Postharvest Characteristics of Cut Flowers of Selected Members of the Family Myrtaceae

A thesis presented in fulfilment of the requirements for the degree

of

Master of Philosophy

at

Massey University

New Zealand

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1999

ABSTRACT

Stages of floral development were described for *Eucalyptus ficifolia* and *Metrosideros collina* 'Tahiti' flowers (Myrtaceae) attached and detached from plants. Vase solution treatments were applied to promote bud opening of cut flowers and to prevent postharvest stamen wilting and abscission in both species. Water uptake and mass of harvested flowers in both species declined rapidly when the pedicels were placed in water (control). Some flower buds did not open after harvest. The decline in water uptake and flower mass was greatly reduced by a vase solution treatment containing 2% sucrose, and 200 ppm hydroxyquinoline citrate (HQC) adjusted to pH 4 using citrate buffer. Vase solutions containing higher sucrose concentrations (more than 6%) and of greater acidity (pH<4) were not beneficial for vase life of both species.

Cut flowers of both species held in the standard solution (2% sucrose, 200 ppm HQC adjusted to pH 4 using citrate buffer) were treated with ethephon (0-10,000 ppm) following pre-treatment with silver thiosulphate (STS) (0-2.0 mM). Ethephon treatments significantly induced stamen wilting, but had no effect on stamen or petal abscission in both species. Pre-treatment with 2 mM STS had no effect on the rate of stamen wilting, but significantly reduced stamen or petal abscission in both species.

Cut flowers of *M. collina* 'Tahiti' held in the standard solution were treated with ethylene (0-5 ppm). Exogenous ethylene significantly promoted abscission of stamens and petals in *M. collina* 'Tahiti'. Treatment with 0.5 and 5 ppm ethylene also induced flower abscission. Ethylene emanation from untreated cut flowers from plants grown in two environments (greenhouse and outside) was also measured. Untreated cut flowers harvested from plants grown outside produced more endogenous ethylene than those from plants grown in the greenhouse. The abscission of *M. collina* 'Tahiti' probably results from a relatively high sensitivity to ethylene.

ACKNOWLEDGEMENTS

I would like to thank the many people who have contributed in their own way to my thesis.

I am very grateful for the efforts of my supervisors in helping me complete this work. Dr. John Clemens provided helpful guidance, patience and encouragement throughout this research project. Dr. John Clemens and Professor Paula Jameson gave valuable writing and editorial comments on drafts of this thesis, and were available and always willing to help solve my problems.

I would like to thank Mrs. Sue Nicholson at Institute of Food, Nutrition and Human Health, Massey University, for providing me facilities for measuring ethylene, and her friendly guidance.

I greatly appreciate the help from Plant Growth Unit staff, especially Mr. Ray Johnstone.

My profound thanks to the staff and my fellow students of the Institute of Molecular BioSciences, who through their support and friendship have given me a most enjoyable time. I would especially thank Mrs. Geraldine Wood, Mrs. Charlotte James, Mrs. Rosanne Churchouse, Robert Henriod, Deming Gong and Celia Zhang.

I gratefully acknowledge the award of the J. P. Skipworth Scholarship (Plant Biology) 1998 to undertake this study.

Finally, I would also like to thank my husband, Deping Song, and son, Roland, for their love, moral support, encouragement and understanding during the course of my study.

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Chapter One
Introduction

Myrtaceae is a large family consisting of about 3,500 species in about 150 genera. The family is distributed throughout Central and South America, Central and South Africa, Europe, Asia, various Pacific Islands (including New Zealand) and Australia. The species are mainly shrubs or trees, rarely climbers. The family Myrtaceae includes many highly ornamental species which are popular in parks and gardens (Elliot and Jones, 1993). For example, *Eucalptus ficifolia*, a large tree native to Australia, is commonly grown in New Zealand, and is arguably the most valued of the flowering *Eucalyptus* (Palmer, 1990; Elliot and Jones, 1993).

Horticulturally, *Metrosideros collina* 'Tahiti' is one of the most useful smaller growing cultivars derived from this Pacific Island species. Brilliant orange-red blooms appear intermittently through the year, but particularly during winter months. Expanding buds through winter have a striking red colour. 'Tahiti' is quite distinct from the more familiar *Metrosideros* in both size and its well-rounded shrubby form. The plant is also becoming important as a flowering pot plant in New Zealand (Elliot and Jones, 1993).

Within the family Myrtaceae, the main postharvest and display problem is flower and floral organ abscission (Joyce, 1993). Although no information is available on the postharvest characteristics of *Eucalyptus* and *Metrosideros* flowers, several genera and species in the family Myrtaceae have been extensively studied. Preliminary investigations revealed that ethylene is an important factor in the postharvest life of a number of native Australian Myrtaceae, including *Chamaelaucium uncinatum* (Joyce, 1988). The most successful treatment for preventing postharvest floral organ abscission of *C. uncinatum* was by blocking perception of ethylene using pulses of silver thiosulphate (STS) (Joyce, 1988). Further, Joyce (1993) stated that flower abscission in *C. uncinatum* induced by water deficit could be reduced by pre-treatment with a pulse of STS. Ethylene also appeared to be involved in flower abscission of *Leptospermum scoparium* (Burge et al., 1996). Ethylene

induced flower abscission under high humidity conditions, while STS treatments prevented ethylene-induced flower abscission in this species (Zieslin and Gottesman, 1983).

The short vase life of *L. scoparium* appears to be principally the result of a rapid decline in water uptake and even more rapid water loss leading to desiccation. Improving the water relations of stems is likely to reveal other limiting factors (such as ethylene sensitivity). Pulsing flowers of *L. scoparium* with STS had little effect on flower senescence. Sucrose reduced the rate of flower senescence and when combined with hydroxyquinoline sulfate (HQS) extended the vase life from 3d to 9d in this species (Burge et al., 1996). Holding solutions containing 1% or 2% sucrose and 8-hydroxyquinoline citrate (8-HQC) at 200 $mg \cdot L^{-1}$ significantly increased the vase life of *Eucalyptus globulus* and *E. cinerea* foliage (Wirthensohn and Sedgley, 1996). Staden and Slootman (1976) observed that placing the stems of *E. fulgens* in citric acid at pH 2.8 for 1 h prevented the flow of latex and extended vase life without stem damage.

The objectives of this research were to extend the longevity of cut inflorescences of *E. ficifolia* and *M. collina* 'Tahiti' and to investigate the role of ethylene in the abscission of floral organs. The hypothesis that improved water balance would extend cut inflorescence longevity was tested by including sucrose and/or acid in the vase solution. Attempts to decrease floral organ abscission were made by attempting to block the effects of ethylene with STS.

Chapter Two
Literature Review

2.1 Flower Senescence Phenomena

Senescence refers to processes that follow physiological maturity and lead to the death of a whole plant, organ, tissue, or cell (Mayak and Halevy, 1980). The flower provides an excellent organ for the study of senescence. Flower senescence is generally rapid and predictable (Borochov and Woodson, 1989). However, the 'flower' includes in many cases a green calyx, achlorophyllous corolla, androecium, gynoecium, and a peduncle. Each of these components is complex in its own right, and they all differ from each other both in structure and physiology.

The interrelationships between the various flower parts may determine their rate of senescence. For example, senescence may occur in one part of a plant and not in another, such as in a flower where the petals and stamens senesce while the ovary starts to grow (Mayak and Halevy, 1980). Pollination of flowers often results in very rapid and controlled senescence of the petals, such as in orchid (Arditti and Flick, 1976) and petunia (Borochov and Woodson, 1989). However, not all flower parts follow the same path of senescence. In some plants, the green sepals persist after the petals have wilted or abscised, and may even continue to photosynthesise (Mayak and Halevy, 1980).

Symptoms of senescence vary in different flowers or different parts of flowers. One of the most obvious symptoms of the final stage of senescence in petals of the morning glory (*Ipomoea purpurea*) is drying and shriveling (Matile and Winkenbach, 1971). In cut *Iris* flowers, visible senescence symptoms start at the edges of the lower whorl of tepals. Edges show inrolling and discolouration. At a late stage of senescence the colour of the tepals completely disappears (van Doorn et al., 1995).

