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FINAL REPORT

PROJECT NO. I-250-82

Storage and Surface Transportation of Cut Flowers and Ornamental Plants

S. Mayak, A.H. Halevy, A. Borochoy, M.S. Reid, A.M. Kofranek

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B A R D

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AND ORNAMENTAL PLANTS

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ABSTRACT

In accordance with the original plan emphasis was placed on evaluation of physiological parameters like ethylene production, responses to ethylene, and ion leakage reflecting membrane permeability. Central to the theme of the research was examination of changes occurring in the membranes. The evaluation of biophysical parameters like fluidity, phase transition temperatures, presence of crystalline liquid or gel phases allowed us to get an insight at the mechanism underlying the increased permeability often observed in senescing flowers but also in flowers exposed to transient stresses, especially those derived from storage. A study of the biochemical changes occurring in the membrane revealed a reduction in the content of PL, however these changes become obvious at the same time as the onset of a rise in ethylene production. Using an original approach allowed to further probe into early biochemical changes. We were able to demonstrate the presence of neutral lipid components capable of ordering lipid fractions transforming them into gel phase. The presence of the neutral lipids was detected well in advance of the rise in ethylene production or any other change we have studied thus far.

The research on the effect of storage was not limited to cut flowers but also included propagation material like corms. Transient water stress is a common occurrence during handling of cut flowers and ornamental plants. Contrary to reports in the literature ethylene is not immediately involved in the response to water stress. However early changes in the content of phospholipids was demonstrated.

The role microorganisms may play in affecting quality of flowers was re-examined. Not all microorganism found in the base of stems of flowers negatively affect quality. Some were found to be virulent while others were virtually without an effect.

The research has been expanded to include a study of processes related to the effect of chilling temperatures, using suitable model plants. Inhibition of cyclosis and changes in cytoplasmic structure was observed in all plants studied. The possibility that these changes might be due a breakdown in compartmentation of intracellular Ca^{+2} was studied using fluorescence probes like chlortetracycline. Decline and increase in the signal corresponding to lowering and raising the temperature of chilling sensitive plants were noticed. This reflect changes in Ca^{+2} content which is correlated with cytoplasmic streaming. This was not evident in chilling insensitive plants. Altering the internal content of Ca^{+2} clearly indicated that Ca^{+2} is playing a central role in the maintenance of cytoplasmic streaming.

D. WORK CARRIED OUT DURING SUPPORT BY BARD NO. I-250-82 IN ISRAEL

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I. PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSES OF CUT FLOWERS AND ORNAMENTAL CORMS TO LOW TEMPERATURE

Physiological response of cut rose flowers to cold storage

J.D. FARAGHER, S. MAYAK AND T. TIROSH

Physiol. Plant. 67:205-210 (1986)

The effects of low temperature storage on the physiology of cut rose flowers (*Rosa hybrida* L. cv. Mercedes) were studied. Extension of cold storage or increase in temperature (from 3 to 8°C) was accompanied by shortening of vase life and advancement of petal senescence, as reflected in an advance in the timing of the rise in ethylene production and an increase in membrane permeability (ion leakage). Although storage at a relative humidity (RH) of 65% reduced petal water content by 20% in comparison with flowers stored at 95% RH, it did not shorten vase life. The progression of petal senescence was measured during storage at 3°C and during aging at 22°C. Both ethylene production rates and membrane microviscosity measured by fluorescence depolarization increased with time at 3°C and at 22°C, but more slowly at 3°C. At 3°C membrane permeability measured by ion leakage did not increase. Following cold storage the rate of ethylene production in the petals was increased by up to eight times the rate in unstored flowers. Silver thiosulphate extended the vase life of both stored and fresh flowers equally by 2 days, but did not increase the life of stored flowers to that of treated fresh flowers. It is concluded that the primary effect of cold storage on roses is to slow down senescence and that the continued slow senescence leads to shorter vase life. The possible occurrence of sequential processes during senescence and the effects of temperature on these processes is

discussed.

Storage environment related to stresses and flower senescence.

S. MAYAK, J.D. FARAGHER

Acta Hortic. 181:33-43 (1986)

The effects of water stress and temperature, components of low temperature storage, were evaluated. Severe transient water stress, such as to induce a loss of 20% or more in fresh weight, results in advanced onset of the rise in rate of ethylene production. At the same time there is an increase in microviscosity of the cell membranes. These changes, however, do not occur during the stress phase, but considerably later, i.e., about 24 to 36 h after termination of stress. The effect of water stress in shortening rose petal longevity can be alleviated if immediately following stress the petals are detached and placed in water. This suggests that the water stress effect is primarily associated with the induction of an obstruction to water uptake by the petals. Water stress in carnations can be relieved if the flowers are pulse-treated with aminooxy acetic acid, an inhibitor of 1-aminocyclopropane-1-carboxylic acid and ethylene biosynthesis. Since the inhibitor exerted its effect while ethylene synthesis was low, this suggests that the low level of ethylene is important in mediating the stress effect. At low temperature ethylene biosynthesis follows a typical climacteric course. However, the rise in the rate of ethylene production is delayed, although maximum rates of production are similar to those observed at ambient temperature. Also, the high rates are maintained for a long time without visible development of symptoms of senescence. During storage, well before the rate of ethylene production starts to rise, X-ray analysis reveals an increase in the phase transition temperature (T_c) of the cell membranes. This physical change is a consequence of an alteration in the lipid molecular species. When neutral lipids extracted from stored flowers are subjected to interaction with phospholipids, the T_c of liposomes prepared from them is increased, thus clearly indicating that alterations in the neutral lipids are among the first changes to occur in response to the storage environment.

Effect of temperature on ethylene biosynthesis in carnation petals.

A. BOROCHOV, H. ITZHAKI AND H. SPIEGELSTEIN

Plant Growth Regulation 3:159-166 (1985)

Carnation petals, at a stage in which they are already producing ethylene, show a sigmoidal dependency of ethylene production on temperature within the range of 0 to 30°C. An Arrhenius plot of these data show a break at ca. 22°C in the straight lines connecting the points. The activity of the ethylene-forming enzyme (EFE), measured both in vitro, using isolated membranes, and in vivo, using petals pretreated with 1-aminocyclopropane-1-carboxylic acid (ACC) shows an exponential dependency on temperature within the same range. Arrhenius plots of EFE activity fail to show any discontinuity.

In contrast, ACC synthase activity measured in vitro shows the same sigmoidal dependency on temperature as that of the intact petals. We suggest, therefore, that ACC synthase activity is the rate-limiting step mediating the influence of temperature on ethylene biosynthesis by carnation petals over the range studied.

Effects of growing and storage temperatures on growth and tuber yield of caladium

A. LAVEE, A. BOROCHOV AND A.H. HALEVY

Scientia Hort. 26:175-182 (1985)

Growth of caladium (*Caladium x hortulanum*) plants was followed during ca. 7 months under prevailing summer conditions in Israel. Weight of daughter tubers increased exponentially with time. Growth ceased when mean night temperatures fell below 12°C in mid-November. Caladium is a plant which is sensitive to low temperatures, and when tubers were stored at temperatures below 20°C, subsequent sprouting rate was slowed. Storing tubers at 2°C for longer than 7 days reduced yield of daughter tubers, but shorter storage resulted in an improved crop. This

was apparently a result of selective damage to the central bud, removing its apical dominance and releasing lateral buds from inhibition.

