THE EFFECT OF SUCROSE-PULSING ON CUT CARNATION AND FREESIA FLOWERS

by

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DISSERTATION

submitted in fulfilment of the requirements for the degree

MAGISTER SCIENTIAE

in

BOTANY

in the

FACULTY OF SCIENCE

at the

RAND AFRIKAANS UNIVERSITY

UNIVERSITY OF JOHANNESBURG

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NOVEMBER 2002

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ABSTRACT

The vase life of cut flowers is determined by various physiological factors that determine the rate of their senescence. A thorough understanding of these factors is required in order to design treatments that will extend the vase life and delay senescence of cut flowers. Senescence of climacteric flowers such as carnations (*Dianthus caryophyllus* L. cv. Nordika and cv. Snow White) and freesias (*Freesia refracta* cv. Athena) is characterized by a climacteric rise in respiration rate and ethylene synthesis during the late stages. The increase in ethylene production is preceded by an increase in the sensitivity of the flowers to ethylene. Pulse treatments with sucrose caused a delay and suppression of the climacteric rise in ethylene synthesis and a delay in the climacteric maximum of the respiration rate. A pulse treatment for 24 hours with a 20% sucrose solution was most effective in extending the longevity of both carnations and freesias. The ability of the receptor molecules to bind ethylene is greatly reduced when flowers are pulse-treated with sucrose. In freesias, the ability to bind ethylene is reduced even further when flowers are treated with STS or 1-MCP. Ethylene synthesis in freesias is suppressed and inhibited when treated with STS or 1-MCP but longevity of the freesias and number of open florets on the stem is not increased. The uptake and distribution of sucrose in the buds of freesias is seen by the distribution of sucrose from the first bud on the stem to the next bud after the bud opens. The distribution of sucrose from one bud to the next results in more buds opening on the stem than that on the stems of STS or 1-MCP treated freesias. It is thus clear from the results of the study, that pulsing senescing climacteric flowers with sucrose increases the vase life of the flowers and suppresses ethylene sensitivity of the flowers, thus delaying the autocatalytic process of ethylene production. It is also evident from the results that the osmolality in the flowers has a direct influence on the metabolic processes of the flowers. In freesias, pulse treatment with sucrose increases the number of open buds on the stem and delays senescence of the florets.

UITTREKSEL

Die vaaslewe van sny-blomme word bepaal deur verskillende fisiologiese faktore wat die snelheid van hul verouderingsverval beïnvloed. ? Deurtastende ondersoek van hierdie faktore word vereis ten einde behandelings te ontwerp wat die vaasleeftyd sal verleng en die verouderingsverval van die blomme sal vertraag. Verouderingsverval van klimakteriese blomme soos angeliere (*Dianthus caryophyllus* L. cv. Nordika en cv. Snow White) en freesias (*Freesia refracta* cv. Athena) gaan gepaard met ? klimakteriese styging in respirasietempo en etileensintese gedurende die finale stadia. Die styging in etileensintese word voorafgegaan deur ? toename in die sensitiwiteit van die blomme vir etileen. Pulsbehandelings met sukrose het ? vertraging en onderdrukking van die klimakteriese styging in etileensintese en ? vertraging in die klimakteriese styging in respirasie tot gevolg. ? Pulsbehandeling vir 24 uur met ? 20% sukrose-oplossing was die mees effektiewe behandeling om die leeftyd van beide angeliere en freesias te verleng. Die vermoë van die etileenreseptore om etileen te bind is heelwat onderdruk deur ? pulsbehandeling met sukrose. In freesias is die vermoë om etileen te bind is selfs verder verminder wanneer die blomme met STS of 1-MCP behandel is. Etileen sintese is onderdruk en vertraag wanneer freesias met STS of 1-MCP behandel is maar die leeftyd en aantal oop blomme op die bloei-steel het nie toegeneem nie. Die opname en verspreiding van sukrose in die blomknoppe op ? freesia bloeiwyse toon duidelik dat sukrose van die eerste knop op die steel na die daaropvolgende knop beweeg nadat die eerste knop oopgegaan het. Die beweging van sukrose van een knop na die volgende het tot gevolg dat meer knoppe oopgaan as in die geval van STS of 1-MCP-behandelde bloeiwyses. Dit blyk duidelik uit die resultate van hierdie studie dat ? pulsbehandeling met 20% sukrose verouderingsverval vertraag en die vaasleeftyd verleng. Die etileensesitiwiteit word onderdruk, en sodoende ook die outokatalitiese stimulering van etileensintese. Dit blyk ook duidelik uit die resultate dat die osmolaliteit van die blomme ? direkte invloed op die metabolisme van die blomme het. In freesias het ? pulsbehandeling met 20% sucrose ? toename in die aantal oop knoppe en ? vertraging in verouderingsverval tot gevolg gehad.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to the following persons and institutions involved in the completion of this study:

- **Prof. C.S. Whitehead** for his understanding, guidance, support, knowledge and passion for the work we do;

- **Mr. T. Fourie** for all the running around he did every week fetching me fresh flowers;

- Rand Afrikaans University, **Department of Botany**, for the availability and use of their laboratories and equipment;

- **Rand Afrikaans University** for their financial support;

- **My family**, for their endless love, encouragement and support;

- **Razia,** for staying a true friend throughout, and for all her support;

- **Our Heavenly Father** without whom nothing is possible.

ABBREVIATIONS

ABA - abscisic acid

ACC - 1-aminocyclopropane-1-carboxylic acid

Ag^{+} - silver ions

AOA - aminooxyacetic acid

ATP - adenosine triphosphate

AVG - aminoethoxyvinylglycine

EFE - ethylene forming enzyme

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HCN - hydrogen cyanide

IAA - indole acetic acid

LSD - least significant difference

MACC - N-malonyl-1-aminocyclopropane-1-carboxylic acid

MCP - 1-methylcyclopropene

MTA - 5-methylthioadenosine

MTR - 5-methylthioribose

STS - silver thiosulphate

INTRODUCTION

1.1 SENESCENCE

Senescence is one of the least well-defined steps in biological development. The gross change of a series of changes leading to the death of an organism has been referred to as senescence. Senescence can be defined as the final phase in the ontogeny of the organ in which a series of normally irreversible events is initiated that leads to cellular breakdown and death of the organ (Sacher, 1973).

The natural life of an organ or organism can be divided into four phases. The first is called cell division, in which the volume of one mature cell is divided into smaller volumes. This occurrence is followed by cell enlargement, in which one or both of the products of cell division increase in volume. The third phase is cell differentiation, in which the cell becomes specialized in one of the various possible organs. Senescence is the fourth and final phase of development (Biale, 1950).

Leopold (1975) defines senescence as the deteriorative processes that are the natural causes of death. Ageing, by contrast, refers to processes accruing maturity with the passage of time. Ageing thus includes a much wider span of physiological processes, which may either weaken the organism or be neutral with respect to the capability of the biological organism to survive. Senescence, in contrast, refers to the changes providing for the endogenous regulation of death (Leopold, 1980).

A striking feature of senescence, whether cellular or organismal, is that it is a correlative phenomenon. Senescence is generally considered to be controlled by hormones (Wareing, 1977; Leopold and Noodén, 1984). Both senescence-promoting and senescence-retarding hormones exist. Ethylene and cytokinins are the most important representatives of these opposing classes. While cytokinins function in a wide range of tissues, it is not a universal retardant of senescence.

Other hormones may have significant regulatory roles in certain tissues. Ethylene is frequently a senescence promoter, but it may not be the primary senescence promoter in many tissues (Noodén and Leopold, 1978). The various parts of a plant influence each other in ways that serve to achieve coordination in their developmental processes (Wareing, 1977). The earliest and most extensive literature on correlative controls centre around flowers. The flower appears to be an excellent model system for the study of the fundamental processes involved in the control of senescence, since its life span is clearly defined and much shorter than that of leaves and fruit. In particular, it was observed that the petals of many flowers were capable of persisting for a long time but quickly faded after pollination. In orchids, petunia, carnation and other flowers, pollination causes the female structure to produce an influence, probably ethylene or a precursor, that is transmitted to the petals and other parts, where it induces senescence (Nichols, 1977; Whitehead *et al.*, 1984; Hoekstra and Weges, 1986; Whitehead and Halevy, 1989). The study of the processes occurring during natural senescence can thus be done in flowers without resorting to artificial "ageing treatments" (Halevy and Mayak, 1979).

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1.2 STRUCTURAL, BIOCHEMICAL AND METABOLIC CHANGES ASSOCIATED WITH SENESCENCE OF FLOWERS

1.2.1 ULTRASTRUCTURAL CHANGES

The most comprehensive studies of the ultrastructural and biochemical processes during petal senescence were carried out in the ephemeral corolla of the morning glory, *Ipomoea tricolor* Cav. The first observed sign of ageing was invagination of the tonoplast. This in turn suggested involvement of autophagic activity of the vacuole, representing the lysosomal cell compartment (Matile and Winkenbach, 1971). Abolition of the compartment of the vacuole and the release of hydrolytic enzymes result in cell death. The presence of cytoplasmic material such as disintegrated mitochondria and different types of membranes in the ageing vacuole support this hypothesis.

The breakdown of the tonoplast is later followed by autolysis of the cell. During maturation and senescence first free single ribosomes disappear followed by those in aggregate clusters and those attached to the endoplasmic reticulum (Matile and Winkenbach, 1971). Vascular occlusions decrease uptake of water and consequently reduce vase life of cut flowers (Burdett, 1970). Stem sterilization or 8-hydroxyquinoline solutions decreased solution flow resistance by controlling microbial growth (Burdett, 1970). Microscopic examinations of longitudinal tangential sections of rose stems have revealed vascular occlusions due to microbial growth and gum deposition (Lineberger and Steponkus, 1976). This indicates a loss of membrane integrity, causing increased permeability and leakage (Halevy, 1981). An increase in apparent free space and membrane permeability during the senescence of several flowers has been demonstrated (Eliam, 1965). A sharp increase in microviscosity of the plasmalemma during ageing of intact rose flowers, cut flowers or isolated petals has also been observed (Borochov *et al.*, 1976). It was further observed that the increase in microviscosity corresponded to an increase in the ratio of free sterol to phospholipid. The free sterol content of the flower remained unaltered during senescence, but the content of phospholipid was reduced. This is attributed to reduced synthesis and increased hydrolysis by phospholipase A, which decreased the level of phospholipids (Halevy, 1981). Such a decline in phospholipids was observed in the *Ipomoea* corolla, even before visible signs of ageing were perceptible (Beutelmann and Kende, 1977). Simons (1973), postulated that the decrease in the level of phospholipids enhanced the permeability of the plasma membrane, causing cell leakage. During senescence, there was a 40% decrease in the level of fatty acids esterified to phospholipids, which corresponded to the loss of phospholipids during the same period. No significant changes were found in the ratio of the fatty acid components (Beutelmann and Kende, 1977). The level and composition of free fatty acids remained unchanged during senescence.

Smith *et al*. (1992) investigated ultrastructural changes associated with carnation petal senescence using ethylene levels of individual petals as a physiological monitor of the senescence process. Limited vacuolar vesiculation was observed in pre-senescent petals, which became more extensive in pre-climacteric tissues, along with dilation of the outer mitochondrial membrane.

Climacteric mesophyll tissue was characterized by widespread cytolysis. Intact cells possessed a highly reduced cytoplasm and vacuoles with electron-dense deposits. Degenerative changes became evident in the vasculature at this stage. These included occlusion of the sieve plate, and membrane abnormalities in the companion cells. Post-climacteric tissue was characterized by loosening of wall fibrillar structure in the vasculature, the appearance of intracellular cytoplasmic debris and cells completely devoid of contents and a loss of fresh weight of the tissue.

The symptoms of loss of fresh weight of the flower tissue, such as drying and shrivelling, are conspicuous in the final phase of senescence, the loss of water occurring even when the ageing petals of cut flowers are held in water, indicating a loss of membrane integrity and consequent increased permeability and leakage (Halevy, 1981).

1.2.2 BIOCHEMICAL CHANGES

Respiration and enzymatic hydrolysis of cellular components are the two major biochemical and metabolic events occurring in the senescing flowers of carnation and freesia. Enhanced peroxidase activity was associated with an increase in the level of peroxides and free radicals, which reacted with cellular constituents (Fridovich, 1975), and are probably involved in the promotion of senescence (Mishra *et al.*, 1976; Brennan and Frenkel, 1977) and in the production of ethylene (Beauchamp and Fridovitch, 1970). A delay in the senescence of carnation flowers was demonstrated by treating them with free radical scavengers such as sodium benzoate (Baker *et al.*, 1977). Significant increases in the activities of ribonuclease, deoxyribonuclease, and hydrolases of cell wall polysaccharides of *Ipomoea* (Wiemken-Gehring *et al.*, 1974) and of ribonuclease in roses (Halevy *et al.*, 1974; Halevy and Mayak, 1974) were reported. The marked development of senescence in flowers was closely associated with a variety of changes, such as blueing of red petals, decrease in protein content, and increase in ribonuclease activity (Halevy and Mayak, 1970; Halevy *et al.*, 1974; Borochov *et al.*, 1976).

