available from other flower parts for transport. Thus a source can be any tissue producing an excess of assimilates which are then available to other plant parts, it is understandable that an application of exogenous sucrose to a flower part will, if the tissue is not highly metabolically active, be exported. This is especially true in the carnation flower as its tissues do not appear to store carbohydrates as starch (DIMALLA AND VAN STADEN, 1980). Any carbohydrate storage would probably be as sucrose in the vacuole (LEIGH, 1984), the petals being anatomically best equipped for such a role with their dominating vacuole and peripheral cytoplasm.

To date the only anatomical study of the translocatory pathway in the cut carnation flower was made by HO AND NICHOLS (1975). Unfortunately their work was confined to the basal uptake of labelled sucrose. Inspired by the work of SACALIS AND DURKIN (1972), who working with roses concluded that absorbed labelled sucrose moved along the phloem, HO AND NICHOLS (1975) found that stem applied labelled sucrose in the carnation moved in both the phloem and xylem. Some sucrose will be expected to move in the xylem as this tissue provides a low resistant pathway to water traffic that supplies transpiration. Thus as water is evaporating continuously from the petal surface cells, so this water is replaced from the stem-immersed sucrose solution. Removal of these xylem orientated sugars can occur anywhere along the vascular tissue, the rate of sugar removal depending on sugar concentration in the tranpiration stream and the length of the pathway (HO AND NICHOLS, 1975). Therefore, for sucrose movement between plant parts, the phloem is likely to be the predominant long-distance pathway.

To reiterate from Chapter 1, the loading of sucrose into the phloem is considered to be the driving force for the mass flow of assimilates from source to sink by decreasing the osmotic potential of the sieve tubes, which in turn generates a water potential gradient and a water influx into the phloem (GIAQUINTA, 1980). This phloem movement of the sugars may thus be another channel via which water uptake to the petals can compensate for transpiratory losses. On the other hand stomatal closure, as induced by the senescence promotory plant hormones, abscisic acid (JONES AND MANSFIELD, 1970) and ethylene (MADHAVAN, CHROMINISKI AND SMITH, 1983), may not only affect the transpiration stream but also inhibit movement in the phloem which results in petal carbohydrate deficiencies. The traffic of substances is not confined to the long-distance xylem and phloem tissues. During petal wilting or irreversible wilting the flux of carbohydrate from the petals may be by the short-distance apoplastic and symplastic channels. Thus the water status of the petals may not be paramount for the movement of carbohydrates. Noteworthy is that the majority of sucrose movement from the petals occurs prior to or as the petals irreversibly wilt (Fig. 3.7).

The speculative nature of this latter discussion exemplifies the situation reached in this research. The movement of the translocatory carbohydrate within the cut carnation flowerhead has been outlined with the exogenous application of labelled sucrose. Together with the circumstantial evidence, it appears that all the flower parts are capable of being carbohydrate sources, including the ovary. It is doubtful in the natural situation that this latter flower part ever

acts as a source. It probably was only due to an excess amount of sucrose being injected into the ovary and the ovary being metabolically incapable of utilising all the added sucrose that radioactivity was exported. In the petals, the question remains as to why a natural excess of carbohydrates would occur? Logically this would happen if they were not being utilised, however, prior to petal senescence there is a vast increase in the respiratory rate (COORTS, 1973). Indeed, senescence has been shown to be a highly active process. Protein synthesis, aerobic metabolism in dark senescence (MARTIN AND THIMANN, 1972), as well as this large respiratory increase and the ATP formation which accompanies it (TETLEY AND THIMANN, 1972; MALIK AND THIMANN, 1980) are requirements for senescence. It may be only when partial uncoupling of respiration from phosphorylation and thus the $NADH_2$ and $FADH_2$ produced from the tricaboxylic cycle are not able to pass through the electron transport system to produce ATP (TETLEY AND THIMANN, 1972) that petal death eventually occurs. Nevertheless, from the first experiment investigating the influence of the presence of petals on ovary development (Fig. 3.2), to the labelled sucrose experiments (Figs. 3.7, 3.9) it is apparent that petal carbohydrates contributed to ovary growth. The calyx plus stem also provided carbohydrates for ovary growth; their photosynthetic ability supplementing the sucrose levels.

The concept that excess assimilates are available for transport implies that sink activity would control the direction of assimilate movement. Much attention has focused on the unloading of this sucrose from the phloem as previously noted in Chapter 1. GEIGER AND FONDY

(1980) proposed a most attractive theory based on the assumption that the unloading of sucrose from the phloem occurs down a concentration gradient. Hence unloading and the transfer of sucrose to nearby consuming cells will occur without further expenditure of energy by sieve elements or their accessory cells. Sink tissues could regulate this passive unloading by maintaining a low concentration of sucrose, that is, by sucrose utilisation. Two enzymes are known to hydrolyse sucrose, namely invertase (β-fructofuranosidase) and sucrose synthase. In the cut carnation flower acid invertase is the most active enzyme (HAWKER, WALKER AND RUFFNER, 1976) and it may well be this enzyme that is hydrolysing the sucrose within sink tissues and maintaining a concentration gradient.

Comparing acid invertase activity between the petals and the ovary when the flower was harvested showed that, at the onset, no significant difference between these flower parts existed (Table 3.4). This situation changed within a day so that higher levels of this enzyme were detected within the petals. On the fifth day, at the first signs of petal irreversible wilting, greater invertase activity was found in the ovary. This situation prevailed until the petals became necrotic. During the final stages of flower senescence (nine days after harvest), the enzyme activity between the petals and the ovary was the same. From these results, sink activity could be related to invertase activity. The petals prior to their irreversible wilting were carbohydrate sinkswhilst the ovary dominated this role after petal senescence.

Table 3.4: Invertase activity (mg glucose g^{-1} fresh mass) in the petals and ovary of a cut carnation flower at harvest. Standard error is indicated.

Time after harvest (days)	Petals	Ovary
0	13,0 ± 1,9	12,9 ± 0,2
1	16,2 ± 0,8	11,1 ± 0,3
3	13,3 ± 3,0	12,9 ± 1,1
4	7,1 ± 1,1	10,3 ± 0,9
5	12,3 ± 2,1	18,5 ± 0,7
6	11,1 ± 3,1	10,7 ± 0,7
7	10,7 ± 3,0	10,4 ± 1,4

HALABA AND RUDNICKI (1981) investigated invertase activity in carnation flowers developing on the parent plant and showed that there was a seven-fold decline in petal invertase activity from expanding petals to petals in the wilted state. It was only at this wilted stage that these workers detected higher invertase activity in the ovary compared to the petals. The joint petals plus ovary invertase activity of these flower parts at this wilted stage was far less than the levels initially detected in the developing petals in the bud stages. This would suggest that invertase activity has little influence in determining the sink activity between the petals and ovary during senescence. However, considering the assimilate movement that is necessary to ensure the formation of a fully opened flower from an

apical bud compared to the enlargement of the ovary as a fruit with seeds, the former growth phenomenon requires a far greater assimilate input. This thus would necessitate the need for higher levels of invertase activity.

With respect to the invertase levels reached in the petals, the invertase activity of the ovary is low, yet it does appear to contribute to sink activity as the flower senesces. This was shown by comparing the acid invertase activity in the ovaries from control flowers with those from silver thiosulphate-treated flowers which did not develop (Table 3.5). The invertase levels were higher in control ovaries than in the silver thiosulphate-treated ovaries. This silver inhibition of invertase activity is in agreement with the results of MEALOR AND TOWNSHEND (1968) who showed that this ion prevented invertase activity by non-competitive inhibition. It thus appears that invertase activity is important for translocated sucrose to be directed to the ovary so as to ensure continued growth.

It is unlikely that the invertase activity of the physiological sink is the sole mechanism by which source-sink relationships are determined within a cut flower. As WARDLAW (1965) pointed out, the phloem is highly pressurised and generally resistant to leakage along its entire length. Thus for the efflux of solute a variable permeable membrane must exist, most probably related to companion cells, or in some cases transfer cells. Sucrose does not readily cross the chloroplast envelope (AP REES, 1984) and requires active loading into

Table 3.5: A comparison of acid invertase activity (mg glucose g^{-1} fresh mass) in ovaries from control and silver thiosulphate-treated flowers. LSD (P = 0,05) = 0,41.

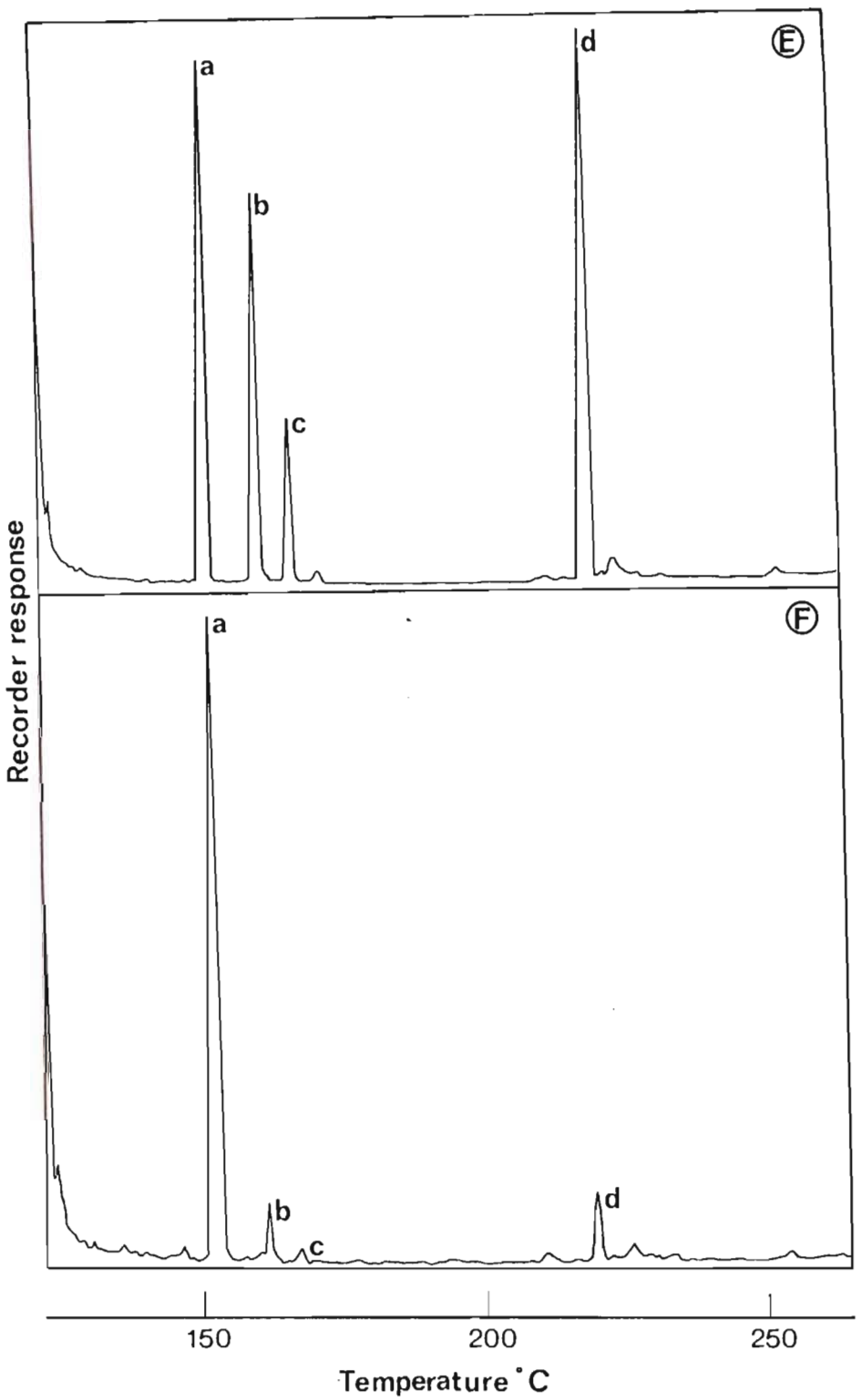
	Time after treatment (days)				
Treatment	0	1	3	6	9
Control	12,96	11,11	12,96	10,70	10,35
STS	12,96	7,11	7,03	8,12	6,31

the vacuole to be confined within the tonoplast for storage. This suggests that a sucrose concentration gradient between cells is not sufficient for sucrose movement through unit membranes. Possible effectors controlling the permeability of membranes to sucrose include the plant hormones (PATRICK, 1976; TANNER, 1980) and specific ions (FONDY AND GEIGER, 1980). The latter suggestion has merit if the loading of sucrose involves a sucrose-proton cotransport system. The driving force for the loading of sucrose would then involve an electrochemical potential gradient of protons across the membranes.

Such a stimulation to regulate the passive unloading of sucrose cannot occur in the nectaries. THORNE AND GIAQUINTA (1984) recognised that the nectaries are the only known flower part in which sucrose is unloaded against a concentration gradient. Analysis of the nectar sugars of the carnation flower indicated that sucrose is the dominant sugar. Also present were fructose, glucose and an unknown

sugar, probably a ribose, rhamnose, by its position in relation to the authentic markers. Nine days after harvest the nectary composition changed so that little sucrose, fructose and glucose were present but the unknown sugar had increased (Fig. 3.10). NICHOLS AND HO (1975a) showed that vascular tissue, mainly phloem tissue, is associated with this nectiferous tissue in the carnation flower. As the stamens protrude through this nectiferous tissue, this vascular tissue probably infiltrated the stamens as well. Even so, the nectaries in the carnation may well be the 'gates' through which plants dispose of excess sugars if the sucrose flux into the phloem exceeds the possibility of assimilation by sinks (FREY-WYSSLING AND HÄUSSERMANN, 1960). Immediately prior to, or during petal wilting, the greatest amount of nectar is produced by the carnation flower. With respect to the above hypothesis, it would appear that the additional petal-carbohydrate contribution into the vascular system exceeds the growth requirement of sink tissues. Thus, indirectly, this suggests that the carbohydrate supply to a sink tissue is not a limiting factor for growth in this situation but rather the regulation of this sink activity.

To summarise, a major event during the senescence of the cut carnation flower is the movement of assimilates, mainly carbohydrates, to the ovary. The source of these carbohydrates appeared to be any tissue producing a greater quantity of these sugars than could be metabolically utilised by the flower part. Initial contributions to this carbohydrate pool were made by the photosynthetically active tissues of the cut flowers. Those sugars phloem-loaded were hypothesised to move via a mass flow gradient to be released from this translocatory



Figures 3.10: A comparison of the sugars present in the nectar from flowers three days after harvest (E) and nine days after harvest (F). a, b, c, d, eluted off the column at 148°C, 158°C, 163°C and 215°C, respectively. a is an unknown sugar; b, c and d co-chromatographed with fructose, glucose and sucrose, respectively.

tissue in places where the permeability of the membrane was adjusted. The unloading of this sucrose could occur without any further energy expenditure as invertase hydrolyses the sink sucrose, maintaining a concentration gradient to the sink tissue to allow this passive sucrose movement. The destination of these available sugars depended on the location of the sink and intensity of metabolic activities within the sink. Those sources closest to the sink provided more carbohydrates than those anatomically distant. To maintain a continuous flow of assimilates the nectaries appeared to act as 'gates' through which excess carbohydrates were released. With the ethylene-inhibitory treatments, such as silver thiosulphate and ethanol, the movement of carbohydrates was directed to the petals instead of the ovary. This may account for the lack of ovary development in these flowers and the extension of petal longevity. The regulating events leading to this redistribution of carbohydrates within the cut carnation flower may provide the key to manipulating flower senescence.

CHAPTER FOUR

HORMONE REGULATION OF SENESCENCE

Introduction

In the cut carnation flower it is generally accepted that the ethylene-emanation prior to petal wilting is crucial for the final senescent event (NICHOLS, 1968; MAYAK AND DILLEY, 1976; CAMPRUBI AND NICHOLS, 1979; DIMALLA AND VAN STADEN, 1980). Additionally, an exogenous application of ethylene results in accelerated flower senescence (NICHOLS, 1968). Because of this, in the cut carnation flower, ethylene is often regarded as the senescence regulating hormone. Hence postharvest treatments are usually assessed on their ability to prevent the final ethylene emanation or ethylene action. However, although the overall response to this hormone causes the flower to senesce, the physiological response of the flower parts differs. The petals, which are responsible for the overall appearance of the flower, irreversibly wilt in the presence of ethylene, whilst in contrast, ovary growth is simultaneously stimulated in the presence of this plant hormone. The rate of ethylene emanation does differ between the flower parts and has been shown to be the highest from the receptacle (VEEN, 1979a). This, however, does not account for the different rates of development between the flower parts.

TREWAVAS (1982) proposed that tissue sensitivity to growth substances is the limiting factor of a growth response and not hormone concentration. This concept of tissue sensitivity will be defined as the number of sites within a tissue to which the hormone binds to elicit its physiological response. The number of these sites can vary as they either do not exist due to genetic constraints or are blocked by competitive inhibition. The sensitivity of a tissue to a hormone may well determine the different development rates between the flower parts. However, prior to attachment to a sensitive site, the presence of other plant hormones, as influenced by the prevailing environmental conditions and their interaction with each other, could alter the capability of the hormone to bind. Examples of one plant hormone altering the action of another are seen with ethylene reducing auxin levels (ERNEST AND VALDOVINUS, 1971; BEYER, 1975a; LIEBERMAN AND KNEGT, 1977) or the cytokinins preventing auxin conjugation (LAU AND YANG, 1973; NOOR-SALEH, 1981). The most publicised case of one hormone affecting another is when high auxin levels stimulate ethylene production (MORGAN AND HALL, 1964; BURG AND BURG, 1966; LIEBERMAN, 1979; YOSHII AND IMASEKI, 1981). Thus it is not surprising that often the plant hormones fulfill similar regulatory functions in different tissues and each of them stimulates a variety of physiological processes. Relating this to carnation flower senescence an interaction of plant hormones, and not the sole action of the ethylene may account for the regulated events that lead to the death of the flower and the production of a new set of seed.

As evident from Chapter 1, one event regulated by plant hormones is the redistribution of carbohydrates, a phenomenon that occurs during cut carnation flower senescence (Chapter 3). Sites of active growth such as shoot apices, fruits and reproductive organs contain high levels of endogenous growth regulators. These high levels of plant hormones may be the cause of sink activity (WAREING, 1973) and thus be responsible for the assimilate influx into these plant parts. In such cases though it is difficult to separate the regulatory activity of plant hormones on metabolic activities with assimilate mobilisation. However, the application of hormones to non-growing tissue can lead to increased movement of assimilates to the point of application. Cytokinins mobilise amino acids towards its site of application (MOTHES, ENGELBRECHT AND KULAJEVA, 1959) whilst indoleacetic acid stimulates labelled sucrose from a site several centimetres from the point of application (BOOTH, MOORBY, DAVIES, JONES AND WAREING, 1962).

Considering the properties of cytokinins and auxins it was hypothesised that these two hormones play a major role in the correlative senescence phenomenon within the cut carnation flower (Chapter 1). The presence of cytokinins and auxins in the ovary may account for the growth and mass increase of this organ. Furthermore, the interaction of ethylene with the auxins and cytokinins prior to reaching their sites of action may modify their physiological responses. This modification of physiological response could occur through the metabolism of these plant hormones into physiologically inactive forms, or alternatively, the protection of these active hormones to carry out their physiological response. Being in an active or inactive form

of a hormone may be related to their capacity to bind to the physiologically active site. Thus in flower senescence the interaction of the plant hormones and their resultant capacity to bind to the available physiologically active sites probably is the means by which the growth difference between the petals and ovary is controlled. The genetically available sensitive sites should only vary with the age of the plant tissue. Because of this, senescence regulation was investigated at the plant hormone level with all chosen flowers uniform in age.