Although chilling damage was evident when growth was considered, none of the accepted physiological processes normally associated with chilling injury, including electrolyte leakage, ethylene and CO₂ production, was correlated with it in caladium.

Low temperature effects on caladium tubers.

A. BOROCHOV, A. LAVEE AND A.H. HALEVY

Acta Hortic. 177:347-352 (1986)

Caladium is a tropical tuberous plant, used as a foliage pot plant. Growing season was found to be limited to the period in which prevailing temperatures were above 12°C. Storage of caladium tubers at temperatures below 20°C, but above freezing, resulted in hampered growth processes; sprouting rate was slower and final growth of leaves and daughter tubers was greatly reduced. Degree of damage was directly related to the duration of exposure to low temperatures and could be assessed by the degree of TTC reduction by the buds. Solute leakage and ethylene production were not related to chilling damage. Maintaining atmospheric high humidity during low temperature storage for 14 days prevented the chilling injury symptoms.

III. BIOCHEMICAL AND BIOPHYSICAL CHANGES DURING SENESCENCE AND IN RESPONSE TO LOW TEMPERATURE

THE FATE OF MEMBRANE PROTEINS DURING FLOWER SENESCENCE

A. BOROCHOV, T. TIROSH, AND S. MAYAK

Acta Hortic. 181:75-80 (1986).

Natural senescence of cut carnation flowers is associated with a decline in several parameters of membrane proteins. These include membrane enzyme activity, membrane protein content and membrane protein thiol groups. When rate of senescence was modified by exposure to ethylene or STS application, membrane protein parameters were changed accordingly.

Solubilization and partial purification of an enzyme converting 1-aminocyclopropane-1-carboxylic acid to ethylene in plants.

Z. ADAM AND S. MAYAK

FEBS Lett. 172:1544 (1984)

The membrane-bound enzyme capable of catalyzing the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene can be solubilized by treatment with detergent. The non-ionic detergent Nonidet P-40, at an optimum concentration of 0.2% and a protein:detergent ratio of 1:2, was found to be an effective solubilizer of the conversion activity. The solubilized enzyme resembles the membrane-bound enzyme with respect to heat sensitivity, pH dependence, Mn^{2+} stimulation and Co^{2+} inhibition. The enzyme can be further purified by means of ion-exchange chromatography with 12-fold increase in specific activity.

Characterization of an endogenous inhibitor of the enzymatic conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in carnation petals

H. ITZHAKI, A. BOROCHOV and S. MAYAK
Israel J. Bot. 36:171-183 (1987)

The membrane-bound enzymatic conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene was demonstrated previously in carnation petals together with an endogenous, soluble inhibitor of this reaction. The aim of the present work was to characterize this inhibitor.

In a standard reaction system using ACC as substrate and intact or detergent-solubilized microsomal membranes from carnation petals as source of enzymatic activity, ethylene production could be inhibited by the addition of various amounts of extract from the petals. Similar effects on ethylene formation were found when petals were treated with extract solution before being fed with ACC. The inhibition was reversible and competitive, with $K_i = \text{eq. } 0.8 \text{ mg FW}$. The presence of the inhibitor did not change the optimal pH of the reaction, but the extent of the inhibition was highly dependent on pH, with maximum inhibition at pH 8.0. No binding to a cation exchanger was detected. The inhibitor was, however, adsorbed onto an anion exchanger, DEAE-Sephadex, in a pH dependent manner, with no further changes in charge below pH 3.2. The inhibitor was dialysable and gel filtration indicated that it has a low MW. In addition, the inhibitor was stable to pH extremes and heat, was not precipitated by TCA, and could be completely separated from proteins by gel chromatography. Thus, supporting a non-proteinaceous nature. Ultraviolet, infra-red and fluorescence spectroscopy of the semi-purified material, together with the fact that it binds to PVPP support our contention that the inhibitory activity may be associated with phenolic compounds.

CHEMICAL CHANGES RELATED TO PHYSICAL PROPERTIES OF MICROSOMAL MEMBRANE LIPIDS DURING SENESCENCE OF ROSE FLOWER PETALS

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ABSTRACT

The early stages of senescence in rose flower petals (*Rosa* hyb. L. cv. Mercedes) involve changes in physical properties of microsomal membrane: increased microviscosity and an increase in the lipid phase transition temperature. To investigate the basis for these changes the chemical changes which occurred in petal microsomal membranes while flowers were held for 2 d at 22°C, or 10 d at 3°C, were measured. No changes were observed in the ratio of protein to phospholipid, the mol% of phospholipid or sterol, the ratio of phospholipid to sterol, or in the mol% of phosphatidylcholine, phosphatidylethanolamine, free fatty acids, triacylglycerol or sterol esters. Further, there were no changes in the fatty acid composition of any lipid classes, except for a small increase in unsaturated fatty acids in phospholipids during 10 d at 3°C and a small increase in saturated free fatty acids at both 22°C and 3°C. Another approach was used to determine the membrane fraction responsible for the changes in physical properties with flower age. The membranes were separated into total lipids, phospholipids and neutral lipids and the degree of fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH), which indicates lipid microviscosity, was measured. With flower age at 22°C or 3°C, fluorescence polarization increased in membranes, total lipids and the phospholipid plus neutral lipid fractions combined, but not in the phospholipids fraction alone. It is concluded that neutral lipids, formed during the early stages of petal senescence, lead to increased membrane microviscosity, and possibly to increased lipid phase transition temperature.

INTRODUCTION

Senescence or rose flower petals (*Rosa* hyb. L) involves changes in the physical properties of microsomal membranes, followed by increased ethylene production, increased membrane permeability, then petal discoloration, wilting and death (Legge, Thompson, Murr and Tsujita, 1982; Faragher and Mayak, 1984; Faragher, Mayak and Tirosh, 1986; Faragher, Wachter and Mayak 1987). This pattern occurs during ageing of both freshly cut flowers held at 22°C and cold-stored flowers during storage at 3°C (Faragher et al. 1986, 1987). The earliest observed events are an increase in microsomal membrane microviscosity and decrease in fluidity, an increase in the gel to liquid-crystalline phase transition temperature of the membrane lipids and a consequent phase separation of lipids (Legge et al. 1982; Faragher et al., 1986, 1987). These parallel changes are different physically, but they are probably related and possibly caused by the same chemical changes (Shinitzky and Henkart, 1979, Quinn,, 1981). The physical changes in membranes are likely to cause the observed increase in membrane permeability, and disfunction of membrane enzymes, transport systems and hormone receptors and hence lead to death (Shinitzky and Barenholz, 1978; Shinitzky and Henkart, 1979; De Gier, Van Echteld, Van Der Steen, Noordham, Verkleij and De Kruijff, 1982; Faragher and Mayak, 1984). The chemical cause of such physical changes in membranes during plant senescence appear to vary. Increased microviscosity and decreased fluidity have been clearly related to decreased phospholipid (PL) and increased sterol (ST) levels (Borochoy, Halevy, Borochoy and Shinitzky, 1978; McKersie, Lepock, Kruuv and Thompson, 1978; Shinitzky and Henkart, 1979; Lurie and Ben-Arie, 1983; Borochoy and Faiman-Weinberg, 1984). In addition, these changes are accompanied by the appearance of neutral lipids (NL) which rigidify the acyl chains of PL (McKersie et al. 1978; McKersie and Thompson, 1979; Barber and Thompson, 1983). On the other hand, increases in lipid phase transition temperature appear to be caused by the appearance of these NL, but they are also accompanied by decreased PL levels in membranes (McKersie et al., 1978; McKersie and Thompson, 1979; Barber and Thompson, 1983). Other chemical changes which could cause the observed physical changes include an increased ratio of protein to lipid, a decrease in saturation or length of the PL acyl chains, changes in the ratio of different PL and an increase in the level of free fatty acids (FFA) (Jain and Wu,. 1977;

Shinitzky and Henkart, 1979; Borochoy and Faiman-Weinberg, 1984).