During the course of petal senescence, a decrease in the level of macromolecular components such as starch (Ho and Nichols, 1977), cell wall polysaccharides (Wiemken-Gehring *et al.*, 1974), proteins (Borochov *et al*,. 1976), and nucleic acids (Matile and Winkenbach, 1971) was noted. The main constituents of the sugar pool of mature petals of carnation and rose were the reducing sugars, rather than non-reducing sucrose (Weinstein, 1951; Nichols, 1968). The majority of the transformation in sugars was accompanied by hydrolysis of starch (Ho and Nichols, 1977). Insoluble pectin, present as the main constituent of the cell wall, was found in different amounts in two varieties of carnation having different vase lives. Astor, a red short-lived cultivar, was characterized by 25% lower pectin content than the yellow long-lasting cultivar, Alice (Jona *et al.*, 1981).

1.2.3 METABOLIC CHANGES

Internal metabolic changes in respiration of cut flowers have been demonstrated. The rate of respiration in many cut flowers reaches its peak at the time of opening of flowers, and decreases as the flowers mature and senesce. Later, there is a second dramatic increase in respiration over a relatively short period, followed by a final decline (Mayak and Halevy, 1980). According to Mayak and Halevy (1980), the second peak in the respiration drift signifies the last phase of senescence. It has been considered to be an analogue to the climacteric rise in respiration of many fruits. The cyanide insensitivity of the respiratory rise in certain flowers (e.g. Araceae), indicates the formation of free radicals with high oxidation potential, which promote senescence in many plant tissues and are associated with increased sensitivity of the tissue to ethylene (Fridovich, 1975; Brennan and Frenkel, 1977). The second peak in respiration of flowers may be employed to assess the effectiveness of senescing-retarding substances, provided the peak in respiration reflects internal metabolic changes associated with ageing. Chemicals that delay the occurrence of the second respiratory peak have been reported to increase the vase life of flowers (Coorts, 1973; Kende and Hanson, 1976). Hew *et al.* (1978), demonstrated a rhythmic pattern of respiration in orchid flowers, CO_2 production starting as soon as the flowers had opened.

The period between amplitudes was about 24 hours, and was not influenced by continued darkness. The amplitude of the rhythm was slightly dampened in cut flowers, but it was partially enhanced by supplying sucrose externally.

The onset of hydrolysis of cellular components such as proteins and carbohydrates is initiated in response to the depletion of free sugars used up in respiration, to supply alternative respirable substrates e.g. carbon skeletons of amino acids or keto acids (Weinstein, 1951). This hypothesis is supported by the observation that an exogenous supply of sugars delays the onset of excessive degradation of protein (Weinstein and Laurencot, 1958).

The gradual decline in respiration and decrease in respiration efficiency of rose petals resulted from the progressive inability of the mitochondria to utilize the substrate (Kaltaler and Steponkus, 1976). A change in respiration control ratio of related mitochondria affects their integrity, thereby reducing their functional capacity. The respiration of isolated mitochondria from cut flowers can be controlled over longer periods of time. The main effect of applied sugar in prolonging vase life of roses was to protect the structure and function of the mitochondria. However, the influence of sugar on these organelles may not have such a specific effect, but a more general protective effect on membrane integrity (Santarius, 1973).

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1.2.4 CHANGES IN PIGMENTS

Discolouration or fading of colour is a common symptom of many senescing flowers. The carotenoids and anthocyanins, two major classes of pigments responsible for different colours in flowers, change significantly during the development and senescence of plant organs. Changes in the composition of carotenoids in *Strelitzia raginae* flowers throughout the development of the plastids from small, colourless plastids through the green chloroplasts to large spindle shaped chromoplasts were monitored. An increase in the concentration of oxygenated carotenoids with age was noted (Simpson *et al*., 1975). Differential changes in the anthocyanin content of senescing flowers have been observed. While its level stays stable in certain flowers (Packet, 1966; Stead and Moore, 1977), in others it declines significantly (Stickland, 1972), whereas in orchids anthocyanins are synthesized continuously.

Changes in the colour of senescing petals are significantly influenced by a change in pH of the vacuole (Stewart *et al.*, 1975). Co-pigmentation with other flavonoids and related compounds is the decisive factor in determining the intensity of the colour in most flowers. In only a few cases is the colour caused by a very low (less than 3) or a very high (more than 7) pH affecting the anthocyanin *per se*. The degree of co-pigmentation is greatly influenced by even slight changes in the pH (Asen *et al.*, 1975).

1.3 MEMBRANE DETERIORATION DURING SENESCENCE

Cellular membranes are selective, dynamic barriers that play an essential role in regulating biochemical events. During senescence there is a progressive loss of membrane integrity. This is most clearly evident from ultrastructural studies showing progressive deteriorative changes in organelles and membranes, and from permeability studies indicating increased leakage of solutes. Senescence is an active process initiated by some combination of internal and environmental triggers, and membrane deterioration is an early and fundamental feature of this process. In senescing carnation flowers changes in permeability reflecting membrane deterioration are initiated during the pre-climacteric period before the climacteric rise in ethylene production (Eze *et al.*, 1986). Various studies demonstrating that fruit ripening and the vase life of flowers can be modulated by free radical scavengers suggest that free radicals are involved in senescence (Baker *et al.*, 1978). Membranes could be expected to be prone to free radical attack inasmuch as unsaturated fatty acids are major components of most membrane lipid bilayers. The consequences of free radical attack on membranes include the induction of lipid peroxidation and fatty acid deesterification (Niehaus, 1978). Microsomal membranes from carnation flowers and from senescing bean cotyledons produce increased levels of superoxide radical (O_2^-) with advancing senescence. The formation of O_2^- has been attributed to a membrane-associated lipoxygenase. A strong correlation exists between changes in microsomal lipoxygenase activity and changes in the O_2^- production during senescence. The formation of O_2^- is sensitive to changes in the availability of substrate for lipoxygenase and O_2^- formation and lipoxygenase activity are affected in a parallel fashion by specific inhibitors of lipoxygenase (Lynch and Thompson, 1984).

The plasma membrane and microsomal membranes sustain a decrease in bulk lipid fluidity with advancing age. Senescing carnation petals show a climacteric-like rise in ethylene production, the decrease in membrane fluidity occurs abruptly and coincident with, or just prior to, the rise in ethylene production (Thompson *et al.*, 1982). During senescence there is a temporal correlation between changes in the bulk lipid fluidity of microsomal membranes and changes in O_2^- production. This indicates that there is a relationship during senescence between free radical production and membrane rigidification (Mayak *et al.*, 1983). The most conspicuous change in lipid composition of senescing plasmalemma and microsomal membranes is a dramatic decline in membrane phospholipid, which become manifest as an increase in membrane sterol:phospholipid ratio. This has been demonstrated in senescing *Ipomoea* flowers, rose flowers, bean cotyledons, bean leaves and carnation flowers (Thompson *et al.*, 1982). These observations imply a role for lipase in membrane deterioration. Thus, as the membrane senesces, it becomes more prone to attack by lipase, such that deesterification can be envisaged as occurring in an essentially autocatalytic fashion. The rise in sterol:phosholipid ratio of senescing plasmamembranes is one of the major factors contributing to the rise in bulk lipid microviscosity. Enhanced levels of sterol relative to phospholipid are known to reduce bilayer fluidity (Noodén and Leopold, 1988).

A model that is intended to serve as a framework for interpreting experimental data for senescing microsomal membranes is illustrated in Figure 1. The focal point of the model is bilayer destabilization. There are three manifestations of this destabilization, i.e. rigidification of bulk membrane lipid, the formation of gel phase lipid, and the formation of nonbilayer lipid configurations, specifically inverted micelles. This destabilization leads to membrane leakiness, advanced proteolytic activity, and a generalized loss of membrane function. Bilayer destabilization is thought to be induced by a sequence of enzymatic reactions that bring about deesterification, resulting in the release of free fatty acids and, through lipoxygenase, the initiation of lipid peroxidation. This sequence of reactions explains the loss of fatty acids that accompany senescence, the increased production of O_2^- and membrane rigidification attributable to free radical-mediated peroxidation (Noodén and Leopold, 1988). Thus, senescence of microsomal membranes appears to be lipid driven.

MODEL

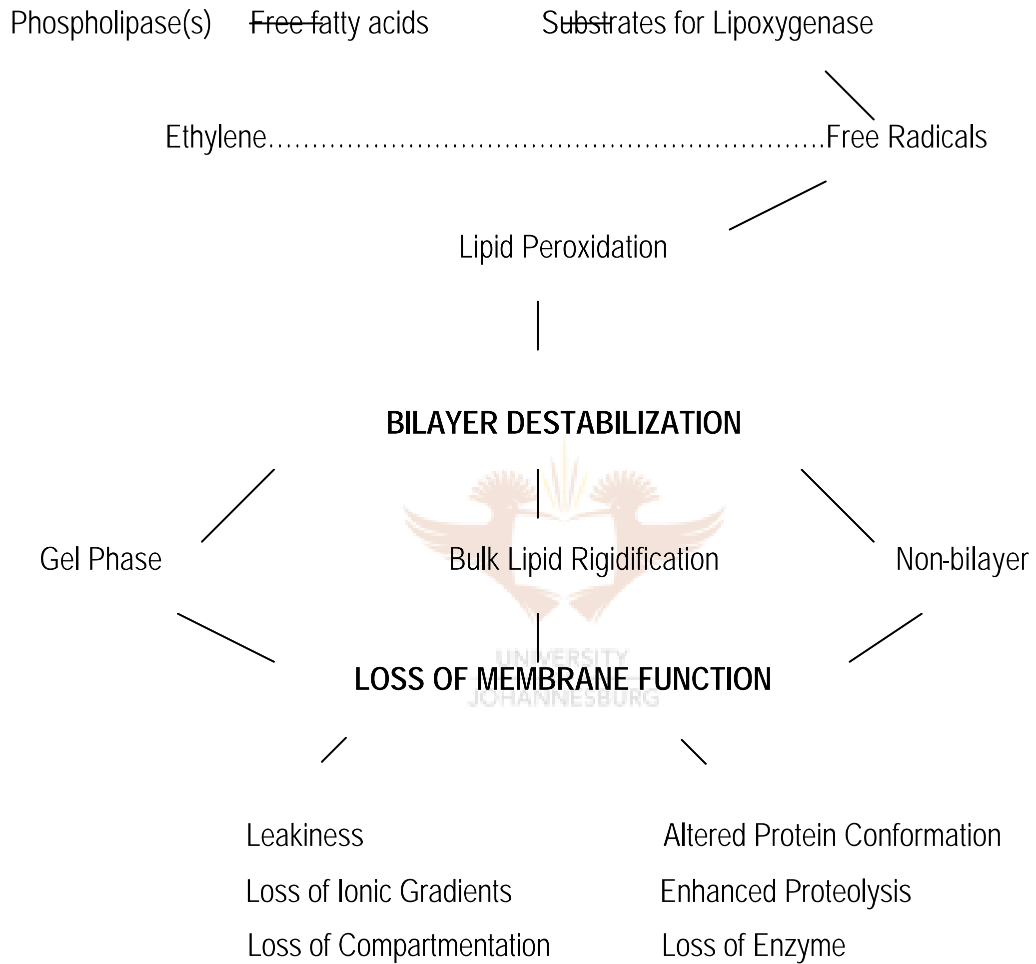

Figure 1: Sequence of events leading to loss of microsomal membrane integrity during senescence (Noodén and Leopold, 1988).

1.4 ETHYLENE

Ageing and senescence of higher plants are genetically as well as environmentally regulated processes intimately associated with hormonal interactions. Of the well-recognized plant hormones, ethylene (C_2H_4) is unique in that it is a simple, gaseous hydrocarbon that is biologically active in trace amounts and produced as a natural product of many plant tissues (Adams and Yang, 1981). This volatile compound is regarded as an endogenous plant hormone involved in the control of many plant growth phenomena of which the stimulation of senescence is the most prominent (Yang and Pratt, 1978).

Senescence of cut carnations is primarily the result of the plant hormone ethylene, produced endogenously by the flower itself or present as a pollutant in the surrounding atmosphere (Nichols, 1979; Reid *et al.*, 1980). In carnations, senescence of the petals is associated with a climacteric-like increase in ethylene production during the final stages. This increase in ethylene production is accompanied by the irreversible inrolling and wilting of the petals (Nichols, 1979; Bufler *et al.*, 1980; Peiser, 1989). The climacteric pattern in ethylene production exhibits two distinct phases. During the first phase ethylene production stays low for the first 4 to 6 days after harvest. This phase is followed by an autocatalytic phase characterized by a log-linear increase in ethylene production. The first visible symptoms of wilting in carnations are observed during, or just after, this rise in ethylene production (Dilley and Carpenter, 1975).