Colour fading (and discolouration) is a common phenomenon in many flowers and parts of flowers during senescence. The two major types of pigments which contribute to the

colour of the flowers are the carotenoids and anthocyanins. An increase in the concentration of oxygenated carotenoids with age was found in *Strelitzia* as well as in rose flowers (Valadon and Mummery, 1969; Simpson et al., 1975). However, much more is known about the change in pigmentation due to anthocyanins. The pigment level stays stable in some flowers (Packet, 1966; Stead and Moore, 1977), and declines drastically in others (Stickland, 1972), whereas in some flowers a dramatic synthesis of anthocyanins is evident (Arditti and Knauft, 1969). In the "Masquerade" rose, the petals are orange-yellow when freshly opened and turn deep red upon senescing. More than a ten-fold increase in anthocyanin level was measured during this period (Shisa and Takano, 1964). The most important factor determining the colour change in senescing petals seems to be a change in the pH of the vacuole (Stewart et al., 1975; Barthe et al., 1991a).

Respiratory metabolism of cut flowers was reviewed by Coorts (1975). The rate of respiration in many flowers rises to a maximum as flowers start to open, followed by a gradual decline as flowers mature and senesce.

Loss of water from the aging petals occurs when the cut flowers are held in water. A decline in water potential (ψH) did not occur in flowers that senesced on cut rose (Mayak et al., 1974; Le Page-Degivry et al., 1991). On the other hand, the drop in FW:DW (ratio of fresh weight to dry weight) and in ψH observed in the later phase of flower vase life reflected the development of water deficit. A direct measure of these two parameters is a good indicator of water status and thus of rose senescence (Barthe et al., 1991b). Flower senescence is accompanied by various functional changes in the properties of petal cellular membranes, including permeability, enzyme activity, solute uptake capacity and hormone binding (Borochov and Woodson, 1989; Borochov et al., 1995). The cell sap properties (pH, conductivity, osmolarity) prove to be good indicators of senescence in cut rose flowers (Durkin et al., 1991).

Members of each of the five groups of plant hormones have been implicated in the regulation of senescence. However, ethylene has been dealt with most extensively with

respect to flower senescence. Ethylene is the major factor regulating the senescence of flowers such as carnation (Nichols, 1971), petunia (Whitehead et al., 1984), and some orchids (Goh et al., 1985), which show a climacteric rise in ethylene production after pollination or at the onset of senescence (Han et al., 1988). In these species, ethylene production was found to increase during senescence and exposure to ethylene accelerated flower senescence. Application of ethylene also catalyzes the synthesis of ethylene by some plant tissues. This phenomenon is termed autocatalysis and is common in senescing flowers (Burg and Dijkman, 1967; Nichols, 1968; Kende et al., 1974). Senescence of these types of flowers can generally be delayed by inhibition of ethylene biosynthesis or ethylene action (Borochov and Woodson 1989; Reid and Wu 1992). There are, however, a number of flowers whose senescence is neither associated with, nor stimulated by ethylene. Woltering and van Doorn (1988) studied the relationship between petal senescence and ethylene sensitivity. They found that petal wilting was the primary senescence symptom in flowers with low sensitivity to ethylene. Ethylene-insensitive flowers include important bulb flowers, such as *Gladiolus*, *Iris*, *Narcissus* and *Tulipa* (Jones et al., 1994), In addition, Eason and Vre (1995) showed that flowers of *Sandersonia aurantiaca* were ethylene-insensitive and STS treatment did not extend the display life of these flowers.

2. 2 Flower and floral organ abscission

Abscission of plant parts is, by definition, due to enzmatic dissolution of the cell walls, and hence is an active physiological process (van Doorn et al., 1997). Abscission of plant reproductive organs, both inflorescences, individual flowers, and flower parts is widespread. Most conspicuous is petal fall but, depending on the species, floral parts such as sepals, styles, and stamens may also fall, and in some flowers the bracts that enclose the unopened bloom abscise (van Doorn et al., 1997).

Abscission of flower and floral organs

The effect of pollination or fertilization on abscission of flower and flower parts differs between species. In many species, pollination and especially fertilization promotes the abscission of flower parts, especially the petals (McGranahan et al., 1994). In some species,

flower shedding is due to the absence of pollination or fertilization. In these species the presence of a single embryo prevented flower fall. It was concluded that in some species pollination, in the absence of fertilization, was sufficient to prevent flower fall (van Doorn et al., 1997). However, Becquerel (1907) described fertilization to be a prerequisite for preventing flower fall in several *Nicotiana* species. Similarly, the unfertilized flowers at the top of the raceme are often shed in legume species (Clifford et al., 1992). Fertilized flowers may also abscise in legumes such as *Glycine max* (Abernathy et al., 1997) and *Vicia faba* (Chapman et al., 1979; Gates et al., 1981). In cocoa (*Theobroma cacao*) up to 98% of fertilized flowers reportedly fall.

The stem segment subtending individual flowers is called the pedicel, and often contains a morphologically distinct abscission zone. The location of the abscission zone in several species has been described by Stephenson (1981), Woltering (1986b), McKenzie and Lovell (1992b). Flower abscission is commercially important as it limits fruit set of plants such as walnut (Catlin and Olsson, 1990), and mungbean (Poehlman, 1991). It is also often a limiting factor in the trade of several flowering potted plants (Van Leeuwen, 1985; Woltering, 1986b).

Abscission of turgid petals is generally a consistent feature at the family level (Woltering and van Doorn, 1988; McKenzie and Lovell, 1992a). Reiche (1885) concluded that, in several species, the petals fell due to fruit growth rather than cell wall dissolution in an abscission zone. In a few species, pollination is assisted by petal abscission. For example, in *Mimulus guttatus* (Scrophulariaceae) the stamens are connected to the corolla, which is shed fully turgid. As they are shed the anthers touch the stigma, which results in pollination (Dole, 1990). Petal abscission is an important factor in the potted plant trade, where several species show extensive petal shattering (Woltering, 1986a). Precocious petal abscission is also a characteristic of many cut flowers (Woltering and van Doorn, 1988). Joyce (1993) and Aloni et al. (1995) observed that external factors such as shaking, wounding, high temperatures, and some gases induce very rapid floral organ abscission, especially of petals in sensitive species.

Depending on flower morphology, stamens may fall separately, or with the petals when they are epipetalous. Generally, stamens that are not attached to the petals wilt and remain attached, irrespective of the type of petal senescence (abscission or wilting) (Cronquist, 1988). The styles of many plants desiccate and remain attached to the developing fruit, but in some families they abscise (McKenzie and Lovell, 1992a). Abscission may occur when styles are still fully turgid, for example in species of *Eucalyptus* (Moncur and Boland, 1989).

The physiology of abscission of flower and floral organs

Abscission of fertilised flowers may also be regulated by the availability of resources (Lee, 1988; Stephenson, 1981). Increasing the available mineral nutrients in *Pisum sativum* decreased flower bud abscission by 75% (Nightingale and Farnham, 1936). Carbohydrate partitioning in structures destined for abscission has only been investigated in a few species. Flower abscission in walnut has also been suggested to be related to competition for carbohydrates (Deng et al., 1991), whereas petal abscission in rose is insensitive to petal carbohydrate levels (van Doorn and Voginovic, 1996).

Cell wall degradation in abscission zones has mainly been studied in leaves, and even for leaves it has not been characterised in detail. Prior to leaf abscission, levels of insoluble wall pectins decreased and the level of soluble pectic acids increased (Osborne, 1989). Evidence for a role of the cellulase enzyme in leaf abscission followed from the work of Sexton et al. (1981) who introduced an antibody to this protein in the abscission layer and found that abscission was prevented. An increase in cellulase activity has been reported to be associated with flower abscission in tobacco (Lieberman et al., 1983), and tomato (Tucker et al., 1984). Two isoforms of the enzyme were found, one with an isoelectric point (PI) of 4.5, the other of 9.5. Gene expression of the latter occurred prior to flower abscission in soybean (Kemmerer and Tucker, 1994).