Materials and Methods

Ovary culture

To observe isolated ovary growth on a controlled medium, a tissue culture technique was used. The ovaries from untreated flowers were removed at the receptacle and placed into distilled water immediately before use (Fig. 4.1a). The medium prepared was a modified MILLER'S (1965) medium. All constituents for this medium (excluding sucrose) were made up as stock solutions. These solutions were obtained by dissolving the required amounts of analytical grade macro-nutrients, micro-nutrients and vitamins in distilled water and making up to the final volume. All stocks were stored in glass containers at 5°C and the light-sensitive vitamins of stock 3 wrapped in aluminium foil to exclude light (Table 4.1). For ovary isolation the medium contained 30 gl^{-1} sucrose unless otherwise stated with no growth regulators. These latter components changed with experiment. After adjusting to a pH of 5,8, ten millilitres of the medium was dispensed into each

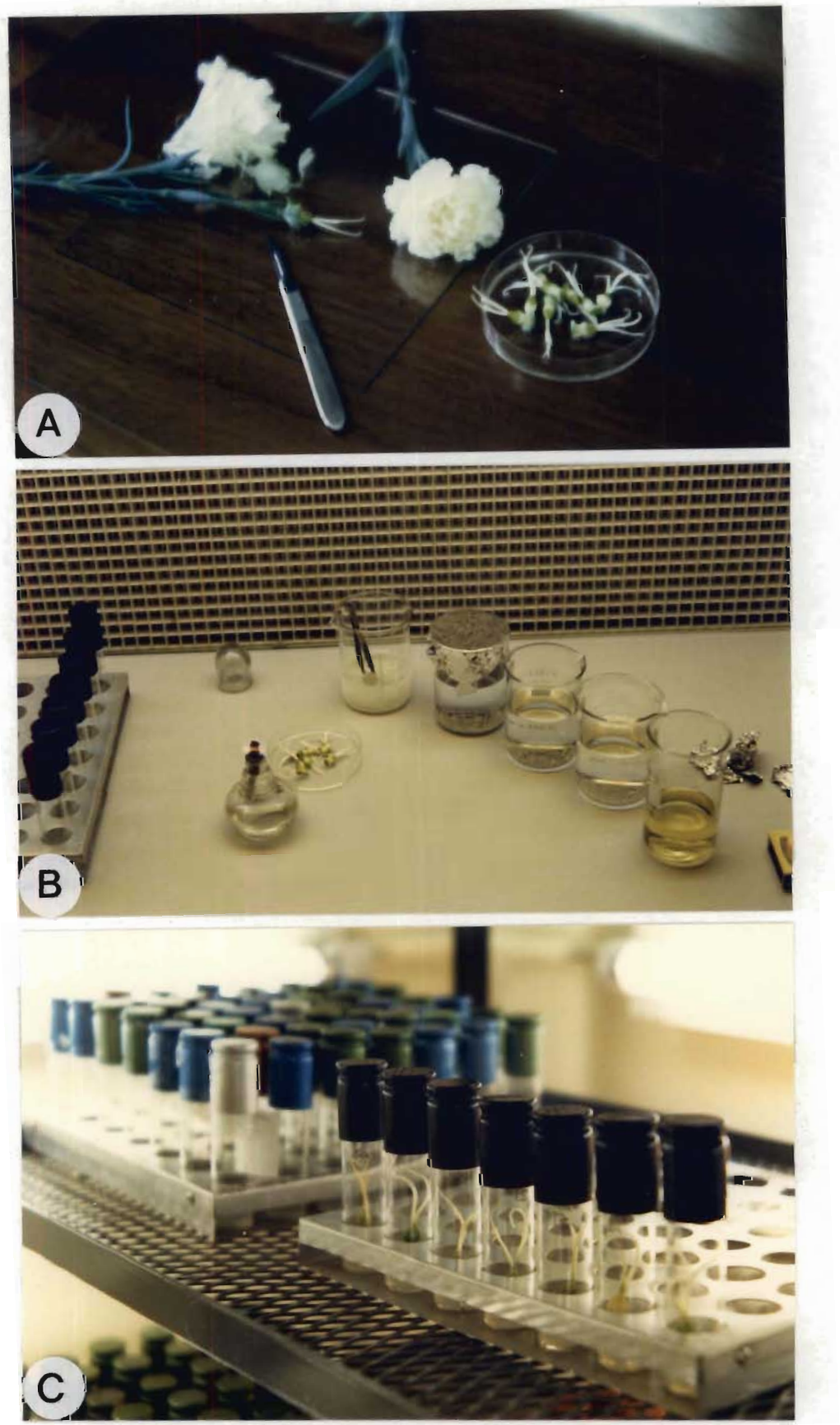


Figure 4.1 : Ovary culture technique. A. Removal of the ovary from the whole flower at the receptacular base. B.Ovaries sterilised on a laminar flow bench, rinsed and placed onto a Modified MILLER's (1965) medium in culture tubes. C. Ovaries incubated in the culture room.

culture tube, the medium was solidified with one percent agar and the sealed tubes autoclaved for twenty minutes at 105 kPa before being transferred onto the laminar flow bench. Under aseptic conditions the isolated ovaries were sterilized for three minutes in a three percent sodium hypochlorite solution (Fig. 4.1b). Following three rinses in sterile distilled water, the ovaries were transferred onto the medium. After being cultured for two weeks at 23°C at a light intensity of 19 $\mu Em^{-2}s^{-1}$ (Fig. 4.1c), the ovaries were removed for analysis.

Extraction and bioassay for cytokinins

For cytokinin analysis, on harvest 5 g of petal tissue, 1 g of ovary tissue and 3 g of calyx plus stem tissue were freeze-dried and separately ground into powder. Each powder was then extracted for twenty-four hours at room temperature with 100 mℓ eighty percent ethanol, the extracts were then filtered and the residues washed with a further 100 mℓ of eighty percent ethanol. The combined ethanolic extracts were taken to dryness *in vacuo* at 35°C and the residues redissolved in 50 mℓ of eighty percent ethanol. The pH of these solutions was adjusted to 2,5 with dilute hydrochloric acid and then passed through a Dowex 50W-X8 cation exchange resin (H^+ form, 20 - 50 mesh, BDH chemicals Ltd) at a flow rate of 10 $m\ell h^{-1}$. One gram of resin per gram fresh mass of material extracted was used. The columns were washed with 50 mℓ distilled water followed by eighty percent ethanol. The combined effluents from the column were considered to comprise the "aqueous extract". The compounds bound to the Dowex were then eluted from the column with 200 mℓ of 5N ammonium hydroxide and collected. The ammonia

Table 4.1 : Basal medium for the soybean callus bioassay adopted from MILLER, 1965.

Stock Solution	Chemical	Mass g l^{-1} stock solution	mℓ stock solution used l^{-1} medium
Stock 1	KH_2PO_4	3,000	100
	KNO_3	10,000	
	NH_4NO_3	10,000	
	$Ca(NO_3)_2.2H_2O$	5,000	
	$MgSO_4.7H_2O$	0,715	
	KCl	0,650	
	$MnSO_4.4H_2O$	0,140	
Stock 2	NaFe EDTA	1,320	10
	$ZnSO_4.7H_2O$	0,380	
	H_3BO_3	0,160	
	KI	0,080	
	$Cu(NO_3)_2.3H_2O$	0,035	
	$(NH_4)_6 MO_7O_{24}.4H_2O$	0,010	
Stock 3	myo-inositol	10,000	10
	nicotinic acid	0,200	
	pyridoxine HCl	0,080	
	thiamine HCl	0,080	
Stock 4	NAA	0,020	10
Additional: Sucrose		30 gℓ^{-1} medium	
Agar		10 gℓ^{-1} medium	
pH adjusted to 5,8 with NaOH			

was removed from this fraction using a rotary evaporator and the residues dissolved in 3 mℓ eighty percent ethanol. These extracts were strip loaded onto Whatmans No. 1 chromatography paper and the constituents within separated with *iso*-propanol : twenty-five percent ammonium hydroxide : water (10:1:1 v:v). After running the chromatogram for approximately ten hours at 23°C, it was removed and thoroughly dried. The paper chromatogram was then divided into ten equal Rf strips. These were cut up and separately bioassayed for cytokinins. Each extraction was repeated at least twice and the bioassay of the whole experiment carried out on the same day. A set of standards was bioassayed with each experiment in which the flasks were placed.

The Rf strips were each assayed for cytokinin activity using the soybean callus bioassay (MILLER, 1965). The callus was obtained from the cotyledons of soybean and maintained by subculturing. To the 50 mℓ erlenmeyer flasks which contained the chromatogram strips, 0,3 g of oxoid agar and 30 mℓ of medium was added. The medium was made up of four stock solutions which were mixed according to the proportions in Table 4.1 and stored at 5°C. The pH of the medium was adjusted to 5,8 prior to addition into each flask. The flasks were then stoppered with cotton wool bungs and covered with aluminium foil. There were autoclaved for twenty minutes at a 105 kPa pressure before being placed into a transfer cabinet which was presterilized by spraying with one percent thymol in ethanol. Protecting the callus with a tinfoil covering, the flasks stood for six hours under ultraviolet light before three pieces of callus (2 mm^2) were cut and placed onto

the solidified basal medium in each flask. The flasks were then incubated in a growth room at a constant 27°C under continuous light of low intensity (cool fluorescent tubes). The fresh mass of all three pieces was simultaneously measured after twenty-one days. Each assay was repeated and the average values of callus growth plotted on a histogram. The confidence limits at the five percent level were calculated for each bioassay. The values of all significant peaks of the extracts were interpolated on the response curve of the standards and expressed as kinetin equivalents.

Results and Discussion

With the exogenous application of ethylene greatly accelerating the senescence of the carnation flower, it was thought that exogenous auxin and cytokinin applications would also have marked physiological effects on cut flower longevity. SACALIS AND NICHOLS (1980) found that high auxin levels delayed carnation flower senescence. This was found to be true with a 500 $mg\ell^{-1}$ stem immersion of 2,4-dichlorophenoxyacetic acid which has the strongest auxin-like activity of the synthetic auxins (Fig. 4.2). With these supraoptimal auxin levels the calyx and stem became necrotic. On the other hand, the weakest indoleacetic acid concentration also extended longevity for a few hours. Overall though, auxin treatments generally accelerated carnation flower senescence most probably due to the high auxin levels stimulating ethylene production (JONES AND KENDE, 1979). Dry mass analyses showed that auxin treatment increased the ovary dry mass compared to the

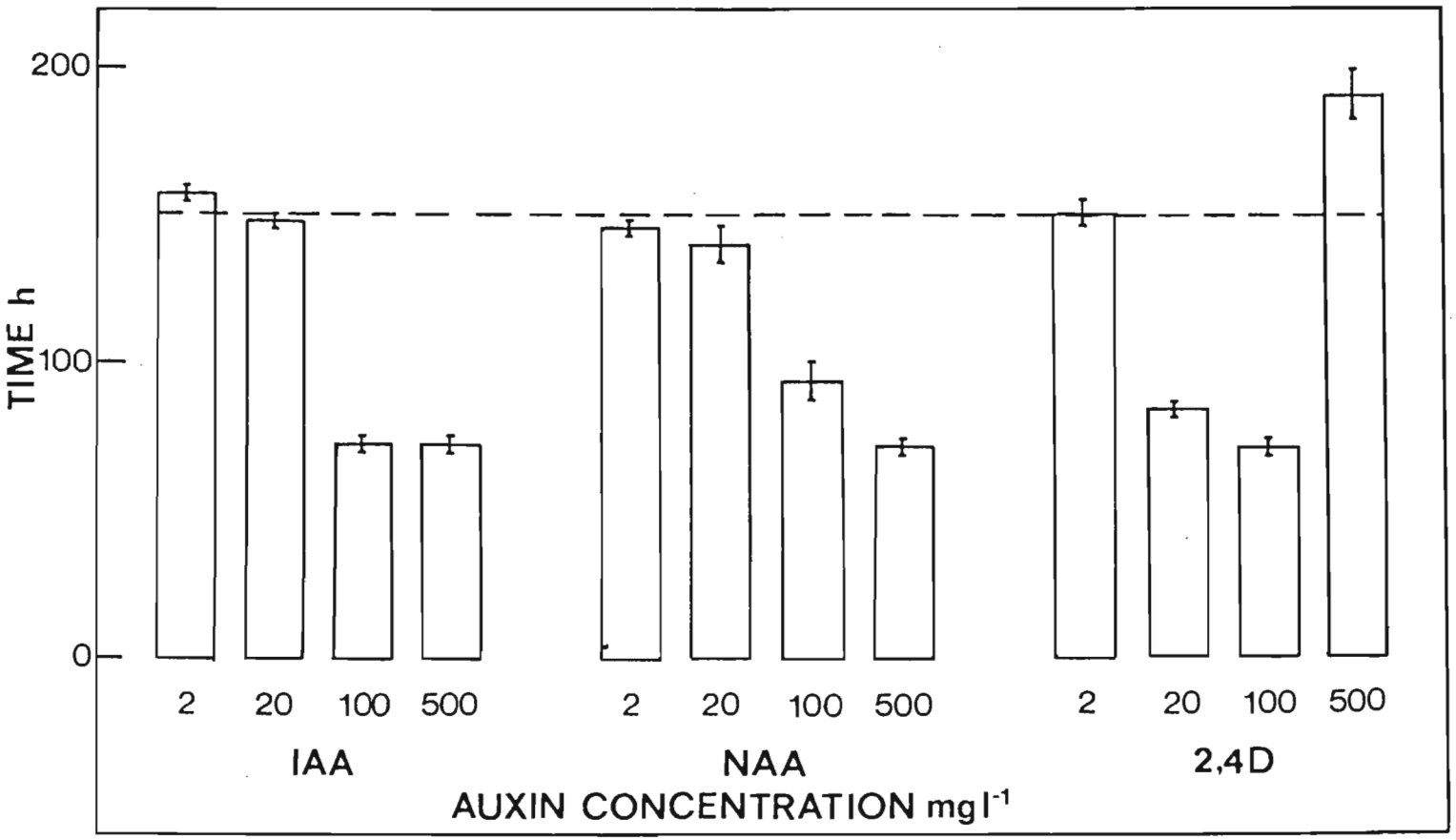


Figure 4.2 : The longevity of cut carnation flowers placed into 2, 20, 100, 500 mgℓ^{-1} indoleacetic acid (IAA), naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4D) staying solutions. Vertical bars indicate the standard error of the mean.

control, a phenomenon that also occurs with ethylene-enhanced carnation flower senescence (Fig. 4.3).

A problem faced with the exogenous application of plant hormones is that with basal uptake the transport passage can be either the xylem or phloem. Xylem is generally a dead tissue and thus will allow the unselective flushing of the hormone into all the flower parts depending on their water requirement. This is not a natural situation especially with the auxins. This hormone group has two main routes of transport. WENT (1942) discovered the polar transport of auxins which was greatest in a basipetal direction. This transport requires energy and thus suggests it is symplastic. Additionally, indoleacetic acid has been chemically identified from phloem sap (ALLEN AND BAKER, 1980) and appears to travel at a similar rate as labelled glucose (MORRIS AND THOMAS, 1978) and accumulates at metabolic sinks. Once in the phloem, however, the auxins do not show polar transport. When the stem of the cut carnation flower was injected with labelled indoleacetic acid, this auxin moved from the stem into the receptacle (Fig. 4.4). A portion of this radioactivity then returned to the stem from the receptacle probably by polar transport, which further continued so that by the ninth day following harvest the stem once more possessed the majority of the radioactivity. Small amounts of radioactivity were detected in the calyx and petals but the ovary did not receive any additional indoleacetic acid. In this case it appeared that the exogenous enhancement of carnation flower senescence was manipulated from the receptacle. This could well be possible if this exogenous auxin application merely stimulated ethylene biosynthesis as the

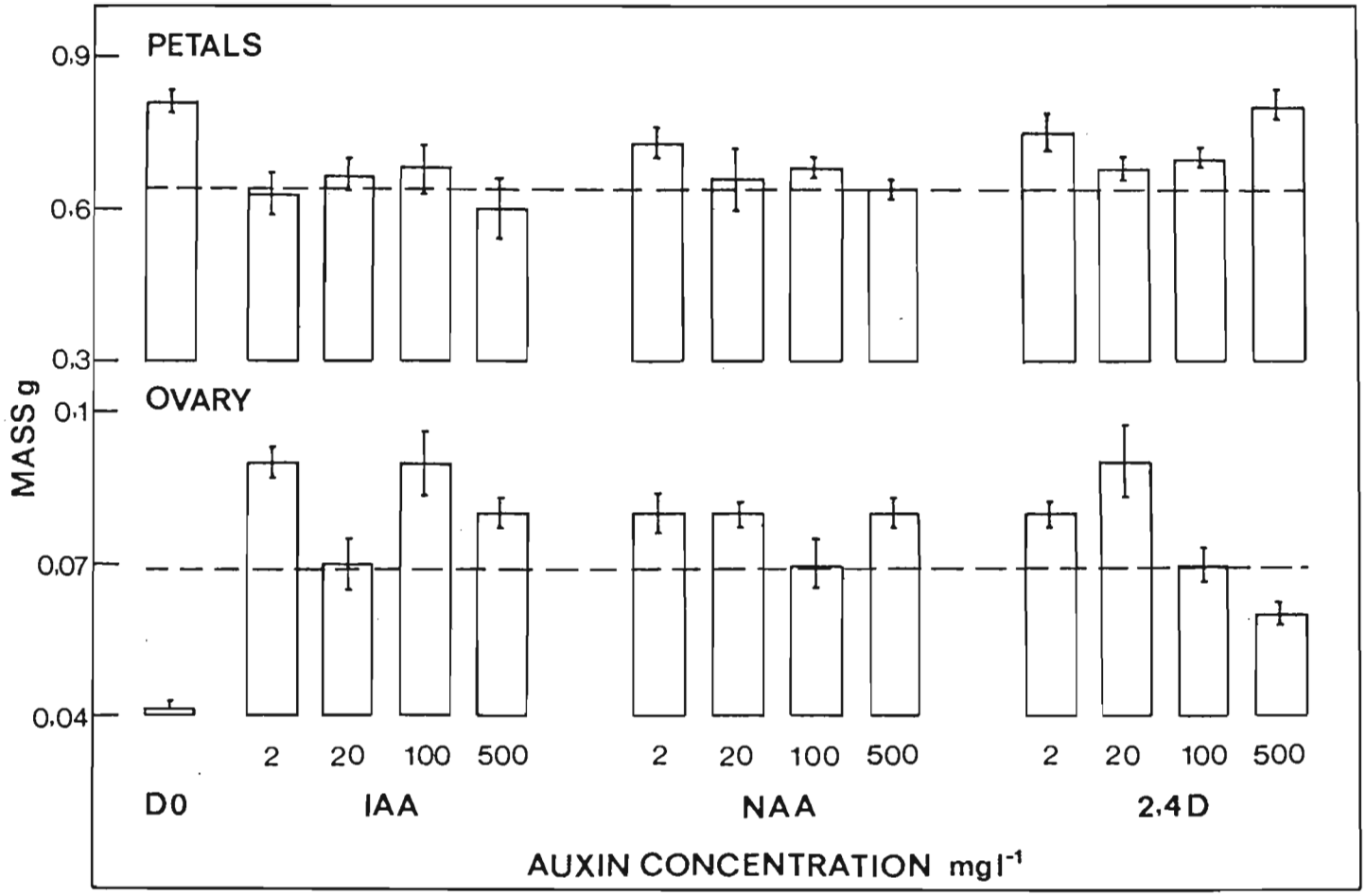


Figure 4.3 : The dry mass of the petals and ovary from flowers placed into 2, 20, 100 and 500 mgℓ^{-1} indoleacetic acid (IAA), naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4D) staying solutions. Vertical bars indicate the standard error of the mean.

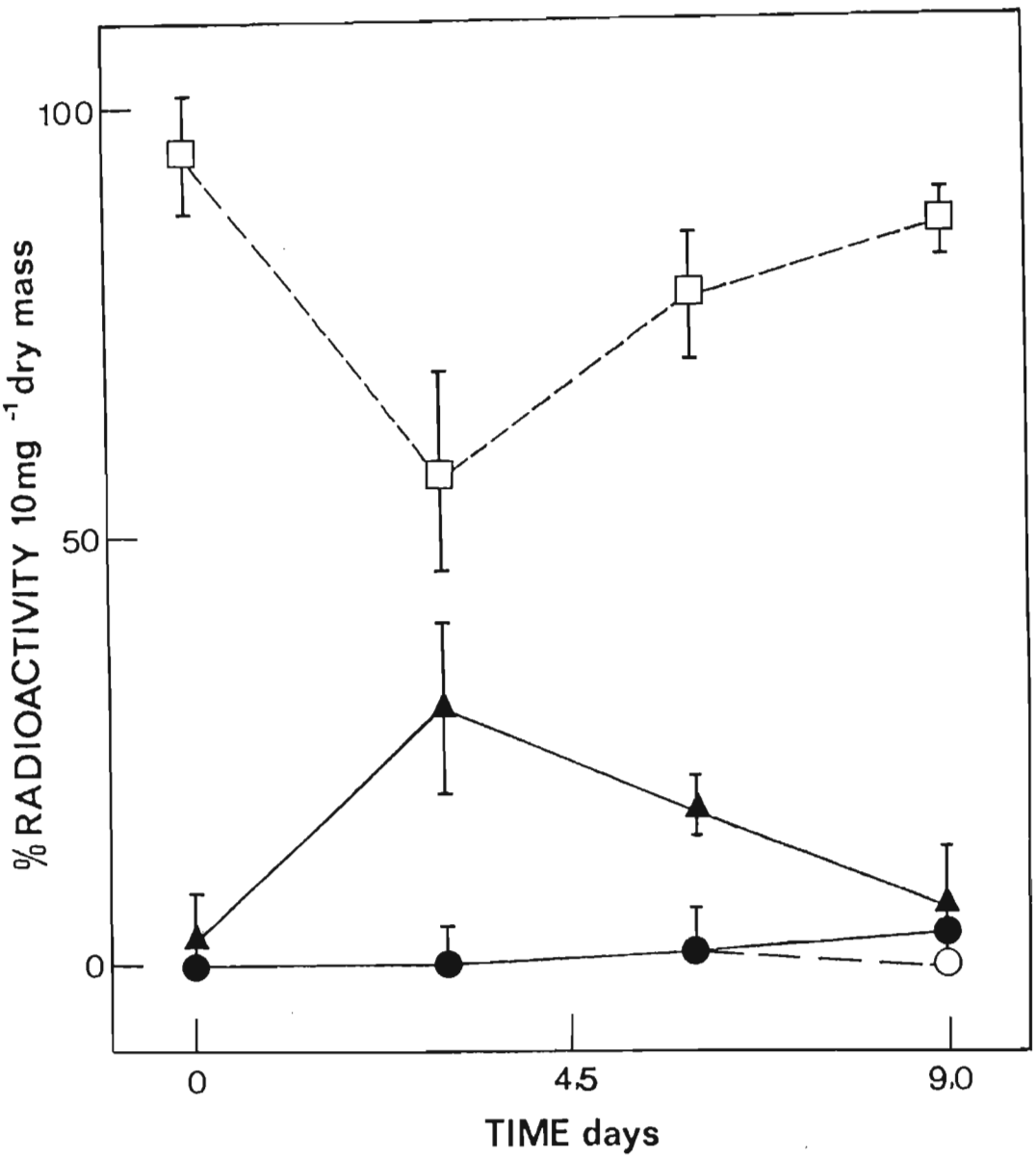


Figure 4.4 : A time-course study of the movement of labelled (^{14}C) indoleacetic acid injected into the stem of the cut carnation flower. Radioactivity was measured in the petals (-●-), ovary (-○-), receptacle (-▲-) and calyx, bracts plus stem (-□-) and expressed as a percentage of the total dpm's measured within each flower. Vertical bars indicate the standard error of the mean.

receptacle is a high ethylene producing tissue (NICHOLS, 1979; VEEN, 1979b).