1.4.1 ETHYLENE BIOSYNTHETIC PATHWAY

Historically, the impetus for unravelling the identity of the endogenous intermediates in the biosynthesis of ethylene came from studies with chemically defined model systems (Lieberman, 1979). Ethylene is readily generated when substrates such as linolenic acid, propanol, methional, or methionine react with free radicals or with transition metals that acts as catalysts (Lieberman and Kunishi, 1968). However, methionine has now been established as the major precursor of ethylene in higher plants.

The first step in the pathway is the conversion of methionine and ATP to S-adenosylmethionine (SAM), catalysed by the enzyme SAM synthetase. SAM is converted by ACC synthase to 1-aminocyclopropane-1-carboxylic acid (ACC) and 5′-methylthioadenosine (MTA). MTA can be recycled to methionine, thus allowing high rates of ethylene production even if methionine concentrations are low. ACC is the immediate precursor of ethylene. Oxidation of ACC by the enzyme ACC oxidase (previously known as the ethylene forming enzyme EFE) results in the production of ethylene, CO_2 and HCN. HCN is thought to be detoxified through conversion to β-cyanoalanine by the enzyme β-cyanoalanine synthase (Manning, 1985).

It has long been known that ethylene production ceases when plant tissue is placed under anaerobic conditions and that there is a definite increase in ethylene production upon re-exposure of the tissue to air. This occurrence indicates the presence of an intermediate that accumulates during anaerobic incubation, which is then subsequently converted to ethylene after exposure to oxygen (Beyer *et al.*, 1984). The metabolism of methionine in apple tissue was investigated under these conditions by Adams and Yang (1981). In air, methionine was efficiently converted to ethylene. In nitrogen, however, it was not metabolised to ethylene but was instead converted to MTA and an unknown compound. This compound was later identified as 1-aminocyclopropane-1-carboxylic acid (ACC) that has now been universally accepted as the immediate precursor to ethylene.

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Other than its conversion to ethylene, ACC can also be metabolised to produce a non-volatile compound known as N-malonyl-ACC (MACC) (Roberts and Hooley, 1988). Although this conversion is reversible and the conjugate does not easily form ethylene, MACC is thought to be an inactive end product rather than a storage form of ACC representing an important mechanism for the regulation of ethylene production (Beyer *et al.*, 1984).

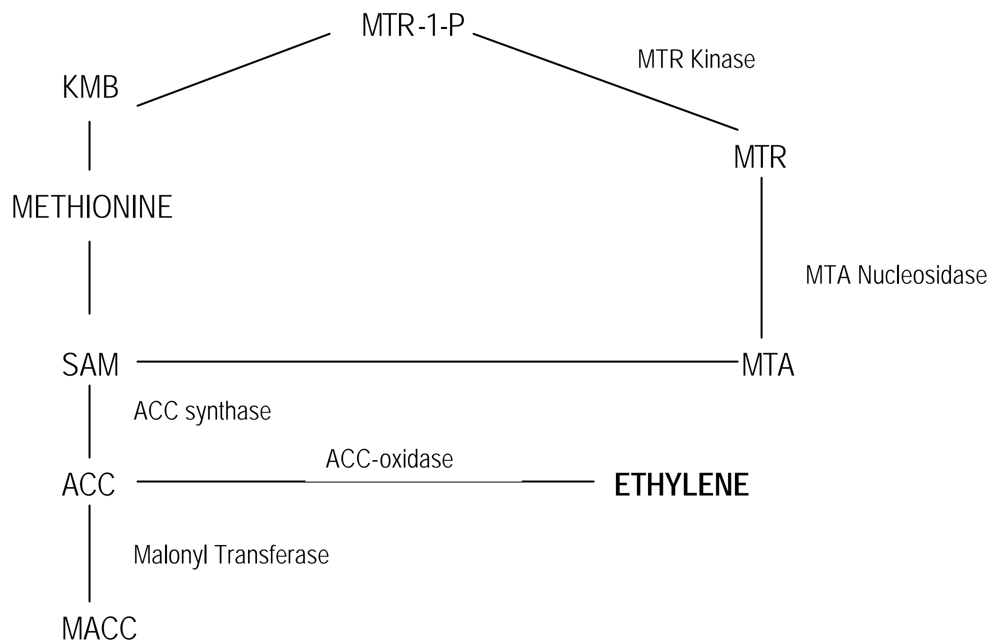

Figure 2: Schematic representation of the ethylene biosynthesis pathway (Noodén and Leopold, 1988)

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1.4.2 PATHWAY ENZYMES

Little is known about the nature of the enzymes involved in the biosynthesis of ethylene. The enzyme which catalyses the conversion of SAM to ACC has been identified as ACC-synthase. Since ACC-synthase requires pyridoxal phosphate for maximum activity and is inhibited by aminoethoxyvinylglycine (AVG) and aminooxyacetic acid (AOA), ACC-synthase is believed to be a pyridoxal phosphate dependent enzyme (Yang, 1980; Beyer *et al*., 1984; Sisler and Yang, 1984; Roberts and Hooley, 1988). Oxygen is directly involved in the conversion of ACC to ethylene (Beyer *et al*., 1984), and low oxygen levels would thus inhibit this reaction. Increased temperature has a negative effect on the activity of the enzymes controlling ethylene biosynthesis (Yang, 1980). The application of ACC to various plant organs, including roots, stems, leaves, fruits and flowers, results in a marked increase in ethylene production, suggesting that the formation of ACC is the rate limiting reaction in the biosynthesis of ethylene (Yang, 1980).

Observations made on the regulation of ethylene production in ripening fruits indicated that the application of ACC to intact pre-climacteric tomato fruit significantly enhanced the ripening process (Yang, 1980). This suggests the possibility that a slight increase in ethylene production caused by ACC application is capable of enhancing the ripening process, as does the application of triggering quantities of exogenous ethylene gas. This capability of tissues to synthesize large quantities of ethylene in response to the application of a low concentration of ethylene is referred to as the autocatalytic effect (Mayak and Halevy, 1994).

Ethylene also inhibits its own biosynthesis by a possible feedback mechanism known as autoinhibition. Although this type of regulation of ethylene production has been known for some time (Vendrell and McGlasson, 1971), the exact target site was only demonstrated after the ethylene biosynthetic pathway was elucidated. Ethylene-treated citrus fruit tissue (Riov and Yang, 1982) and ethylene-inhibited, indole-3-acetic acid (IAA)-treated mung bean hypocotyls (Yoshii and Imaseki, 1982) were found to have markedly decreased amounts of ACC synthase.

Furthermore, the conjugation of ACC to MACC is markedly increased when leaves (Philosoph-Hadas *et al.*, 1985; Gupta and Anderson, 1985) and fruits (Liu *et al.*, 1985) are exposed to ethylene. Since ethylene does not cause the afore mentioned effects in cell-free systems, the mechanisms of these ethylene effects must be indirect. In this regard, the definitions of autocatalysis and autoinhibition should be taken in a physiological rather than a biochemical sense.

1.4.3 ETHYLENE BINDING

Ethylene, like most plant hormones, must bind to a specific receptor molecule in order to carry out its physiological action (Sisler, 1980; Goren *et al.*, 1984; Sisler and Yang, 1984; Brown *et al.*, 1986). The ethylene binding sites appear to be located in the membranes of the endoplasmic reticulum (Beyer *et al., 1984;* Sisler and Yang, 1984), the golgi bodies (Beyer *et al.*, 1984) and the plasmalemma (Sisler and Yang, 1984). According to Beyer *et al.*, (1984), binding is heat-labile and only partially destroyed by proteolytic enzymes, suggesting that the receptor molecules may be buried within the membranes.

In *Phaseolus vulgaris*, the protein that binds ethylene has been purified and characterized. It appears to be a membrane-bound hydrophobic protein (Sisler, 1980; Beyer *et al.*, 1984), with a high affinity and specificity for ethylene. However, there are certain arguments against the protein being an ethylene receptor. Receptor kinetics demands a high rate of dissociation and association, whereas these rates are slow in the proteins extracted from *Phaseolus vulgaris*.

Two new studies on ethylene sensors put the receptor into sharper focus (Schaller and Bleecker, 1995; Wilkinson *et al.*, 1995). Plants sense ethylene by a protein kinase cascade (Schaller and Bleecker, 1995). CTR 1 and ETR 1, two *Arabidopsis* genes essential for ethylene signalling, encode a putative RAF-like serine-threonine protein kinase and a putative histidine protein kinase (Kieber *et al.*, 1993). Without functional ERT 1 plants do not bind ethylene effectively (Ecker, 1995). The ERT 1 protein has been proposed as the ethylene receptor (Chang *et al.*, 1993). However, ERT 1 was not the only ethylene receptor identified. A second ethylene sensor, ERS, was isolated from *Arabidopsis* (Hua *et al.*, 1995). An ERS mutant confers dominant ethylene insensitivity to wild-type *Arabidopsis* (Hua *et al.*, 1995).

Experimental work undertaken by Brown *et al.*, (1986), showed that ethylene binding in carnation petals remained high in pre-senescent flowers and only began to decline after the initiation of the senescence processes. In the case of tobacco leaves, ethylene binding changes during leaf development and senescence (Goren *et al.*, 1984). During the progression of senescence in carnations, the molecular organization of the membranes becomes altered. As the tissue ages, the fluidity of the cellular membranes decrease, thereby altering the deposition of the ethylene binding sites in the membranes. This in turn results in a modification of their ability to bind ethylene. Such changes are thought to be brought about by a vertical displacement of the proteins in the lipid bilayer of the membrane. As a result, conformational changes are induced which lead to an altered protein function. Furthermore, the breakdown of cellular membranes during the senescence of the petals leads to an extensive loss of phospholipids and this can be expected to change the number of available binding sites in the tissue (Brown *et al.*, 1986).

1.4.4 EXOGENOUS ETHYLENE

Exogenous ethylene can evoke various responses in plant tissue. Exposure of carnation flowers to ethylene results in an increased production of autocatalytic, endogenous ethylene concurrently with an accelerated senescence and wilting of the petals (Nichols, 1968; Wilkins and Swanson, 1975; Sisler and Yang, 1984).

Senescence of carnations can be pre-empted by as little as 30nl/litre of ethylene. A 9 to 12 hour treatment is sufficient to cause experimental flowers to wilt within two days, while less than a third need only 3 to 6 hour exposure time to respond in the same way (Mayak *et al.*, 1977). This demonstrates the inherent variability of the flowers in relation to their sensitivity to ethylene, that exists in a given, presumably uniform population (Mayak and Kofranek, 1976; Mayak *et al.*, 1977). Flowers from given populations develop early stage wilting symptoms when treated with ethylene which later disappear when ethylene treatment is stopped. This suggests that ethylene is required beyond the appearance of early visual symptoms to uphold the "senescence syndrome" (Mayak and Kofranek, 1976).

Treatment of green bananas with ethylene during the pre-climacteric phase will accelerate their ripening. However, the extent to which ripening is accelerated depends largely on the duration of exposure to ethylene. Ripening occurs faster in fruit exposed to ethylene for a 24 hour period than in fruit treated for shorter periods. It appears that the acceleration of ripening by treatment of ethylene is related to the ability of the tissue to bind ethylene (Whitehead and Bossé, 1991).

In mume fruit discs and banana slices, ethylene production is decreased by exogenous ethylene. This is paralleled by a decrease in ACC synthase activity. It is assumed that the shock of excising or injuring internal tissue may lower the concentration of ethylene receptors, ethylene affinity or the rate of response reactions resulting from receptor binding.

This may result in the inhibition of ACC synthase synthesis (Philosoph-Hadas *et al*., 1985; Sawamura and Miyazaki, 1989), due to the control mechanism caused by the feedback repression of the synthesis of the enzyme, leading to decreased ethylene production (Sawamura and Miyazaki, 1989). ACC conjugation to MACC may also lower the levels of endogenous free ACC, and thus ethylene production (Philosoph-Hadas *et al*., 1985).

1.4.5 ETHYLENE SENSITIVITY AND FLOWER SENESCENCE

The development of sensitivity to growth substances such as auxins, cytokinins and ethylene appears to precede the developmental process that it induces (Trewavas, 1982; Whitehead and Vasiljevic, 1993). In many flowers, ethylene plays an important role in the initiation and regulation of the processes that accompany corolla senescence. Senescence of flowers such as *Dianthus caryophyllus* (carnation) and *Petunia hybrida* is characterised by a climacteric rise in ethylene production during the final stages (Nichols *et al*., 1983; Whitehead *et al*., 1984) and an early increase in the sensitivity of the corolla to ethylene, which precedes the onset of the ethylene climacteric (Whitehead and Halevy, 1989; Whitehead and Vasiljevic, 1993). This increase in sensitivity during the pre-climacteric phase is associated with an increase in the ability of the petal tissue to bind ethylene to its membrane-bound receptor sites (Brown *et al*., 1986; Whitehead and Vasiljevic, 1993). The decrease in ethylene binding that commences just prior to the climacteric rise in ethylene production could be attributed to the saturation of binding sites by ethylene (Whitehead and Bossé, 1991), or changes in the membrane composition (Brown *et al*., 1986), or both.