The role of ethylene in abscission is well established. Exposure of plants to exogenous ethylene causes flower fall in several species (Hoyer, 1985a, b; van Leeuwen, 1985; Rewinkel-Jansen, 1986). The rate of ethylene production often increased prior to flower and floral organ abscission, for example, in flowers of *Lathyrus odoratus* (Mor et al., 1984), and in petals of *Digitalis* (Stead and Moore, 1983), and *Rubus* (Burdon and Sexton, 1993). Silver thiosulphate (STS), an inhibitor of ethylene action, reduced flower abscission to zero in several species (van Leeuwen, 1985; Hoyer, 1985a, b; Joyce, 1989; Sexton et al., 1995), and prevented petal abscission in numerous species (Woltering and van Doorn, 1988; McKenzie and Lovell, 1992b).

Recently, some cyclic olefins have been found to inhibit ethylene action; one of these, diazocyclopentadiene (DACP), inhibited flower abscission in sweet pea (Sexton et al., 1995) and another, 1-methylcyclopropene (MCP), had the same effect on flower abscission in Geraldton waxflower (Serek et al., 1995). Aminoethoxyvinylglycine (AVG) was reported to prevent petal shattering in potted *Pelargonium* × *hortorum* (Miranda and Carlson, 1982), and raspberry (Burdon and Sexton, 1993).

Auxins may also be involved in flower drop. Flower removal in tomato (Roberts et al., 1984) and pepper (Wien and Zhang, 1991) resulted in rapid pedicel abscission which may relate to auxins. Floral abscission in *Cleome hassleriana* was found to be partially controlled by the presence of anthers, which apparently had their effect by virtue of their continued production of auxin (Koevening, 1973). Exogenously applied auxins can prevent or delay flower abscission in Geraldton wax flowers (Joyce, 1989). At certain concentrations auxin may increase rather than decrease abscission, but it is well established that excess auxin stimulates ethylene production (Abeles et al., 1992). McKenzie and Lovell (1992b) found hastening of petal abscission by auxin in *Crocosmia*.

The possible regulating effect of other hormones is as yet unclear. Application of gibberellin to the pedicel of cotton flower after severing the flower hastened pedicel abscission (Chatterjee, 1977). Flower fall of *Capsicum annuum* could nevertheless be

completely overcome by a combination of gibberellic acid and a cytokinin (Wien and Zhang, 1991). Abscisic acid (ABA) concentrations in petals of cut roses increased prior to abscission. This rise also occurred earlier in a cultivar showing early abscission compared to one with late abscission (Mayak et al., 1972), but McKenzie and Lovell (1992b) found that ABA application had no effect on petal abscission in *Crocosmia.*

2. 3 Postharvest characteristics of flowers in the family Myrtaceae

There are relatively few studies on the postharvest characteristics of flowers in the family Myrtaceae. These studies, which are summarized below, include members such as *Chamaelaucium* spp. (Geraldton waxflower), small numbers of *Leptospermum* spp., *Thryptomene* spp., and *Verticordia* spp.

Ethylene

Ethylene production by healthy isolated flowers of several cultivars of Geraldton waxflower was found to be low. However, whole sprigs of waxflower produced more ethylene as they aged (Joyce et al., 1993). The absence of an ethylene production peak indicates that Geraldton waxflower flowers are non-climacteric in nature (Olley et al., 1996). Ethylene does not appear to be involved in normal senescence or flower abscission of *Thryptomene calycina* (Olley et al., 1996). However, ethylene appears to be involved in flower abscission of *L. scoparium* (Zieslin and Gottesman, 1983). While *Verticordia nitens* is sensitive to ethylene (it causes flower abscission), *V. chrysantha*, *V. densiflora* and *V. plumosa* are insensitive (Joyce and Poole, 1993).

Water balance

Water deficit developing in Geraldton waxflower sprigs in the vase results in desiccation of flowers. Pressure/volume analysis shows that flower tissues are more elastic than leaf tissue, which explains why water deficit effects are often noted on leaves first (Joyce and Jones, 1992). Maintaining the water balance of Geraldton waxflower is important in extending vase life (Joyce and Jones, 1992). Flower closure and wilting in cut *T. calycina* branches commenced 2-3d after harvest, and was associated with a rapid decline in water

uptake (Jones et al., 1993a). Longevity was increased by measures expected to remove or prevent blockage of xylem vessels. These included recutting stems under water, or adding antibacterial compounds (e.g. hydroxyquinoline citrate (HQC), hydroxyquinoline sulfate (HQS) and citric acid) to the vase solution (Joyce, 1988 and Jones et al., 1993a). In contrast, Burge et al. (1996) found stem cutting or heat had little effect on water uptake, while solutions with HQS and cycloheximide (CHI) extended the vase life of *L. scoparium* flowers. Vase life of cut stems of *Verticordia* spp. was improved with a solution of CHI and citric acid (Jones, 1991).

Carbohydrates

The vase life of Geraldton waxflower (Joyce, 1988), *T. calycina* (Jones et al., 1993a), *L. scoparium* (Burge et al., 1996) and *Verticodia* (Jones, 1991) flowers can be increased by supplying sucrose from the vase solution. Further, sucrose increased flower opening in *L. scoparium* (Gottesman, 1982).

Abscission

Flower fall is the most important postharvest problem with cut Geraldton waxflower. Exogenous ethylene elicited flower and bud abscission in Geraldton waxflower (Joyce, 1988 and 1989). However, Thomas et al. (1992) believed that the serious flower fall observed upon unpacking of some export air consignments of Geraldton waxflower was probably caused by fungal pathogens. The flower abscission of *T. calycina* becomes a significant problem during postharvest handing of branches cut late in the flowering season (Jones and Faragher, 1991). Floral abscission also increased in association with *Botrytis cinerea* infection and was inhibited when *T. calycina* branches were stored in a controlled atmosphere of 1% O_2, and 5% CO_2 (Joyce et al., 1993).

Storage

Geraldton waxflower 'Purple Pride' and 'Alba' can be stored dry at 0.5-2°C for about two weeks without dramatic loss in quality, or appearance of fungal rot (*Botrytis cinerea*) which limits storage life (Joyce, 1988; Seaton and Joyce, 1989; Jones and Faragher, 1991).

T. calycina stores for three weeks at 1°C without significant decline in vase life, provided that bunches are treated with a fungicide effective against *Botrytis* during storage and adequately rehydrated after storage (Jones and Faragher, 1991). *V. grandiflora* can be stored dry for three weeks at 1°C without significant decline in vase life, but *V. nitens*, *V. monadelpha* and *V. plumosa* deteriorate rapidly if stored for longer than one week. *Verticordia* appear prone to fungal infection during storage (Jones and Faragher, 1991).

2. 4 Water relations of cut flowers

The extent of the vase life of some cut flowers is affected by hormonal balance (Borochov and Woodson, 1989), whereas in many others flowers the limiting factor is water stress. The water relations of cut flowers have been briefly reviewed by Halevy and Mayak (1981) and van Doorn (1997).

Natural senescence of flowers is determined usually through colour changes, flower closure, petal wilting, or petal abscission. When flower shoots are cut and placed in water, symptoms of natural senescence are often not observed, but symptoms of water stress, such as premature wilting of the flowers and leaves are expressed. Examples of flowers that show water stress are rose, *Gypsophila, Astilbe, Bouvaraia* and *Acacia*. Other flowers, such as *Tulipa, Freesia* and *Iris*, do not show this early water stress (van Doorn, 1997).

2.4.1 *Transpiration*

Transpiration is the loss of moisture from living tissues by evaporation through the plant surface. It is the major factor causing weight loss of fresh products (van Doorn, 1997). Cut flowers lose water from all tissues depending on environmental and internal factors.