The exogenous application of various cytokinins on carnation flower longevity has been well documented. As reviewed in Chapter 1, cytokinin supplements have been shown to generally extend cut carnation longevity (MACLEAN AND DEDOLPH, 1962; HEIDE AND OYDVIN, 1969; MAYAK AND KOFRANEK, 1976). The effect of a series of kinetin concentrations, with the standard experimental conditions (Chapter 2), on carnation longevity was determined. It was found that lower concentrations of kinetin accelerated senescence whilst 0,02 $mg\ell^{-1}$ kinetin extended flower longevity (Table 4.2). This varied response of kinetin on flower longevity could be due to the translocation of this growth substance to the site of ethylene action as MAYAK AND DILLEY (1976) and EISINGER (1977) observed that cytokinin-treatment lowered tissue-sensitivity to ethylene. Monitoring basally applied benzyladenine, KELLY (1982) found that the majority of radioactivity remains within the stem with small amounts of radioactivity detected within the petals and ovary. Higher concentrations of cytokinins may be needed to overcome this stem cytokinin accumulation so as to reach the petals. Furthermore, many workers have observed that the effectiveness of these exogenous cytokinin-treatments is dependent on concentration applied and environmental conditions (WATERS,1964; HALEVY AND WITTWER, 1965; HEIDE AND OYDVIN, 1969; MAYAK AND DILLEY, 1976). This suggests that cytokinin action within the carnation flower may be indirect. LESHAM, WURZBURGER, GROSSMAN AND FRIMER (1981), proposed that cytokinins are involved in antioxidative mechanisms as well as radical scavengers.

Ethanol has also been proposed to be a free radical scavenger (BAKER, 1983) and is extremely effective in delaying carnation flower senescence (HEINS, 1980). Other indirect actions of cytokinins to delay the senescence process in the cut carnation flower may involve the soluble reducing sugars (HOLZAPFEL, WILD AND ZERBE, 1983). Kinetin was shown to increase the sugar content of *Sinapis alba* primary leaves. This kinetin action on the sugar levels is modified with quality of light. The highest increase of sugars was observed under blue light, the smallest under red light but starch synthesis showed a reversed trend in relation to light quality (HOLZAPFEL, WILDE AND ZERBE, 1983). Whilst this information once again relates cytokinins to carbohydrate metabolism (refer to Chapter 1), it also demonstrates a case where the environment alters a growth event through a plant growth regulator. Unfortunately, the means by which kinetin was affected by the quality of light was not investigated. Nevertheless, in the *S. alba* primary leaf system, the sugar content was enhanced by the presence of kinetin. This kinetin action may either be through photosynthesis, as kinetin stimulates chloroplast development (FLETCHER AND McCULLAGH, 1971) or due to a mobilisation of the carbohydrates. Additional sucrose together with a kinetin treatment enhanced flower longevity and significantly increased petal and ovary dry mass (Table 4.2).

Although such exogenous hormone treatments indicate the physiological activity of these hormones entering via the stem, these latter experiments do not impart any information as to the physiological effect of a hormonal balance between the different flower parts. The

Table 4.2 : Effect of exogenous kinetin application on carnation flower longevity, petal and ovary dry mass.

Kinetin Conc. ($mg\ell^{-1}$)	Longevity (h)	Petal Dry Mass (g)	Ovary Dry Mass (g)
0	163 ± 20	0,52 ± 0,02	0,053 ± 0,004
0,004	144 ± 14	0,51 ± 0,04	0,080 ± 0,011
0,01	158 ± 10	0,53 ± 0,05	0,048 ± 0,005
0,02	177 ± 6	0,49 ± 0,03	0,055 ± 0,005
0,01 ± 2% sucrose	187 ± 5	0,66 ± 0,05	0,068 ± 0,008

hormonal balance between the flower parts may contribute the vital information as to the regulatory control of these plant hormones in senescence.

To determine the effect of high concentrations of auxins and cytokinins in the ovary on assimilate mobilisation and flower senescence, this flower part was injected with indoleacetic acid and zeatin or a combination of both these natural hormones. The dry mass and movement of the applied sucrose from the petals was quantified after seven days. The mixture of the two plant hormones injected into the ovary created the largest dry mass loss from the petals and increase in the ovary (Fig. 4.5). This resulted in accelerated flower senescence. Ovaries injected with indoleacetic acid or zeatin, respectively, did not stimulate a greater dry mass in the ovary than shown by the control flower. As for petal dry mass, the lowest mass was shown by the mixture of zeatin and indoleacetic acid followed by zeatin injected into the

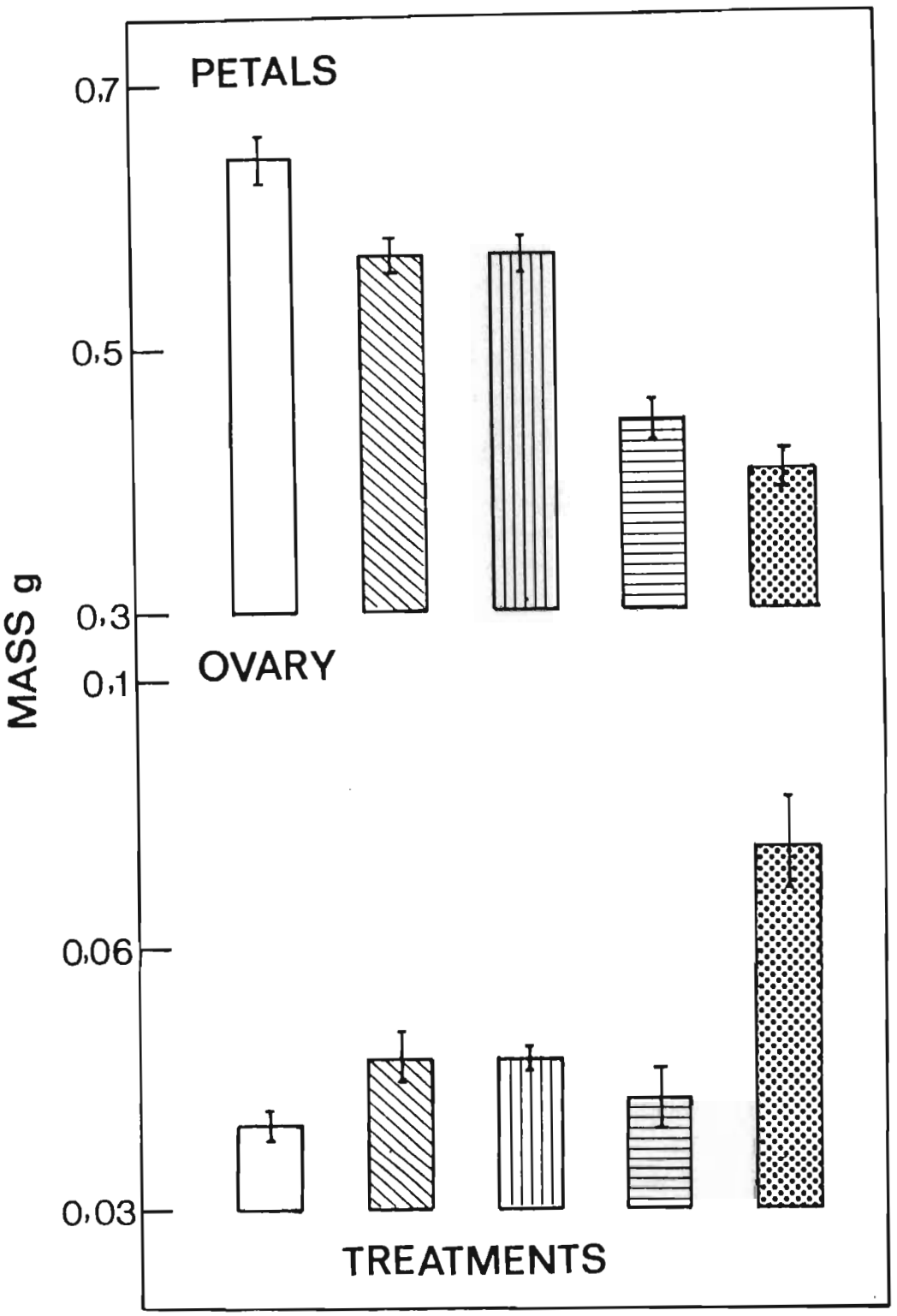


Figure 4.5 : Petal and ovary dry mass at day 0 ( ) and seven days later. The ovaries of these flowers were injected with 1 µℓ of distilled water ( ), 175 mgℓ$^{-1}$ indoleacetic acid ( ),219 mgℓ$^{-1}$ zeatin ( ) and a mixture of both these hormones ( ) on day 0. Vertical bars indicate the standard error of the mean.

ovary by itself. Indoleacetic acid-treatment did not significantly differ from the control. Complementing these dry mass results, the combination of zeatin and indoleacetic acid 'attracted' the most radioactivity to the ovary from the sucrose-labelled petals (Fig. 4.6). Zeatin and indoleacetic acid, respectively, showed only slight increases of radioactivity in the ovary compared to the control. This experiment thus suggests that the accumulation of cytokinins and auxins together in the ovary may be crucial in stimulating sink activity.

To further investigate this phenomenon, the ovary was isolated *in vitro* and a series of experiments investigating sink establishment carried out. The first question asked was whether the 'greening' and enlarged appearance of the ovary on senescence was caused by the influx of sucrose or regulated by plant hormones. High levels of sucrose were made available to the ovaries dissected from the carnation flowers at harvest. After a two week incubation period the ovaries were then removed and dry mass and chlorophyll content quantified (Table 4.3).

Table 4.3 : Dry mass accumulation and chlorophyll content (transmittance) of the whole ovary cultured on different levels of sucrose for fourteen days.

Sucrose concentration ($g\ell^{-1}$)	0	30	60
Dry mass (g)	0,052 ± 0,06	0,056 ± 0,05	0,061 ± 0,01
Chlorophyll content (%)	78 ± 10	87 ± 15	91 ± 9

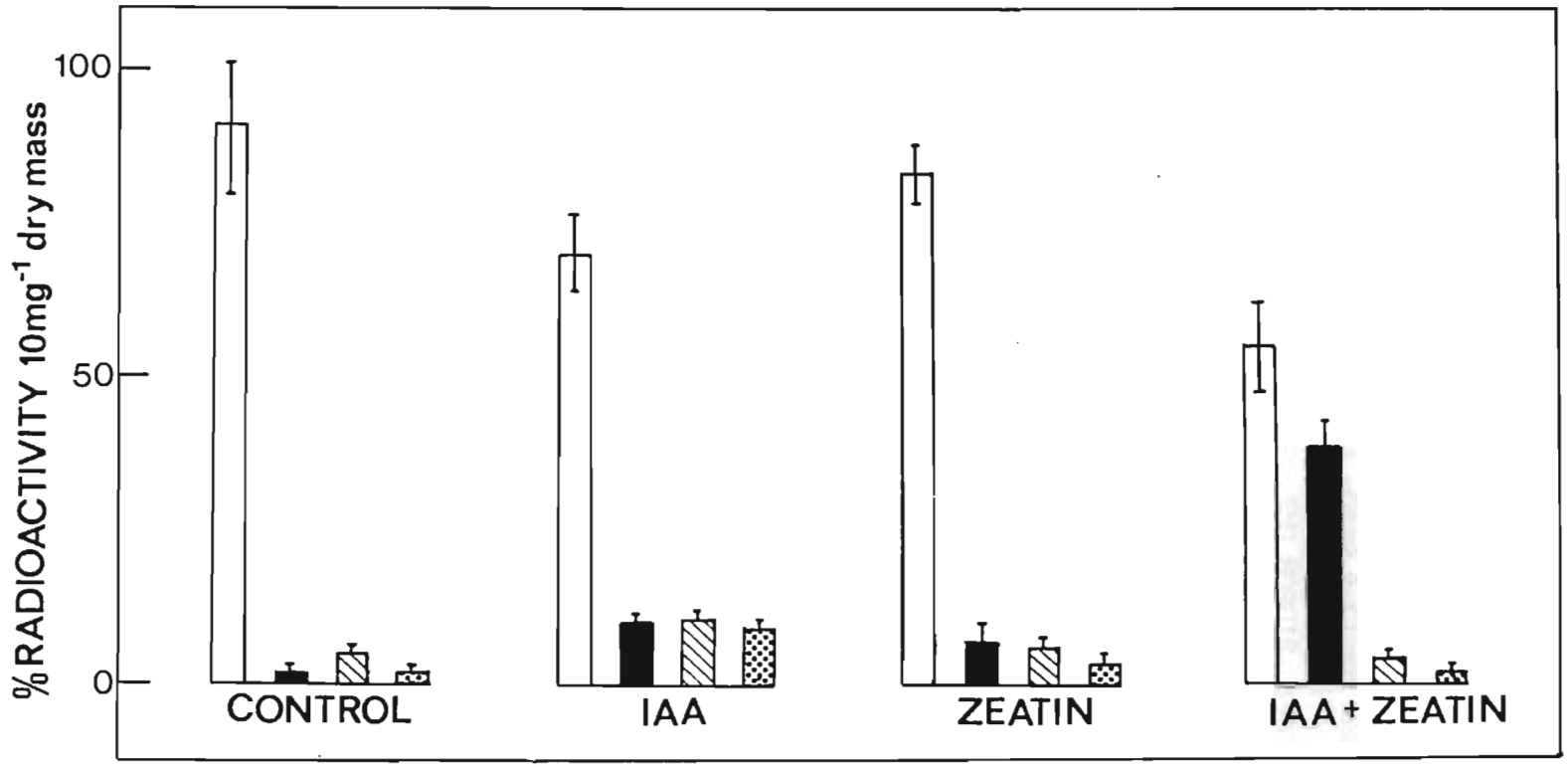
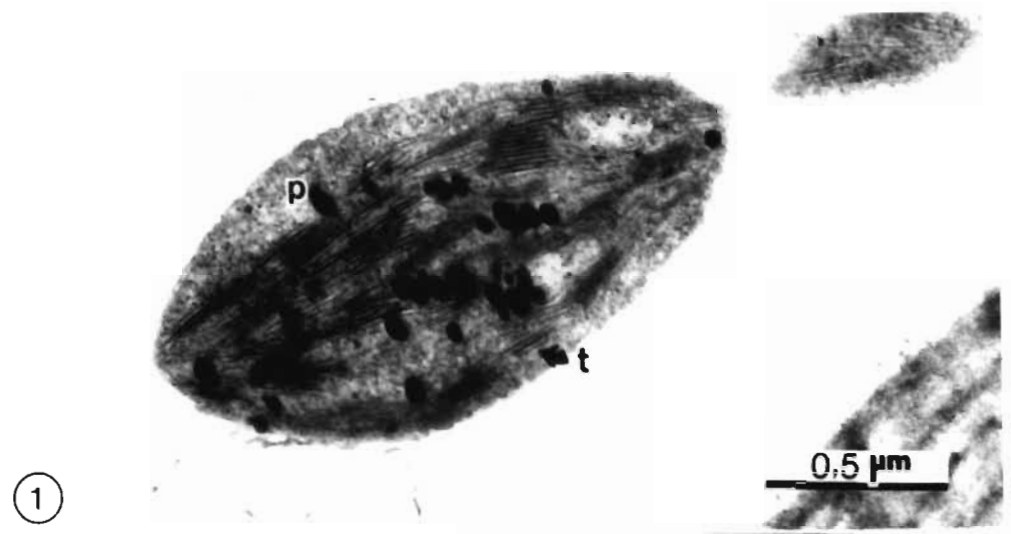


Figure 4.6 : The radioactivity detected seven days after the cut carnation flowers were treated. On day 0 the ovaries of these flowers were injected with 1 $\mu\ell^{-1}$ of distilled water (CONTROL), 17,5 $mg\ell^{-1}$ indoleacetic acid (IAA), 21,9 $mg\ell^{-1}$ zeatin and a mixture of both these hormones (IAA + ZEATIN). Radioactivity was measured from the petals, cold and labelled (□), ovary (■), calyx plus bracts (▨) and receptacle (▩). The vertical bars indicate the standard error of the mean.

Electron microscopic investigations of the chloroplasts in the ovary wall treated with naphthalene acetic acid showed that this hormone stimulated chloroplast development (Plate 4.1). After two weeks of culture on a naphthalene acetic acid-rich medium, the chloroplasts were large and filled with densely stacked thylakoids. Kinetin-treatment also stimulated chloroplast development but not to the extent as seen in the auxin-treated chloroplasts. The thylakoids were not systematically arranged into grana and many plastoglobuli were present (Plate 4.1). The cytokinins are regarded as regulatory hormones in chloroplast development, The application of cytokinin can stimulate chlorophyll formation in etiolated leaves or cotyledons (BEEVERS, LOVEYS, PEARSON AND WAREING, 1970; FLETCHER AND McCULLAGH, 1971; FLETCHER, TEO AND ALI, 1973; DEI, 1983). Furthermore, it is well established that kinetin treatment retards senescence in detached green leaves of a variety of species (RICHMOND AND LANG, 1957; STOBART AND SHEWRY, 1972). This is apparently achieved by maintaining protein synthesising systems and regulating RNA synthesis. In the carnation ovary wall, the chlorophyll levels appear to be controlled by the stimulation of chloroplast development (Plates 2.1,2.2). Silver thiosulphate treatment (Plate 2.4) hindered chloroplast development as well as preventing an increase of endogenous cytokinin within the ovary (VAN STADEN AND DIMALLA, 1980). The absence of this cytokinin activity within silver thiosulphate-treated ovaries could account for the lack of ovary wall chloroplast development. However, the cytokinins may not be the sole hormone involved in chloroplast development as was indicated by the stimulation of chloroplast activity

Plate 4.1 : Chloroplasts from the walls of carnation ovaries cultured on a modified MILLER's (1965) medium that contained:

1. No plant growth regulator additives, control. Thylakoids (t) stacked into grana and plastoglobuli (p) were present within the chloroplast.
2. 1 $mg\ell^{-1}$ kinetin. The thylakoids (t) remain stacked into grana and plastoglobuli (p) were present within the chloroplast.
3. 2 $mg\ell^{-1}$ naphthalene acetic acid. The chloroplasts have increased in size with the extent of the thylakoid system stacking into grana (G) also increasing. Plastoglobuli (p) were still present.



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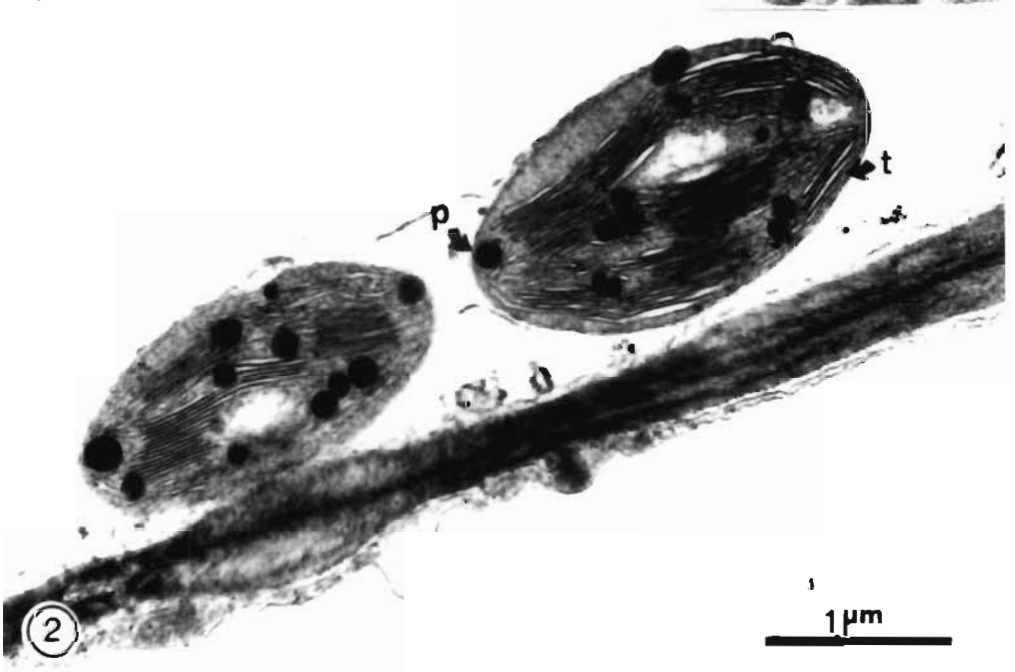

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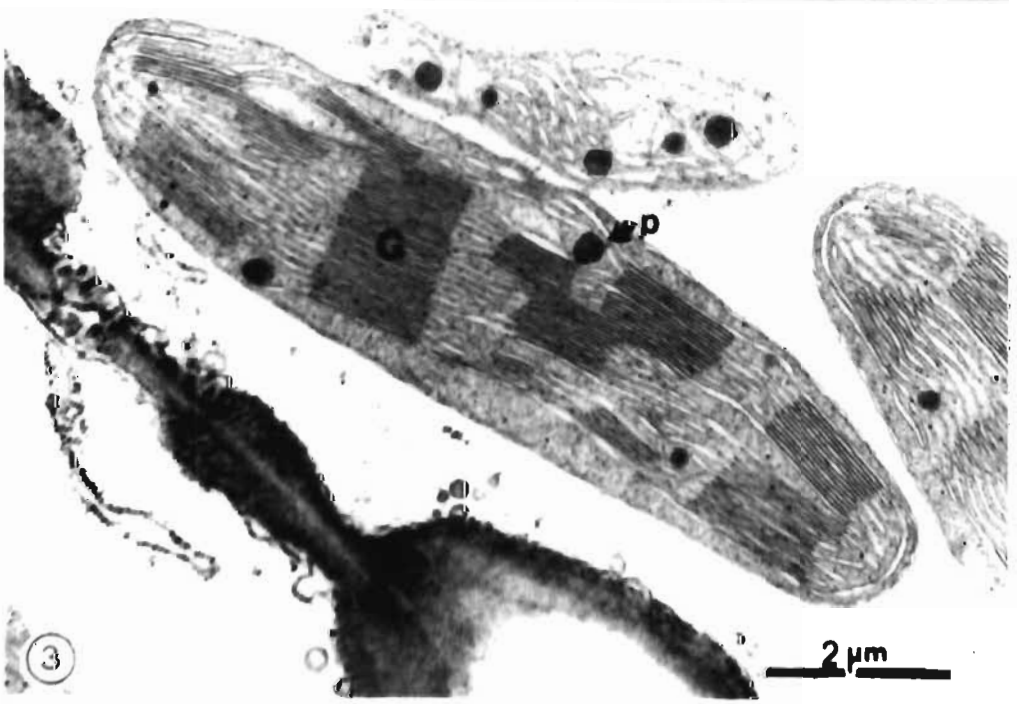

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with naphthalene acetic acid and ethylene in the whole flower. A synthetic auxin was used in these tissue culture experiments as it is stable during autoclaving which is not the case with indoleacetic acid. In the natural situation, as stated in Chapter 1, the cytokinins have been shown to protect indoleacetic acid from conjugation but in the presence of naphthalene acetic acid cytokinins would not have to protect this more stable synthetic auxin. So in the natural situation a balance of auxins and cytokinins may be necessary for chloroplast development in the ovary wall. In carrot cultures, a kinetin to auxin ratio has been shown to influence chloroplast development (as indicated by 'greening'); ratios of 10:1 and 50:1 induced maximal 'greening' whilst a ratio of 1:1 had little effect (BANDIERA AND MURPURGO, 1970). This close relationship between auxins and cytokinins was first realised in experimental studies on organogenesis from callus (SKOOG AND MILLER, 1957). This organogenesis begins with cytodifferentiation. A combination of auxins, cytokinins and sugars influences the translocatory tissue produced (WETMORE AND RIER, 1963). Indoleacetic acid plus sucrose induced the complete differentiation of xylem and phloem in callus tissues; the greater the sucrose level, the more phloem present. Cytokinins increased the density of developing xylem (ELLIS AND BORNMAN, 1971). Thus although chloroplast development has been observed to be stimulated by auxins and cytokinins, these two plant hormones may also be differentiating other tissues and organelles not, as yet, measured.