In senescing rose flower petals, measured after ca. 5d at 22°C, there was a decrease in membrane PL levels which apparently caused the observed decrease in microviscosity (Borochoy et al. 1978). However, we are interested in the initial events of senescence, when there are physical changes in the membranes, but before any increase in petal ethylene production, for example during the first two days of ageing at 22°C (Faragher et al. 1986, 1987). During this period there have been no measurements made of chemical changes in rose petal membranes.

The aim of the present work was to investigate the chemical changes which may cause physical changes in microsomal membranes during the initial stages of rose petal senescence. Two approaches were used, the first was to analyse the membrane lipid composition and the second was to measure microviscosity (by fluorescence polarization) of different lipid fractions extracted from the membranes. Senescence was studied during 2 d flower vase life at 22°C and during 10 d cold storage at 3°C.

Materials and Methods

Flowers of the rose cv. Mercedes were used. Measurements were made on petals of flowers which had been treated as follows: (i) Fresh, immediately after cutting; (ii) 2d at 22°C in water, at 70% relative humidity (r.h) with continuous white fluorescence light of 3 W m^{-2} irradiance; or (iii) 10d at 3°C, without water, at 95% r.h.

Membrane and Lipid Extraction. Microsomal membranes were extracted from petals as described previously (Faragher et al. 1986). Membranes were extracted from four replicate groups of 5 to 20 flowers each. Total lipids were extracted from membranes by a modified Folch method (Renkonen, Kosunen and Renkonen, 1963).

Measurement of fluorescence polarization and phase transition temperature of lipids. Fluorescence polarization in membranes and isolated lipids (as liposomes) was measured using 1,6-diphenyl-1,3,5-hexatriene (DPH, Fluka) as described previously (Borochoy et al., 1978; Shinitzky and Barenholz, 1978). The concentrations of membranes and lipids used were those which gave minimum values of microviscosity and thus minimized light scattering: membranes from 0.08 flowers per 10^{-6}

m³ and liposomes from 0.16 flowers per 10⁻⁶m³. The gel to liquid-crystalline phase transition temperature of isolated membrane lipids was measured by wide-angle, x-ray diffraction analysis as described previously (Faragher et al., 1987). The phase transition temperature was taken to be the highest temperature at which gel phase lipid was detectable.

Measurement of membrane lipid, PL and protein. To measure membrane lipid weight, the lipid extract was added to a preweighed test tube and evaporated under nitrogen at 60°C and then in air at 110°C to constant weight. PL was measured by the method of Bottcher, Van Gent and Pries (1961). Protein was measured by the method of Bensadoun and Weinstein (1976) with BSA as standard.

Lipid analysis. Internal standards containing the synthetic n-heptadecanoic acid, diheptadecanoyl phosphatidylcholine and triheptadecanoin (Sigma) were added to the membrane lipid extract before analysis. The lipid components, PL, ST, FAA, triacylglycerides (TG) and sterol esters (SE) were separated from each other by TLC on silica gel (Kieselgel G60, Merck) using petroleum ether (60-80°C)-diethyl ether-acetic acid (75:25:1 v/v) as the developing solvent. Individual phospholipids, were separated from NL by TLC using chloroform-methanol-water (65:35:2 v/v) as the developing solvent. Lipids were extracted from the silica gel with the hydrolysing-methylating medium and for ST, with chloroform-methanol (2:1 v/v). At this stage, fractions which did not contain an internal standard had a known amount of n-heptadecanoic acid added. The lipids were hydrolysed and methylated in 5% H₂SO₄ in methanol and extracted into petroleum ether. The fatty acid methyl esters were analysed by GLC as described by Sklan, Budowski and Volcani (1971). The amounts of each fatty acid were calculated from the known amount of internal standard. The amounts of each lipid class were calculated from the sum of weights of component fatty acids. ST were measured by the colorimetric method of Searcy and Bergquist (1960), with sitosterol (Sigma) as standard.

Membrane and lipid fractions for measurements of fluorescence polarization. Fluorescence polarization was measured in the following membrane fractions: membranes, total lipids, PL and PL plus NL. The amounts of total lipids, PL and PL plus NL which were compared, or mixed together, were extracted from an equal number of flowers. The

total lipids were separated into polar lipids (mostly PL) and NL by adsorbing the PL on activated silicic acid. A sample of the lipids in chloroform was diluted to $10 \times 10^{-6} \text{ m}^3$ with chloroform and to this was added 1 g of activated silicic acid (100 mesh). The mixture was shaken and then centrifuged at 1000 g and the supernant collected. Another $10 \times 10^{-6} \text{ m}^3$ of chloroform was added to the precipitate and the above procedure repeated twice. The chloroform fractions containing NL were combined and evaporated to small volumes under nitrogen. To remove the adsorbed PL from the silicic acid $10 \times 10^{-6} \text{ m}^3$ of methanol was added and the mixture was held at 50°C for 30 min under nitrogen and shaken. the mixture was centrifuged at 10,000 g and the methanol fraction containing PL collected. This procedure was repeated twice and the PL fractions were evaporated to small volumes under nitrogen. The purity of NL and PL fractions was ascertained by TLC. For measurements of fluorescence polarization the lipids were formed into multiamellar liposomes by bath sonication of lipid extracts into Hepes buffer (10 mol m^{-3} , pH 7.5).

RESULTS

Membrane physical properties. During ageing of rose flowers for 2 d at 22°C , or for 10 d at 3°C , there were changes in the physical properties of petal microsomal membranes (Table 1). There was an increase in polarization of fluorescence from the DPH probe which indicates increased microviscosity, or decreased fluidity, of the lipids in the membranes (Shinitzky and Barenholz, 1978). There was an increase in the gel to liquid-crystalline phase transition temperature of the lipids isolated from the membranes.