In rose and other cut flowers it was demonstrated that, after cutting, water loss decreased sharply due to stomatal closure (Mayak et al., 1974), and cut flowers lacking stomata on any part of the flowering stem lose considerably less water per unit fresh weight than other cut flowers (van Doorn, 1997). Van Doorn (1997) suggested rapid weight loss related to stomatal transpiration, whereas slow weight loss was due to cuticular

transpiration. This indicates that the main pathway of water loss and flower weight loss is through stomata. Similarly, Slootweg and van Meeteren (1991) pointed out that an increase in the duration of stomatal opening of cut rose in vases may lead to higher water loss. Excessive transpiration results if stomata on leaves of rose cultivars lack a relatively rapid closing mechanism (Donnelly and Skelton, 1989). In some cut flowers, water loss may still occur after stomatal closure. This cuticular water loss also occurs through the flowers, and for a given cuticle the rate of transpiration relates to its thickness (van Doorn, 1997). The other factor affecting the rate of transpiration is the boundary layer of still air on the transpiring surface (van Doorn, 1997). Nobel (1991) pointed out that provided that the stomata are fully open, the resistance of the boundary layer may be the limiting factor for the rate of transpiration.

Light also promotes water loss by causing stomatal opening (Halevy and Mayak, 1981). For example, Carpenter and Rasmussen (1973) reported that roses held under constant light lost five times more water than those held in complete darkness.

2.4.2 *Water uptake*

Water balance is determined by water uptake and transport, water loss and the capacity of the flower tissue to retain its water. The rate of water uptake by flowers typically decreases with time (Halevy and Mayak, 1981). In cut rose flowers, it has been shown that the loss of petal turgidity and fresh weight was preceded by a decreased rate of water uptake (Burdett, 1970). The rate of water uptake of freshly cut flowers may initially be high when the plant has a low water potential at cutting. The rate of uptake will reach a steady state corresponding to the rate of transpiration but, depending on the species, the rate of uptake may subsequently decrease (van Doorn, 1997). Some cut flowers, such as *Thryptomene calycina* (Jones et al., 1993a), *Limonium* 'Chorus Magenta' (Burge et al., 1998), and *Leptospermum scoparium* (Burge et al., 1996), exhibit rapidly decreased water uptake rate to low values with time. Jones et al. (1993a) reported that the rate of water uptake declined further than transpiration, the longevity of *T. calycina* being correlated with the rapid decrease in water content of the cut branches. In other cut flowers, e.g. *Heliconia (H.*

psittacorum), there was little water uptake even shortly after cutting and placement in water (Donselman and Broschat, 1986).

Occlusions in the water conducting system can inhibit water uptake of cut flowers. Such occlusions are part of a wound reaction. Cutting can lead to the deposition of material in the lumen of the xylem conduits, exudation at the cut surface and the formation of tyloses in the conduit lumen. Occlusions may also relate to microbial growth or may be due to air bubbles (van Doorn et al., 1995, van Doorn, 1997). When roses are stored dry, the growth of bacteria occurs at the cut surface and in the xylem interior, which may lead to blockage following reimmersion in water (van Doorn, 1990).

The rate of water uptake of cut flowers also depends on many other factors, such as transpiration pull, temperature and composition of the solution. When the rate of transpiration declines but tends to be higher than the rate of water uptake, a negative water balance results leading to turgor loss on cut flowers. For maintaining a positive water balance, it is important that water loss be minimised and water uptake be enhanced (van Doorn, 1997). Van Doorn (1997) suggested that water loss can be minimised by controlling three factors: minimising the water vapour pressure difference between a flower and its environment, reducing surface area, and reducing flower surface permeance.

Several techniques can improve water uptake of cut flowers:

1. Re-cutting the stem under water.

Air embolism occurs when small air bubbles enter the stem of cut flowers at cutting (Reid, 1992). Re-cutting the stem under water can remove the air bubbles and promote water uptake. e.g. *Chrysanthemum* (van Meeteren, 1992) and *Leptospermum scoparium* (Burge et al., 1996).

2. Water temperature.

The response to water temperature is species and cultivar dependent (van Meeteren, 1992; Slootweg, 1995). Sacalis (1993) advised the use of warm water for rehydration of many flowers, including *Dahlia, Gladiolus*, *Protea*, *Syringa* and *Tulipa*. Rehydration in dry-stored stems of cut chrysanthemum flowers may increase with increasing water temperature (van Meeteren, 1989). Warm water flows more rapidly through cut flowers after dry storage (van Doorn, 1997). In Holland the use of warm water (about 50°C) is also advised for rehydration of *Phalaenopsis* flowers (van Doorn, 1997). The mechanism of action of warm water is not clear, but Slootweg (1995) explained that more air embolisms in stems could dissolve in water while the warm water is cooling down. Van Meeteren (1992) and Slootweg (1995) found that chrysanthemum and rose flowers took up water more rapidly in cold water than warm. It has been suggested that air embolism in the stems can dissolve quickly in low water temperature, because cold water can contain more air. Water uptake of some cut flowers, e.g. lily and greenhouse-grown sunflowers (Slootweg, 1995) does not respond to water temperature.

3. Effect of solutes in vase water.

Sugar in the solution is often reported to decrease transpiration (van Doorn, 1997), and improve water uptake by increasing the osmotic potential of the petals (Halevy, 1976). Burge et al. (1996) found that flower senescence of *L. scoparium* was reduced and the vase life extended by adding sugar to the vase solution. Sugars are often used in preservative solutions to extend the vase life of cut flowers. Preliminary research showed solutions containing 0.5% or 1% sucrose significantly increased the vase life as well as the percentage of buds that opened fully of *Heuchera sanguinea* (Han, 1998). The main effect of sugars on longevity of harvested flower stems is due to improved water balance status. The rate of water uptake is determined principally by the water potential of the flower tissue (van Meeteren, 1992). Sugars can also contribute the maintenance of the respiration rate and membrane integrity (Halevy and Mayak, 1979).

Low pH has been shown to be favourable for rehydration of dry-stored rose and chrysanthemum (Durkin, 1979a, b; 1980). Rehydration of flowers in retail premises with a citric acid solution at pH 3.5 is recommended by Sacalis (1993) for some species of flower. Citric acid at pH 3.5 improved stem hydraulic conductivity and prevented bacterial proliferation in the vase solution for *T. calycina* (Jones et al., 1993a). When acidity is effectively buffered around pH 3 it results in inhibition of bacterial growth in rose stems (van Doorn and Perik, 1990).

Hydroxyquinoline citrate (HQC) or hydroxyquinoline sulfate (HQS) in the vase solution has been shown to reduce stomatal opening, and hydroxyquinoline compounds are often used as antimicrobial agents (van Doorn, 1997). Most antimicrobial substances including HQC or HQS, salts of copper and zinc, and sodium hypochlorite can control microbial growth without being toxic to cut flowers (van Doorn, 1997). For example, HQS greatly improved the water balance and vase life of stems of *L. scoparium* (Burge et al., 1996). Keeping the pH at a low level by either adding a phosphate-citrate buffer (pH 3.1) or including HQC in the vase solution was incapable of stopping bacterial growth, but the combination of these two treatments was effective (van Doorn, 1997).

Wetting agents improve water uptake of cut flowers by decreasing the surface tension of water (Myers, 1991). The use of Tween-20 in the vase water was reported by Durkin (1980) to improve water uptake of chrysanthemum flowers. A pulse treatment of Agral-LV (active ingredient: alkylphenoxypolyethoxy ethanol with an average ethoxy chain length of 8.5) applied to roses prior to dry storage was effective in promoting water uptake after dry storage (Perik and van Doorn, 1988). Pulsing with Triton X-100 (a phenoxy type of surfactant) also increased the length of vase life of roses, *Bouvardia* and *Astilbe* (van Doorn et al., 1993), as well as sunflowers (*Helianthus annuus*) (Jones et al., 1993b).

2. 5 Ethylene biosynthesis and metabolism

Ethylene is a simple gaseous hydrocarbon that exerts profound effects on plant growth and development. It has been implicated in the promotion of seed germination,

diageotropism, flowering, abscission, senescence, fruit ripening, and pathogenesis responses (Mattoo and Suttle, 1991; Abeles et al., 1992).

It is now established that the major route of ethylene biosynthesis in higher plants involves the metabolic sequence shown in Figure 2.1.