The auxins also regulate invertase levels. Both naphthalene acetic acid (SACHER AND GLASZIOU, 1962; GAYLER AND GLASZIOU, 1969) and indoleacetic acid

(RUSSEL AND MORRIS, 1982) are effective in stimulating invertase activity. To increase invertase activity the process of synthesis must exceed destruction particularly as estimated half-times for the decay of acid invertase ranges from two hours (GLASZIOU, WALDRON AND BULL, 1966) to forty-eight hours (ROBERTS, 1982). Carnation ovaries cultured on an auxin-rich medium showed the greatest invertase activity compared to control and ovaries cultured on a kinetin-containing medium (Table 4.5). Kinetin treatment also significantly increased invertase activity which may be due to the prevention of auxin conjugation. The increased levels of invertase activity as a result of indoleacetic acid can be related to the increased assimilation of labelled sucrose from the medium (Table 4.5). It would thus appear that in this system auxins play an important role in mobilising sucrose via invertase activity in the ovary. However, such a relationship between invertase activity and labelled sucrose is not apparent with kinetin treatment. Here the invertase levels were significantly higher than the control but the labelled sucrose uptake by the ovary was less than that by control ovaries (Table 4.5). Thus invertase activity does not appear to be the sole determining factor in sink activity. These results tend to suggest that the auxins could be acting more directly on the transport process (PATRICK AND WAREING, 1973) and not only via invertase activity.

A question which should be asked is why was it that the sink-stimulating effect of auxins was undetectable with the injection of additional indoleacetic acid into the ovary of the whole flower compared to the vast mobilising effect of a mixture of indoleacetic

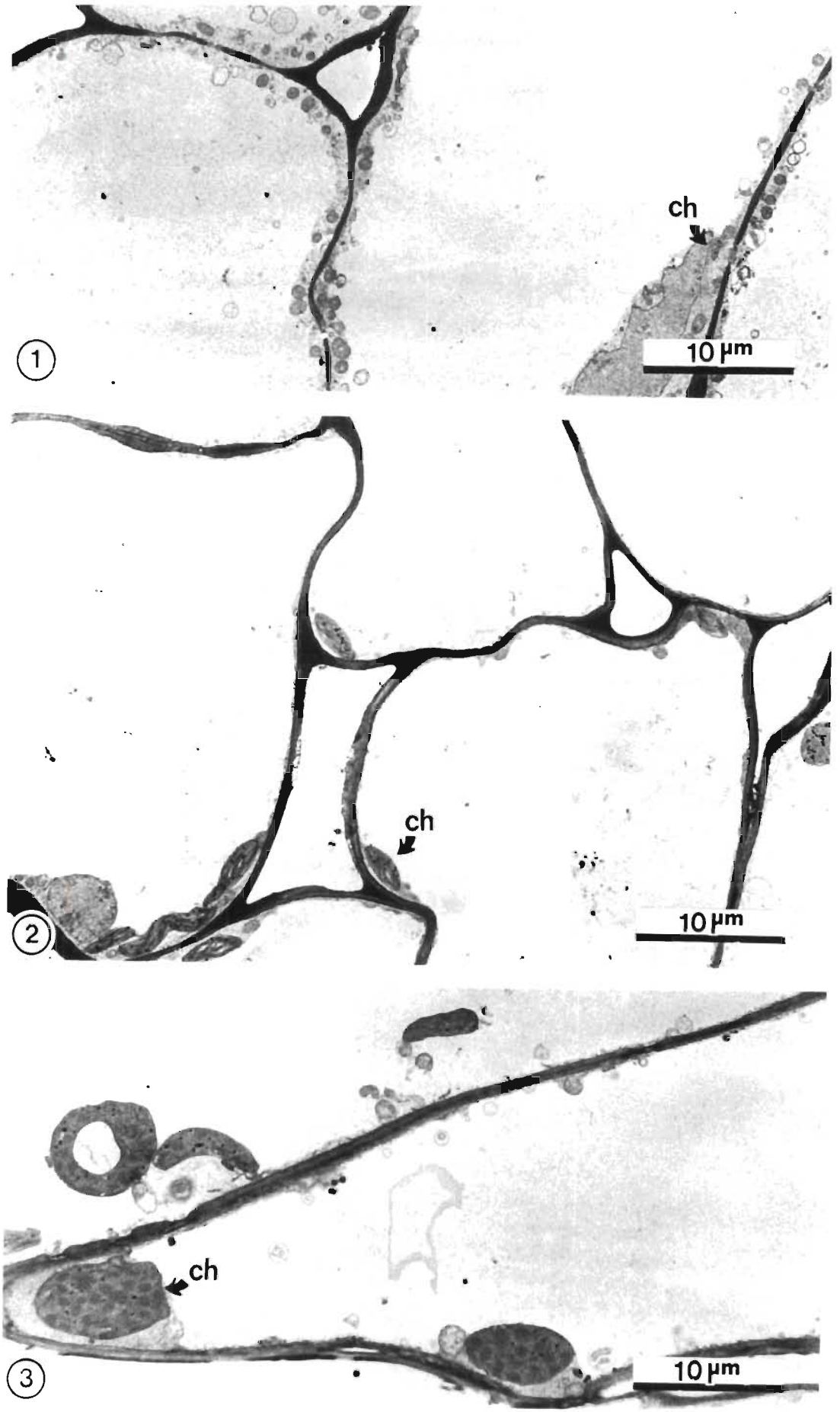
Table 4.5 : Labelled sucrose uptake and invertase activity (mg glucose g^{-1} fresh mass) of ovaries cultured in the presence of 1 $mg\ell^{-1}$ kinetin and 2 $mg\ell^{-1}$ naphthalene acetic acid (NAA).

Treatment	Control	Kinetin	NAA	LSD (p=0,05)
^{14}C-sucrose uptake (dpm)	5782	3961	9672	2188
Invertase activity	7,32	12,31	13,12	0,59

acid and zeatin (Fig. 4.6)? In the whole flower, the emanation of ethylene may play a major role in that it reduces the endogenous indoleacetic acid levels in different plant tissues (RIOV, DROR AND GOREN, 1982). Cytokinins present or additional cytokinin added exogenously may 'protect' the indoleacetic acid against such destruction by suppressing the conjugation of indoleacetic acid (LAU AND YANG, 1973). This would result in a higher level of free indoleacetic acid and a lower level of indoleacetic acid conjugates. This interaction of the auxins and cytokinins against ethylene action could produce the resultant growth of the ovary. This hypothesis places auxin as the sink-regulating hormone which may well be the case. Evidence for such a role is accumulating. Auxins are able to: stimulate invertase activity (GLASZIOU, WALDRON AND BULL, 1966) in cultured ovaries (Table 4.5), effect the unloading of sucrose from the apoplastic pathway (WAREING, 1978) and be involved in cell expansion (WENT, 1942). This cellular expansion was observed when the ovaries were cultured on naphthalene acetic acid (Plate 4.2). in comparison with the cells

Plate 4.2 : A comparison of the size of the cells and the chloroplasts within the cells from the wall of carnation ovaries cultured on a modified MILLER's (1965) medium that contained:

1. No plant growth regulator additives, control. Chloroplasts (Ch) are indicated.
2. 1 $mg\ell^{-1}$ kinetin. Note the size increase of the chloroplast (Ch) and the number of plastoglobuli within this organelle.
3. 2 $mg\ell^{-1}$ naphthalene acetic acid. The size of both the cells and chloroplasts (Ch) had markedly increased.


ch
10 µm
1
ch
10 µm
2
ch
10 µm
3

from control and kinetin-treated ovary walls, the cells with auxin treatment were greater in size. NICHOLS (1976) showed that ovary growth during flower senescence was mainly due to cell enlargement, so this increase in cell size may be regulated by auxin levels. It is well established that cell extension is not a mere physical stretching of the cell wall and therefore auxin must effect some modification of the wall preceding extension itself.

Whilst exogenous hormone applications indicate the possible activity of the hormone, this experimentation does not heed hormone interaction nor the sensitivity of the tissue. Furthermore, the amount absorbed by the plant and reaching the site of action will differ between plants and influence the growth response. Thus although regulatory patterns by the plant hormones during carnation flower senescence are indicated, further research is necessary to confirm this.

With endogenous cytokinin levels being determined with paper chromatography, little identification of the different cytokinin forms is possible save the determination of their polarisation. Thus although PALNI, PALMER AND LETHAM (1984) pointed out that cytokinin activity differed with cytokinin metabolite, so little is generally known of the cytokinins in the cut carnation flower that the aim of this endogenous cytokinin study was merely to gain an overall impression of the physiologically active cytokinin levels as the flower senesces. Cytokinin metabolism within a senescing carnation flower would be a study within itself once the regulation significance of this plant hormone has been established.

Prior to analysis of the endogenous cytokinin levels of the flower parts, the technique was checked to see if any cytokinin-like compounds were eluted into the aqueous fraction from the Dowex exchange resin. This exchange resin generally separates the nucleotides into the aqueous fraction with the nucleosides, free bases, glucosides remaining in the ammonium fraction. In this ammonium fraction the cytokinin activity was detected at Rf 0,6 to 0,8. The non-polar cytokinins that co-elute in these Rf's include zeatin and ribosylzeatin. In the aqueous fraction no cytokinin-like activity was detected signifying a possible absence of nucleotides in the carnation flower. From this experiment on, endogenous cytokinin levels were only measured from the ammonium (Dowex 50) fraction, the aqueous fraction being discarded (Fig. 4.7).

In the cut carnation flower, the petals and ovary appear to be the flower parts most involved in determining the rate of the senescence process. Because of this, primarily, endogenous cytokinin analyses were confined to these two flower parts. The first experiment determined the endogenous cytokinins levels of the petals and ovary at harvest, and again three, six and nine days later. The cytokinin content of the ovary per gramme fresh mass was far greater than in the petals (Fig. 4.8). This cytokinin activity in the ovary reached its highest measured level three days after harvest and had declined by the next sampling six days after harvest. During this period the petals had irreversibly wilted. These results are similar to that earlier reported by VAN STADEN AND DIMALLA (1980). They suggested that this peak of cytokinin activity in the ovary created a strong sink which enhanced flower senescence. The steady increase of cytokinins in the petals is

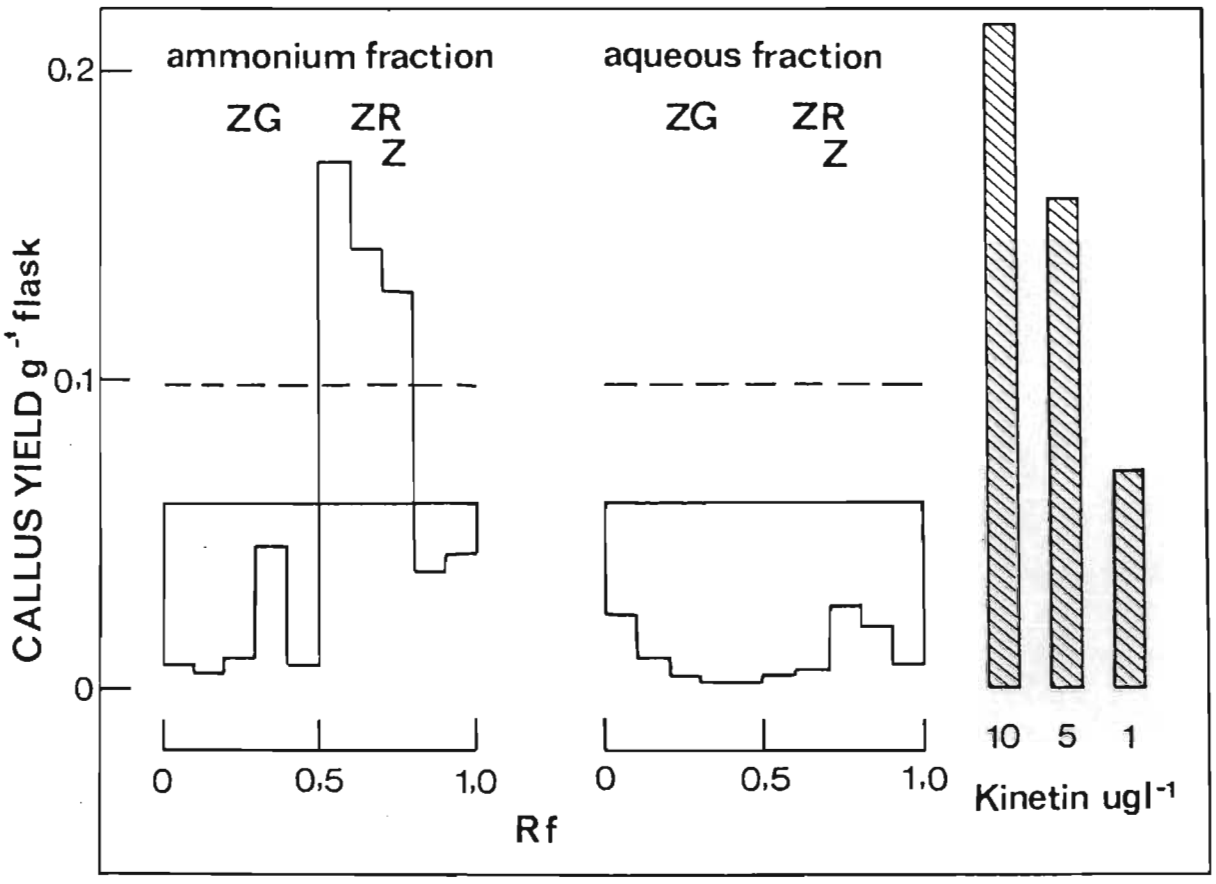


Figure 4.7 : Soybean callus bioassay of extracts from the equivalent of 50 g of carnation flowerhead material. The ammonium and aqueous Dowex 50 purified eluants were individually separated using *iso*-propanol : 25 percent ammonia solution : water (10:1:1 v/v). The broken line indicates the significance limit at the 0,1 percent level. ZG = zeatin glucoside; ZR = zeatin riboside; Z = zeatin.

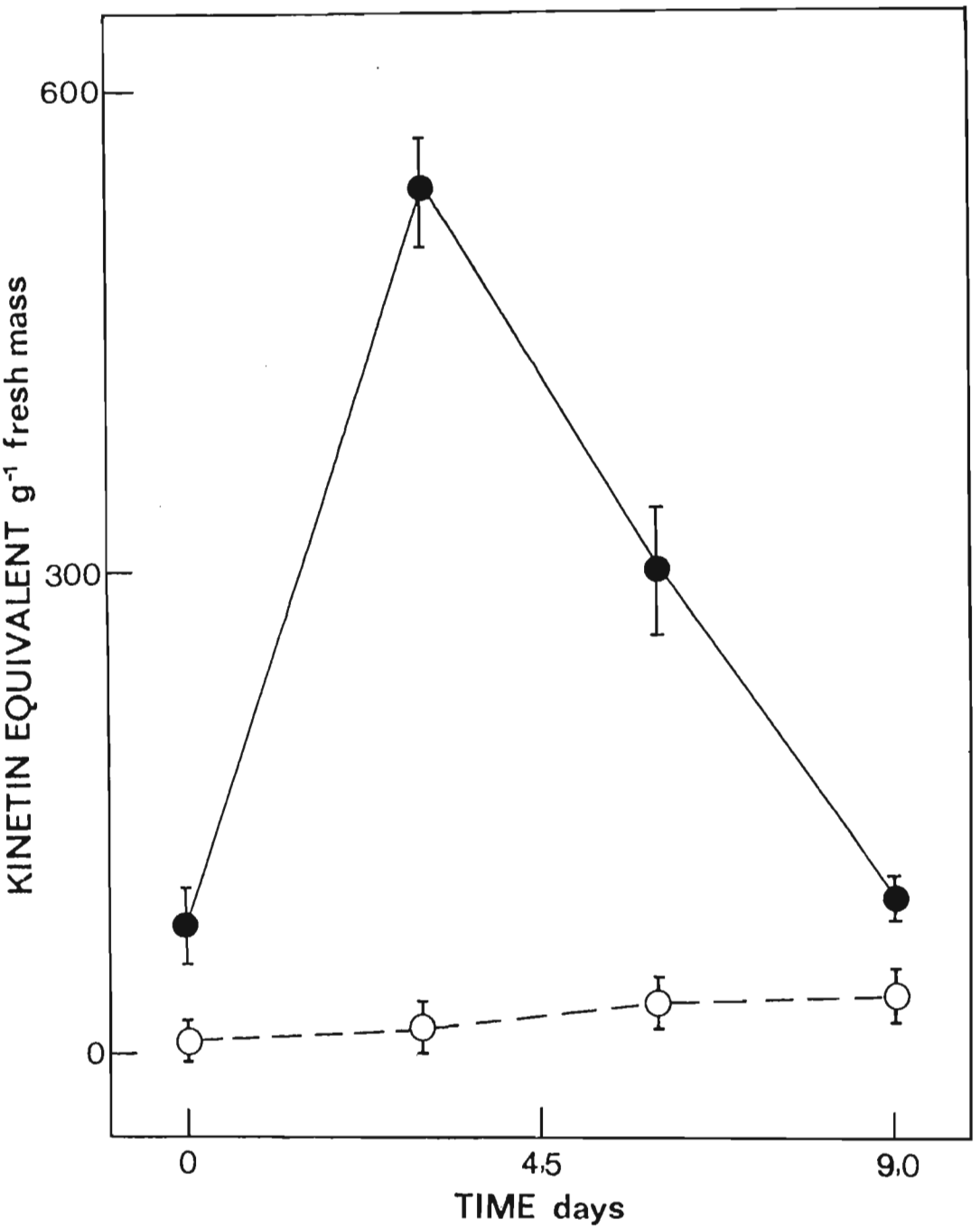


Figure 4.8 : Cytokinin activity expressed as kinetin equivalents in the petals (-○-) and ovary (-●-) from cut carnation flowers over a period of nine days from harvest. Dowex 50 purified extracts were separated using *iso*-propanol : 25 percent ammonia solution : water (10:1:1 v/v) and the activity recorded using the soybean callus bioassay. The biological activity was expressed for those peaks with significant cytokinin-like activity. Vertical bars indicate the standard error of the mean.

not a fair reflection of the true situation. During petal senescence fresh mass rapidly declines, and with the kinetin equivalents being related to per gramme fresh mass, the final decline of endogenous cytokinins is overshadowed by the decrease in fresh mass. The possibility that when the petals do senesce, the endogenous cytokinin levels may be too low to 'protect' the petals from ethylene action (MAYAK AND DILLEY, 1976; EISINGER, 1977) therefore still exists.

The three stages of flower development prior to harvest were analysed for endogenous cytokinins (Fig. 4.9). Day 0 was measured twenty-four hours after harvest. Significant are the higher cytokinin levels in the petals compared to the ovaries of the flower prior to harvest (Fig. 4.10). The high cytokinin levels of the youngest bud stage may be involved in tissue differentiation as these processes are regulated by the cytokinins. Thus it appears that prior to harvest the majority of cytokinin activity is confined to the petals, which then moves to the ovary as the flower senesces. These cytokinins within the petals may maintain petal longevity as suggested by MAYAK AND HALEVY (1970). However, the greater levels of endogenous cytokinins in the ovary may protect this organ from ethylene action whilst the lower cytokinin content in the petals leaves them susceptible to ethylene action. Thus MAYAK AND HALEVY (1970) are probably correct in their theory that petal cytokinin levels are involved in flower longevity. Ethylene-sensitive flowers may thus be those flowers which genetically maintain low petal cytokinin levels as seen in the carnation flower.