Although the response of flowers to ethylene is clearly dependent on the availability of binding sites, this is not the only factor involved in the determination of the ability of the tissue to bind ethylene. The affinity of these binding sites for ethylene also plays an important role in the control of ethylene sensitivity. Brown *et al*. (1986) have shown a single type of binding site in both pre- and post-climacteric petals. However, the affinity of the receptor for ethylene decreases in older petals.

Differences in ethylene sensitivity due to differences in the affinity of the receptor molecules for ethylene are also known to exist between different carnation cultivars. The extended vase life of some cultivars is the result of either an inability to produce ethylene, or a reduced capacity to respond to the hormone (Wu, Zacarias and Reid, 1991; Brandt and Woodson, 1992).

According to Whitehead *et al.*, (1984), ethylene sensitivity is increased after pollination. This occurrence is followed by an increase in ACC and ethylene synthesis. However, the increase in ethylene sensitivity is independent of ACC and ethylene synthesis, since it occurs in pollinated flowers treated with aminoethoxyvinylglycine (AVG), an inhibitor of ACC-synthase. The application of the short-chain saturated fatty acids, octanoic and decanoic acid, to the stigmas of non-pollinated flowers of *Petunia hybrida* resulted in an increased ethylene sensitivity similar b that observed in pollinated flowers (Whitehead and Halevy, 1989). It appears that the fatty acids may be involved in stimulating the sensitivity in the corolla to ethylene. These acids are synthesised in the styles of the pollinated flowers from where they are transported to the corolla to bring about an increase in ethylene sensitivity. It has also been suggested that short-chain saturated fatty acids may be involved in various other plant growth processes ranging from inhibition of pollen and seed germination (Berrie *et al.*, 1979; Iwanami and Iwadare, 1979; Metzger and Sebesta, 1982) to auxillary bud growth (Tso, 1964) and stomatal movement (Willmer *et al.*, 1978).

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Although the exact mechanism whereby these acids act to increase ethylene sensitivity is unknown, their site of action appears to be the cellular membranes (Babiano *et al.*, 1984), into which they become incorporated (Berrie *et al.*, 1975; Iwamani and Iwadare, 1979), resulting in modifications of the activities of cellular exchange systems and an increase in membrane permeability (Berrie *et al.*, 1979; Hyodo and Tanaka, 1982). It seems possible that such changes could cause an increase in the ability of the tissue to bind ethylene. According to Whitehead and Bossé (1990), the increase in ethylene sensitivity caused by these acids is associated with an increase in the ability of the tissue to bind ethylene. It appears that short-chain saturated fatty acids ranging in chain length from C_7 to C_{10} are the "sensitivity factor" responsible for the increase in ethylene sensitivity during flower senescence (Whitehead, 1994).

The existence of a transmissible "sensitivity factor" was demonstrated in petunia when it was shown that application of an exudate collected from excised pollinated styles to the stigmas of unpollinated flowers resulted in accelerated senescence. A drastic increase in the sensitivity of the flowers to ethylene was observed. The control was flowers treated with an exudate from unpollinated flowers (Whitehead and Halevy, 1989). The "sensitivity factors" were revealed when the exudates from pollinated carnation and petunia styles were analysed for their fatty acid contents. Large quantities of short-chain saturated fatty acids (chain length C_7 toC_{10}) were found (Whitehead and Halevy, 1989; Whitehead and Vasiljevic, 1993). Application of these acids to the stigmas of the unpollinated flowers resulted in the stimulation of ethylene sensitivity in the corollas similar to that observed in pollinated flowers. It was also shown that when the acids were applied to the styles, they were transported rapidly from the stigma to the corolla without accumulating in any of the floral parts along the way (Whitehead an Halevy, 1989).

The relationship between short-chain saturated fatty acids and ethylene sensitivity is confirmed by the rapid increase in short-chain fatty acid levels which accompany the sudden increase in ethylene sensitivity during the first 12 hours after pollination (Whitehead, 1994). The action of these acids is thought to lie in their effect on cellular membranes, since treatment of flowers with these acids resulted in changes in the physical properties of the membranes (Halevy *et al.*, 1996). This could lead to an increase in membrane permeability and ethylene sensitivity.

1.5 ETHYLENE ANTAGONISTS

1.5.1 SILVER THIOSULPHATE (STS)

Treatment with silver has widely been used as a preservative measure for cut flowers (Whitehead and De Swardt, 1980). Earlier experimental work indicated that silver ions move very slowly in the stems of cut carnations. Since silver ions must penetrate to the site of ethylene action, it must be converted to a form which can be taken up and transported much more readily than in the ionic form.

It was later demonstrated that silver moves more readily in stems if it is present as the silver thiosulphate complex (STS) formed by the combination of $AgNO_3$ and $Na_2S_2O_3$ in specific molar ratios (Reid *et al.*, 1980; Farhoomand *et al.*, 1980). Treatment of freshly harvested petunia flowers (pollinated or non-pollinated) with STS delays their senescence (Whitehead *et al.*, 1984). Similarly, the wilting of carnation petals is delayed by STS treatment of the flowers (Veen and Van de Geijn, 1978). Silver acts as an anti-ethylene agent by inhibiting the ethylene surge that usually occurs 7 to 8 days after harvest. This in turn results in the suppression of the climacteric respiratory rise (Veen, 1979).

Bufler *et al.* (1980), maintain that STS completely prevents the climacteric rise in ethylene production, without affecting basal ethylene production. Silver, being a heavy metal, acts as a non-competitive enzyme inhibitor. Its presence in the cell results in the suppression of respiration and the delay of senescence (Whitehead and De Swart, 1980). Investigations into the distribution of STS after treatment indicate that STS accumulates in the receptacle of the flower. Ultrastructurally, silver deposits are associated primarily with the cell wall and the intracellular spaces of the receptacle tissue (Veen *et al.*, 1980). In etiolated pea seedlings, silver inhibits both ethylene action and the incorporation of ethylene into the tissue. In carnation flowers the silver ions inhibit ethylene incorporation, which occurs in the reproductive and receptacle tissues just before the surge in ethylene production occurs. Thus, the inhibitory effect of silver on the ethylene surge may be associated with the prevention of ethylene binding in the tissue (Veen, 1979).

It has been stated by many authors that silver is in fact an inhibitor of ethylene action. Abeles and Wydoski (1987), define an inhibitor of ethylene action as "a compound that has no physiological effects of its own at the concentration used to block ethylene action." As mentioned before, ethylene binds to a receptor site embedded within the cell membrane. It forms a complex with its receptor molecule in order to carry out its physiological action. Silver may substitute for copper (Beyer, 1976), the metal core of the ethylene receptor site, and thereby interfere with ethylene binding.

The longevity of flowers is strongly influenced by the presence of exogenous ethylene, which speeds up the process of senescence. STS counteracts the effect of exogenous ethylene completely by rendering the tissue insensitive to ethylene (Veen and Van de Geijn, 1978; Veen, 1979). An increase in the concentration of silver ions progressively reduces the ability of exogenous ethylene to induce senescence (Beyer, 1976).

1.5.2 1-METHYLCYCLOPROPENE (1-MCP)

1-Methylcyclopropene (1-MCP), formerly designated as *Sis-X*, is an effective inhibitor of ethylene responses in carnation flowers in either the light or the dark. It would appear that the mechanism of 1-MCP action is its ability to bind irreversibly to the ethylene receptors or at least remains bound for many days (Sisler *et al.*, 1996). Flowers treated with exogenous ethylene 10 days after the chemical treatment were not sensitive to ethylene. 1-MCP is the first gaseous product found to be an irreversible antagonist of ethylene in the dark, and that acts at very low concentration. 1-MCP also appears to interact with the ethylene production mechanism and is able to stop irreversibly the autocatalytic production of ethylene, but this is not thought to be a direct effect on ethylene production (Sisler *et al.*, 1996). The sensitivity of flowers to ethylene has been shown to increase with age of the carnations, whereas the ethylene binding capacity decreases. Old flowers require a higher concentration of 1-MCP to be protected against exogenous ethylene than younger flowers (Sisler *et al.*, 1996). The results found by Sisler *et al.* (1996), indicate that 1-MCP is bound to the physiological ethylene receptor, but likely competes with endogenous ethylene for binding. The extremely low amount of 1-MCP necessary to inactivate the receptor, and the fact that it remains bound for a long period of time, suggests it to be very useful compound for controlling ethylene responses (Sisler *et al.*, 1986).

Serek *et al.* (1995) investigated the effects of 1-MCP on cellular senescence symptoms in petunia flowers following exposure to ethylene. Cut petunia (*Petunia hybrida*) flowers that were exposed to ethylene for 12 hours at concentrations of 1-12ppm wilted sooner than their untreated counterparts. This effect was abolished by a 6 hour pre-treatment with 1-MCP.

Immediately following the ethylene treatment, decreases in petal fresh weight and total protein content were measured, along with higher electrolyte leakage, and lower membrane lipid fluidity and protein content. When applied alone, 1-MCP had little impact on these parameters. However, when the flowers were treated with 1-MCP prior to the ethylene treatment, ethylene had no effect. The results of the study indicated that while ethylenes effects on wilting were obvious 3 days after the treatment, cellular parameters were already affected at the end of the treatment. Since 1-MCP repressed these early ethylene effects, it was concluded that it interferes with ethylene action in petunia flowers at a rather early stage, long before wilting (Serek *et al.*, 1995).

Studies over the last few years have shown that 1-MCP can delay fruit ripening (Serek *et al.*, 1995; Golding *et al.*, 1998) as well as flower senescence (Porat *et al.*, 1999). Feng *et al.* (2000) found that 1-MCP is a potent inhibitor of avocado fruit ripening which exerts its effect via inhibition of ethylene action. The advantage of using inhibitors of ethylene action over inhibitors of ethylene production lies in the ability of the inhibitors of ethylene action to protect the tissue against both endogenous and exogenous ethylene, thus providing better overall protection.

1.5.3 AMINOOXYACETIC ACID (AOA)

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Ethylene production can be suppressed by inhibitors of ACC–synthase, the enzyme responsible for the conversion of SAM to ACC in the ethylene biosynthetic pathway. Aminooxyacetic acid (AOA) is an example of such an inhibitor, which, when included in the holding solution, prolongs the longevity of carnation flowers (Yang, 1980). Ethylene production remains at basal levels, never displaying the climacteric rise observed during the normal progress of senescence. It would appear that the mechanism of AOA action is related to its ability to inhibit endogenous ethylene production (Fujino *et al.*, 1980). Mayak *et al.* (1985), found this to be true when investigating the effect of AOA on temporary water-stress induced ethylene production in carnation flowers. They found that the treatment with AOA resulted in resistance by the flowers to water-stress by inhibiting ethylene biosynthesis.

However, AOA does not seem to have an effect on protecting carnations against the action of exogenous ethylene (Fujino *et al*., 1980). This is in contrast with the anti-ethylene effect of silver ions and 1-MCP.

1.6 THE ROLE OF APPLIED SUGARS

Sucrose is included in most preservative formulations, but other metabolic sugars like glucose and fructose are similarly effective. Lactose and maltose are only active in low concentrations, while non-metabolic sugars such as mannitol and mannose are inactive or harmful (Aarts, 1957; Halevy and Mayak, 1974). The optimal concentration of sucrose varies with the treatment and the type of flower. Generally for a given flower the longer the exposure to the chemical solution, the lower the concentration required. Therefore high concentrations are used for pulsing, intermediate for bud opening, and low for holding solutions.

The final stages of flower development are characterised by a decline in the content of carbohydrates and dry weight of petals (Aarts, 1957; Coorts, 1973). The flower is a heterogeneous organ, composed of floral parts each of which may be at a different physiological development stage. Generally, the senescence and wilting of the petals determine the longevity of the flower. Reducing sugars rather than sucrose were noted as the main constituents of the sugar pool of mature petals (Kaltaler and Steponkus, 1974; Nichols 1973). The changes in sugars are accompanied by starch hydrolysis (Ho and Nichols, 1977). The gradual decline in respiration in ageing flowers may be caused by short supply of readily respirable substrates, mainly sugars. It was suggested that the content of these substrates may indicate the potential life of the flower at a specific temperature (Nichols, 1973). This is supported by the observation of a relationship between potential keeping life and dry matter content of the cut flower at the time of harvest. The respirable substrate pool is composed mainly of sugars. The size of the pool is affected by the rate of hydrolysis of starch and other polysaccharides (Ho and Nichols, 1977; Nichols, 1976) and translocation to the petals (Nichols and Ho, 1975) from one side, and respiration and translocation out of the flowers to other plant parts from the other.