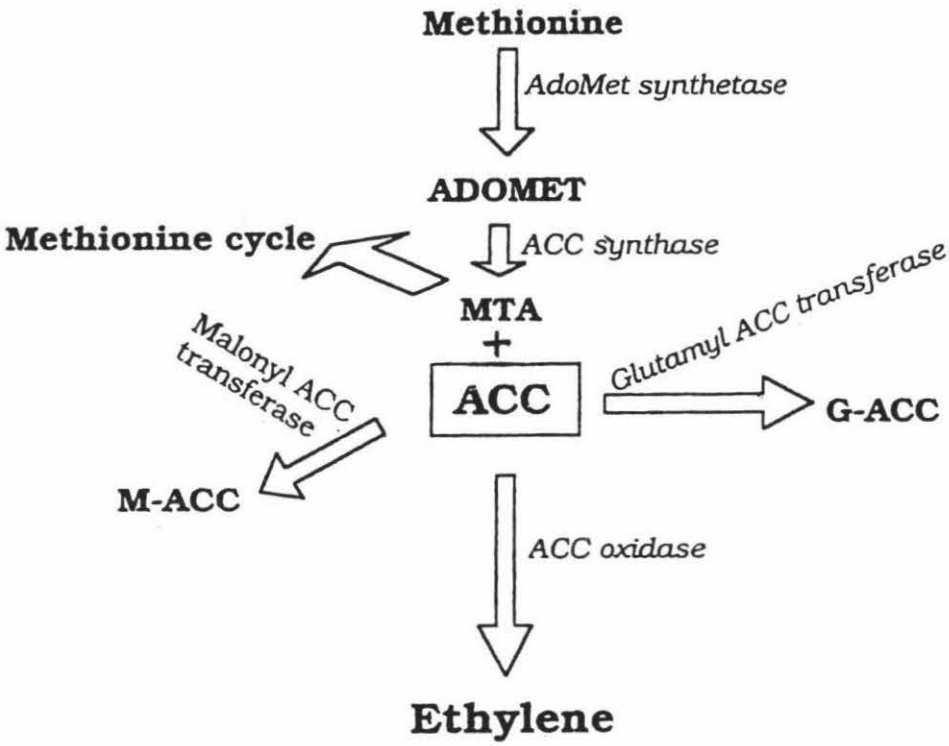

Figure 2.1 The major pathway of ethylene biosynthesis in higher plants and the enzymes involved. Also shown are routes to the methionine cycle and conjugation reactions that prevent ACC being directly available for ethylene production (Fluhr and Mattoo, 1996).

The key enzymes involved in biosynthesis of ethylene include the enzyme which converts methionine to AdoMet [ATP: L-methionine S-adenosyltransferase (AdoMet synthetase, EC 2. 5. 1. 6), Giovanelli et al., 1980], and AdoMet to ACC [AdoMet methylthioadenosinelyase (ACC Synthase, EC 4. 1. 14); Kende, 1989]. The sulphur atom from methionine is recycled back to methionine via methylthioribose so the level of methionine in the tissues does not become a limiting factor. The enzyme ACC synthase

specifically utilizes AdoMet as a substrate and this has been shown to be a pyridoxal enzyme, since it is strongly inhibited by aminooxyaceticacid (AOA) which is a inhibitor of ethylene biosynthesis. ACC synthase is thought to be the major rate-limiting enzyme in the ethylene biosynthetic pathway and plays a key role in regulating ethylene production (Kende, 1993). The enzyme catalysing the step from ACC to ethylene was originally termed the ethylene forming enzyme (EFE) (John, 1991). Three key developments led to the isolation and identification of the enzyme which catalyzed ACC to ethylene. Firstly, Hamilton et al. (1990) sequenced the pTOM13 clone from tomato, the deduced amino acid sequence of which was similar to flavanone 3-hydroxylase (EC 1, 14, 11, 9). It was proposed that this clone may represent the gene encoding the ethylene forming enzyme. Second, transgenic tomato plants harboring an antisense pTOM13 gene produced little ethylene, indicating that its gene product was essential for ethylene biosynthesis. Third, Ververidis and John (1991) applied the experimental conditions used to isolate and assay flavanone 3-hydroxylase activity in recovering in *vitro* an authentic soluble EFE activity from melon fruit (Smith et al., 1992). Subsequently, similar activities were recovered from avocado fruit (McGarvey and Christoffersen, 1992), and pear (Vioque and Castellano, 1994). The enzyme was shown to require CO_2 for activation (Smith and John, 1993). The enzyme was identified as 1-aminocyclopropane–1-carboxylate (ACC) oxidase, and is responsible for the final stage in the biosynthesis of ethylene (John, 1991). A large body of evidence is available to indicate that ACC oxidase activity also increases in some plant tissues in response to internal or external factors that induce ethylene production (Kende, 1993).

Delaying fruit ripening and flower deterioration by reducing ethylene biosynthesis or perception has been a major goal of postharvest physiologists. Three different strategies of genetic engineering have been employed to reach this aim, namely inhibiting the expression of genes encoding ethylene biosynthetic enzymes by transformation with their respective antisense genes (Kende, 1993), antisensing the gene coding for an ethylene receptor or a transforming with a mutant form of an ethylene receptor gene. While the majority of work has been focused on delayed ripening in fruit, especially tomato (Hamilton et al., 1990 and Oeller et al., 1991), mutant forms of the ethylene receptor gene from *Arabidopsis* have been

used to delay senescence or wilting of flowers. For example, recent work has involved modifying perception of ethylene in *Petunia* flower, using the etr1-1 gene (Raven et al., 1999).

In recent years, much research has focused on the positive and negative feedback regulation of ethylene biosynthesis. Positive feedback of ethylene on the expression of genes encoding enzymes of ethylene biosynthesis was found in carnation petals (Woodson et al., 1992). A negative feed-back loop in ethylene biosynthesis can be uncoupled by inhibitors of ethylene action. As such, STS and silver nitrate, antagonists of ethylene action, actually promoted ethylene synthesis in green tomato fruit tissue (Atta-Aly et al., 1987).

It is well established that many microorganisms can metabolise ethylene, but until relatively recently it was believed that higher plants lacked the means to metabolise ethylene (Sanders et al., 1986). Beyer (1984) was able to demonstrate that ethylene metabolism was widespread in the plant kingdom and, more over, that changes in the magnitude of the activity observed in particular tissues correlated well with changes in the sensitivity of these tissues to ethylene during the life cycle. Immature morning glory (*Ipomoea tricolor*) flower buds are insensitive to applied ethylene and lack the ability to metabolise ethylene. However, as the buds develop they become responsive to ethylene (which accelerates senescence of the petals) and there is a parallel increase in ethylene metabolism (Beyer, 1978). Similar correlations have been observed in peas, carnations (flower fading), cotton leaves (abscission) and leaves of *Vicia faba* (senescence) (Beyer, 1977, 1979b; Hall et al., 1982).

It has now been shown that some plants are capable of oxidising ethylene to CO_2 (the process referred to as "OX") and/or incorporating it into tissue (referred to as "TI") (Beyer, 1977). The rate of ethylene metabolism also varies between tissues of the same plant. The receptacle of the *Dianthus* flower has a significantly higher TI than petals and peduncle (Beyer, 1977). Similarly, TI of petals is greater than reproductive portions of the flower of *Ipomoea*, and both are greater than that of stem tissue (Beyer and Sundin, 1978). The final product of ethylene oxidation was thought to be CO_2, but it was later

demonstrated that ethylene oxide was a major metabolite of OX (Sanders et al., 1986). Ethylene oxide may be further metabolised and retained in the tissue (TI) as ethylene glycol and its glucose conjugate (Blomstrom and Beyer, 1980). Experimentally, it has been shown that the "OX" pathway increased rapidly prior to abscission while the "TI" pathway did not. It was shown that the increased oxidation of ethylene took place in the abscission zone but not elsewhere (Beyer, 1984). Beyer (1979a, 1977) showed that metal chelators, substances such as CS_2 (carbon disulfide) and COS (carbonyl sulfide) all inhibited metabolism. There is evidence that Ag^+ inhibited TI and that added CO_2 inhibited OX (Beyer, 1979b).

2. 6 Ethylene sensitivity

Many processes in plant development are known to be controlled not only by the level of plant hormones but also by the sensitivity of the tissue to these hormones (Trewavas, 1982). Sensitivity of tissues may be changed by a change in the number of receptors, a change in the affinity of receptors, or a change in the subsequent chain of events (Davies, 1987). However, sensitivity to ethylene is also known to change under certain environmental conditions, such as water stress, so that a change in ethylene production may not be necessary for ethylene regulation, for example, in abscission (Brown, 1997).