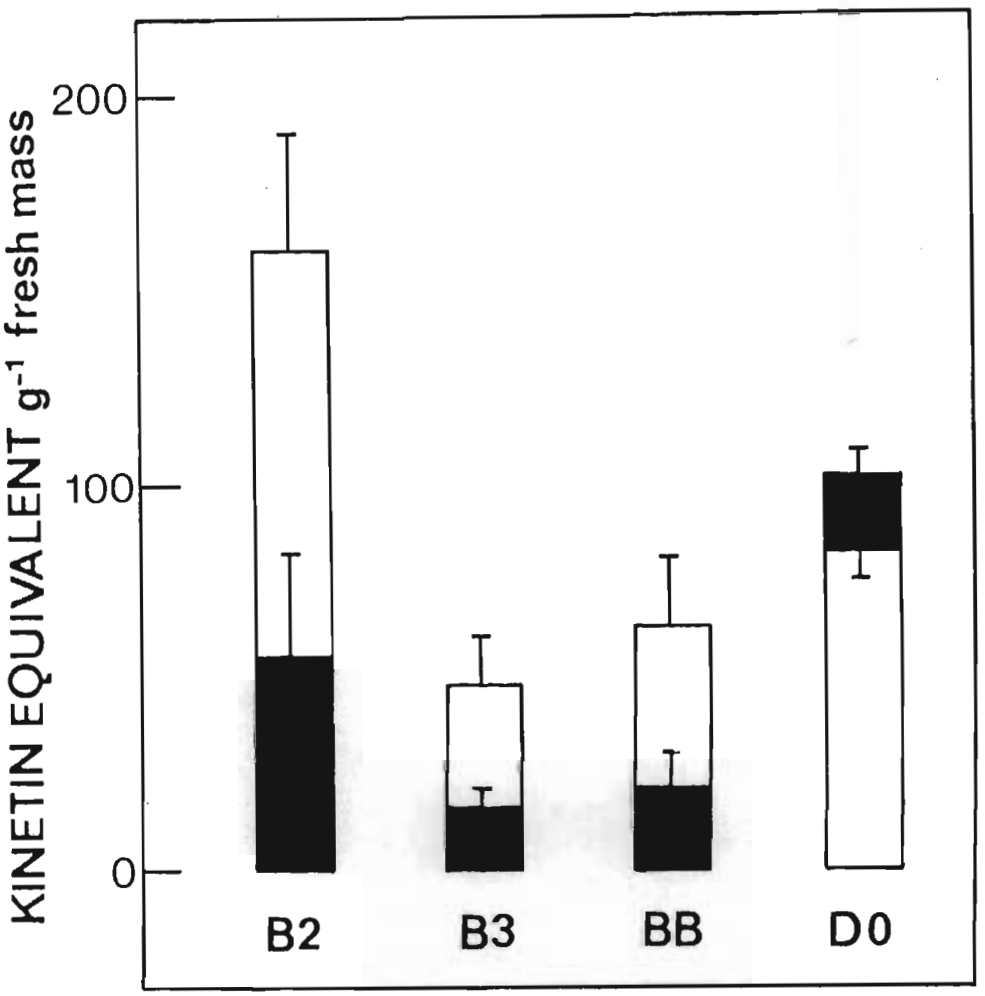


Figure 4.10 : Cytokinin-like activity expressed as kinetin equivalents in the petals (□) and ovary (■) from buds 2 cm in length (B2), buds 3 cm in length (B3), buds in which the petals are emerging from the calyx (BB) and open flowers twenty-four hours after harvest (D0). Vertical bars indicate the standard error of the mean.

Figure 4.9 : The three stages of bud development prior to harvest that were analysed for endogenous cytokinins. The youngest bud was two centimetres in length (B2), the next was three centimetres in length (B3) and in the oldest bud the petals could just be seen breaking through the calyx (BB).

Having suggested that a possible interaction of cytokinins, auxins and ethylene occurs prior to their attachment to a receptor site, the effect of exogenous auxin and ethylene on the endogenous cytokinin levels was determined. In the petals (Fig. 4.11), the cytokinin content in the naphthalene acetic acid and 2-chloroethyl-phosphonic acid-treated petals initially declined as did the control cytokinin content. Relating these kinetin equivalents to petal fresh mass once again created an artefact showing the petals to have a vast increase in endogenous cytokinins following petal irreversible wilting. Of importance is that although naphthalene acetic acid and 2-chloroethyl phosphonic acid-treatments accelerated petal senescence, there was no signigicant

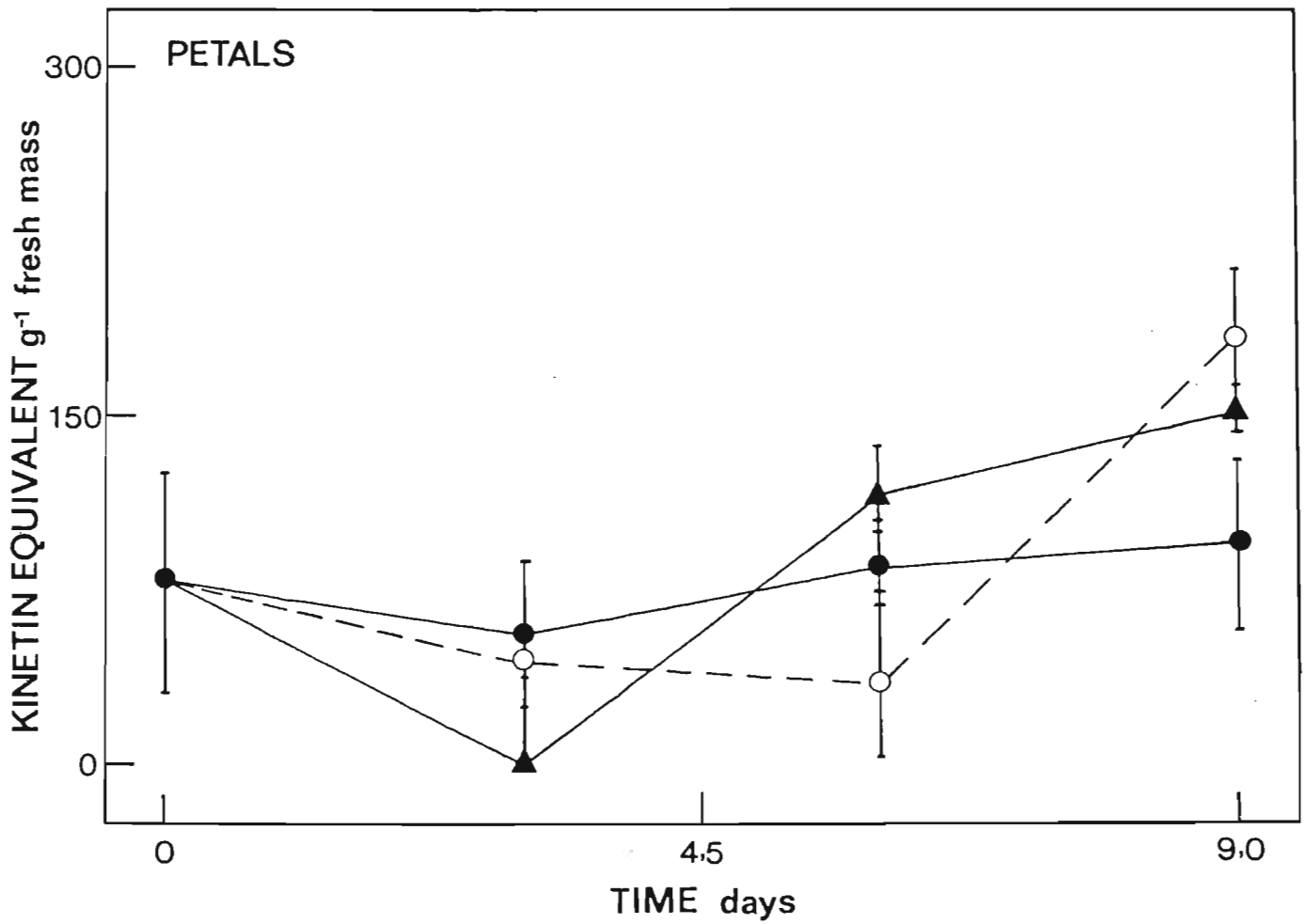


Figure 4.11 : Cytokinin-like activity expressed as kinetin equivalents in the petals from control (-●-), 2-chloroethyl phosphonic acid (-○-) and naphthalene acetic acid (-▲-) treated flowers. Dowex 50 purified extracts were separated using *iso*-propanol : 25 percent ammonia solution : water (10:1:1 v/v) and the activity recorded using the soybean callus bioassay. The biological activity was expressed for those peaks with significant cytokinin-like activity. Vertical bars indicate

effects on the cytokinin levels. In the ovary (Fig. 4.12) similar endogenous cytokinin levels were measured from the treated flowers; their cytokinin content reaching the highest level on the sixth day after harvest. With the calyx and stem (Fig. 4.13), the control and naphthalene acetic acid-treatment maintained their endogenous cytokinin levels but the 2-chloroethyl-phosphonic acid treatment reduced the calyx and stem cytokinin levels so that no activity could be detected by the ninth day. Overall it appears that the presence of additional auxins and ethylene do not significantly change the pattern of endogenous cytokinin activity during carnation flower senescence. If 2-chloroethyl phosphonic acid is not interfering with the stem plus calyx endogenous cytokinin levels, then the loss of cytokinin activity in the stem may accelerate senescence. EISINGER (1977) found that the longer the carnation stem, the greater the extension of flower longevity which he suggested could be related to the presence of cytokinins.

On the other hand, VAN STADEN AND DIMALLA (1980) compared endogenous cytokinin levels of control flowers with flowers treated with the senescing-delaying treatment, silverthiosulphate. The most noticeable difference between these two treatments was the absence of the peak of cytokinin activity that occurs in the ovary after five days from harvest. It thus appears that the significance of this ovary cytokinin rise in the control, 2-chloroethyl phosphonic acid and auxin treated flower ovaries (Fig. 4.13) could be related to ethylene emanation prior to petal wilting and/or the carbohydrate movement within the flowers as it senesces (Figs. 3.6, 3.7, 3.8). It is essential that the exact interaction between these hormones receives further attention.

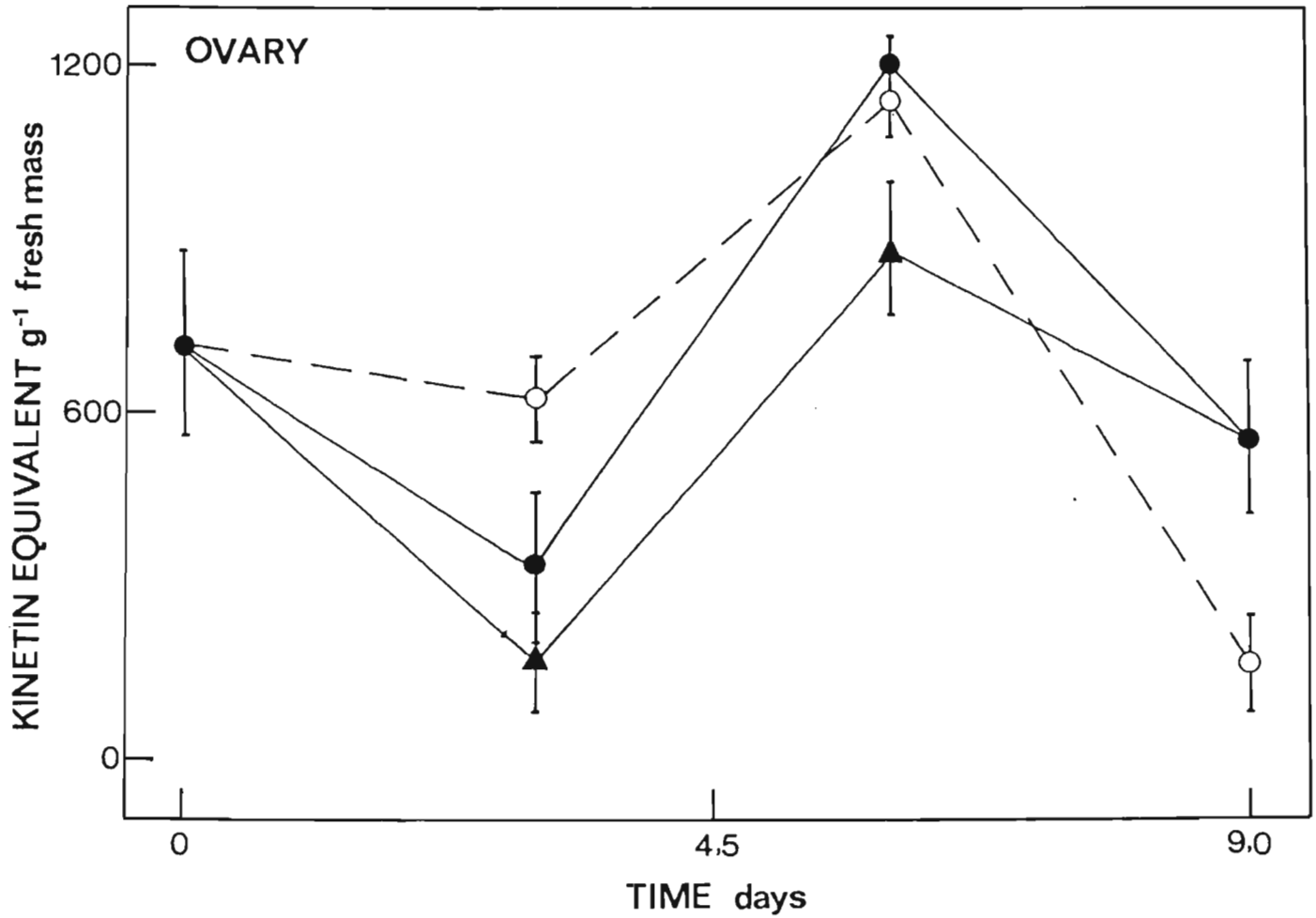


Figure 4.12 : Cytokinin-like activity expressed as kinetin equivalents in the ovary from control (-●-), 2-chloroethyl phosphonic acid (-○-) and naphthalene acetic acid (-▲-) treated flowers. Dowex 50 purified extracts were separated using *iso*-propanol : 25 percent ammonia solution : water (10:1:1 v/v) and the activity recorded using the soybean callus bioassay. The biological activity was expressed for those peaks with significant cytokinin-like activity. Vertical bars indicate the standard error of the mean.

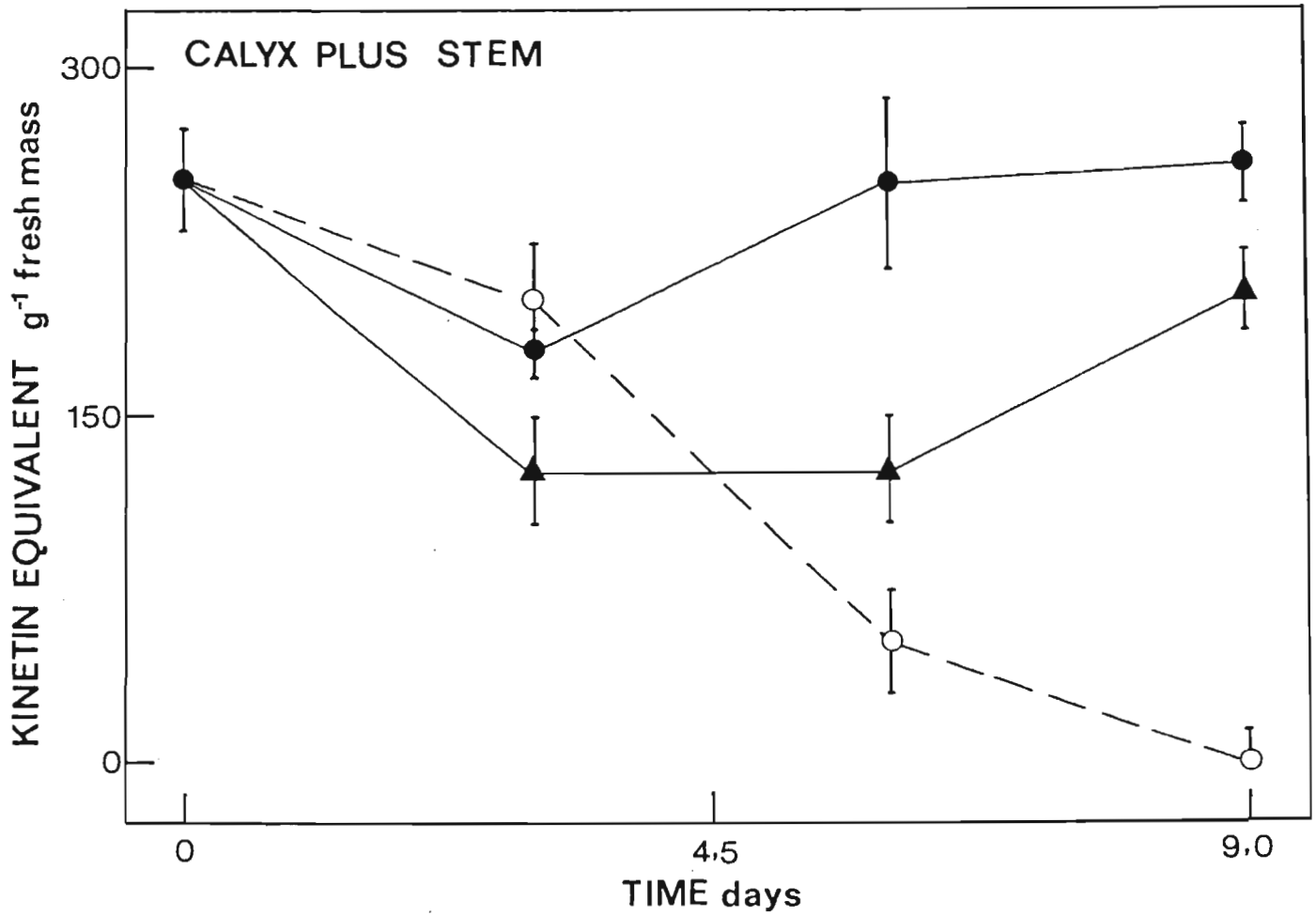


Figure 4.13 : Cytokinin-like activity expressed as kinetin equivalents in the calyx plus stem from control (-●-), 2-chloroethyl phosphonic acid (-○-) and naphthalene acetic acid (-▲-) treated flowers. Dowex 50 purified extracts were separated using *iso*-propanol : 25 percent ammonia solution : water (10:1:1 v/v) and the activity recorded using the soybean callus bioassay. The biological activity is expressed for those peaks with significant cytokinin-like activity. Vertical bars indicate the standard error of the mean.

CHAPTER FIVE

QUESTIONING SILVER ACTION

Plant hormone research is undergoing a quiet evolution (WEYERS, 1984). Although it is well established that development in plants is intimately related to plant growth substances, since their discovery researchers have tried to relate plant growth substance action to that of animal hormones from which the concept was taken. To date the mechanism of hormone action remains to be elucidated. Because of this, plant physiologists are now questioning basic concepts of hormone physiology even to the extent of their existence. With botanists being quick to borrow the hormone concept defined by an animal physiologist, STARLING (1905), it was not surprising that the adaption of this theory to plant systems created many problems. The idea of specific chemicals that could link growth, development and function of different tissues was an overwhelming attraction in the absence of a plant nervous system. For the action of plant hormones, it was thus natural to draw a parallel from mammalian hormones. This suggests that protein receptors are an obligatory part of the cellular apparatus which mediates the growth substance and its biological response (TREWAVAS, 1981). With no examples forthcoming in the plant system, the question arises as to whether this assumption of protein receptor sites has hindered progress in revealing the action of plant hormones.

Research into the action of the silver ion is still in its infancy but it appears that through this ion, the controversy of a plant hormone

action may be solved. The structural difference between the plant hormones demands a cautious note that the mechanism of action may differ depending on the hormone. Thus this discussion is confined to ethylene action. Unravelling this mechanism of action may reveal the discrepancies in our concept of plant hormone action.

The relatively recent physiological use of silver in plants may be due to it being rarely found in a natural soluble form and that it does not pose a threat to plant life in the trace amounts usually present. However, with the increase in available silver ions from cloud seeding (KLEIN, STIFFLER AND TELLER, 1975) mining operations and the disposal of silver-contaminated effluent (KOONTZE AND BERLE, 1980), the problem of silver toxicity has recently been recognised by physiologists as an environmental hazard (Nathanson AND BLOOM, 1976; PETTERSON, 1976). Nevertheless, the usefulness of this ion, as in the words of BEYER (1976a), 'to block specifically the action of a plant hormone in the intact plant ..... is unparalleled in plant biology'. Since this disclosure, the silver ion has received considerable attention being used extensively for economic gain. With suggestions that the silver ion attaches to the binding site of ethylene action (VEEN, 1983), this element has physiologically become one of the most powerful tools to investigate ethylene action and locate its binding site (BEYER, 1976b; AHARONI, ANDERSON AND LIEBERMAN, 1969).

The ethylene-inhibitory action of the silver ion was first thought to be due to the free silverions 'scavenging' the ethylene before it

reached its site of action. Reason for such a hypothesis was probably because historically the silver ion had been used in gas chromatography to complex with olefins (BEDNAS AND RUSSEL, 1958; ATKINSON, RUSSEL AND STUART, 1967). However, BEYER (1976a) demonstrated that the concentration of ethylene entering and leaving a chamber containing silver-treated plant tissue was always the same. Nevertheless, the molecular requirements of the ethylene action site were found to be similar to those governing silver-olefin complexes (BURG AND BURG, 1967). Hence BEYER (1975b) suggested that ethylene must bind to a metallic receptor site for ethylene action and the metal probably involved was copper.