Membrane Composition. The ratios of the major membrane components, lipid, PL and protein, did not change during 2 d at 22°C or 10 d at 3°C (Table 2). Further, the ratios of the major lipid classes in the membranes, PL, ST, FFA, triacylglycerols (TG) and sterol esters (SE) and the ST:PL ratio did not change during these times (Table 3). The fatty acid composition of the total membrane PL did not change during these times (Table 3). The fatty acid composition of the total membrane PL did not change during 2d at 22°C (Table 4). However, during 10d at 3°C there were decreases in the proportions of 16:0 and 18:1 fatty acids, an increase in 18:3 and a decrease in the ratio of saturated to

unsaturated fatty acids (Table 4). The relative amounts of the two major PL, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) did not change with flower age (Table 4). These two together made up ca. 90% of the PL; the contents of the minor PL were not measured. In the FFA there were small increases in the saturated fatty acids (16:0, 18:0) and a decrease in the unsaturated acids (18:2, 18:3) with flower age at 22°C and 3°C, but the overall ratio of saturated to unsaturated fatty acids did not increase significantly (data not shown). The fatty acid composition of the membrane TG and SE fractions did not change with flower age at 22°C or 3°C (data not shown).

Fluorescence polarization in membrane fractions. Since the membrane analysis did not indicate a chemical cause of the physical changes we tried an alternative approach to identify the membrane fraction reasonable. The membranes were separated into total lipid, polar lipid (mostly PL) and NL fractions and fluorescence polarization was measured in each fraction and in mixtures of fractions. Fluorescence polarization in lipids of whole membranes and in the total isolated lipids increased with flower age (Table 5). In the isolated PL, there was no increase in polarization with flower age, but when the PL fraction was added back to the PL (PL + NL) there was an increase with age (Table 5).

DISCUSSION

The changes described in the physical properties of microsomal membranes are the earliest observed events in rose petal senescence. The increase in lipid microviscosity (measured by fluorescence polarization) and phase transition temperature occur simultaneously and although they are physically different they are probably related and may well have the same chemical basis. For example, changes which rigidify the PL acyl chains would lead to both increased microviscosity and increased phase transition temperature, e.g., decreased PL:ST ratio, increased fatty acid and saturation, presence of proteins or certain NL (Shinitzky and Henkart, 1970; Quinn, 1981; Thompson et al. 1983).

In ageing rose petals the changes which lead to increased fluorescence polarization and phase transition temperature occur in the membrane lipids themselves since the physical changes were measured in isolated lipids as well as in whole membranes (Tables 1 and 5 and Faragher et al. 1987). The increase in fluorescence polarization appears to be caused by quantitative or qualitative changes in the NL fraction. It seems likely that the increase in lipid phase transition temperature is caused by the same change. This result is consistent with other findings that the increase in membrane lipid phase transition temperature during senescence of bean cotyledons is caused by the appearance of certain NL which rigidify the unsaturated acyl chains of PL (Barber and Thompson, et al. 1983). Such a mechanism would be expected to also increase lipid microviscosity, or decrease fluidity, a change which also occurs in bean cotyledons (McKersie et al., 1978). In bean cotyledons the active NL were tentatively identified as long-chain compounds, products of free radical-mediated lipid peroxidation, associated with increased peroxidase and decreased glutathione (reduced), superoxide dismutase and catalase levels (Barber and Thompson, 1983; Thompson et al., 1983; Pauls and Thompson, 1984). In roses there is evidence that endogenous ethylene is, at least in part, responsible for the increase in lipid phase transition temperature (Faragher et al., 1987). Ethylene could conceivably induce changes in enzyme activity which lead to lipid peroxidation.

We carried out preliminary experiments to investigate which NL compounds were responsible for the increase in fluorescence polarization in rose petal membrane lipids. We found that NL fractions which either co-chromatographed with ST, or were more polar than ST, on TLC caused an increase in fluorescence polarization with flower age. However, the identification of the active NL compounds requires considerably more work.

Our analyses failed to reveal major changes in lipid composition during flower ageing, for 2 d at 22°C or 10d at 3°C. For example, there were no changes in PL content or PL:ST ratio during these times. However, during later stages of rose petal senescence, after ca. 5d at 22°C, there was a decrease in the PL content of protoplast membranes and this apparently led to the increase in plasmalemma microviscosity which occurred at that time (Borochoy et al., 1978). Thus, it seems likely that during the first days of aging of cut roses changes in membrane physical properties result from changes in NL and that later there is decrease in membrane PL content which leads to a further increase in microviscosity.

The analyses also showed that there was an increase in unsaturated fatty acids of PL during 10d at 3°C, but not at 22°C. This would be expected to decrease lipid microviscosity and indeed there was a small, but non-significant ($P > 0.05$), decrease in fluorescence polarization of PL from flowers stored for 10d at 3°C. There were small increases in saturated fatty acids in the FFA. The likely effect of this is unclear but it could possibly increase lipid transition temperature (Jain and Wu, 1977). There were no changes in the protein content of membranes with flower age, but the absolute levels of protein measured are unusually low, presumably due to the method used.

Cold-storage was used as a second ageing treatment, for comparison with the events of senescence at 22°C. Previous experiments have shown that the events which occur during cold-storage are essentially the same as, but slower than, those which occur during senescence at 22°C (Faragher et al. 1986, 1987). This was confirmed here for the increases in membrane lipid fluorescence polarization and transition temperature and the changes in NL which apparently caused the increase

in fluorescence polarization. Cold-storage had an additional effect, to increase the unsaturation of fatty acids in the membrane PL. This is a typical cold-adaptation process in membranes (Quinn, 1981). However, this probably had little physiological impact since there was still an overall increase in fluorescence polarization in membrane lipids during 10 d at 3°C. Taken together these results support our general conclusion that the major effect of cold-storage on rose flowers is to allow continued ageing and senescence to occur and that cell membranes are a site of primary changes during ageing in cold storage (Faragher and Mayak, 1984; Faragher et al. 1986, 1987).

The probable sequence and mechanisms of senescence in rose petals can be summarized as follows. The earliest observed events are a quantitative or qualitative change in NL, possibly as a result of lipid peroxidation. These NL, and the later decrease in PL, lead to increased membrane microviscosity which will decrease the activity of membrane enzymes and transport systems and hence promote cell death (Shinitzky and Henkart, 1979). The NL may also cause the observed increase in lipid transition temperature and consequent lipid phase separation which would lead to the observed increase in membrane permeability (Faragher and Mayak, 1984, Faragher et al. 1987). Ethylene within the petals causes increased lipid transition temperature and increased membrane permeability (Faragher and Mayak, 1984; Faragher et al. 1986) and the mechanism of ethylene action could well involve formation of rigidifying NL.

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Table 1. Effects of flower age at 22°C and 3°C on physical properties of petal microsomal membranes and lipids.

Flower treatment	Fluorescence polarization ($'P'$)	Phase transition temperature ^{a,b} (°C)
Fresh	0.271 x	6.0 y
2d at 22°C	0.290 z	24.0 z
10d at 3°C	0.280 y	24.4 z

^a Fluorescence polarization was measured in membranes and phase transition temperature was measured in isolated lipids.

^b Means of four replicates; values in a column followed by a letter in common are not significantly different ($P < 0.05$, Duncan's Multiple Range Test).

Table 2. Effects of flower age at 22°C and 3°C on phospholipid (PL), protein and lipid content of petal microsomal membranes.