Ethylene accelerates flower and corolla abscission in many plant species. In surveys of horticulturally important flower species, the tendency to wilt or abscise was shown to fall into plant families (Woltering, 1987). Flowers in those families that abscised, rather than wilted, also tended to exhibit the highest ethylene sensitivity. Variations in sensitivity to ethylene have been reported for different genotypes of flowers, such as orchids (Goh et al., 1985), carnations (Woltering et al., 1993) and petunia (Porat et al., 1993). *Dendrobium* flowers, as with most orchids, are known to be rather sensitive to ethylene and pollination induced a further increase in the sensitivity of the flowers to ethylene (Porat et al., 1994). Woltering (1986a, 1987) reported that most of the tested leaves attached to foliage plants were less sensitive to ethylene than most of the tested flowers attached to flowering plants. Ethylene sensitive species might therefore become as economically important as the

insensitive ones when adequately treated with inhibitors of either ethylene production or ethylene action.

Unpollinated cyclamen flowers are insensitive to ethylene and eventually lose turgor and wilt, while pollinated flowers are ethylene sensitive and abscise the turgid corolla (Halevy et al., 1984). In some cases, changes in ethylene sensitivity may be related to auxin flux. At least for flowers which abscise at the pedicel, regulation by auxin and ethylene appears to be similar to that of leaf abscission zones (Oberholster et al., 1991). Changes in ethylene sensitivity during development are probably at least as important as changes in endogenous ethylene concentration in regulating petal abscission.

Maturity influences the sensitivity of ethylene and ethephon-promoted abscission of leaves. Although it is generally accepted that greater leaf maturity is associated with increased ethylene sensitivity, this is not always the case (Woolf et al, 1995). However, there are well documented changes in sensitivity to ethylene in ripening fruit (insensitive to ethylene when immature, and responding to ethylene when mature) (Suttle and Hultstrand, 1991).

The molecular basis for the changes in ethylene sensitivity leading to abscission competence is unknown. Whatever the endogenous messenger (declining auxin, pollination signals, or other factor), one possible mechanism for the tissue specific change in ethylene sensitivity is an increase in ethylene receptors or other response elements (Brown, 1997.) The function of many genes (for example, ETR1, eTAE1) in ethylene signal transduction is not understood, but only the ERS-type genes, which are subject to developmental regulation, are likely to influence ethylene sensitivity by changes in their expression (Brown, 1997).

Interestingly, in a recent report the suggestion was made that short-chain saturated fatty acids could cause the increase in ethylene sensitivity. It appears that, in carnation, 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 senescence (Whitehead and Vasilevic, 1993).

2. 7 Interaction of ethylene and other plant hormones

The auxin/ethylene interaction in abscission is well documented and recent work by Peck and Kende (1997) proposed a model for the sequential regulation of the ethylene biosynthetic enzymes. Indole-3-acetic acid (IAA) causes an increase in ACC synthase transcript abundance leading to an increase in ACC synthase activity. The newly formed ACC is converted to ethylene by a low, constitutive level of ACC oxidase. The ethylene produced then causes an increase in the levels of ACC oxidase via a positive feedback loop (autocatalytic). Ethylene eventually reduces ACC synthase transcript via a negative feed back loop, ACC activity levels decrease leading to the cessation of IAA-induced ethylene production.

In carnation flowers, several plant hormones have been shown to influence ethylene metabolism (reviewed by Cook and van Staden 1988). Auxins are thought to promote petal senescence through the stimulation of ACC-synthase activity (Nichols, 1971; Wulster et al., 1982). It was suggested that cytokinin-induced senescence is modified through an interaction with the gynoecium that led to ACC accumulation and premature ethylene production in both the gynoecium and the petals (Woodson and Brandt, 1991). In all flower parts endogenous levels of ACC were reduced with gibberellic acid (GA_3) treatment. This was most pronounced in the petal bases (Saks and van Staden, 1993a and 1993b). As already mentioned, petal bases are important regulatory sites for ethylene production. Using detached carnation petals as a model, Mor et al. (1983) showed that pre-treatment with cytokinin blocked the conversion of applied ACC to ethylene as well as the in *vivo* production of ACC and ethylene. Further, cytokinin application negated the ACC-stimulated senescence of floral tissues of broccoli (*Brassica oleracea* var Italica) (Clarke et al., 1994).

2. 8 Inhibitors of organ abscission

In horticulture, various methods have been employed to reduce either ethylene production or the effects of exposure to ethylene. Antagonists and absorbents of ethylene can be used to reduce its effects on plants. For example, 'Purafil' (alumina coated with concentrated $KMnO_4$), mercuric perchlorate [$Hg(ClO_4)_2$] and potassium permanganate ($KMnO_4$) (Lemos and Blake, 1996), and Lemos and Blake (1996) reported: "although all the absorbents which were used significantly reduced the leaf abscission in nodal cultures of *Annona squamosa* L., they were unable to stop the process and stimulate new healthy growth. The 'Purafil' was the most effective absorbent tested and reduced leaf abscission".

Ethylene inhibitors may be divided into two groups. One group acts via inhibition of ethylene biosynthesis. The inhibitors of ethylene biosynthesis used interfere in one of two different positions of the biosynthetic pathway. 1) Application of aminooxyacetic acid (AOA) or L-α-2-aminoethoxyvinyl glycine (AVG) is thought to interrupt ACC production by inhibiting the pyridoxal phosphate which is required by ACC synthase for activity (Yang and Hoffman, 1984), and thus avoids the production of the ethylene precursor. 2) Application of cobalt ions (as $CoCl_2$), interferes with the ACC oxidase complex (Lau and Yang, 1976) and prevents ethylene formation from ACC.

AVG and AOA have been placed among the most powerful ethylene biosynthesis inhibitors (Yang and Hoffman, 1984), while the efficacy of cobalt ions has been questioned (Khalid et al., 1991) and it seems that the effectiveness of this or other compounds varies among species and tissues used. AVG has been used successfully to increase the shelf life of ethylene-sensitive cut flowers (Spikman, 1989) held in air. It was observed that cobalt chloride, AVG and AOA can reduce leaf abscission, but that AVG and AOA reduced significantly the leaf abscission percentage when compared with $CoCl_2$ (Lemons and Blake, 1996). Commonly these compounds reduce but do not abolish ethylene production (Lemons and Blake, 1996).

Another group of ethylene inhibitors acts via inhibition of ethylene action. These normally including silver nitrate ($AgNO_3$), STS, CO_2, 2,5-norbornadiene (2,5-NBD) and analogous cyclic olefins (Yang, 1987). Silver has been found to inhibit the binding of ethylene in plant tissues such as *Dianthus* petals (Sisler et al., 1985). However, Ag^+ did not inhibit ethylene binding in *vivo* in *Pisum* epicotyls (Sanders et al., 1991). Sisler and Yang (1984) proposed that silver ions interfere with the ethylene receptor complex by removing an essential ligand of the binding receptor, resulting either in a biologically inactive complex or in a receptor that loses its capability to bind ethylene.

Lemos and Blake (1996) described two approaches used to inhibit the action of ethylene in nodal explants of *A. squamosa*: 1) competing for the ethylene binding site with 2,5-NBD and 2) altering the binding site with silver ions. 2,5-NBD and Ag^+ reduced very significantly the percentage of leaf shedding from almost 70% in the control to less than 5% in *A. squamosa* (Lemos and Blake, 1996). Silver thiosulphate is widely used for reduction of senescence and leaf abscission in cut flowers. Aloni et al. (1995) showed that STS effectively reduced abscission of pepper flowers, and proposed that STS inhibited pepper flower abscission by blocking ethylene action in the abscission zone. STS was reported to prevent or reduce leaf drop in several foliage plants (Auer and McDonnell, 1984; Reid, 1985), prevent leaf abscission from harvested holly and mistletoe (Joyce et al., 1990), and prevent flower abscission in Geraldton waxflower (Joyce, 1989). Among the cycloolefins, NBD is the best competitive inhibitor of ethylene action (Sisler and Wood, 1988), and its effective range of action is between 500 and 2000 $\mu l \cdot L^{-1}$ (Sisler et al., 1990). The action of NBD can be reversed by adding more ethylene to the gas phase (Bleecker et al., 1987). Many systems of ethylene action have been shown to be inhibited by 2,5-NBD including flower senescence (Hyodo et al., 1990) and fruit softening (Blandenship and Sisler, 1989).