Translocation within the flower from the petals to the ovary was also demonstrated in senescing flowers (Hsiang, 1951; Nichols, 1976). This transport is promoted by pollination and ethylene. Supplying cut flowers with exogenous sugar maintains the pool of dry matter and respirable substrates, especially in petals, thus promoting respiration and extending longevity (Coorts, 1973; Rogers, 1973). Several metabolic sugars are active in this respect, but non-metabolic sugars are not (Aarts, 1957; Halevy and Mayak, 1974). The application of ^{14}C-sucrose to cut roses revealed that sucrose was incorporated into protein, ethanol soluble carbohydrate, starch, and ethanol insoluble material (Ho and Nichols, 1977), though the incorporation into polysaccharides declined along with flower maturation. At the same time, an absolute reduction in starch content was measured. It indicated a dynamic balance in which active anabolic processes were taking place simultaneously with catabolic processes (Ho and Nichols, 1977).

It is known that sucrose improves water balance in cut flowers (Halevy and Mayak, 1974). This was attributed to the effect of sugars on the closure of stomata and reduction of water loss (Marousky, 1971). However, this cannot account for the great increase in water uptake in sucrose treated flowers (Halevy, 1976). The water deficit of sucrose-treated rose flowers was initially higher than that of control flowers held in water since less water was absorbed presumably due to the lower water potential of the sugar solution (Borochov *et al.*, 1976). Later, most probably after the supplied sugar reached the flower head, an improvement in water balance was observed. The improvement of water balance was also associated with a reduced endogenous level of abscisic acid (Borochov *et al.*, 1976), which is a typical response to reduction of water stress. Shortly after application of sucrose, increased amounts of reducing sugars appeared in the stem (Kaltaler and Steponkus, 1974; Sacalis and Chin, 1976). This indicates that the stem possessed high sucrose inversion capacity (Chin and Sacalis, 1977). Both the xylem and phloem are involved in the translocation of sugars (Ho and Nichols, 1975). Radial movement of sugars in the stem was demonstrated (Ho and Nichols, 1975), thus providing ready access between the xylem and phloem along the stem. The glucose that enters the xylem may move radially to the phloem where it combines to form sucrose, and be transported to the flower bud. The supplied sugar may also reduce naturally occurring starch hydrolysis and lipid degradation in cut rose stems held in water.

The translocated sugars accumulate in the flowers, increasing their osmotic concentration, and improving their ability to absorb water and maintain their turgidity (Halevy, 1976; Halevy and Mayak, 1974). Maintenance of improved water status seems to be the most important aspect of longevity (Halevy, 1976; Rogers, 1973). It therefore seems that one of the main effects of applied sucrose on flower longevity results from their contribution to the osmotic adjustment of the flowers.

1.7 RESPIRATION IN SENESCING CUT FLOWERS

1.7.1 PATTERNS OF RESPIRATION

The respiratory behaviour of cut flowers has not been investigated as extensively as has that of fruits and detached leaves. The available data, however, exhibit both climacteric and non-climacteric respiratory drifts in these organs. Nichols (1968), demonstrated that the respiratory drift of cut carnations is similar to that of climacteric fruits in that there is an initial sharp decline after harvesting, followed by a steep rise to a peak and then a second decline.

Climacteric commodities respiratory rate remains constant, or shows a gradual decrease, after it is harvested until a minimum value, known as the pre-climacteric minimum, is reached. The pre-climacteric phase is that phase from when the commodity is harvested till the pre-climacteric minimum is reached. It is followed by an increased respiratory rate, the climacteric phase, and reaches a peak value known as the climacteric maximum (indicates the most wilted flowers). A second decrease in the respiratory rate occurs when senescence is finally reached and this is the post-climacteric phase. This pattern of respiration in climacteric commodities is known as the climacterium (Fig 3).

1.7.2 CAUSES OF THE CLIMACTERIC RISE IN RESPIRATION

Respiration of detached plant organs can be stimulated by several factors, such as the addition of uncouplers of oxidative phosphorylation, chilling temperatures, wounding, addition of sugars, and irradiation (Millerd *et al.*, 1953; Theologis and Laties, 1978). The climacteric rise in respiration has been variously attributed to the decrease in organizational resistance (Blackman and Parija, 1928), enhancement of protein synthesis (Hulme, 1954), the presence of natural uncouplers of oxidative phosphorylation (Millerd *et al.*, 1953), and an attempt by the tissue to maintain metabolic homeostasis (Romani, 1984). The relationship between the rate of respiration and protein synthesis is not always closely linked. For instance, in most climacteric fruits, the incorporation of amino acids into proteins decreases sharply long before the climacteric peak (Richmond and Biale, 1966; Baker *et al.*, 1985). In addition, apart from the requirement of protein synthesis for ripening, there is no common metabolic denominator to explain the climacteric rise in respiration that occurs in certain commodities. The only common metabolic feature of climacteric commodities is their ability to both produce ethylene (C_2H_4) and to respond to exogenous application of the gas by an increase in respiration and eventual senescence. Considerable effort has been expended in attempting to prove whether the rise in C_2H_4 evolution precedes or follows that of respiration (Rhodes, 1980; Biale and Young, 1981). The results of these investigations show that in several fruits the rise in C_2H_4 precedes (Pratt and Goeschl, 1968), coincides with (Biale and Young, 1981), or follows (Kosiyachinda and Young, 1975) that of respiration. However, it should be borne in mind that because the sensitivity of the tissue to C_2H_4 increases with maturity (Burg, 1962), the levels of C_2H_4 already present in the tissue may be adequate to trigger both the rise in respiration and C_2H_4 evolution. Brief exposure to C_2H_4 of early-maturity avocados induces a transient increase in respiration but not ripening (Rhodes, 1980). Sfakiotakis and Dilley (1973), treated immature apples with propylene, a mimic of C_2H_4 (Burg and Burg, 1967), which increased the rate of respiration but failed to cause ripening. Exogenous application of C_2H_4 enhances respiration of non-ripening tomato mutants but does not induce any of the overt changes of ripening, such as softening and lycopene formation (Herner and Sink, 1973). Further, the rise in respiration requires the continuous presence of C_2H_4 and can be invoked several times in succession.

A similar response to exogenous C_2H_4 has been observed with non-climacteric citrus fruits (Rhodes, 1980; Biale and Young, 1981). McMurchie *et al.* (1972), treated bananas (a climacteric fruit) and oranges (a non-climacteric fruit) with propylene. Propylene elicited a rise in respiration in both fruits, but only in the bananas did it engender production of endogenous C_2H_4. To use the authors' terminology, oranges (non-climacteric fruit) lack the system II of C_2H_4 synthesis, which the authors consider to be associated with the induction of the autocatalytic production of C_2H_4. In climacteric fruits, the application of exogenous C_2H_4 leads to the induction of C_2H_4 biosynthesis, which in turn, because of the diffusivity barriers to gas exchange, raises the internal concentration of C_2H_4 to high levels, and these probably saturate the system (Solomos, 1987). It was shown by Nichols (1968), that the rise in respiration of cut carnations was preceded by a rise in C_2H_4 evolution. Mayak *et al.* (1977) also observed that treatment of cut carnations with propylene induces both an increase in respiration and C_2H_4 synthesis. Addition of aminoethoxyvinylglycine (AVG), an inhibitor of C_2H_4 biosynthesis, to the holding solution of cut carnations prevents the rise in both C_2H_4 and CO_2 evolution, although the carnations finally wither. Application of exogenous C_2H_4 leads to a sharp increase in CO_2 evolution. Similarly, the addition of silver ions, an inhibitor of C_2H_4 action (Beyer, 1976), abolishes the rise in respiration in cut carnations, and the application of exogenous C_2H_4 fails to increase the respiration rate. Thus, the inhibition of both the biosynthesis and action of C_2H_4 eliminates the rise in respiration without preventing eventual senescence. The sharp climacteric rise in respiration during senescence of cut flowers appears to be an aspect of C_2H_4 action and not of senescence as such (Noodén and Leopold, 1988).

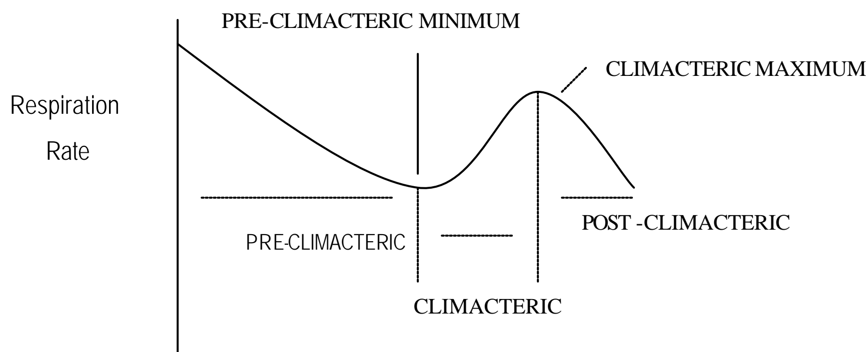

Figure 3: The Climacterium

1.8 AIM OF THE STUDY

Flowers are an excellent model for studying the process of senescence, since flowers have a relatively short vase life. It is thus possible to study senescence without applying artificial "senescence-inducing" treatments, as is used in studies of leaf senescence (Halevy and Mayak, 1979). Senescence in climacteric flowers is characterized by a climacteric rise in respiration rate and ethylene synthesis during the late stages. A pulse treatment with sucrose is commonly used to increase the longevity of cut flowers. As indicated before, sucrose exerts its effect on senescence through the internal water balance and metabolic processes such as respiration and ethylene synthesis action. In this study, the effect of pulsing with sucrose on flower longevity was studied and compared in freesia inflorescences and carnation flowers. From the results obtained, it was attempted to find explanations for:

- the effects sucrose has on a single flower (carnation) and an inflorescence (freesia)
- the effect of sucrose pulsing on flower longevity
- the effect of sucrose on the physiological processes of the flowers
- and the effect STS and 1-MCP has on freesias

The main aim of the study therefore was to establish what is the main effects of sucrose pulsing on different types of senescing climacteric flowers. In carnation, the effect is limited to the senescence of a single flower, while in freesia its effect is also extended to the opening of closed buds along the inflorescence axes.

PART I - CARNATIONS

CHAPTER 2

METHODS AND MATERIALS

2.1 PLANT MATERIAL

White carnation flowers (*Dianthus caryophyllus* L. cv. Nordika and *Dianthus caryophyllus* L. cv. Snow White) were obtained from a local grower and were freshly harvested at the beginning of each experiment. Flowers were harvested at the commercial stage of partially open and their stems were trimmed to 25cm. All the leaves were removed. The flowers were then placed in glass jars containing the test solutions. The flowers were treated and kept in a growth room at a constant temperature of 22±1°C, under continuous illumination from cool white fluorescent lights (1.8 Wm^{-2} PAR) for the duration of the experiments. The day on which the flowers were harvested and received was taken as Day 0.

2.2 DETERMINING CHANGES IN THE APPEARANCE OF SENESCING FLOWERS

The progress in senescence in the flowers was monitored by assessing the changes in the appearance of the flowers by ascribing a numerical value to each individual flower according to the following scale (Plate 1):

0 – flowers almost fully expanded showing a yellowish tinted centre;
1 – mature fresh looking fully expanded flowers with a complete white centre;
2 – senescent flowers showing symptoms of petal inrolling;
3 – old flowers in which the petals had lost turgidity;
4 – old flowers in which the flowers show signs of desiccation.

PLATE 1: STAGES OF SENESCENCE OF CARNATION FLOWERS

STAGE 0 - flower almost fully expanded showing yellowish tinted centre

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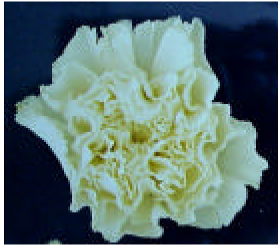
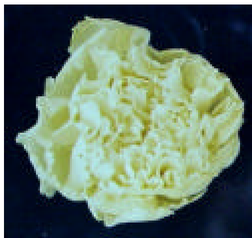
STAGE 1 – mature fresh looking fully expanded flower with a complete white centre

STAGE 2 – senescent flower showing symptoms of petal inrolling



STAGE 3 – old flower in which the petals had lost their turgidity



STAGE 4 – old flower in which the petals show signs of desiccation

Flowers were scored at daily intervals for the duration of the experiment and the final day was taken when the flower reached stage 4. A mean score was calculated according to the number of replications that was used in each treatment. Day 1 of the experiments was taken after pulse treatment of the flowers.