Flower treatment	PL/lipid ^a ($\mu\text{mol mg}^{-1}$)	PL/protein ^a ($\mu\text{mol mg}^{-1}$)	Protein/lipid ^a ($\mu\text{g mg}^{-1}$)
Fresh	0.55 x	58 z	9.5 z
2d at 22°C	0.49 z	62 z	7.9 z
10d at 3°C	0.58 z	66 z	8.8 z

^a Means of four replicates; values in a column followed by a letter in common are not significantly different ($P < 0.05$, Duncan's Multiple Range Test).

Table 3. Effects of flower age at 22°C and 3°C on lipid composition of petal microsomal membranes

Flower treatment	Lipid composition					
	PL (mol %)	ST	TG	FFA	SE	ST:PL (mol:mol)
Fresh	37 z	24 z	6 z	12 z	21 z	0.65 z
2d at 22°C	39 z	22 z	6 z	11 z	22 z	0.58 z
10d at 3°C	37 z	22 z	6 z	12 z	23 z	0.61 z

^a Means of four replicates; values in a column followed by a letter in common are not significantly different ($P < 0.05$, Duncan's Multiple Range Test).

Table 4. Effects of flower age at 22°C and 3°C on the fatty acid and phospholipid composition of microsomal membranes phospholipids^a

Flower treatment	Fatty acid composition ^b					PC:PE ^{a,b}	
	16:0 (ug %)	18:0	18:1	18:2	18:3	Sat:Unsat ^c (ug:ug)(mol:mol)	
Fresh	22 z	8 z	5 z	52 z	12 z	0.44 z	0.78 z
2d at 22°C	22 z	10 z	5 z	53 z	11 z	0.46 z	
10d at 3°C	17 z	8 z	1 z	56 z	18 z	0.34 z	0.82 z

^a The total phospholipids were separated into individual phospholipids and the ratio of phosphatidylcholine (PC): phosphatidylethanolamine (PE) was measured.

^b Means of four replicates; values in a column followed by a letter in common are not significantly different ($P < 0.05$, Duncan's Multiple Range Test).

^c Sat:Unsat is the ratio of saturated to unsaturated fatty acids.

Table 5. Effects of flower age at 22°C and 3°C on fluorescence polarization in petal microsomal membranes, lipids and lipid fractions^a

Flower treatment	Fluorescence polarization ('P')			
	Membranes ^b	Total lipids ^b	PL ^{a,b}	PL + NL ^{a,b}
Fresh	0.271 x	0.274 x	0.205 z	0.284 x
2d at 22°C	0.304 z	0.318 z	0.224 z	0.318 z
10 d at 3°C	0.280 y	0.302 y	0.220 z	0.303 y

^a The total lipids were separated into phospholipid (PL) and neutral lipid (NL) fractions.

^b Means of four replicates; values in a column followed by a letter in common are not significantly different $P < 0.05$, Duncan's Multiple Range Test).

IV. EFFECT OF TRANSIENT WATER STRESS

Transient water stress is a common occurrence during handling of flowers and ornamental plants. It is also an effector during the course of post-storage transportation and marketing. Thus the effect of transient water stress was studied in detail.

Transient water stress in carnation flowers: effect of amino-oxyacetic acid

S. MAYAK, A. BOROCHOV AND T. TIROSH

J. Exp. Bot. 36:171-183 (1987)

A short and temporary water stress imposed on cut carnation flowers (Dianthus caryophyllus L., cv. White Sim) flowers advanced senescence symptoms, including ethylene production and wilting. Pretreatment with amino-oxyacetic acid (AOA) resulted in an increase of the resistance of the flowers to water stress: water loss during stress was reduced, recovery was more rapid and wilting was delayed. Water stress accelerated the decrease in level of membrane phospholipids, but pretreatment with AOA counteracted this effect. Since the content of membrane sterols was not affected by the treatments, the mole ratio of sterol to phospholipid increased in water-stressed flower petals but not in stressed flowers pretreated with AOA. Membrane permeability and fluidity were also adversely affected by water stress and AOA: water stress alone resulted in an increase in permeability and a decrease in fluidity, but in AOA-pretreated stressed flower petals these parameters were similar to those of nonstressed control flower petals.

On the basis of these results two main conclusions can be drawn: (a) Water stress induces alterations in the physical and compositional properties of carnation petal membranes. (b) Pretreatment of the flowers with AOA influences petal membrane traits, most probably via modifications in phospholipid turnover, in a way which counteracts the effects of water stress.

Effect of water stress on ethylene production and on membrane microviscosity in carnation flowers

T. COKER, S. MAYAK, AND J.E. THOMPSON

Scientia Hortic. 27:317-324. (1985)

Carnation flowers subjected to two 12-h stress periods separated by a 12-h recovery period were visually indistinguishable from non-stressed flowers for up to 60 h after the termination of stress. During this time-period, no differences were observed in water saturation deficit (WSD), ethylene (C_2H_4) production or degree of depolarization for microsomal membranes ('P'). The only detectable effect of the stress periods was accelerated onset of senescence. After the onset of senescence, as determined visually by petal inrolling and fading of colours, there was a simultaneous increase in the three parameters (WSD, C_2H_4 production and 'P'), suggesting that they are interrelated.

In flowers subjected to continuous stress, the usual visual symptoms associated with senescence were not observed, but flower petals dried out and shriveled. The WSD of the petals and 'P' values reflecting degree of depolarization of microsomal membranes began to rise within 24 h of the start of stress. However, C_2H_4 production did not increase until after 48 h, thus apparently ruling out a simple cause and effect relationship between WSD, 'P' and C_2H_4 production. Generally, the measurements of C_2H_4 production in both petals and green tissue in response to stress confirmed reports suggesting that the accelerated onset of senescence in response to stress is not directly mediated by C_2H_4 .

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Title of the research
STORAGE AND SURFACE TRANSPORTAION OF CUT FLOWERS
AND ORNAMENTAL PLANTS

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Project's commencement Date: Dec. 1983

Type of Report: Initial Second Year Final

Signature

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Institution's Authorized Official

E. WORK CARRIED IN THE USA

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Research was concentrated on the response of ornamental crops to postharvest storage and related treatments.

1. THE RESPONSE OF PLANTS TO CHILLING TEMPERATURES

Storage of pot plants and cut flowers, almost always, involved periods at low temperatures but above freezing. This is often resulted in chilling injuries to the tissue. A study of the mechanisms involved was undertaken using foxglove, episcia, veronica watermelon and tomato as model plants with different degrees of chilling sensitivity:

Response to chilling stress in plant cells I. changes in cyclosis and cytoplasmic structure

C.M. WOODS, M.S. REID AND B.D. PATTERSON

PROTOPLASMA 121:8-16 (1984).

Cytoplasmic structure and rates of cyclosis in trichomes from chilling-sensitive watermelon (*Citrullus vulgaris* L), tomato (*Lycopersicon esculentum* Mill.) and *Episcia reptans* plants and from chilling-resistant foxglove (*Digitalis purpurea*) and *Veronica persica* were examined with differential interference contrast optics (DIC) as the temperature of the microscope stage was lowered. Below the chilling threshold, the rate of streaming in chilling-sensitive species fell markedly. At chilling temperatures the complex network of transvascular strands in the cytoplasm disappeared and the cytoplasm became vesiculated. During rewarming of the chilled cells, the vesicles fused into pleiomorphic blebs, which gradually stretched out into fully functional strands. These events were not seen during the chilling and rewarming of chilling-resistant plant cells.