The literature pertaining to all the possible cellular sites of attachment is reviewed here to gain an overall perspective of the possible sites to which this silver ion could bind to inhibit ethylene action. Silver has a century of history as a histological reagent and histochemically has been used to identify a variety of chemical sites. Unfortunately, silver occurs as a solution and precipitates within the plant raising the question of which physical form is physiologically active. VEEN, HENSTRA AND DE BRUYN (1980) assumed that the silver precipitated for its ethylene inhibition. Alternatively, KOONTZE AND BERLE (1980) considered the precipitation of silver to render the silver ion non-toxic to plant metabolism. Unfortunately there is little evidence to substantiate either view but as the majority of literature on cellular sites of silver accumulation obviously involves precipitation this aspect will be primarily covered. Furthermore, it must be realised that the silver may not necessarily complex with all metabolically active substances. Such precipitation, however, may interfere with transport

pathways, blocking cation recognition sites needed for ion uptake as well as symplastic transport (HIGINBOTHAM, 1973).

In many situations involving metal impregnation, it is not always known just which molecular substate is responsible for the staining produced. Silver binds in two distinct forms: firstly, silver as silver ions attach to tissue ligands and secondly, silver nuclei, so named by analogy to the photographic situation as the nuclei probably comprise of silver atoms. On application to plant tissue, the silver ion is initially adsorbed, dissolved or bound weakly, all fast processes that can be reversed easily. However, further reactions do occur binding the silver into one of the two above forms. Silver nuclei formation although slow, due to the bound metallic species being reduced into crystals of free metal, is not readily lost. Often the presence of a reducing agent, exogenous or endogenous, influences the formation of these free metal crystals. In this form silver accumulation is easily observed at the light microscope level. In the alternate case, silver bound to a tissue ligand has sufficient electron scattering ability to be detected with the electron microscope.

As with most staining techniques, the use of silver nitrate was pioneered on animal tissue. VON RECKLINGHAUSSEN (1862) investigating sectioned lymphatic tissue found that after silver nitrate impregnation and exposure to light, cell outlines were revealed. Twenty years later living plant material was also shown to possess silver reducing properties (LOEW AND BOKORNY, 1882). Since then silver accumulation

has been associated with many organelles and enzymatic reactions within the plant.

Historically, living chloroplasts were one of the first organelles noted to reduce silver (MOLISCH, 1918) and a variety of substances were named the reducer. These ranged from chemical constituents involved in the photosynthetic process itself (GAUTHERET, 1934; BROWN, MOLLENHAUER AND JOHNSON, 1962), to an aldehyde or peroxide (MOLISCH, 1918), an oxyflavon (GAUTHERET, 1934), essential oils (WIELER, 1936) and ascorbic acid (GIROUD, RATISMAMANGA AND LEBLOND, 1935). Ascorbic acid in its reducing form, ascorbate, appears to be the most likely reducer of silver nitrate to metallic silver in the chloroplast. With the discovery of the human dietary importance of this vitamin, silver nitrate was then used extensively to locate all possible plant sites of ascorbic acid (MAPSON, 1958). BARNETT AND BOURNE (1941), nevertheless, questioned the specificity of silver attachment to ascorbate and found that ascorbic acid is the only plant originating compound capable of this reducing capacity at low pH levels (JENSEN AND KAVALJIAN, 1955). Furthermore, using chromatographic techniques to separate ascorbate from tannins and flavanoid pigments, all of which are presumed to have a reducing capacity, NAGAI (1951) found that ascorbate was the most commonly occurring substance in plant tissues reduced by silver nitrate in the cold at neutral or acid pH.

RABINOWITCH (1956) separated the silver reduction by chloroplasts into a photochemical reaction sensitised by chlorophyll whilst in the

dark ascorbic acid appeared essential for silver ion reduction. Electron microscopy of angiosperm chloroplasts demonstrated that silver was associated specifically with the stroma lamellae (BROWN, MOLLENHAUER AND JOHNSON, 1962), suggesting that chlorophyll is probably not directly involved in silver reduction. The specificity of silver attachment within the chloroplast indicates that varying reducing conditions for silver may exist within this organelle. The conditions may include membrane permeability differences to silver ion complexes or the presence of silver-reducing tissue ligands. In the chloroplast there are two separate photosystems controlled by different enzymes (PARK AND SANE, 1971). Photosystem I is thought to be in both the stroma and granal lamellae whilst photosystem II is confined to the granal lamellae. The stroma lamellae system is probably involved with cyclic photophosphorylation. It may thus be that in light the silver ion complexes with these enzymes.

Whilst investigating the silver reducing property of chloroplasts, BROWN, MOLLENHAUER AND JOHNSON (1962) observed that silver particles also associated with the cell wall. Specifically, silver was detected in the middle lamellae, the inner surface of the cell wall and within the plasmodesmata. REID (1941) found that ascorbic acid was associated with the surface of root tip cells and, with ascorbic acid oxidase being attached to the cell wall (GOODWIN AND MERCER, 1983) this may explain the presence of reduced silver associated with the cell wall. Ascorbic acid oxidase is a specific copper protein, the only one of five oxidases capable of directly catalysing ascorbic acid and oxygen.

The cell wall is the structural position of many enzymes, for example, β-fructofuranosidase (invertase), DNA-ase, RNA-ase, phosphatases and many other oxidases besides ascorbic acid oxidase. Of these enzymes, invertase has been shown to be inhibited by silver nitrate (MEALOR AND TOWNSHEND, 1968). The reaction with the enzyme is instantaneous and shows non-competitive inhibition, that is the silver and sucrose bind at different sites on the enzyme. Due to the initial binding of various impurities to the silver ion, the silver must exceed microgram concentrations for invertase inhibition (MEALOR AND TOWNSHEND, 1968). Thus in those tissues treated with silver ions, the hydrolysis of sucrose will be prevented.

Enzymes containing sulphydryl groups are also inhibited by the silver ion (MAHLER AND CORDES, 1971; KLEIN, 1978; KOONTZE AND BERLE, 1980). This affinity between silver and sulphydryl groups was recognised through electron microscopy staining procedures. SWIFT (1966) showed that silver deposits accumulating in yeast cell walls after silver methenamine treatment marked the presence of free sulphydryl groups and disulphide linkage sites within proteins. Initially this reaction was thought to demarcate aldehyde groups but when an aldehyde-blocking agent was added prior to silver treatment, silver accumulation was not prevented. This silver reduction in the vicinity of the cell wall could be due to the presence of sulphur-containing structural or catalytic proteins or amino acids.

One of the most renowned histochemical tests using silver nitrate signifies the presence of chloride ions with the production of a white silver chloride precipitate. Together with its tissue fixation properties, silver nitrate provides a promising method of preventing the highly mobile chloride ions from displacement (KOMNICK AND BIERTHER, 1969; VAN STEVENINCK AND CHENOWETH, 1972; VAN STEVENINCK, VAN STEVENINCK, HALL AND PETERS, 1974). Once again, the specificity of the method was questionable. Comparing diffraction patterns of the silver thiosulphate deposits with silver chloride standards proved unsatisfactory due to the instability of silver chloride under an intense electron beam. Yet when comparing plant tissues directly treated with silver nitrate with those that were initially frozen rapidly with the silver ions then applied under anhydrous conditions, the silver chloride distribution was identical. In barley seedlings, the main pathway of chloride ions from cell to cell depicted by silver nitrate treatment was through the plasmodesmata (VAN STEVENINCK AND CHENOWETH, 1972). As chloroplasts have a high salt content, especially potassium and chloride ions compared with the cytoplasmic phase, silver deposits were also detected in these organelles (VAN STEVENINCK AND CHENOWETH, 1972). In some cases the nuclei have also contained silver deposits (CHAYEN, DAVIS AND MILES, 1953; ZIEGLER AND LUTTGE, 1967) but these have been disregarded as artefacts (MAPSON, 1958; BROWN, MOLLENHAUER AND JOHNSON, 1962).

The many potential sites of silver attachment within plant tissues somewhat destroys the illusion of using the silver ion as a tool to identify a site of ethylene action. However, this revelation that a hormone could have the capacity to bind to more than one site within

a cell opens the possibility that this may be the channel via which tissue sensitivity changes. For example, if the silver ion prevents ethylene from binding to sulphydryl groups, chloride ions, ascorbate and invertase and only one of these sites is physiologically active, the efficiency of the ion blocking ethylene action will be reduced depending on the other non-physiological sites present in the tissue at that time. Of importance though is the possibility that one of these silver-reducing sites may well be physiologically involved in ethylene action.

To discover this site of ethylene action using the silver ion, the first approach taken was to elucidate the path of translocation and cellular accumulation of this ion within the cut carnation flower. Hence the flower part and tissue within this flower part that produced the vast physiological impact this silver ion had on carnation flower senescence may be isolated and concentrated upon.

As reviewed in Chapter 1, silver nitrate initially was used as a bacteriocide together with sucrose in a postharvest treatment to extend the vaselife of cut carnation flowers (AARTS, 1957). However, HALEVY AND KOFRANEK (1977) found that petal-applied silver nitrate increased carnation flower longevity to a greater degree than via basal uptake. The bacteriocidal properties of silver nitrate were thus not the cause of this treatments capacity to extend carnation longevity and it was realised that this ion had potent ethylene action-inhibitory properties (BEYER, 1976a). This property of the silver ion to block ethylene action was utilised fully in the carnation flower once VEEN AND VAN DE GEIJN (1978) had overcome the poor translocation of this

ion by complexing it with the thiosulphate anion. Noteworthy is that even in this complex the silver ion maintains its ethylene inhibitory capacity.

The first experiments that located the site of silver action within the carnation flower were not initiated for this purpose. VEEN AND VAN DE GEIJN (1978) used labelled silver (^{110m} Ag) thiosulphate to determine the rate of silver translocation through the stem to the flowerhead. They noted that after a twenty-four hour silver thiosulphate pulse, the greatest accumulation of radioactivity was detected in the receptacle. Similar experiments with labelled (^{110m} Ag) silver thiosulphate treatments were carried out in other cut flowers primarily to determine if silver also reached the flowerheads (NOWAK, 1979; NOWAK AND VACHAROTAYAN, 1980; LE MASSON AND NOWAK, 1981; NOWAK, 1981). The *Gerbera* inflorescence, in which the silver thiosulphate treatment was successful in delaying senescence, was the only floral structure to show a similar silver accumulation in the receptacle (NOWAK, 1979). In the cut carnation flower, the receptacle is the flower part that produces the most ethylene (NICHOLS, 1977a; VEEN AND VAN DE GEIJN, 1978). As this endogenous ethylene production is fully blocked by a pretreatment with silver thiosulphate, VEEN, HENSTRA AND DE BRUYN (1980) hypothesised that the silver ion could be used as a tool to find the site of ethylene biosynthesis and its site of action inside the plant cell. Thus these workers investigated the receptacle ultrastructurally and detected electron dense silver deposits in the cell wall and the intercellular spaces mainly confined to xylem tissues. When these particles were analysed with X-ray micro-analysis the

presence of silver and sulphur was detected (VEEN, HENSTRA AND DE BRUYN, 1980). Similar results were found in the 'green' portions of an unevenly silver thiosulphate infiltrated tomato fruit. However, with minimal quantities of silver thiosulphate used the particles accumulated preferentially in the phloem walls and associated parenchyma rather than the xylem as detected in carnation tissue (HOBSON, NICHOLS, DAVIES AND ATKEY, 1984). The distribution of ions demarcates an apoplastic route for the movement of silver ions. But once again these electron-dense particles were shown by X-ray microanalytical techniques to consist of silver and sulphur. As the silver accumulated in different tissues with a short silver thiosulphate treatment in the tomato, the question arose as to whether the high accumulation of silver in the carnation receptacle after a twenty-four pulse may be due to an accumulation of excess silver. Thus the distribution of silver thiosulphate (^{110m} Ag) after a ten minute pulse was investigated to confirm the accumulation of this receptacle silver (^{110m} Ag) in delaying carnation senescence. By labelling the silver thiosulphate complex on the silver (^{110m} Ag) a time-course study was conducted to establish the movement of the silver thiosulphate complex within the cut carnation flower over a period of nine days. This time-course study was repeated with the sulphur (^{35}S) of the thiosulphate anion complex labelled. Furthermore, the possible sites of silver attachment to block ethylene action were considered in relation to cut carnation flower senescence.

Materials and Methods

To determine the translocation of labelled silver thioshulphate

The harvested carnations were treated with silver thiosulphate. This silver thiosulphate was either (^{110m} Ag) labelled (specific activity 37 MBq 1.1 mg Ag $m\ell^{-1}$) or labelled on the sulphur (inner S-S^{35}) molecule (specific activity 40,7 MBq 27,6 mg Ag $m\ell^{-1}$) contained within the thiosulphate complex. Following a ten minute pulse the carnations were placed into distilled water. Over a period of nine days groups of the flowers were removed and divided into petals, ovary, receptacle and calyx plus stem. Radioactivity was measured, as previously described (Chapter 3), using oven dried material.

To determine the translocation of petal-applied silver nitrate

A 0,01 Molar silver nitrate solution was spiked with labelled silver (^{110m} Ag) nitrate. This solution was carefully painted on to the petals with a brush. Over a period of nine days the movement of this petal-applied radioactivity to the other flower parts was measured. Once again the radioactivity was determined, as previously described (Chapter 3), using oven-dried material.

Light microscopy

The receptacle tissue was prepared as for electron microscopy (Chapter 2). Monitor sections were cut for light microscopy and stained

using a one percent toluidine blue in one percent borax : one percent pyronin Y (1:1 v:v) and photographed.

Scanning electron microscopy and microanalysis

The receptacle was isolated from the whole flower. Directly after the receptacle was sectioned along the transverse plane with a dissecting blade, it was placed into liquid nitrogen and then freeze-dried for twenty-four hours. On removal the section was mounted onto a specimen stub and coated with carbon using a vacuum evaporator. The specimens were then viewed and photographed on the scanning electron microscope, JEOL T-200.

For microanalysis the samples were taken to Mr P Evers, Electron Microscope Unit, Natal University, Durban. The sections were observed with the scanning electron microscope (JEOL 35 plus KEVEX energy-dispersive analyser, type 7000/77).

Results and Discussion

Following a ten minute labelled silver thiosulphate (^{110}Ag and ^{35}S) stem pulse, radioactivity was detected in all the flower parts (Fig. 5.1). This distribution is similar to that demarcated by the uptake of eosin stain via the transpiration stream. This was expected as the silver anionic complex had been developed to overcome the negatively charged sites of the xylem vessels and this, as shown, move more or

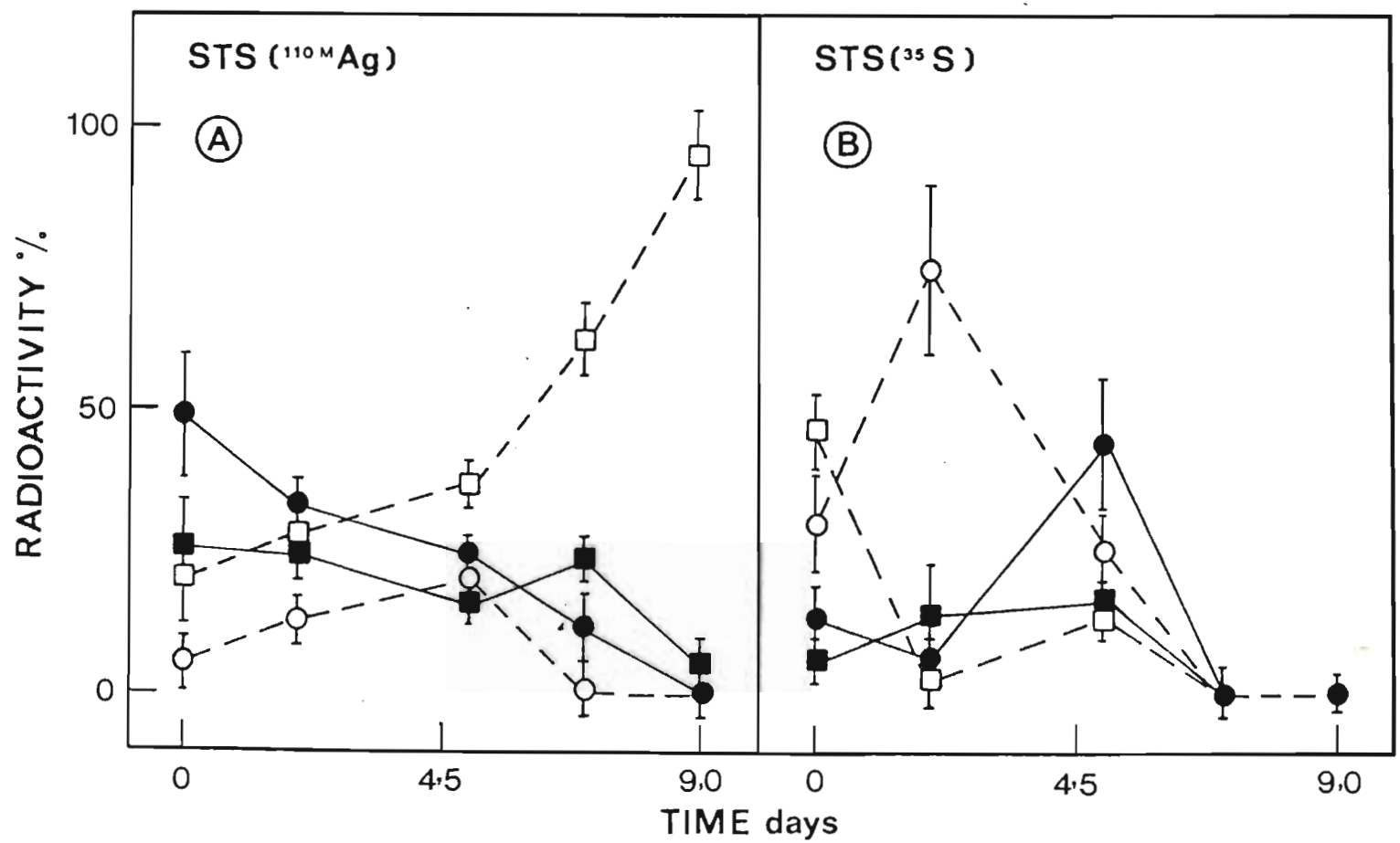


Figure 5.1 : The movement of labelled, ^{110m}Ag (A) or ^{35}S (B), silver thiosulphate within the cut carnation flower after a ten minute pulse. Radioactivity was measured in the petals (-●-), ovary (-○-), calyx plus stem (-■-) and receptacle (-□-) and expressed as a percentage of the dpm's measured within each treated flower. Vertical bars indicate the standard error of the mean.

less freely with the water flux within the carnation flower (VEEN AND VAN DE GEIJN, 1978). If silver attachment to a specific site is necessary to inhibit ethylene action or production then the translocation of the silver ion within the transpiration stream is probably of little significance regarding ethylene metabolism. However, this silver thiosulphate-xylem passage appears essential to facilitate silver transport to the site(s) of ethylene production within the carnation flower (VEEN, 1979a).

After the initial silver thiosulphate-flux into the flower, a gradual decline of labelled silver was detected within all the flower parts save the receptacle in which the radioactivity gradually increased (Fig. 5.1A). This movement of labelled silver from the peripheral flower parts to the receptacle suggests that the silver within the cut carnation become phloem-mobile, a phenomenon detected when silver nitrate was applied to leaves (KOONTZE AND BERLE, 1980). To determine the possibility of the silver moving from the petals to the receptacle once it had dissociated from the anionic complex, labelled silver nitrate (^{110m} Ag) was applied to the carnation petals and its movement monitored (Fig. 5.2). A day after silver nitrate application, twenty percent of the labelled silver was detected in other flower parts besides the labelled petals, indicating that silver nitrate can become phloem mobile in carnation flowers. The low percentage of the total radioactivity moving from the petals may be due to inefficient absorbance, as indicated by the 'blackening' of the petals. However, of the silver translocated from the petals, the majority was again shown to accumulate within the receptacle. Thus for this transport to occur it appears that the silver

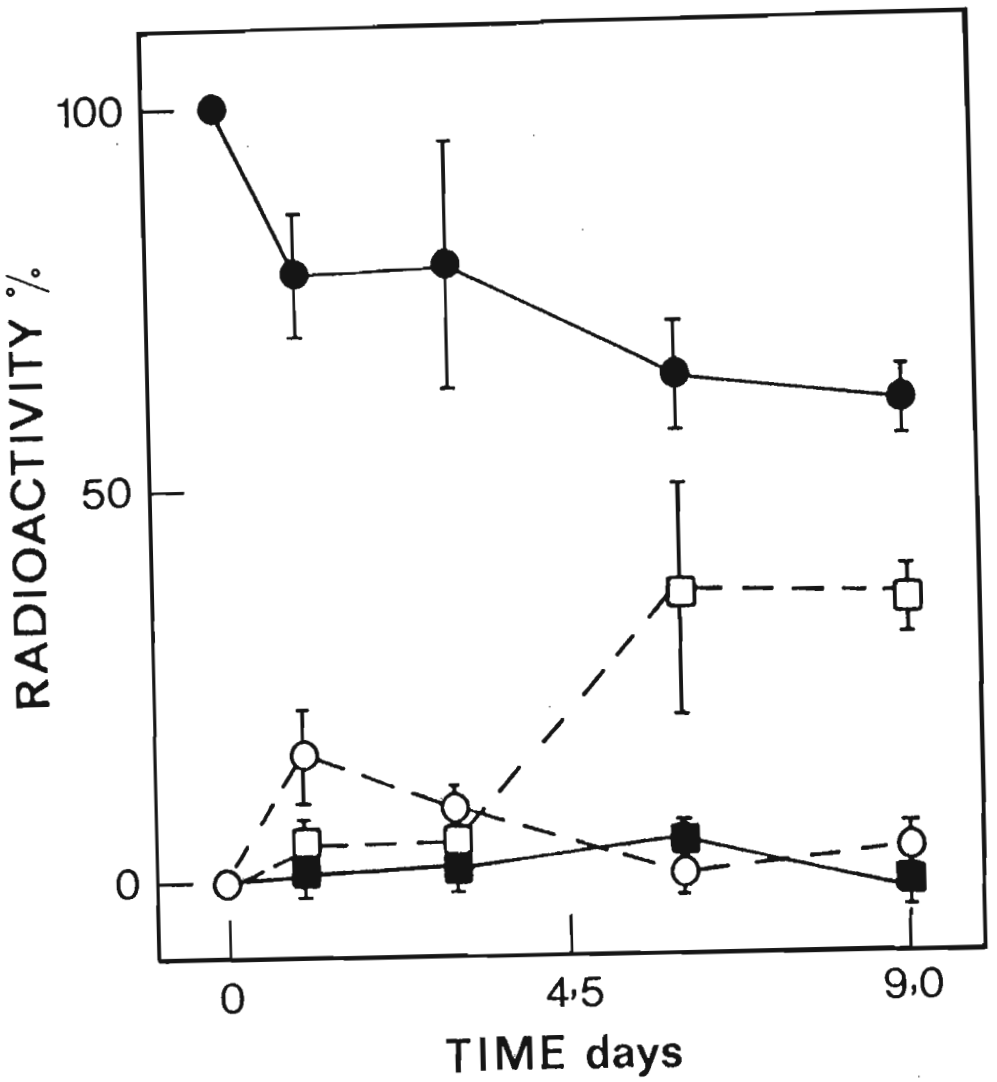


Figure 5.2 : The movement of petal applied labelled silver nitrate (^{110m} Ag NO_3) within a cut carnation flower. Radioactivity was measured in the petals (-●-), ovary (-○-), calyx plus stem (-■-) and receptacle (- □ -) and expressed as a percentage of the total dpm's measured within each flower. Vertical bars indicate standard error of the mean.

need not remain attached to the thiosulphate anionic complex but can dissociate within the petals and still move to the receptacle. In labelling the silver thiosulphate on the silver (^{110m}Ag) or thiosulphate (^{35}S), it was hoped that the flower part and time of dissociation would be located by comparing the similarity or dissimilarity of the translocation of these two isotopes (Fig. 5.1). After the initial influx of silver thiosulphate (^{35}S) into all flower parts, no transport similarity with silver thiosulphate (^{110m}Ag) was apparent. Contamination of silver-free labelled anionic complexes moving randomly through the cut flower could have masked any significant relationship. However, these free anionic complexes may play an important silver translocatory role. Their presence could 'wash' any unattached silver (GURR, 1958) towards the receptacle where the silver then may become attached to a strong reducing site. This would explain the lack of radioactive silver detected in the petals and ovary.