2.3 TREATMENTS USED IN THE EXAMINATION OF FLOWER LONGEVITY

In order to examine the effect of different treatments on ethylene sensitivity and flower longevity, flowers were treated as described below at the onset of each experiment.

a) Control

Control flowers were trimmed and kept in distilled water for the duration of the experiment.

b) Sucrose

The effect of sucrose concentration on the longevity of senescing cut carnations was determined by placing the stems in solutions containing 10%, 20% and 30% sucrose. The flowers were then pulsed for 6, 12, 24 and 48 hours for each sucrose concentration respectively. Ten carnations were used for each pulse treatment. The flowers were then placed in glass jars containing distilled water for the duration of the experiment. The experiment was repeated three times

c) STS

Stems were placed in a solution containing $AgNO_3$ and $Na_2S_2O_3$ in a 4:16 mM ratio, for 15 minutes (Reid *et al.*, 1980). The flowers were then placed in glass jars containing distilled water for the duration of the experiment.

d) 1-MCP

The effect of 1-MCP on the longevity of the cut flowers was determined by placing the flowers in a sealed glass chamber along with a glass beaker containing a 5ml buffer solution and a level scoop (0.07 g) of Ethylbloc® obtained from Biotechnologies for Horticulture Inc., USA. Flowers were exposed to 1-MCP for a time of four hours. The flowers were then taken out and placed in glass jars containing distilled water for the duration of the experiment.

2.4 MEASUREMENT OF THE CHANGE IN OSMOLALITY IN CARNATION PETALS

Flowers were treated by placing the stems in 10, 20 and 30% sucrose and pulsed for 6, 12, 24 and 48 hours for each sucrose solution. To determine the osmolality in the carnation, petals from the outermost whorl of an intact flower were removed and squashed using a household garlic crusher and the sap from the petals collected. A volume of 50μl of juice was transferred into an Eppendorph tube and the osmolality measured using a cryoscopic osmometer. Osmolality was calculated as μosmol per 1 gram of fresh weight. Measurements were done at 24 hour intervals for each treatment. Three replicates were used for each treatment.

2.5 DETERMINATION OF THE TOTAL DISSOLVED SOLIDS (TDS) IN CARNATION PETALS

The flowers were treated as described in Section 2.3. The petals from the outermost whorl of an intact flower were removed and squashed using a household garlic crusher to release the sap from the petals. A refractometer was used to determine the Total Dissolved Solids (TDS) in the sap and was measured as the BRIX %. Three replicates were used for each treatment.

2.6 MEASUREMENT OF ETHYLENE SENSITIVITY

To determine the effect of sucrose-pulsing on ethylene sensitivity in carnation flowers, flowers were treated for 24 hours in 10, 20 and 30% sucrose solutions prior to exposure to exogenous ethylene. After the 24 hour pulse treatment, flowers were sealed in glass chambers and exposed for up to 8 hours to a flowing air stream containing 3,5 μl ethylene L^{-1}. The progress of senescence in the different flowers was then monitored by assessing changes in the appearance of the flowers as set out in Section 2.3. Flowers were scored at 24 hourly intervals. Ten flowers were used for each treatment.

2.7. DETERMINATION OF THE RESPIRATION RATE OF CARNATION PETALS

To determine the effect of sucrose pulsing on the respiration rate of carnation flowers, they were pulse-treated for 24 hours in 10, 20 and 30% sucrose solutions. A single petal was removed from the outermost whorl of the intact flower at 24 hour intervals after treatment and respiration was measured using a Gilson differential respirometer. Each petal was weighed and gently placed into a glass respirometer flask. A piece of folded filter paper and 200μl of 20% KOH (potassium hydroxide) was placed into the central well prior to the onset of respiration measurements.

The flasks were then sealed to the flask attachments of the Gilson respirometer and immersed in the water bath set at 25 °C for the duration of the experiment. Three replicates were used for each treatment. The respiration rate was calculated as µl oxygen produced per 1 gram of fresh weight per hour.

2.8 MEASUREMENT OF ETHYLENE BINDING IN CARNATION FLOWERS

Ethylene binding was measured using [U-^{14}C]-ethylene as described by Brown *et al.* (1986) and Whitehead and Vasiljevic (1993). Flowers were exposed to labelled ethylene by placing them into sealed 1 litre glass jars containing a 10 ml glass beaker with 20 µl (37KBq) of a ^{14}C-ethylene-mercuric perchlorate complex and a magnetic stirrer. The ^{14}C-ethylene was released into the flask by injecting 2 ml of a 4M LiCL solution through a rubber septum into the beaker while stirring. A duplicate set of experiments was run where unlabelled ethylene was introduced into the flask to a final concentration of 1000 $\mu l.l^{-1}$ prior to the injection of LiCl. After incubation for 2 hours at room temperature (22°C), the flowers were removed from the flasks and immediately placed individually into a flask fitted with a scintillation vial containing a 1,5 cm^2 piece of glass fibre filter paper wetted with 300µl of mercuric perchlorate solution to trap the ethylene released by the plant tissue. The flasks were sealed and placed in the dark at room temperature for 24 hours. After 24 hours, the scintillation vials were removed from the flasks and 10 ml of Ready Value scintillation cocktail added to each vial. The radioactivity in each vial was counted in a liquid scintillation counter. The difference in count rates between samples exposed to ^{14}C-ethylene in the presence or absence of unlabelled ethylene was used as a measure of physiological ethylene binding. The experiment was conducted at 48 hour intervals for five days. Three replicates were used for each treatment.

2.9 MEASUREMENT OF ETHYLENE PRODUCTION BY CARNATION FLOWERS

Petals were removed at regular intervals from the outer whorls of flowers. Each individual petal was sealed in an 8 ml glass tube and incubated at 22±1°C. After 30 minutes a 1 ml gas sample was withdrawn from the headspace and the ethylene concentration measured on a gas chromatograph equipped with a flame ionisation detector and a 1 m X 3 mm 80-100 mesh alumina column. Helium was used as a carrier gas and the oven temperature was kept at 80°C (Whitehead and Vasiljevic, 1993). Ethylene production was calculated as nl ethylene per 1 gram fresh weight per hour.

CHAPTER 3

RESULTS

3.1 EFFECT OF SUCROSE PULSING ON THE LONGEVITY OF CARNATION FLOWERS

The results of this study showed that pulsing carnation flowers for 24 hours with a 10, 20 or 30% sucrose solution resulted in an overall increase in vase life (Fig. 4). Pulsing with 30% sucrose for 24 hours caused necrosis of the petal edges, while pulsing with 10% sucrose was not as effective as the 20% sucrose pulse. The 48 hour pulsing proved to have a negative effect on the flowers as they had the shortest vase life overall and showed early signs of petal edge necrosis. Pulsing for 6 and 12 hours did not increase the flower vase life, and in some cases even resulted in a slight decrease in longevity.

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3.2 ETHYLENE SENSITIVITY

Exposing carnation flowers to exogenous ethylene at 3,5 µl ethylene l^{-1} for 8 hours accelerated their senescence (compare Figs. 4 and 5). Treatment with sucrose decreased the sensitivity of the flowers to exogenous ethylene. The 30% sucrose treatment showed the most significant decrease in ethylene sensitivity (Fig. 5).

3.3 CHANGES IN OSMOLALITY AND TOTAL DISSOLVED SOLIDS OF CARNATION PETALS AFTER SUCROSE PULSING

The results of Figure 6 show that the osmolality of the sap expressed from flowers pulsed with 10%, 20% and 30% sucrose for 6 hours was much lower than that of the control. The higher the concentration of sucrose used for pulsing, the lower was the osmolality of the sap. These results were confirmed by measurement of the TDS in the sap (Fig. 10), indicating that the sap of treated flowers contained less solutes than the control. The extent to which the TDS decreased after pulsing also depended on the concentration of the sucrose in the pulsing solution.

Pulsing with sucrose for 12 hours had a similar effect on sap osmolality and TDS than that for 6 hours (Figs. 7 and 11). However, the extent to which the osmolality and TDS were decreased by pulsing was much less significant than for the 6 hour pulsing period. After 4 days, the osmolality of the 10% and 20% sucrose pulsed flowers was higher for the 12 hour pulsing period than for the 6 hour pulsing period (compare Figs. 6 and 7). The osmolality of the 30% sucrose pulse treatment remained much the same for the two pulsing periods.

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Treatment with 10, 20 and 30% sucrose solutions for 24 hours immediately after harvest generally did not cause significant changes in the osmolality and TDS of the sap expressed from senescing carnation flowers (Figs. 8 and 12). However, treatment for 48 hours did, in most cases, cause a significant increase in osmolality and TDS of the sap (Figs. 9 and 13). The flowers also showed early signs of petal edge necrosis.

3.4 RESPIRATION RATE OF CARNATIONS AFTER SUCROSE PULSING

Carnation flowers show a typical climacteric pattern in respiration during senescence (Fig. 14). The climacteric peak was reached 4 days after harvest. Pulsing with sucrose for 24 hours resulted in a stimulation of respiration and a delay in the climacteric peak to day 7 for 20 and 30% sucrose and day 9 for 10% sucrose.

3.5 ETHYLENE BINDING

In control flowers, ethylene binding increased until day 4 after harvest when the climacteric peak was reached (compare Figs. 14 and 15). Pulsing with 20% sucrose suppressed ethylene binding and resulted in a delay in the peak in ethylene binding.

3.6 COMPARISON OF ETHYLENE PRODUCTION AND RESPIRATION BY SENESCING CARNATIONS

Figure 16 shows a comparison between changes in respiration rate and ethylene production in senescing carnation flowers. Carnations show a climacteric pattern in both their respiration and ethylene production rates during senescence. In this experiment, the climacteric peak in respiration rate was reached on day 6, while the peak in ethylene production followed one day later on day 7.

PART II - FREESIAS

CHAPTER 4

4.1 PLANT MATERIAL

White freesia inflorescences (*Freesia refracta* cv. Athena) were obtained from Hadeco, a local grower and were freshly harvested at the beginning of each experiment. The inflorescences were harvested at the commercial stage of closed tight buds and the stems were trimmed to 30cm. All the lower leaves were removed. The inflorescences were placed in glass jars containing the test solutions. Inflorescences were treated as indicated below and kept in a growth room at a constant temperature of 22±1°C under continuous illumination from cool white fluorescent lights (1.8 Wm^{-2} PAR) for the duration of the experiments. The day on which the flowers were harvested and received was taken as Day 0.

4.2 STAGES OF BUD OPENING

Freesia has an inflorescence with an average of 10 flower buds occurring on the flowering axis. The buds were classified according to their stage of opening and were scored according to the following scale (Plate 2):

1 - White open floret
2 - White closed floret
3 - Closed white/yellow bud
4 - Tight yellow/green bud
5 - Tight green bud

The inflorescence was regarded as senesced when the first floret on the axis had wilted.

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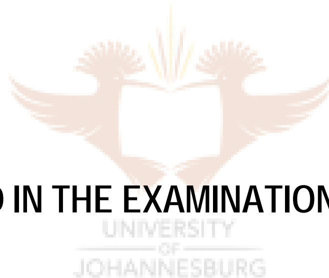
PLATE 2: DIFFERENT STAGES OF BUD OPENING OF FREESIAS

1 - White open floret
2 - White closed floret
3 - Closed white/yellow bud
4 - Tight yellow/green bud
5 - Tight green bud



4.3 TREATMENTS USED IN THE EXAMINATION OF FLOWER LONGEVITY

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In order to examine the effect of different treatments on ethylene sensitivity and flower longevity, freesias were treated as described below at the onset of each experiment.

a) Control

Control flowers were trimmed and kept in distilled water for the duration of the experiments.

b) Sucrose

The effect of sucrose on the longevity of senescing freesias and ethylene sensitivity and production by the freesias was determined by placing the cut end of the stems in a solution containing 20% sucrose in distilled water. The inflorescences were pulsed for 24 hours, and transferred to glass jars containing distilled water for the duration of the experiments.

c) STS

Inflorescences were treated with STS as described in Section 2.3. In some cases inflorescences were pulsed with 20% sucrose prior to treatment with STS.

d) 1-MCP

The effect of 1-MCP on the longevity of the cut flowers was determined as described in Section 2.3. Some inflorescences were pulsed with 20% sucrose prior to treatment with 1-MCP.

4.4 MEASUREMENT OF THE CHANGE IN OSMOLALITY IN FREESIA FLORETS

For the measurement of the osmolality, only open florets were used. Florets were picked from the stem when fully open. All open florets were picked from the stems and the osmolality was measured as described in Section 2.4. Osmolality was calculated as μosmol per 1 gram of fresh weight. Measurements were done at 24 hour intervals for each treatment, and each experiment was repeated 3 times.

4.5 DETERMINATION OF ETHYLENE PRODUCTION BY FREESIA BUDS

Ethylene production by the freesia florets was determined over a period of five days. Each floret was weighed, gently rolled and slid into a 10ml test tube, which was then flushed with ethylene free air and sealed with a rubber septum. The ethylene concentration in the head space of the tube was measured after 30 minutes as described in Section 2.9. Ethylene production was calculated as nl ethylene per 1 gram fresh weight per hour. Three, single replicates were used for each treatment.

4.6 MEASUREMENT OF ETHYLENE BINDING IN FREESIA BUDS

Ethylene binding was determined as described in Section 2.8 for inflorescences treated with sucrose, STS and 1-MCP as described in Section 4.3. The experiment was done for one day only for each treatment. Three replicates were used for each treatment.