Similar inhibition of cyclosis and changes in cytoplasmic structure were observed in cells from all species studied when they were treated with the action inhibitor, cytochalasin B, or with uncoupling agents. Phalloidin had no detectable effect. Cyclosis in colchicine-, nocodazole-, trifluralin- and IPC-treated cells was not affected for many hours and did not cause the structural changes seen with chilling. The possible role of action in these low-temperature effects on cytoplasmic structure and function is discussed.

RESPONSE TO CHILLING STRESS IN PLANT CELLS II. REDISTRIBUTION OF INTRACELLULAR CALCIUM

C.M. WOODS, V.S. POLITO, AND M.S. REID
PHOTOPLASMA 121, 17-24 (1984).

We have investigated the possibility that the rapid low temperature effects upon cyclosis and subcellular structure might be due to a breakdown in compartmentation of intracellular calcium, leading to an increase in cytoplasmic Ca^{+2} . Changes in fluorescence of chloretracycline (CTC), a probe for membrane-bound Ca^{+2} were monitored in the corners of individual trichome cells (effective spot size ca. 800 square microns) with the aid of a Zeiss epifluorescence microscope linked to a Zeiss Zonax analyzing system. A consistent decrease in signal was observed as cells of chilling-sensitive *Lycopersicon esculentum* Mill. (cv. ace) were cooled below their threshold temperature for chilling sensitivity. On rewarming, as the temperature rose above the chilling threshold, there was an increase in fluorescent signal. In contrast, trichomes of *Digitalis purpurea* (chilling-resistant) showed no such changes. The uncoupling agent, CCCP, and the Ca^{2+} -chelator, EGTA, induced marked decreases in the fluorescent signal in cells from both species. A more direct approach to testing the hypothesis was to examine the effect of modulating cytoplasmic Ca^{+2} with the aid of the Ca^{+2} -ionophore A 23187 and a Ca^{+2} concentration series in EGTA buffer. Above 10^{-8} M external free Ca^{+2} , streaming began to be inhibited, full inhibition occurring at 5×10^{-6} M Ca^{+2} . The strand structure started to disappear when the Ca^{+2} rose above 10^{-7} M. Disappearance of strands was accompanied by an increase in the number of cells with vesiculated

cytoplasm, an effect analogous to that of chilling temperatures on these wells. The phenothiazines, trifluorperazine and chlorpromazine (10^{-5} M) had similar effects. However such effects were not seen with R 24571 or N(6-aminoethyl)5-chloro-1-naphthalenesulfonamide (W7) until concentrations were reached that orders of magnitude above their IC50 for calmodulin.

DIURNAL CHILLING SENSITIVITY AND DESICCATION IN SEEDLINGS OF TOMATO

A.I. KING AND M.S. REID

J. Amer. Soc. Hort. Sci. 112:821-824 (1987).

Seedlings of six cultivars of tomato (*Lycopersicon esculentum* Mill.) showed differences in chilling sensitivity depending on the time during the diurnal light/dark cycle when they were placed at low temperature. In all cultivars, sensitivity was highest when the chilling exposure started at the end of a dark period, although the magnitude of the diurnal variation differed among the cultivars. When roots were removed prior to or just after chilling, injury also appeared earlier in seedlings whose chilling was started at the end of a dark period. Seedlings chilled starting at different times during the diurnal cycle had similar stomatal conductance values during chilling. It appears that diurnal changes in chilling sensitivity are not due to altered root function during or following chilling exposure, nor to variations in desiccation during the chilling period resulting from diurnal differences in stomatal conductance.

II. VASCULAR BLOCKAGE

During and following storage, vascular blockage of stems occur. The role of microorganisms and air embolism in this process was evaluated in the following works:

EVALUATION OF THE ROLE OF VASE MICROORGANISMS IN THE POSTHARVEST LIFE OF CUT FLOWERS

D. ZAGORY AND M.S. REID

Acta Hortic. 181:211-217 (1986).

Three of twenty-five microorganisms isolated from carnation vase solutions reduced carnation (*Dianthus caryophyllus* L. cv. Improved White Sim) vase life when added individually to vase solutions. Some of these same microorganisms reduced the vase life of rose (*Rosa hybrida* cv. Cara Mia) and chrysanthemums (*Chrysanthemum x morifolium* Ramat. cv. May Shoesmith). Neither differences in initial microbial inoculum nor change in vase solution pH were correlated with vase life of carnations. Repeated cutting of the stem base did not ameliorate the effects of the microorganisms. Silver thiosulfate pretreatment of cut carnations delayed the effects of an ethylene producing bacterium but not of non-ethylene producing bacterium.

Using isogenic bacterial lines produced through transposition mutagenesis, we are testing hypotheses to explain the activity of these bacteria against cut flowers.

ROLE OF VASE SOLUTION MICROORGANISMS IN THE LIFE OF CUT FLOWERS

D. ZAGORY AND M.S. REID

J. Amer. Soc. Hort. Sci. 111:154-158 (1986)

Out of 25 microorganisms commonly occurring in carnation (*Dianthus caryophyllus* L. 'Improved White Sim) vase solutions, 3 greatly reduced carnation vase life when added individually to vase solutions. Some of these microorganisms also reduced the vase life of roses (*Rosa hybrida*

'Cara Mia'), chrysanthemums (*Chrysanthemum x morifloium* Ramat. 'May Shoesmith') and other cultivars of carnation ('Improved Red Sim' and 'Improved Pink Sim'). The microorganisms that reduced flower vase life were tentatively identified as a yeast, a fluorescent pseudomonad and a nonfluorescent pseudomonad. A 10-fold reduction in the initial inoculum level affected neither the final microbial concentrations nor the vase life of the test flowers. The effects of the microbes were not correlated with their effects on solution pH.

SENESCENCE AND VASCULAR BLOCKAGE OF DETACHED PLANT PARTS

W.G. VAN DOORN AND M.S. REID

Proc. 10th annual Symposium in Plant Physiology, Univ. of California, Riverside, Ed.w.W. Thomson et al. (1987)

Plant senescence is frequently studied in detached systems, such as leaf discs, and explants. In cut flowers, wilting commonly is considered as a symptom of senescence, but may simply reflect an inability of the stem to conduct water. Since unfavorable water relations may influence senescence processes, we have examined some factors influencing the water relations of cut flowers of two rose cultivars (*Rosa hybrida* cvs Sonia and Cara Mia). Although flowers of both cultivars took up about 50 ul of air in the first 10 min after cutting, they differed in their ability to bypass the resulting emboli when they were placed in water. Sonia roses showed a large transitory increase in water uptake, after which uptake continued at a substantial rate. In Cara Mia flowers, water conductivity remained below the rate of transpiration, which explains why flowers of this cultivar often wilt rapidly. If their stems were recut under water, Cara Mia flowers showed a similar uptake pattern to that of Sonia flowers. During a study of extended water relations in Sonia flowers, it was found that stem water conductivity fell as bacterial populations in the stem increased. Bacteria were largely confined to the lower 5 cm of the stem. Micrographs showed plugging of vessels and vessel pits by bacteria and by bacterial products in such flowers. These results indicate that the ability of cut plant parts to take up

water may be substantially affected, first by air emboli formed in the vascular system after detachment, and then by the rapid growth of bacteria. Because of the drastic effect of the resulting poor water relations on hormonal levels and plant function, the results of studies of senescence using detached plant parts should be interpreted with care.