The receptacle is that part of the stem that bears all the flower parts and thus is also the metropolis of all flowerhead vascular tissue. Anatomically the vascular tissue in the receptacle acquires a 'star' arrangement in transverse section (Plate 5.1 A,B) as this is the point where the vascular traces divide into the various flower parts. The central pith tissue appears to be aerenchyma (Plate 5. 1 B,C). Silver accumulation within the receptacle was also suggested to block the xylem vessels by precipitating proteins and thus inhibiting water transport (NOWAK AND VACHAROTAYAN, 1980). However, after a twenty-four hour silver thiosulphate pulse, obstructions in the receptacle vascular tissue could not be observed (Plate 5.1D). Furthermore, attempts to

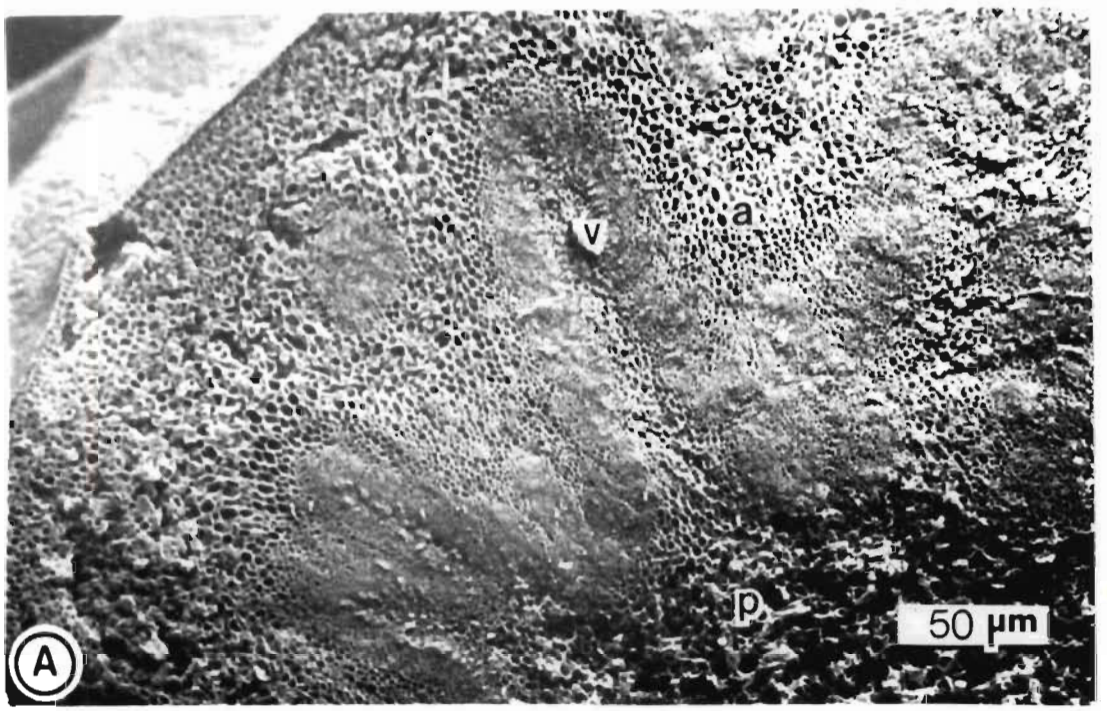
Plate 5.1 : The carnation receptacle in transverse section.

A. A scanning electron micrograph showing the central pith tissue (p) surrounded by the vascular tissue (v) forming a 'star' shape. Aerenchyma-like tissue (a) surrounds the vascular tissue.

B. A light microscope micrograph of the vascular tissue (v) in which xylem vessels (x) are present, surrounded by the aerenchyma-like tissue (a).

C. A scanning electron micrograph of the pith region of a receptacle taken from a flower treated three days previously with silver thiosulphate. Note the structure of the tissue with large air-spaces between the cells.

D. A scanning electron micrograph of the vascular region from a receptacle taken from a flower treated three days previously with silver thiosulphate.


v
a
p
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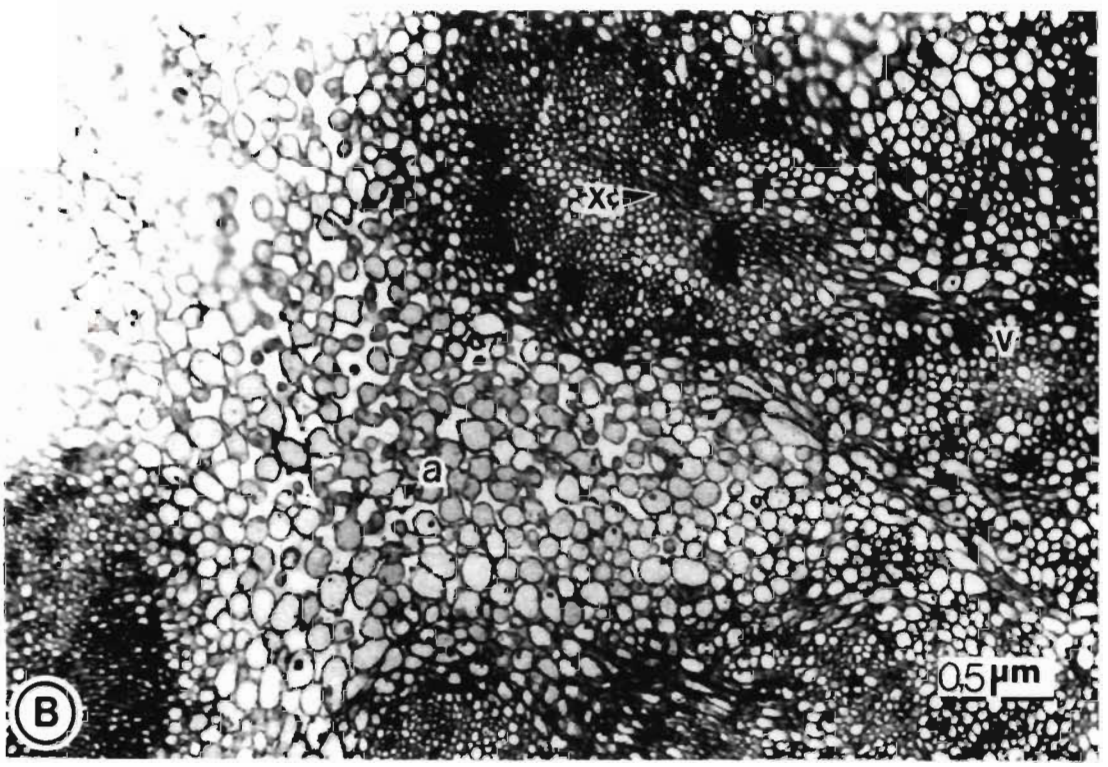
A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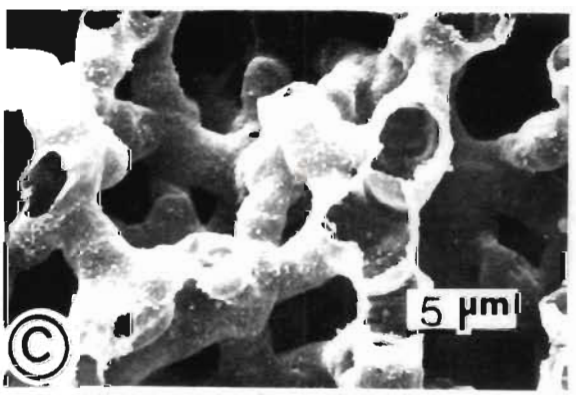
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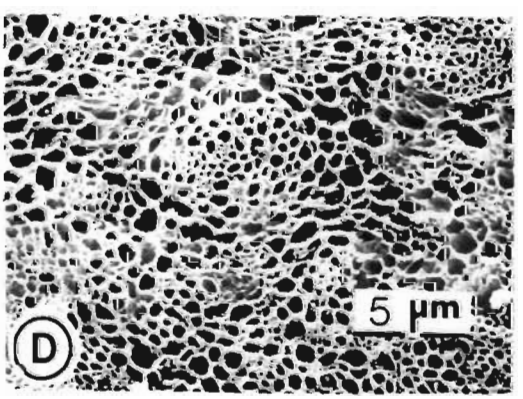
5 µm
D

repeat the X-ray microanalysis with a scanning electron microscope failed. Silver was not detected in the receptacle tissue even after a twenty-four hour pulse (Fig. 5.3). High levels of calcium, silicon potassium and copper were present. If silver was within this tissue, its strong binding properties make it difficult to accept that preparatory methods could have washed the silver from the receptacle. Overall the importance of this silver accumulation in the receptacle in delaying the senescence of silver thiosulphate-treated cut carnation flowers is not understood. Whether the receptacle is the site from which the silver delays carnation senescence is a point in question. Alternatively the receptacle may act as a store of silver to avoid silver toxicity in the remaining flower parts. KOONTZE AND BERLE (1980) suggested that the precipitation of silver as a sulphide (agreeing with the X-ray microanalysis results) or reduced to metallic silver renders the ion non-toxic in an unbound form. This implies that the senescence-delaying effect or ethylene inhibition of the silver ion occurs, not in the receptacle, but in another flower part.

The speculative nature of this latter discussion indicates that there are too many outstanding questions remaining unanswered to gain anything further from silver translocatory studies. Knowledge of the actual sites of silver attachment related to the physiological activity of this silver ion need to be known before progress can be made. Thus an initial question that could be asked is what would the resultant physiological activity be if the silver ion bound to the identified histochemical sites, sulphydryl groups, ascorbic acid, invertase and chloride ions.

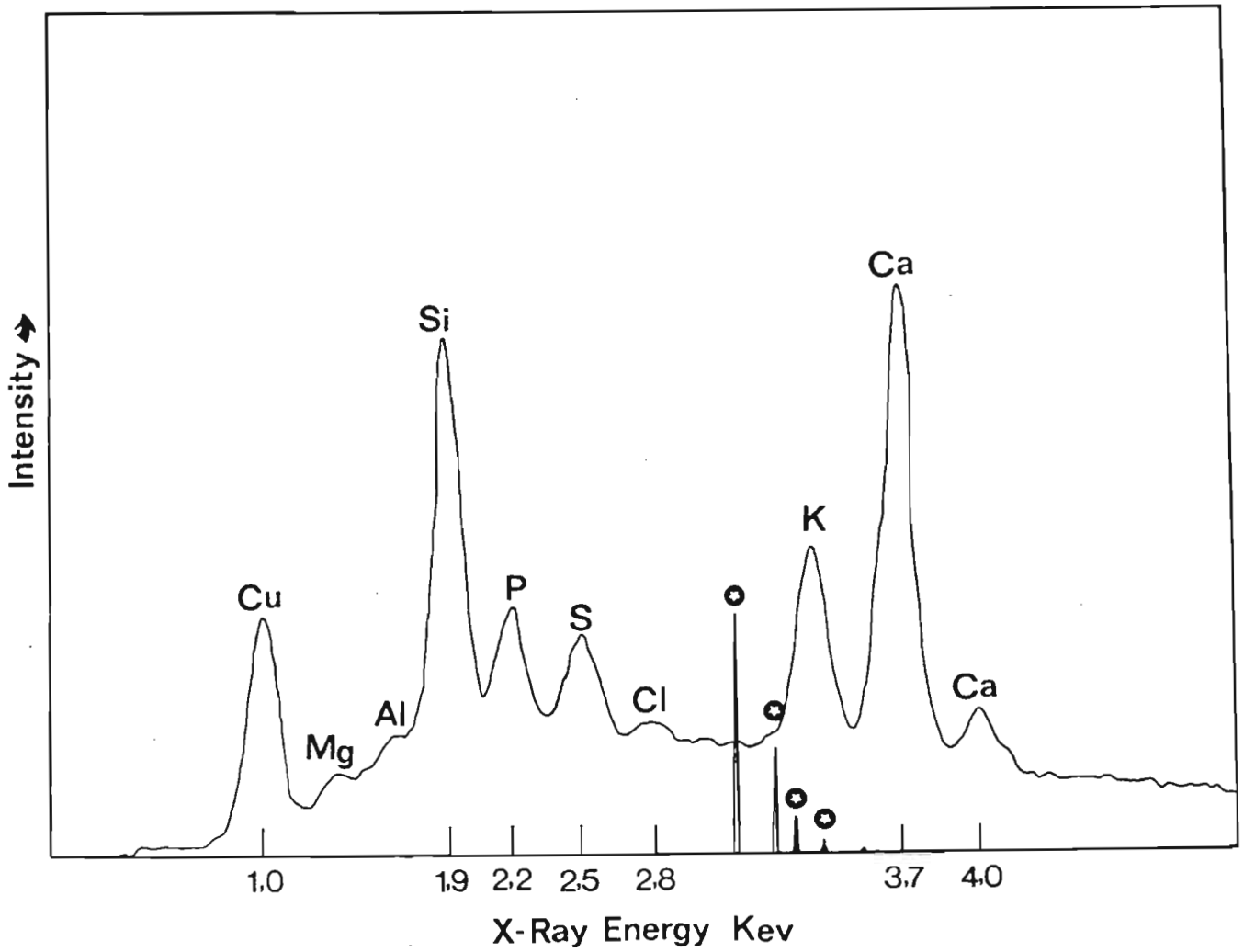

Figure 5.3 : Microanalysis using the scanning electron microscope of the carnation receptacle after a twenty-four hour silver thiosulphate pulse. ✪ indicates the silver spectrum.

Acid invertase activity is inhibited by the silver ion binding non-competitively to this enzyme (MEALOR AND TOWNSHEND, 1968). As a result silver thiosulphate-treatment of the cut carnation flower could prevent the hydrolysis of sucrose. If active in sink tissues this silver action would prevent a sucrose gradient to develop in this tissue. Such a relationship occurred in the ovaries from silver thiosulphate-treated carnation flowers (Table 3.5). Twenty-four hours after silver-treatment the level of invertase activity was lower compared to the control ovary and the carbohydrates did not accumulate in this silver-treated ovary to the degree seen in the control ovary (Fig. 2.7). Sink activity in the ovary with silver thiosulphate treatment was only detected within the first twenty-four hours after treatment, thereafter the petals remained the dominant carbohydrate sink. In this initial period after silver thiosulphate treatment, silver was present within the ovary. This silver does not remain within the ovary but moves back into the receptacle. However, even with this movement of silver from the ovary, this flower part does not show any sink activity (Fig. 3.6). A direct relationship between the presence of silver and carbohydrate sink activity thus does not appear to occur. To further investigate this invertase-silver relationship in the carnation flower, the direct effect of silver thiosulphate and silver nitrate on carnation petal invertase was measured (Fig. 5.4). In the whole petals, silver thiosulphate treatment did not significantly alter invertase activity whilst silver nitrate treatment increased invertase activity. This latter result was probably due to the poor translocatory properties of this silver compound. To overcome this, petal discs were used to increase the area of silver uptake. Once again silver thiosulphate

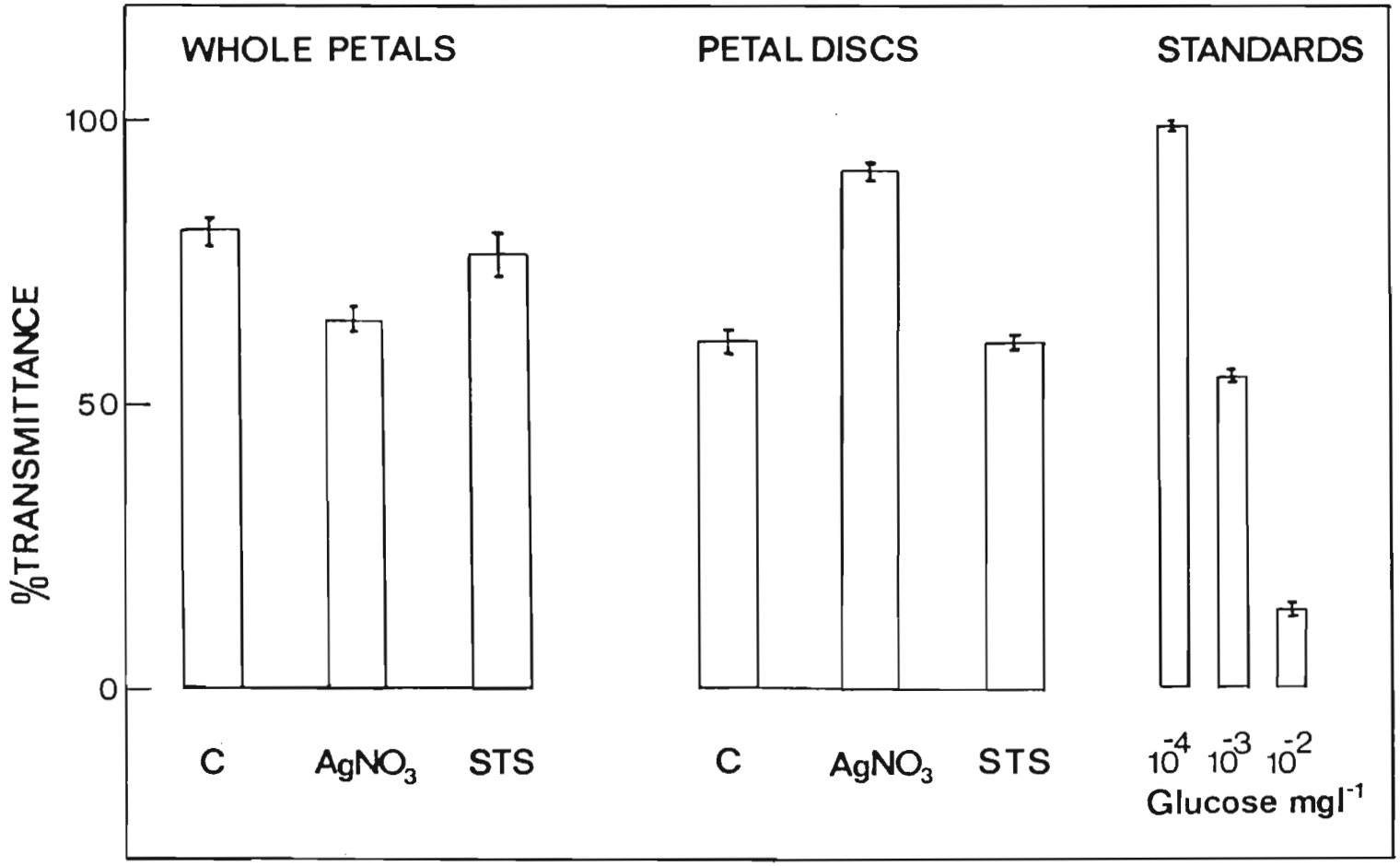

Figure 5.4 : Invertase activity (transmittance) in whole petals and petal discs treated with silver thiosulphate (STS) and silver nitrate ($AgNO_3$) solutions. Vertical bars indicate the standard error of the mean.

and the control did not significantly differ but silver nitrate treatment inhibited invertase activity. It thus appears that in the silver thiosulphate form, the silver ion is incapable of affecting invertase activity. Such a result is apparent within the cut carnation flower as in this system,with silver thiosulphate treatment, the petals are the dominant carbohydrate sink suggesting that invertase activity is not interfered with. Thus if silver thiosulphate physiologically affects the petals, its action could not be through invertase inhibition. In the ovary, however, invertase activity is reduced by a silver thiosulphate treatment. Either this inhibition of invertase activity is an indirect effect of silver thiosulphate treatment or this silver complex is previously altered so that it is able to non-competitively bind to invertase. The former hypothesis appears to be the most likely because of the gradual withdrawal of silver from the ovary into the receptacle.