4.7 DETERMINATION OF THE RESPIRATION RATE OF THE FREESIA BUDS

Inflorescences were treated as described in Section 4.3. The respiration rate of the first five florets and buds was measured at 24 hour intervals for a period of five days only. Each bud/floret was weighed, gently placed into a glass respirometer flask and the respiration rate measured in a Gilson differential respirometer as described in Section 2.7. Three replicates for each bud/floret were used for each treatment. The respiration rate was calculated as µl oxygen produced per 1 gram of fresh weight per hour.

4.8 MEASUREMENT OF SUCROSE UPTAKE AND DISTRIBUTION IN FREESIA BUDS

The stems of individual inflorescences were placed into glass conical test tubes containing 2 ml 20% sucrose with the addition of 720 KBq ^{14}C-sucrose. The inflorescences were left in the ^{14}C-sucrose solution under continuous lighting at 22±1°C for a period of 24 hours, after which they were removed and placed into distilled water. Five inflorescences were used for each day of the experiment. Florets and buds were picked and placed individually into test tubes containing 100% ethanol and boiled at 90°C for 4 hours. The extract was then placed into a scintillation vial, 10 ml of Ready Value scintillation cocktail was added and the radioactivity counted in a liquid scintillation counter. The first five florets and buds of the inflorescence were used. The experiment was repeated 3 times.

CHAPTER 5

RESULTS

5.1 CHANGES IN OSMOLALITY IN FREESIA BUDS AFTER SUCROSE PULSING

On the first day of the experiment, only the first and second florets were open on the floral axis. At this stage, the bud number corresponded with the stage of development (bud 1, stage 1; bud 2, stage 2; etc.). The osmolality of the sap in the florets of the sucrose-treated inflorescences was much higher than the control (Fig. 17). In both treated and control inflorescences, the osmolality of the second floret was higher than in the first. On the second day, 4 florets had opened in the treated inflorescences compared to 2 in the control (Fig. 18). In both the control and treated inflorescences the osmolality of the first two florets had decreased when compared to day 1 (see Figs. 17 and 18). The osmolality of sap from treated florets remained much higher than the controls.

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On day 3 after treatment, 4 florets had opened in control inflorescences compared to 5 in the sucrose treatment. There appeared to be an equal distribution of solutes in the sap of the different florets (Fig. 19). The osmolality of the sap from treated florets was higher than the control, but lower in the first 4 florets than on day 2 (compare Figs. 17 and 18). On day 4, the first florets of the control showed signs of wilting. After 5 days, 8 florets had opened on the treated inflorescences compared to 5 on the control. The osmolality of the sap from sucrose treated florets was still higher than that of the control (Fig. 20). In both control and treated inflorescences the osmolality of the sap from the first two florets was lower than on day 3. However, the osmolality of florets 3 and 4 had increased in the sucrose-treated inflorescences.

5.2 SUCROSE UPTAKE AND DISTRIBUTION IN THE FREESIA BUDS

On the first day after pulsing with ^{14}C-sucrose, the level of radioactivity was much the same in the first five buds of the inflorescence (Fig. 21). However, on the second day the level of radioactivity had increased in all the buds. The level of radioactivity was highest in the first two buds, indicating that ^{14}C-sucrose was absorbed from the stem. After 3 days, the level of radioactivity in the first two buds did not change, but was much higher in buds 3 to 5. The highest level of radioactivity was found in bud 3, followed by buds 4 and 5 respectively. On day 4, the level of radioactivity had decreased in bud 3 and increased in buds 4 and 5. The level of radioactivity was now highest in bud 5, followed by buds 4 and 3 respectively. After 5 days the levels of radioactivity had decreased in all buds when compared to day 4.

5.3 ETHYLENE PRODUCTION BY FREESIA BUDS

Figure 22 shows the rate of ethylene production of different buds along the floral axis of freesia inflorescences during senescence. On day one, the stage of development corresponded to the bud number. In bud 1 and 3 ethylene production peaked on day 3 after harvest and then dropped to lower levels on day 4 and 5. In bud 4, ethylene production was highest on day 4, while bud 5 showed a single peak in ethylene production on day 3. A 24 hour pulse treatment with 20% sucrose resulted in a delay of the peak in ethylene production in day 3 to 5 (Fig. 22). However, treatment with both STS and 1-MCP caused a marked suppression in ethylene production in all buds (Figs. 24 and 25).

5.4 ETHYLENE BINDING

The ethylene binding capacity of buds at different stages of development along the axis of freesia inflorescences is shown in Figure 26. The stages of development of the different buds at the time of measurement corresponded to those described in Section 4.2. Bud number 1

was most mature at stage 1, followed by bud 2 at stage 2, bud 3 at stage 3, bud 4 at stage 4 and bud 5 at stage 5. Ethylene binding was highest in fully open florets (stage 1). This was followed by a decrease in ethylene binding by bud 2 (white closed floret, stage 2). Bud 3 (closed white/yellow bud, stage 3) showed the lowest capacity to bind ethylene. Thereafter, ethylene binding capacity increased again in bud 4 (tight yellow/green bud, stage 4), followed by a further increase in bud 5 (tight green bud, stage 5). Fully open florets and tight green buds had the highest ethylene capacity. Treatment with 20% sucrose, STS and 1-MCP resulted in a drastic decrease in ethylene binding by buds at all stages of development (Fig. 26).

5.5 EFFECT OF 20% SUCROSE, STS AND 1-MCP ON BUD OPENING

The effect of a 24 hour pulse treatment with 20% sucrose, STS and 1-MCP on flower longevity and bud opening was assessed by scoring the number of buds that opened to stage 1 (white open floret) against the time it took for the first open floret to show the first signs of wilting. In control inflorescences, an average of four buds had opened over a period of three days before the first bud started to wilt (Fig. 27). Treatment with STS and 1-MCP resulted in a reduction in the rate of bud opening and an extension in the longevity of the first bud from 3 to 4 days. No significant difference was observed in the final number of open florets in an inflorescence.

Although pulsing with 20% sucrose did not affect the rate of bud opening, it did result in a marked extension of longevity in the first floret and an increase in the number of open florets in the inflorescence (Fig. 28). Approximately 8 buds had opened by day 5 before the first floret showed signs of wilting. Combined treatments with sucrose and STS or sucrose and 1-MCP resulted in a decrease in the rate of bud opening when compared to the sucrose treatment on its own. However, addition of STS or 1-MCP to the sucrose treatment did not affect the longevity of the first floret or the final number of open florets in the inflorescence.

5.6 CHANGES IN THE RESPIRATION RATE OF FREESIA BUDS

The first floret in the inflorescence showed a typical climacteric respiration pattern during senescence with a peak on day 3 after harvest (Fig. 29). Bud 2 showed a decline in respiration rate and no peak was observed. The peak in respiration rate observed in buds 3, 4 and 5 is not associated with the respiratory climacteric, but with bud growth and opening. These buds just started to open, or had not yet opened on day 2 (Fig. 27). The rate of respiration increased again after reaching a minimum on day 3.

Pulsing with 20% sucrose for 24 hours initially resulted in a marked stimulation of respiration (compare Figs. 29 and 30). In the first and second florets the rate of respiration continued to decline from a high on day 1 (Fig. 30). However, the rate of respiration in buds 3, 4 and 5 again increased after reaching a low on day 3.

In STS-treated inflorescences, the initial rate of respiration for all buds was also higher than in the control (Fig. 31). The respiration rates of the first two florets peaked on day 3. In buds 3, 4 and 5 the rate of respiration decreased from a high on day 1 and reached a minimum on day 4.

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Treatment with 1-MCP resulted in a decline in respiration rate from day 1 to day 2, followed by an increase to a peak on day 4 in all buds (Fig. 32). All buds showed the typical climacteric pattern in respiration rate.

In treated inflorescences the rate of respiration of the first floret peaked one day later than in the control. The peak was also much higher than in the control 360 $\mu lO_2/g/h$ compared to 270 $\mu lO_2/g/h$, see Figs. 29 and 32).

5.7 COMPARISON BETWEEN RESPIRATION AND ETHYLENE PRODUCTION DURING SENESCENCE OF THE FIRST FLORET OF A FREESIA INFLORESCENCE

Figure 33 shows the changes in respiration and ethylene production rates of the first floret in a freesia inflorescence during senescence. Freesia florets showed a typical climacteric pattern in both ethylene production and respiration during senescence. The peak in ethylene production coincided with the climacteric peak in respiration rate.

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PART III

CHAPTER 6

DISCUSSION

Carnations and freesias are popular cut flowers. The carnation flower is a single flower with petals arranged in multiple whorls. In freesia, 7-10 flower buds are arranged in an inflorescence and each individual floret has 2 whorls of 3 petals each. Senescence of climacteric flowers such as carnations, is characterized by a climacteric pattern in respiration rate accompanied by a climacteric-like rise in ethylene production during the final stages (Halevy and Mayak, 1981; Maxie *et al.*, 1973; Nichols, 1968; Whitehead and Vasiljevic, 1993). This rise in ethylene production in climacteric tissues may precede, coincide or follow that of respiration. The results of this study confirm the climacteric nature of carnation senescence. In the cultivars used for this study (*Dianthus caryophyllus* L. cv. Nordika and *Dianthus caryophyllus* L. cv. Snow White) the peak in ethylene production followed that of respiration. Examination of senescence of the first open floret in a freesia inflorescence (*Freesia refracta* cv. Athena) revealed that its senescence was also accompanied by a typical climacteric pattern in both respiration and ethylene production rates. However, ethylene production started to increase in the pre-climacteric stage and peak concomitant with the peak in respiration rate. These results clearly establish the climacteric nature of freesia flowers.

Sucrose is commonly used in flower preservatives to extend longevity (Coorts, 1973; Halevy and Mayak, 1979). It is applied either in the vase solution, or as a pulse treatment prior to shipping. Pulsing is done by placing the cut stems in a high concentration of sucrose for a short period of time. The concentration of sucrose and pulsing time is dependent on the species and cultivar, as well as the conditions under which pulsing is done. In general, the higher the sucrose concentration and pulsing temperature, the shorter the time required for pulsing. Cut flowers are treated with sugars to increase the pool of respirable substrates (Coorts, 1973; Ho and Nichols, 1977; Nichols, 1973), delay the onset of hydrolysis of structural

cell components (Parups and Chan, 1973), decrease ethylene production and sensitivity (Mayak and Dilley, 1976), enhance the effect of cytokinins (Beevers, 1976) and improve the water balance (Halevy and Mayak, 1974). Sucrose is taken up from the vase medium and transported in both the xylem and phloem (Ho and Nichols, 1975). According to Halevy and Mayak (1974) sugars are transported to, and accumulate in the flowers where it increases the osmotic concentration and improves the ability of the flowers to absorb water and maintain turgidity. This appears to be one of the main contributions of applied sugars to increase the longevity of cut flowers (Halevy, 1976). In this study, pulsing cut carnation flowers with 10%, 20% or 30% sucrose resulted in an increase in flower longevity only if pulsing was done for 24 hours. Pulsing with 20% sucrose for 24 hours was most effective in extending flower longevity. Pulsing for 48 hours resulted in a decrease in longevity at all of the sucrose concentrations used in this study. Damage and browning of the petals occurred when the sucrose concentration exceeded 20% and when the pulsing period exceeded 24 hours. These results confirm those of other researchers in the field who also found a 24 hour pulsing treatment with 20% sucrose to be the most effective in increasing vase life and limiting petal damage to a minimum (Halevy and Mayak, 1979; O'Reilly, 1996). Although the freesia inflorescence differs from the carnation flower in that it consists of multiple florets arranged on a single flowering axis, pulsing with 20% sucrose for 24 hours also resulted in a marked increase in flower longevity. In addition to a 2 day increase in the longevity of the first floret, pulsing also caused a drastic stimulation of bud opening in the inflorescence, resulting in a much improved decorative appearance of the whole inflorescence.

The senescence process in climacteric flowers is mediated by the plant hormone ethylene. Ethylene is an important regulator of processes in plant growth and development, including fruit ripening, leaf abscission, seed germination and senescence (Van Altvorst and Bovy, 1995). Senescence of climacteric flowers such as carnations is associated with a climacteric increase in ethylene production during the later stages (Maxie *et al.*, 1973). This ethylene production is autocatalytic, which means that exposure to ethylene stimulates ethylene biosynthesis (Woodson and Lawton, 1988). Irrespective of the increase in ethylene synthesis, senescence in climacteric flowers is also coupled to an increase in ethylene sensitivity (Halevy and Mayak, 1981; Whitehead and Halevy, 1989; Whitehead and Vasiljevic, 1993).