III. ABSCISSION

Abscission of leaves and petals is frequently the terminating process of plant life. This phenomenon is inhibited by an exogenous application of several growth regulators including STS:

ANATOMICAL AND PHYSIOLOGICAL EFFECTS OF SILVER THIOSULFATE ON ETHYLENE-INDUCED ABSCISSION IN COLEUS

L. MORRISON BAIRD, M.S. REID, AND B.D. WEBSTER
J. of Plant Growth Regul. 3:217-225, 1984.

Petioles of explants of *Coleus blumei* Benth. exposed to 20 μ l/l ethylene abscised within 36 h. Pretreatment of explants with 4 mM silver thiosulfate (STS) inhibited ethylene-induced abscission. Delaying treatment with STS reduced its effectiveness in retarding ethylene-promoted abscission, suggesting that some events leading to abscission are initiated during the first hours of ethylene treatment. Microscopic study of abscission zones of ethylene-treated explants showed greatly increased amounts of rough endoplasmic reticulum, disruptions of the plasma membrane, and some cell separation in the region of the middle lamella. Pretreatment with STS prevented ethylene-induced reorganization of the endomembrane system and the subsequent middle lamellar dissolution.

THE ROLE OF PLANT HORMONES IN THE POSTHARVEST LIFE OF CUT FLOWERS

D.M. GOSZCZYNSKA, R.M. RUDNICKI, M.S. REID,
Acta Hortic. 167, (1985)

The present state of information as to the role of each of the five major classes of plant hormones in the development and senescence of cut flowers is reviewed. In addition, the status of the 'ovary factor' and the role of growth regulators in pollination-induced senescence are discussed. Much information has been accumulated in the last five years showing the importance of ethylene in the senescence of many flowers. The control of its biosynthesis during their senescence is now well understood, and the use of silver ion to inhibit its action has radically change the handling and possible longevity of such flowers. The role of the other growth regulators in flower senescence remains controversial, and with the exception of auxin dips to delay floret and petal abscission, their practical use is minimal. A study of the many species of flowers not sensitive to ethylene may reveal more as to the role of other regulators in flower senescence.

GROWTH REGULATORS AND ABSCISSION IN ORNAMENTALS

M.S. REID, D.M. GOSZCZYNSKA,
Acta Hortic. 167, 1985

The loss of leaflets, leaves, petals, florets or flowers can be a serious problem during production and marketing of many cut flowers and potted plants. Recent advances in our understanding of the physiology and biochemistry of these events, and of the role of different natural growth regulators in their expression is reviewed. The anionic silver thiosulfate complex has become an important tool in preventing abscission in ornamental plants. The action of this material and its practical applications are discussed.

IV. ETHYLENE BIOSYNTHESIS AND RESPONSE TO ETHYLENE

STS is a very potent inhibitor of ethylene action which, in turn, is known to be intimately involved in flower senescence. It was logical, therefore, to pursue studies of ethylene synthesis in relation to flower development and senescence:

Control of ethylene synthesis during development and senescence of carnation petals

C.S. WHITEHEAD, A.H. HALEVY AND M.S. REID
J. Amer. Soc. Hort. Sci. 109:473-475 (1984)

Ethylene synthesis by detached preclimacteric carnation (*Dianthus caryophyllus* L. cv. White Sim) petals was stimulated slightly when they are infiltrated with a high concentration of 1-aminocyclopropane-1-carboxylic acid (ACC). Similar infiltration of petals at daily intervals did not change their age-dependent pattern of ethylene evolution. Ethylene production increased more than 1000-fold during senescence, but the ACC concentration in petals increased only 3-fold (to 3 nmol.g⁻¹ fresh weight). The concentration of conjugated ACC was generally lower than that of free ACC, but the pattern of changes in its concentration was similar to that of the changes in ACC concentration over time. Unlike in many other tissues, the rate of ethylene production in aging carnation petals is not limited by the availability of free ACC. The pattern of ethylene production by individual petals was similar, whether the petals were removed from the outer or the ontogenetically younger inner portion of the flower.

1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) - TRANSMITTED STIMULUS IN POLLINATED FLOWERS?

M.S. REID, D.W. FUJINO, N.E. HOFFMAN, AND C.S. WHITEHEAD
J. Plant Growth Regul. 3:189-196 (1984).

Ethylene production and senescence of petals of pollinated carnation flowers were not prevented by removal of the ethylene produced by the gynoecium, suggesting that these events are a response to movement from the gynoecium of some stimulus other than ethylene gas.

Application of 1-aminocyclopropane-1-carboxylic acid (ACC) to the stigmas caused an initial increase in gynoecium and petal ethylene production similar to that reported for pollinated flowers. This response was not seen in flowers whose stigmas were treated with indoleacetic acid (IAA). When [2-¹⁴C] ACC was applied to the stigmas of carnation flowers, radioactive ethylene was produced both by the gynoecia and by the petals. The possibility that ACC, transported from the stigmas to the petals, is responsible for the postpollination changes in carnation flowers is discussed.

CONTROL OF EARLY ETHYLENE SYNTHESIS IN POLLINATED PETUNIA FLOWERS

J.C. Pech, A. Latche, C. Larrigaudiere and M.S. Reid
Plant Physiol. Biochem. 25(4):431-437 (1987)

Ethylene production by stigmas of petunia (*Petunia hybrida* L. cv. Pink Cascade) increases within five minutes of the application of pollen. This rapid increase is prevented by prior application of an inhibitor of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase [(2-aminoethoxyvinyl)-glycine, AVG], and by an inhibitor of protein synthesis (cycloheximide) but not by inhibitors of DNA-dependent RNA synthesis (α-amanitin, actinomycin-D). Activity of ACC synthase in the stigma increases following pollination, but remains low in the style. The ethylene-forming enzyme (EFE) is constitutive in the stigma, and has highest activity at anthesis. The EFE is shown to be located very close to the surface of the stigma. The high osmolarity of the

stigmatic exudate (>4 Osm) appears to restrict diffusion of ACC from the pollen to the stigmatic EFE. Application of ethylene to the stigmas of unpollinated flowers does not result in the acceleration of senescence which follows pollination.

The binding of ethylene to carnation petals was studied in relation to the proposed role of this process in ethylene action:

EFFECT OF ANTAGONISTS OF ETHYLENE ACTION ON BINDING OF ETHYLENE IN CUT CARNATIONS

E.C. SISLER, M.S. REID AND S.F. YANG.

Plant Growth Regul. 4:213-218 (1986).