Veen (1986) proposed a model to explain the nature of the ethylene reception and the effects of inhibitors both of ethylene production (2,5-NBD) and ethylene action (Ag^+) (Fig. 2.2). This model implicates a receptor consisting of a regulatory unit (sub-unit A) and one or more sub-units (sub-unit B). Ethylene binding to sub-unit A results in a

conformational change allowing sub-unit A to regulate enzymatic sub-unit B. The competitive binding of 2,5-NBD at the same site, prevents ethylene binding and thus the conformational change does not occur and ethylene action is inhibited. When Ag^+ competes with the copper for the coupling site between sub-units A and B, it blocks the conformational changes of sub-unit B and inhibits ethylene action.

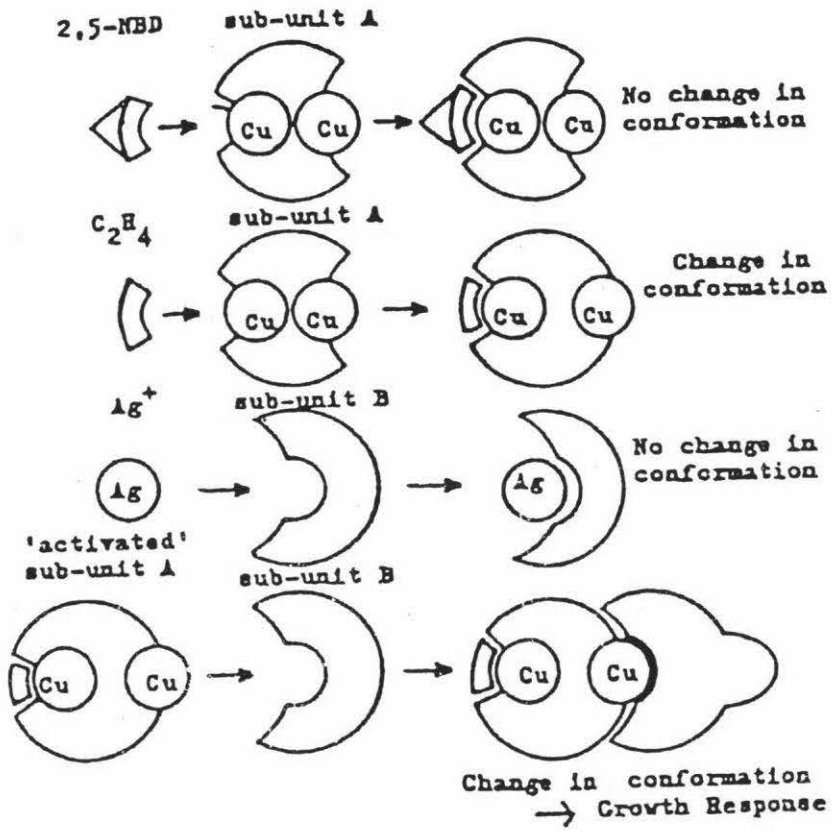

Figure 2.2 Model of mechanism of action of ethylene inhibitors 2,5-NBD and Ag^+ (Veen, 1986).

Recently, one exciting new approach is the use of a gaseous binding-site competitor, 1-methylcyclopropene (1-MCP), which appears to give ethylene protection equivalent to that obtained from STS. This material is presently being registered for use on cut flowers and other horticultural crops, because silver is a heavy metal and its disposal causes environmental concerns (Newman et al., 1998). Treatment with 1-MCP at very low

concentrations effectively eliminates the effects of ethylene on ethylene-sensitive potted plants and cut flowers (Serek et al., 1994, 1995; Sisler et al., 1995). For example, 1-MCP can prevents abscission of plant organs from flowering branches of Geraldton waxflower (Serek et al., 1995). Since 1-MCP is very effective in inhibiting the response of phlox flowers to ethylene, has no toxic effects and probably acts like STS (Porat et al., 1995), it may serve as an excellent alternative to STS, which is considered by many as an environmental hazard (Nell, 1992).

Chapter Three
Materials and Methods

3.1 General statistical analysis methods

In this research, experiments were laid out in a randomised block design (RBD). Data were repeated measure every day. So the model used in the analyses was the model for a randomised block design with repeated measure, Day (Time). Data were analysed by analysis of variance (ANOVA) of the Statistical Analysis System (SAS Inst. Inc., Cary, NC., USA, 1989) (SAS) with repeated measure, Day. Difference among treatments were analysed further with Duncan's Multiple Range Test (p=0.05) using statement **means** in PROC GLM of SAS, and statement **lsmeans** computing the Least-Squares means for interactions (factorial means) was used to compare relationship between two factors. Untransformed data are presented throughout. In general, some common transformation scales should be used for all observations in the data, such as percentage was transformed by arcsin or logarithm (log). Count numbers were transformed by log (1+count). Therefore the assumptions underlying ANOVA using raw data, log transformation data and arcsin transformation data in PROC PLOT of SAS for obtaining residual plots were checked. The result showed there was no difference between each residual plot, and residual plots showed a form of randomness, with nearly equal negative residuals and positive ones. These identified the assumption of normality on the residuals and the homogeneity of variance are satisfactory for raw data, transformation is not necessary.

3.2 Holding solution treatments for cut inflorescences of *Eucalyptus* and *Metrosideros*

3.2.1 Experiment 1: Effect of sucrose and hydroxyquinoline citrate (HQC) on stamen senescence, water balance and vase life characteristics in cut flowers of *Eucalyptus ficifolia*.

Inflorescences of *E. ficifolia* were harvested (8.00am-9.00am) (13/02/98) from trees growing on the Massey University campus. Sufficient inflorescences were harvested to

allow the later collection of individual flowers at the desired stage of development. Inflorescences were placed in flasks holding distilled water at room temperature, and were transported to the laboratory. Individual flowers at Stage 4 (Fig. 3.2b) were cut from the inflorescences under distilled water as required using a razor blade, assigned to one of ten blocks, and placed in Eppendorf tube vials (2.5ml) containing one of five holding solutions. Holding solutions (A-E, respectively) were distilled water, a solution in distilled water of HQC (200ppm), and the same HQC solution containing sucrose at three concentrations (2, 4, 6% w/v). Vials with flowers, and vials with flowers temporarily removed, were weighed (2 dp), arranged randomly within the ten blocks and placed in the vase life evaluation room. This was maintained at constant 20 °C, 70-80% relative humidity, 12 hours photoperiod at a photon flux density of 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Philips TLD 36w/48 cool white fluorescent tubes) as recommended by Halevy (1976).

Flowers were observed over the following five days. Daily measurements were made of flower mass, transpiration and water uptake and five vase life characteristics [stage of development (Table 3.1), pollen shedding, stamen wilting, stamen abscission and pedicel damage]. Before adding any of the solution A-E to a vial the weight of the flower plus vial was measured, and the weight of the vial with the flower removed was than recorded. These two weights allowed calculation of the water uptake from the vial, the weight change of the flower, and the amount of water transpired from the flower over the previous 24 h. When the level of the solution in the vial had dropped so that more solution needed to be added, enough of the appropriate solution (A-E) was added to the vial and the flower plus vial was then reweighed (flower+vial). Flowers and vials were replaced in the vase life room.

Statistical design and analysis: The experiment was laid out in a randomised block design (RBD), the model of statistical analysis was the model for a randomised block experiment with one factor (vase solution treatment), and data were analysed by ANOVA of SAS with repeated measure, Day.

3.2.2 Experiment 2: Effect of sucrose, HQC and pH of HQC solution (controlled by citric acid-sodium citrate buffer) on the water balance, stamen wilting and vase life characteristics in cut flowers of *Eucalyptus ficifolia*.