Although ethylene synthesis is not directly connected to ethylene sensitivity (Halevy and Whitehead, 1989), ethylene sensitivity can be suppressed through treatment with compounds such as STS, 2,5-Norbornadiene and 1-MCP that effectively block the binding of ethylene to its membrane associated receptors (Veen, 1979; Sisler *et al.*, 1985; Whitehead and Vasiljevic, 1993). Suppression of ethylene binding by these compounds result in an increase in the longevity of climacteric flowers. Exposing cut carnations to exogenous ethylene reduces longevity (Nichols, 1977; Thompson, *et al.*, 1982). Treatment with ethylene as done in this study, resulted in a drastic decrease in longevity by more than 70%. However, pulsing with sucrose for 24 hours at concentration ranging from 10% to 30% prior to treatment with ethylene resulted in a marked delay in senescence. The extent to which ethylene sensitivity was suppressed, depended on the concentration of sucrose used for pulsing. Higher concentrations of sucrose were more effective than lower concentrations. Although sucrose pulsing enhanced longevity of ethylene treated flowers, it cannot prevent ethylene action completely as shown in the results. Thus, treating with sucrose suppressed the sensitivity of the flowers to ethylene and conferred "resistance" of the flower to ethylene treatnment, as was found in research by Dilley and Carpenter (1975) and O'Reilly (1996).

The increase in ethylene sensitivity associated with senescence of carnation flowers is accompanied by an increase in the ability of the petal tissue to bind ethylene to its membrane associated receptors (Brown *et al.*, 1986; Whitehead and Vasiljevic, 1993). This binding of ethylene to a specific receptor molecule is a crucial step in its action to initiate a physiological response (Goren *et al.*, 1984; Sisler, 1980; Sisler and Yang, 1984).

Treatment with compounds such as octanoic acid, that stimulate ethylene sensitivity, also stimulate ethylene binding in carnation flowers (Whitehead and Vasiljevic, 1993). However, treatment with compounds such as silver, norbornadiene and 1-MCP, effectively reduce ethylene sensitivity by competitively binding to the binding sites, thus blocking ethylene binding and preventing a physiological response (Goren *et al.*, 1984). Brown *et al.* (1986) and Whitehead and Vasiljevic (1993) showed that ethylene binding in senescing carnation flowers increased in pre-senescent flowers concomitant with the increase in ethylene sensitivity, and only began to decline rapidly when maximum sensitivity was reached during the later stages of senescence.

The results of this study clearly confirm these findings. In addition, these results also show that a 24 hour pulse with 20% sucrose resulted in a suppression of ethylene binding by the flower tissues as well as a delay in the peak of ethylene binding. It appears that sucrose exerts its suppressing effect on ethylene sensitivity by reducing the ability of treated flowers to bind ethylene. It is highly unlikely the sucrose would reduce binding by competitive binding to the ethylene receptor molecules. However, it is possible that it could do so by its general stabilising effect on other senescence processes, such as the degradation of cell membranes (Coorts, 1973; Parups and Chan, 1973).

Ethylene plays an important role in many diverse aspects of plant growth and development, ranging from seed germination to rooting, flowering, senescence, ripening, etc. (Taiz and Zeiger, 1991). Ethylene production by young tight green buds (stage 5) is low initially, and remains low for most of the display life of the inflorescence. However, ethylene binding in these buds is very high, even higher than in, mature open florets, indicating a high sensitivity for the hormone. Ethylene binding decreases as the bud develops and is lowest in stage 3 (closed white/yellow buds). This decrease in ethylene binding suggests a decrease in ethylene sensitivity and coincides with an increase in the rate of ethylene production in stage 3 buds. From stage 3 onward, ethylene binding increases again to reach a maximum when the florets are fully open (stage 1). However, ethylene production does not increase with this increase in ethylene sensitivity. It can be concluded from these results that ethylene sensitivity in a freesia inflorescence is at its lowest when buds are at stage 3 of development, and increases in less or more developed buds. Open florets and tight green buds show far higher sensitivity to ethylene than any other stages of development.

Treatment with the inhibitors of ethylene binding, STS and 1-MCP, rapidly resulted in a drastic reduction in ethylene binding by all buds and florets, confirming that these compounds are very effective in blocking the ethylene receptors. Pulsing with 20% sucrose for 24 hours was equally effective in reducing ethylene binding in buds of all stages, suggesting that sufficient sucrose was taken up and distributed along the flowering axis of the inflorescence to facilitate a very effective reduction in ethylene binding. Although the sucrose, STS and 1-MCP had similar effects on ethylene binding, their effects on ethylene synthesis were quite different. In stage 1 open florets, ethylene production was stimulated from the onset by treatment with sucrose.

In immature buds (stage 2 to 5) treatment with sucrose initially resulted in an inhibition of ethylene synthesis, followed by a stimulation as the buds continued to mature. These results indicate that ethylene synthesis in freesias is stimulated by sucrose in mature floral tissues and inhibited in immature tissues.

Treatment with STS and 1-MCP, however, was very effective in inhibiting ethylene synthesis in buds at all stages of development, ranging from mature open florets (stage 1) to tight green buds (stage 5). Since 1-MCP has no known direct effect on ethylene synthesis and effectively inhibits ethylene binding, it appears that the inhibiting effect of STS and 1-MCP on ethylene synthesis was mediated largely through reduction in the autocatalytic stimulation of ethylene on its own synthesis. In addition to the effect of STS and 1-MCP on increased longevity, these compounds also resulted in a decrease in the rate of bud opening when compared to control inflorescences, even when inflorescences were first treated with sucrose. However, when inflorescences were treated with a combination of sucrose and STS or 1-MCP, the latter two compounds had no additional effect on the longevity of the inflorescence when compared to those only treated with sucrose. It appears from these results, that ethylene not only accelerates senescence of mature open florets in freesia inflorescences, but that it also stimulates the maturation and opening of immature buds. Ethylene, therefore, appears to perform a dual role in freesia inflorescences; the stimulation of bud opening as well as the acceleration of floret senescence.

Carnation flowers and mature open (stage 1) freesia florets both exhibit a climacteric pattern in respiration rate during senescence. In both, pulsing with sucrose for 24 hours resulted in an increase in longevity. Supplying cut flowers with exogenous metabolic sugar maintains the pool of dry matter and respirable substrates, especially in the petals, thus promoting respiration and extending longevity (Coorts, 1973; Mayak and Halevy, 1974). Pulsing cut carnation flowers for 24 hours with 10, 20 and 30% sucrose resulted in a marked stimulation of respiration during the first 3 days, as well as a delay in the climacteric peak in respiration rate. This stimulation of respiration can possibly be ascribed to an increase in the internal carbohydrate pool, which serves as a ready source of respirable substrate. The delay in the timing of the climacteric peak in respiration is directly related to the extension of flower longevity (Mayak *et al.*, 1978).

Similar effects were observed in sucrose treated stage 1 freesia buds. Just as in the carnation, the stimulation of respiration could be ascribed to an increase in the pool of respirable substrates (Coorts, 1973; Mayak and Halevy, 1974).

The rate of respiration of many flowers rises to a maximum as flowers start to open, followed by a gradual decline as the flowers mature. This decline is then followed by the climacteric rise as flowers start to senesce (Mayak *et al.*, 1978). Immature freesia buds (stage 3 to 5) show this typical rise in respiration rate as the florets start to open during the first few days after harvest. The extent of this first rise in respiration rate appears to be dependent on the stage of bud development. Immature buds appear to be metabolically more active than mature ones, and the more immature the bud, the higher the rise in respiration rate. This first rise is followed by a second more gradual rise as the florets mature and start to senesce. As in mature open florets, pulsing with sucrose also stimulated the rate of respiration, possibly due to an increase in the pool of respirable substrate. Although treatment with STS delayed senescence, it did not suppress the rate of respiration or the timing of the climacteric peak in fully open florets (stage 1). In immature florets further down the axes (stage 3 to 5), the initial rate of respiration was greatly stimulated and the second rise in respiration rate delayed by one day, clearly showing the retarding effect of silver on both bud development and floret senescence. The stimulation of respiration by STS suggests that the level of silver in the tissue was not sufficient to reduce the rate of metabolism, thus extending the longevity of the tissue. It could be possible that the slowing down in the rate of bud development and senescence by STS could be ascribed mainly to its effect on ethylene action and synthesis.

The effect of 1-MCP on respiration was somewhat different from STS. Both compounds resulted in deceleration in the rates of bud development and senescence. However, initially the rate of respiration was not stimulated to the same extent as in the case of STS. As in the case of STS, the first peak in respiration in stages 3 to 5 during day 2 completely disappeared when compared to the fully developed first floret, but unlike STS all buds (stages 1 to 5) showed a peak in respiration rate on day 5. The delay in the peak in respiration in the first 2 buds (stage 1 to 2) can be ascribed to the suppression in the rate of senescence, while the delay in the respiratory peak in the last 3 buds (stage 3 to 5) could be ascribed to a slowing down in the rate of bud development.

Since 1-MCP has no other known direct effect on plant metabolism, its retarding effect on bud development and floret senescence may clearly be ascribed to its affect on ethylene action. Due to the similarity in the effects of 1-MCP and STS on development and senescence, the results obtained with 1-MCP provide some support for the aforementioned conclusion that STS acts mainly through its effect on ethylene action.

According to Halevy and Mayak (1974) treatment of cut flowers with metabolic sugars increases their osmotic concentration and improves their water balance. In this study, pulsing cut carnations with 10 to 30% sucrose for less than 24 hours resulted in a decrease in the osmolality of petal sap and no improvement in flower longevity. The extent to which the osmolality was decreased was concentration dependent. The higher the concentration of sucrose used, the greater the reduction in osmolality. A lower osmolality is an indication of a lower solute concentration, suggesting that these short pulse treatments resulted in rapid water uptake by the tissue, thus causing a dilution of the cell content. These results were confirmed by measuring the total dissolved solids (TDS) in sap expressed from petals by means of a hand held refractometer. This measurement is a direct measurement of the amount of solutes in the sap and is commonly used in the fruit industry as an indication of the sugar content of fruit juice. On the other end of the scale, pulsing carnations with the same solutions for periods exceeding 24 hours resulted in a marked increase in osmolality and TDS. This increase in osmolality was accompanied by a decrease in flower longevity and necrosis of the petal edges, indicating that the higher solute concentration in the sap resulted in osmotic stress and early senescence. Pulsing for 24 hours, however, did not result in drastic changes in osmolality and TDS, and was effective in increasing flower longevity. These observations belie the notion that an increase in osmotic concentration is required to improve water balance and thus increase longevity (Halevy and Mayak, 1974). The results of this study clearly show that the most effective treatments are those that had no significant effect on tissue osmolality and the amount of solutes in the cell sap. It appears that no great fluctuations in osmolality occur in carnation petals during senescence and that any effective treatment with sucrose should not significantly alter tissue osmolality. From these results it is possible to deduce that, contrary to the conclusions of Halevy (1976), the contribution of applied sugars to the maintenance of the tissue water balance is not one of its major effects to increase flower longevity.

In freesia, pulsing with 20% sucrose for 24 hours did result in an increase in petal osmolality. However, the osmolality of the first open florets decreased with time to a value close to the original value of the first untreated open florets (approximately 250 μosmol/g). Measurement of petal osmolality in both treated and untreated inflorescences as floret opening progressed along the floral axis with time, showed a decrease in the osmolality of open florets as subsequent florets started to open. Tracing the accumulation of ^{14}C-sucrose in the inflorescence also showed an accumulation of sucrose in younger buds with time. The movement of solutes along the floral axis occur in a wave-like fashion, from older to younger buds. These results suggest that sucrose taken up by the inflorescence during pulsing accumulates in the first open florets from where it is translocated to younger florets to facilitate opening. The mechanism by which the facilitation process is done remains unclear, but the distribution of ^{14}C-sucrose suggests that it is a metabolic related mechanism rather than a simple osmotic process. However, the osmotic concentration of the tissue does appear to affect floral longevity, since treated florets where the osmolality did not fall below the original control value lasted longer than untreated florets where the osmolality did decrease with time.

The results of this study clearly shows that pulsing with 20% sucrose for 24 hours is effective in increasing flower longevity in cut carnations and freesias. Sucrose concentration and pulsing time is of utmost importance, and for optimum results should not affect the osmolality of the petal tissue. Treatments with sucrose reduces the sensitivity of both flower types by decreasing the binding of ethylene to its receptor molecules, thereby preventing its autocatalytic production. It appears that the promoting effect of sucrose pulsing on flower longevity is not primarily exerted through its effect on tissue water balance, but rather on tissue metabolism.

CHAPTER 8

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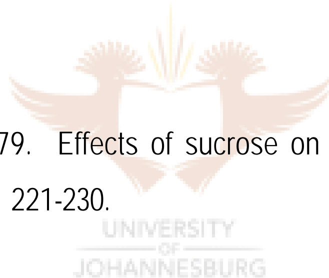

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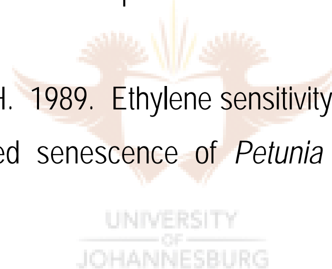

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