Five hours after cut carnations had been treated with a pulse of 1 or 4mM silver thiosulfate (STS), in vivo ethylene binding in petals was inhibited by 22 and 29%, respectively. When binding was measured 4 days after the 4mM STS treatment, binding was inhibited by 81%, 2,5-Norbornadiene, which substantially delays carnation senescence, inhibited ethylene binding by 41% at a concentration of 1000 μ l/l. The K_d for ethylene binding in carnations was estimated to be 0.1 μ l/l in petals and 0.09 μ l/l in leaves. The concentration of binding sites was estimated to be 6.0×10^{-9} mol/kg of petals and 2.0×10^{-9} mol/kg of leaves.

V. FLOWER DEVELOPMENT

It is a common practice to store cut flowers at a tight bud stage. This is followed with chemical treatments intended to achieve a proper development of the flowers. The control mechanisms of flower development differ in different flower types:

CONTROL OF CUT FLOWER OPENING

M.S. REID AND R.Y. EVANS.

Acta Hortic. 181:45-54 (1986)

The postharvest life of many flowers includes a period during which the flower opens from the bud or near-bud state. New techniques for handling of cut flowers may include greater use of bud harvest and storage, and would therefore require better understanding of the mechanisms of bud opening. These mechanisms vary for different flowers, and include: growth of the petals (as in carnations, chrysanthemums and roses); reorientation of the petals (as in many members of the Compositae); growth of the pedicel (as in iris); reorientation of the pedicel (as in bird-of-paradise); untwisting of the petals (as in cyclamen), and abscission of the calyx to allow unfurling of fully developed petals (as in poppies). These processes are sensitive to, and may even be controlled by, a range of environmental influences, notably temperatures, water supply, carbohydrate supply, and light.

In roses, normal development of the flower is a rhythmic phenomena, largely manipulated by environmental conditions:

CONTROL OF PETAL EXPANSION DURING DIURNAL OPENING OF ROSES

R.Y. Evans and M.S. Reid

Acta Hortic. 181:55-63 (1985)

Time-lapse cinematography was used to examine the opening of cut rose flowers. Flowers opened in a rhythmic fashion, starting before dawn

and continuing rapidly for about 5 hours. No further opening then occurred until the following dawn. This rhythmic opening appears to be controlled by the day/night rhythm - it could be abolished by placing flowers in continuous light or dark.

The daily pulses of flower opening were associated with increased flower diameter, fresh weight, dry weight, and petal surface area. Petal growth occurs sequentially, beginning in the outermost whorl of petals and proceeding to the inner whorls.

Petal expansion appears to be due to a pulsed increase in concentrations of soluble sugars in the petal cells, presumably from hydrolysis of petal starch. The starch content of the outermost whorl declined substantially shortly before the onset of petal expansion, whereas the starch content of inner whorls remained constant during this time.

The understanding of the opening process lead us to an establishment of a detailed protocol for the treatment of rose flowers, harvested at a tight bud stage:

STUDIES OF THE DEVELOPMENT OF TIGHT CUT ROSE BUDS

D.M. GOSZCZYNSKA, M.S. REID

Acta Hortic. 167:101 (1985)

Tight rose (Rose Tea Hybrids) buds of cultivar Sonia were developed to commercial maturity in a vase solution containing 8-hydroxyquinoline citrate (300 ppm) and sucrose (2%). In very tight buds (19:13 mm and showing no colour) development included substantial growth of the peduncle. The subsequent vase life of flowers obtained from buds was similar to that of those harvested at commercial maturity. The buds' development was strongly inhibited by low concentrations of ethylene, but this effect could be prevented by pretreating the buds with the anionic silverthiosulfate complex.

IV. HANDLING PROCEDURES OF CUT FLOWERS

These findings opened the way to the formulation of pulse treatments of chemicals to several cut flowers, resulting in appreciable improvement in quality.

PULSE TREATMENTS WITH SILVER THIOSULFATE AND SUCROSE IMPROVE THE VASE LIFE OF SWEET PEAS

Y. MOR, M.S. REID AND A.M. KOFRANEK

J. Amer. Soc. Hort. Sci. 109:866-868 (1984).

Senescence of sweet pea (*Lathyrus odoratus* L.) flowers was associated with a climateric rise in ethylene (C_2H_4) production. Pretreatment for 8 min with 4 mM silver thiosulfate (STS) double the vase life of the flowers and enhanced opening of buds on the spike. An overnight pulse at 20°C with 4% sucrose also promoted bud opening. A combined STS and sucrose treatment improved flower quality by promoting bud opening of spikes cut with tight florets, and by delaying floret senescence and abscission in both fresh and stored flowers. Aminooxyacetic acid (AOA) was less effective than STS in extending the vaselife of sweet peas.

POSTHARVEST HANDLING OF LILY OF THE NILE FLOWERS

Y. MOR, A.H. HALEVY, A.M. KOFRANEK AND M.S. REID

J. Amer. Soc. Hort. Sci. 109:494-497 (1984)

A range of postharvest treatments was applied to cut flowers of the Lily of the Nile, *Agapanthus orientalis* Hoffmanns cvs. Mooreanus (blue) and Albidus (white), in an effort to improve flower bud opening, vase life, and to reduce floret abscission. Basal treatment of partially opened flowers (2 to 5 florets open) with solutions containing 10-20% sucrose and a bactericide improved bud opening. A 60% to 120% increase in vase life and a substantial reduction in bud abscission (20-20% of the control) was obtained by treating the

flowers with the anionic silver-thiosulfate complex (STS) as a basal 'pulse' (4 mM Ag^+ , 3h), and spraying the inflorescence with 30 ppm naphthalene acetic acid (NAA) prior to the sucrose pulse. These treatments also were effective in flowers subjected to short-term cold storage (4 days at 1°C).

F. DESCRIPTION OF THE COOPERATION

During the course of this project, a continuous coordination and exchange of information between the two laboratories took place. This include intensive correspondence and visits of the investigators in each other's laboratory: M.S. Reid visited Israel and S. Mayak stayed in Davis during 1985. In each of these visit reserch was conducted and information was exchanged.

Already in an early stage of the project it was agreed that in order to avoid overlapping of efforts, most of the in-vivo studies of ethylene synthesis and action and also the studies related to chilling will be done in Davis, while the work related to in-vitro studies of ethylene synthesis and that on effects of senescence and stresses on flower metabolism and membranes will be done mostly in Rehovot. As seen in the body of the report, we succeeded in keeping our work in this frame, together with producing a large volume of scientific work.

G. RESEARCH ACHIEVEMENTS

The research addressed several major questions which are related to the postharvest of ornamental plants. Ethylene biosynthesis by flowers was studied in depth, and also its mode of action. As a result, new techniques were developed for the application of ethylene inhibitors to cut flowers and pot plants. In fact, most of the carnation exported from Israel today are pretreated with STS using protocols which were developed during this project. Storage of cut flower storage is gaining popularity with growers in Israel and the US. Without any doubts it relates to more reliable methods that were developed to store flowers. Our present knowledge of the effects of low temperature and water stress helped to achieve this end. The most important achievement of this project is in the better understanding we have now of the basic mechanisms which control flower senescence. We are sure that it will improve our capabilities to control these processes and also to apply this know-how to other economically important plant organs.

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