Ascorbic acid is an oxidising agent but when dissociated into ascorbate and hydrogen ions, the ascorbate is the reducing agent that binds to the silver ion. Many physiological events involve the dissociation of ascorbic acid which were comprehensively reviewed by CHINOY (1984). Ascorbic acid is closely correlated with photosynthesis and is abundant in chlorophyllous tissues. In the photosynthetic process ascorbic acid may act as an electron donor or catalyst as when added with magnesium ions, flavin mononucleotides and vitamin K, this process is enhanced. Ascorbic acid is also thought to function as a protector of some essential components against chloroplast inactivation.

Thus the reduction of ascorbate by the silver ion could result in the retardation of the photosynthetic process and chloroplast inactivation. In the silver thiosulphate-treated cut carnation flower the ovary wall chloroplasts (Plate 2.4) showed little development from the day the flower was harvested (Plate 2.11) compared to the control (Plate 2.2). Yet on the other hand, when the petals were removed from a silver thiosulphate-treated carnation flower, the chlorophyll content of the ovary increased (Fig. 3.3). Additionally, a silver nitrate treatment prevented chlorophyll loss in tobacco leaf discs (AHARONI, ANDERSON, LEIBERMAN, 1979) and in citrus rind (PURVIS, 1980). Furthermore, adding ascorbic acid to the silver thiosulphate solution used to pulse the carnation flowers, a precipitate does not form. On the other hand, in the silver nitrate solution a precipitate formed on the addition of ascorbic acid. Thus, once again, in the silver thiosulphate complex, the silver ion appears to be unaffected by ascorbic acid.

The only identified site of silver attachment that may involve a protein receptor site for ethylene action is the sulphydryl group. Silver binding to this group could affect a variety of events from enzyme activity to structural proteins or causing a deficiency of a vital amino acid. PLICH (1983), using apple fruit cylinders, recognised this close relationship between silver and sulphydryl groups. In relating it to ethylene biosynthesis he proposed that the silver bound to the sulphydryl group of methionine, this caused a deficiency of this amino acid precursor and hence ethylene biosynthesis was prevented. Interesting was that PLICH (1983) found that the application of the

silver ion in the thiosulphate form was ineffective in inhibiting ethylene emanation. In this complex, it appears that plant orientated sulphydryl groups involved in ethylene biosynthesis could not compete with the thiosulphate bond to which silver was attached. Sulphydryl groups are involved in ethylene biosynthesis as indicated by using sulphydryl-protecting compounds, such as dithioerytritol or L-cysteine, and sulphydryl reagents such as N-ethylmaleimide, iodoacetic acid or p-chloromercuribenzenesulphonic acid. The sulphydryl-protecting agents together with silver nitrate maintained ethylene emanation levels similar to the control, whilst sulphydryl reagents mimicked the silver nitrate ethylene inhibition. Therefore, the dissociation of silver from the nitrate ion and this free silver binding to a sulphydryl group appears a means to reduce ethylene emanation.

The differential effects of silver thiosulphate and silver nitrate on ethylene production were also recognised by LIS, KWAKKENBOS AND VEEN (1984). They showed that silver nitrate prevented ethylene biosynthesis from apple cylinders by the toxic action of the silver ion on their protoplasts. In the thiosulphate form silver appears to be less toxic in plant systems (DENNIJS AND VISSER, 1980) unless the plant tissues are exposed to long incubation periods with this silver complex.

This differential effect of silver nitrate and silver thiosulphate may explain the dilemma over the production of ethylene often occurring when silver thiosulphate treatment inhibits ethylene action. As suggested above, in the thiosulphate form plant orientated sulphydryl groups involved in ethylene biosynthesis could not compete with the

silver thiosulphate bond. Thus it may only be in cases where this bond is previously broken by another stronger reducing agent that silver binds to the ethylene-forming sulphydryl group. Hence, the hypothesis proposed by VEEN (1979a) that the silver ion is not always able to reach the site of ethylene biosynthesis may be correct, not in anatomical terms but biochemically.

It is unfortunate that the experiments of PLICH (1983) and LIS, KWAKKENBOS AND VEEN (1984) were not related also to ethylene action as the question remains as to the possible role of the sulphydryl group in this physiological response. However, these experiments have shown that a sulphydryl group is involved in ethylene biosynthesis and it is via this group that the silver ion interferes with ethylene biosynthesis. In the carnation flower, the precipitate in the receptacle containing silver and sulphur may be related to the inhibition of ethylene biosynthesis by a silver thiosulphate treatment. The receptacle is the flower part in the carnation that produces the most ethylene (VEEN, 1979a).

There has been further investigation into the silver inhibition of ethylene biosynthesis. VEEN in 1983 hypothesised that the ethylene surge that preceded carnation petal wilting can itself be a consequence of ethylene action. Closer investigation of the ethylene biosynthetic pathway showed that silver thiosulphate treatment prevented the natural ACC rise that occurs in carnation flowers. This lack of ACC appears tp be the determining factor in the silver inhibition of ethylene emanation

as exogenous ACC application to silver thiosulphate-pulsed flowers results in a production of ethylene even greater than the control (VEEN AND KWAKKENBOS, 1983). Thus in the carnation flower the thiosulphate complex is probably removed from the silver ion so that it can complex with an ethylene-forming sulphydryl-containing enzyme. If this is true, this enzyme must then act prior to ACC formation in the ethylene biosynthetic pathway.

However, since the rate of ethylene production in vegetative tissues is mainly controlled by indoleacetic acid (BURG AND BURG, 1966; FUCHS AND LIEBERMAN, 1968; LAU AND YANG, 1973) the silver inhibition of ethylene production may not be directly to an ethylene biosynthetic enzyme. Alternatively it may be related to auxin levels. Auxin appears to induce the continuous *de novo* synthesis of ACC synthetase, which in turn increased the amount of ACC and hence ethylene production. The simultaneous application of indoleacetic acid with the silver ion results in large increases in ethylene production showing that the silver ion does not interfere with auxin stimulated ethylene production (AHARONI, ANDERSON AND LIEBERMAN, 1979). Furthermore, AHARONI (1985) demonstrated that silver-pretreated leaf discs contained more free labelled indoleacetic acid than untreated ones. This was due to decreased decarboxylation of indoleacetic acid which resulted in more ethylene being produced by the silver-treated discs. Noteworthy then is that the silver ion and ethylene have antagonistic effects on the level of indoleacetic acid within plant tissue; whilst ethylene reduces the indoleacetic acid levels so the silver ion is conserving the indoleacetic acid level. Hence, by preserving the indoleacetic acid present in a

plant system with the silver ion, this may result in ethylene production if the auxin level becomes sufficiently high.

The last identified silver-binding site to be discussed is the chloride ion. By interfering with the chloride ions, the silver ion could affect the electrochemical potential of membranes interfering with active transport processes between cells. Considering sucrose translocation within the cut carnation, if the sucrose moves through the phloem down a concentration gradient to sink tissues and the permeability of the membrane is adjusted, then active offloading of the sucrose is not required (Chapter 3). Silver thiosulphate treatment of the cut carnation flower, as expected, does not affect sucrose translocation into the petals (Fig. 3.6). Alternatively, this positive silver ion could adjust the pH of the tissues as potassium and calcium ions have been proposed as components capable of altering the pH of the free space, that is, the area between cells. A physiological event related to pH is cell extension (BRUMMER AND PARISH, 1983). Thus through adjusting cellular pH, the silver ion may prevent cell extension and hence ovary enlargement during the senescence of the silver-treated carnation flower. The ovary increases in size during senescence by cell enlargement (NICHOLS, 1976). Thus the interference of the silver ion with the chloride ions may well physiologically effect the carnation flower senescence process but no evidence as to its involvement with ethylene action has arisen.

Another factor to take into consideration is the interaction of the silver ion with other plant hormones. It may be through affecting these other plant hormones that ethylene action is affected. On the other

hand, by preventing ethylene action and ethylene biosynthesis, the silver ion may alter the levels or action of the other plant hormones.

Generally silver thiosulphate treatment of the cut carnation flower prevents the plant hormone levels from rising. NOWAK AND VEEN (1982) showed that endogenous abscisic acid levels naturally increase in the cut carnation flower but were absent after a silver thiosulphate pulse. Likewise, the cytokinin peak of activity in the ovary detected during carnation flower senescence was prevented with silver thiosulphate application (VAN STADEN AND DIMALLA, 1980). Indoleacetic acid levels were preserved by the silver ion preventing their decarboxylation (AHARONI, 1985) but no evidence has been given on their levels increasing. Ethylene emanation with silver thiosulphate treatment appears to depend on ACC levels present prior to treatment. As yet the relationship between gibberellins and silver thiosulphate has not been investigated. Although circumstantial, the above evidence suggests that *de novo* synthesis of plant hormones is prevented by silver treatment.

It would be short-sighted to consider that the whole question of carnation flower senescence revolves around ethylene biosynthesis and action and that the answer to the silver-ethylene relationship would resolve all the mysteries of the senescence process. Not all cut flowers are as sensitive to ethylene nor is the longevity of all cut flowers delayed to the extent seen in cut carnation flowers by a silver thiosulphate treatment. Silver treatment is successful on *Lilium* flowers (SWART, 1980); the *Dendrobium* (NOWAK AND VACHAROTAYAN, 1980);

Cattleya (BEYER, 1976b) and *Cymbidium* orchid flowers (VAN STADEN AND DAVEY, 1980); anthurium (PAULL AND GOO, 1982); gladioli (MOR, HARDENBURG, KOFRANEK AND REID, 1981), gerbera inflorescence (NOWAK, 1979), miniature carnations (REID, FARNHAM AND McENROE, 1980) and preventing floret abscission in snapdragon inflorescences (FARNHAM, REID AND FUJINO, 1981; NOWAK, 1981). Noteworthy is the contrasting effect of a silver thiosulphate treatment on roses. In this cut flower, the longevity of the bloom is not greatly extended with a silver thiosulphate treatment (DE STIGTER, 1981). Furthermore, with the simultaneous addition of ethylene and silver thiosulphate, the silver thiosulphate treatment did not nullify the ethylene action, an action that silver thiosulphate is effective in inhibiting in carnation flowers. The reason for this may be due to the morphology of the rose, which possesses an hypanthium or alternatively, it may be important that the storage carbohydrate within the rose is starch.

In conclusion, it appears that the silver accumulation in the carnation receptacle after a silver thiosulphate treatment may be important in the senescing-delaying effect of this treatment. This silver accumulation could inhibit ethylene biosynthesis and action or render the silver ion non-toxic to the surrounding tissues. However, the other flower parts also may be physiologically affected by the silver ion. Sites to which silver has been shown to bind in plant tissues include ascorbic acid, invertase, chloride ions and sulphydryl groups. For the silver thiosulphate to be reduced by ascorbic acid, invertase and the sulphydryl groups, it appears as if another reducing agent must first

weaken the bond of this complex. Each identified site shows the possibility of having a role in carnation flower senescence but whether it is related to ethylene action is a question, as yet, untouched.

GENERAL DISCUSSION

Senescence is a broad term which incorporates many metabolic events and structural changes within the cells of plant parts undergoing this process. Although it is a commonly held view that senescence represents a descent into chaos, in terms of these metabolic and structural changes, senescence is in fact a tightly controlled process in which the sequence of events is usually highly ordered until the terminal stages are under way (SEXTON AND WOOLHOUSE, 1984). Furthermore, the variety of senesence strategies shown by flowers indicate that senescence is genetically programmed and has arisen as a result of natural selection. The question often asked is what switches the programme on? Considering the development of the carnation flower, as soon as the floral stimulus is received the apex becomes determinate in growth. Thus, genetically speaking, flower senescence could be initiated as early in development as the genetic recognition of the floral stimulus.

Plant hormones regulate flower senescence according to the genetic messages received or sensitive sites available, the environmental influences on their endogenous levels and their interaction; but these chemicals cannot totally prevent the ultimate goal of the genetic programme, the death of the plant part. This statement does not belittle the vital role these hormones play in regulating this process. As TREVAWAS (1981) pointed out, the sessile mode of plant life has problems unique to itself which arise from the fact that adverse environmental conditions are unavoidable. So when the death of a

plant part occurs the plant may be affected by losing photosynthetic organs or the rate of transpiration being reduced. Furthermore, if the assimilates or limiting chemical constituents rare in the area occupied by the plant are lost from the plant as a whole, the survival capacity of that plant in adverse environmental conditions is reduced. Thus the controlled recovery of these chemical constituents during correlative senescence is a vital aspect of the sessile existence of a plant. Another advantage of a senescence period prior to the death of a plant part is the opportunity of a plant to mobilise waste products into the plant part to be lost. The senescence strategy, therefore, determines the efficiency by which these latter two processes, in relation to environmental conditions, are able to function. The regulation of this strategy is the role of the plant hormones.

The senescence of the carnation flower ultimately results in the death of all the flower parts except the seeds, when pollinated. The rate of the senescence of these organs differs and is, therefore, a good example of correlative senescence. Evidence that the genetical control of senescence in this flower does not include correlative senescence is seen by silver thiosulphate and ethanol treatments to the cut flower altering the rates of development of the different flower parts. The general senescence strategy of the carnation flower starts with the petals irreversibly wilting, the ovary 'greening' and increasing in size and the other flower parts later becoming necrotic. The conservation of assimilates for seed development (when pollinated) will naturally determine the continuation of the carnation as a species.

This senescence pattern, however, persists even if the ovary is not pollinated; the difference between an unpollinated flower and a pollinated flower is that the former flower lives longer. Wounding, as in severing the cut flower from the parent plant, and high temperatures both, like pollination, accelerate the timing of senescence although the actual senescence processes do not alter. With silver thiosulphate and ethanol treatments, petal longevity is greatly extended to the detriment of ovary growth. Flower death is nevertheless ultimately achieved. The difference in the correlative senescence pattern of silver thiosulphate and ethanol treated cut carnation with the naturally senescing flower appears to be regulated at the hormone level.

The onset of petal irreversible wilting is heralded by an ethylene rise above the basal level. This ethylene emanation can be accelerated by pollination, wounding and high temperatures. Thus because the timing of senescence only differs and not the process itself, the common regulator controlling this event must be ethylene. Further evidence that ethylene is a regulating hormone in carnation flower senescence is seen by this process being accelerated with the exposure of the flower to exogenous ethylene. The ethylene rise above the basal level is prevented by silver thiosulphate and ethanol treatment and thus may account for the petals remaining turgid.

Carnation flower senescence does not only involve deteriorative processes but also developmental processes prior to the death of the flower. It is in these developmental processes that other plant hormones

play important roles. Thus, although ethylene is a major influence in carnation flower senescence, the other plant hormones such as the auxins, gibberellins, cytokinins and abscisic acid are also thought to regulate correlative senescence within the flowerhead.

To further investigate this hormonal regulation of cut carnation flower senescence it was necessary to relate hormone action to a physiological activity during this process. From the physical appearance of the flower, the most obvious choice was assimilate partitioning and the stimulation of ovary growth. In the presence of ethylene the ovary became the dominant sink of carbohydrates. This sink activity was related to acid invertase activity which maintained a sucrose gradient into the ovary for the passive movement of sucrose. However, the immobility of sucrose through membranes suggests that the maintenance of a sucrose gradient does not solely determine sink activity. The permeability of the membranes into the sink, especially from the phloem, could be altered such that sucrose would passively enter these tissues. Plant hormones may regulate sink activity through altering the permeability of these membranes.

The sources of sucrose from within the cut carnation flower appear to be any flower part that produces or contains an excess of carbohydrates above the metabolic needs of that tissue. For the first days following harvest, the carnation flower is able to contribute to its carbohydrate pool via photosynthesis, the stem plus calyx being the most effective flower parts. The amount of source-sucrose reaching the ovary will depend on its anatomical proximity to this sink and the carbohydrate

supply from the other flower parts. Of the flower parts tested, the petals and stem, each showed that they were potential sources when the ovary was the sink. Such a conclusion could not be reached on dry mass analyses alone. The carbohydrate pool content alters with contributions via photosynthesis and the gradual depletion due to respiratory activities.

The inhibition of the ethylene rise altered the movement of carbohydrates. The petals became the dominant sink in the flowerhead so that the ovary was deprived of carbohydrates. Although carbohydrates are necessary for ovary growth, they do not regulate the development of this organ. For its increase in size and chlorophyll content, the ovary requires hormonal stimulation. The question asked is whether ethylene is the sole hormone involved in this regulatory role?

Concomitant with the ethylene fluctuations during senescence, the cytokinin-like activity of the ovary also changes. A rise in endogenous cytokinin-like activity in the carnation ovary occurs when ethylene is present. This ethylene may be a natural rise, formed with 2-chloroethyl phosphonic acid or from an auxin treatment. Such cytokinin-like activity in silver thiosulphate treated flowers is absent from the ovary (VAN STADEN AND DIMALLA, 1980). Thus it appears that like the rise in ethylene production is part of the senescence regulatory pattern in carnations, so too may the cytokinin activity in the ovary contribute to regulating the senescence strategy of this bloom.

To further question such an hypothesis exogenous cytokinins were applied to the ovary. The expected acceleration of petal wilting and

mobilisation of assimilates, in this case, were not detected. However, the combination of cytokinins and auxins greatly enhanced the latter processes. This implies that cytokinins alone in the ovary are incapable of regulating sink activity. For ovary development high auxin levels concomitant with the rise in cytokinins may be the necessary combination for sink activity.

Additional indirect evidence of the regulatory role of auxins in ovary development was observed when this hormone was applied to isolated ovaries in culture. These ovaries increased their overall growth, dry mass, chlorophyll content, chloroplast development and cell size (this latter phenomenon being the means by which the ovary enlarges in the cut carnation flower). This auxin treatment stimulated the greatest ovary growth activity in comparison with the kinetin treatment and control. In retrospect, a facet that may have contributed to this phenomenal ovary growth is the stability of naphthalene acetic acid, the auxin used. In the literature examples are cited where the cytokinins are capable of protecting the auxins from conjugation and thus maintaining them in an active form. In addition, ethylene enhances indoleacetic acid conjugation so the high levels of cytokinin-like compounds in the ovary may be 'protecting' auxin activity during the ethylene rise. As the cytokinin levels are low in the petals, the natural ethylene rise could inactivate the auxins in the petals and thus enhance petal senescence.

Another view as to why ethylene could not be the only hormone involved in flower senescence is through its role, or undetectable role

in assimilate partitioning. Although the greatest stimulation of carbohydrate partitioning arises as the result of the presence of ethylene, there is no direct evidence to suggest that ethylene has any regulatory role in this process. There is, however, a continuing controversy over whether the presence of high levels of hormones in developing fruits (the ovary being an undeveloped fruit) promotes the metabolic activity which generates a sink demand or whether there is a distinct phenomenon of hormone-directed transport operating (NOODEN, 1980). Much evidence does exist for hormone-directed transport but this is also often inconclusive (WOOLHOUSE, 1983). An alternative means by which hormones could stimulate sink activity of the ovary is by inducing the differentiation of channels of transporting tissue, a possible activity of the auxins (SACHS, 1975). This is an aspect that must be considered in further studies. The ramifications, agriculturally, of being able to regulate carbohydrate partitioning within a crop are infinite with the common result being an increase in productivity and growth efficiency.

An alternative approach to elucidate the action of ethylene during the senescence of the cut carnation flower was taken. This involved analysing the action of the silver ion in plant tissues to discover the site of ethylene action as the silver ion is a potent inhibitor of ethylene action. Histochemically and histologically there are many sites of silver binding within plant tissues. One such site was the enzyme invertase, however neither silver thiosulphate nor ethylene appear directly involved with its activity, further substantiating the indirect role of ethylene on carbohydrate partitioning. Other sites

of silver binding identified included ascorbic acid, sulphydryl groups, and chloride ions. Each has their possibilities but no one site was confirmed as a definite site of ethylene action. This study, however, did question the concept of hormone-binding and agreed with TREVAWAS (1981) that a hormone may not necessarily act through binding to a protein, a concept taken from animal physiology. Furthermore, sensitivity may be determined by the sites available for ethylene binding but not all of these sites may be physiologically active.

Ethylene-sensitivity differs between flowers, and yet all flowers senesce. What is the physiological advantage of being ethylene-sensitive? Another flower that is extremely sensitive to this hormone is the orchid. The insect-attracting appearance of the flower is maintained as long as the pollinia remain untouched or higher temperatures are not reached. Following either of these phenomena, the flower 'blushes' and rapidly dies. It thus appears that these ethylene-sensitive flowers maintain their insect-attracting appearance as long as possible for pollination to take place. Thereafter senescence is rapid to conserve carbohydrates. Petals in the carnation flower may be simply"flags that can be waved to attract the right insects for pollination to take place"(GROUNDS, 1980) but they also contribute carbohydrates to the stimulated ovary growth. Such a rapid mobilisation is convenient in the carnation flower what with the carbohydrates being stored as sucrose and not as immobile starch.

Therefore, to conclude, although the carnation flower is an ornamental crop, and an economically important one at that, it provides

an excellent system to solve many physiological problems. The extension of the petal longevity is of prime importance to the floriculturalist but there are other important facets that can be investigated in this system, such as, assimilate partitioning and its possible hormone regulation, chloroplast development, correlative senescence, ethylene action, silver-inhibition of ethylene action, hormone interaction and carbohydrate translocation; aspects all covered in this thesis. These investigations into the senescence strategy of the carnation flower are however far from complete.

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