Inflorescences of *E. ficifolia* were harvested (26/02/98) using the same procedure described for Experiment 1. Eleven treatments were applied to individual cut flowers as different holding solutions contained in Eppendorf tube vials. Holding solutions were distilled water (control), solutions in distilled water of HQC (100, 200 and 400 ppm), the pH of these HQC solutions being 5.0, 4.9 and 4.6, respectively; and solutions containing HQC (200 ppm) with the pH nominally adjusted to 3, 4 and 5 using different proportions of sodium citrate (0.1 M) and citric acid (0.1 M) as described by Lillie (1948). After mixing with the HQC, the pH values of these solutions were pH 3.3, 4.3 and 5.15, respectively; and solutions containing HQC (200 ppm) with sucrose at four concentrations (0.1, 0.5, 1 and 2% w/v). The flower vials were arranged as a randomised block design. Using a polystyrene base to hold them upright, vials were arranged randomly within five blocks and placed in the vase life evaluation room. Flowers were observed daily over the following 4-5 days, and water use measurements taken each day, as described in Experiment 1.

Statistical analysis: The experiment was laid out in a randomised block design. The model of statistical analysis was the model for a randomised block experiment with one factor, and data were analysed by ANOVA of SAS with repeated measure, Day for water relations of the experiment. Because of some flower buds not full opening, stamen wilting and abscission were recorded only for opening flowers. So these average of data were analysed by ANOVA of SAS without repeated measure, Day.

3.2.3 Experiment 3: Effect of sucrose and pH of HQC solution (controlled by citric acid – sodium citrate buffer) on water use and vase life characteristics in cut cymules of *Metrosideros collina* 'Tahiti' flowers.

Seven stages of floral development were described for individual flowers of *Metrosideros* (Fig. 3.1).

Cymules (three flowers on each pedicel) of *M. collina* 'Tahiti' bearing flowers at Stage 2 (Fig 3.1B) were harvested (9.00am-10.00am) (10/08/98) from pot plants growing in the greenhouse at the Plant Growth Unit. Enough flowers were harvested each day for one block until the fifth day. Individual cymules were recut under distilled water to avoid air embolisms in the xylem of the flower pedicels before being placed in 25ml beakers of holding solution in the laboratory. Holding solutions treatments were solutions containing HQC (200 ppm) with the pH adjusted to 3, 4 or 5 using proportions of sodium citrate (0.1M) and citric acid (0.1M) as described by (Lillie, 1948). After initial mixing with the HQC, the pH values of these solutions were 2.65, 3.76 and 4.78, respectively. Solution containing sucrose at three concentrations (1, 2 and 4% w/v) were prepared for every solution with the pH adjusted 3, 4 and 5. The flower beakers were arranged as a A×B factorial randomised block design, arranged randomly within five blocks and placed in the vase life evaluation room (in section 3.1.1). A distilled water was also included as a control.

Flowers were observed over the following eight days. Daily measurements were made of solution uptake (described in Section 3.2.1) and six vase life characteristics (stage of development, pollen shedding, stamen wilting (described in Fig. 3.3), stamen abscission, petal abscission and pedicel damage).

Statistical analysis: In this experiment, treatments were laid out in a A×B factorial randomised block design. The model used in the analyses was the model for a randomised block experiment with two factors. Such as the model included terms for each of the two factors and their interaction. Treatments and control were laid out in a randomised block design for comparing each treatment and control, so another model used was as described in Experiment 1.

3.3 Effect of ethephon and a pre-treatment with silver thiosulphate (STS) on stamen abscission and wilting in cut flowers of *Eucalyptus* and *Metrosideros*

3.3.1 Experiment 4: Effects of STS and ethephon on vase life characteristics of *E. ficifolia* flowers.

Inflorescences of *E. ficifolia* were harvested (17/03/98) using the same procedure described for Experiment 1. Sixty flower buds at Stage 4 (Fig. 3.2b) were placed in vials containing one holding solution (200 ppm HQC, 2% sucrose and citrate buffer at pH 4). The vials holding the flowers were placed in the evaluation room. After 24 h, all flowers had opened fully and were at Stage 5 (Fig. 3.2c). Flowers were sprayed to incipient runoff with one of two concentrations of STS (0 and 2 mM). Flowers were allowed to dry before being replaced in the vase life room. After 24 h, flowers were sprayed to incipient run off with one of five levels (0, 10, 100, 1,000 and 10,000 ppm a.i.) of ethephon (Ethrel 48 Rhone-Poulenc New Zealand Limited). Tween 20 (0.5% v/v) was added to both STS and ethephon solutions. Flower vials were arranged as a randomised block design with six blocks and placed in the evaluation room.

Flowers were observed every day over the following 5-6 days. Seven vase life characteristics (pollen shedding, style length, stigma receptivity stage, stamen abscission, stamen wilting, stage of development and pedicel damage) were scored daily.

Statistical analysis: The experiment was laid out in a A×B factorial randomised block design. The model used was as described in the Experiment 3.

3.3.2 Experiment 5: Effects of STS and ethephon on vase life characteristics of *Metrosideros collina* 'Tahiti' flowers.

Cymules of *Metrosideros collina* 'Tahiti' bearing flowers at Stage 2 (Fig. 3.1B) were harvested (9.00am-10.00am) (02/08/98), and the cut ends of the pedicels placed in beakers containing a solution of HQC (200 ppm) and sucrose (2% w/v), with the pH adjusted to 4.0 using a citric acid-citrate buffer. The beakers holding the cymules were placed in the evaluation room. After 24 h all flowers had opened fully and were at Stage 3 or 4 (Fig. 3.1C or 3.1D). Flowers were dipped into one of two concentrations of STS (0 and 2 mM), and flowers were allowed to dry before being replaced in the vase life room. After a further 24 h, and when all flowers were at Stage 4 (Fig. 3.1D), flowers were dipped into

one of five concentrations (0, 10, 100, 1000 and 10000 ppm a.i.) of ethephon (Ethrel 48, Rhone-Poulenc Ltd. Wellington, N.Z.).

Flowers were harvested over three days starting 31 July 1998, two blocks being harvested each day. Flower beakers were arranged as a randomised block design within six blocks and placed in the evaluation room.

Flowers were observed every day over the following eight days. Measurements (stage of development, pollen shedding, stamen wilting (Fig. 3.3), stamen abscission, petal abscission and pedicel damage) were taken each day. After eight days all flowers had senesced. The data were analysed as described in Section 3.3.1.

3.4 Ethylene treatment and ethylene production for cut cymules of *Metrosideros collina* 'Tahiti'

3.4.1 Experimental apparatus

The standard apparatus used for each replicate of each experiment consisted of a 1 L glass jar containing a 20 or 25 ml beaker. Cymules of *Metrosideros collina* 'Tahiti' bearing flowers at Stage 2 (Fig. 3.1B) harvested from plants growing both in the greenhouse and outside (9.00am-10.00am) (30/10/98~11/11/98). The cut ends of the pedicels were cut under distilled water, and the cymules placed in beakers containing a solution of HQC (200 ppm) and sucrose (2%), with the pH adjusted to 4.0 using a citric acid-citrate buffer. Cymules were suspended above the solution held in beakers using food wrap. Total stamen and petal numbers of each cymule was recorded. The beakers with cymules were placed into each glass jar, 5 g of sodalime (NaOH) was added to each jar and the jars were sealed. Seals were fitted with a rubber septum to allow for injection of gaseous ethylene, or for withdrawal of gas from the head space for analysis. Ethylene levels were measured with a 3400 gas chromatograph (oven 80 °C, injector-detector 120 °C) fitted with FID detector and 2-m Poropak N column (made in USA). A CIG Betagrade gas mixture (0.1 μL C_2H_4/L in N_2) was the standard. All experiments were run under conditions of constant temperature

(20 °C) and constant 12 h light. Over the time period of each experiment stamen and petal drop were recorded at 24h intervals. At the time of each recording, each jar was lifted 2 cm from the bench top and dropped onto the surface of the bench to give unit a uniform jolt. Eight replicates of the Experiment 6 and seventeen replications of Experiment 7 were always placed in random complete blocks on the bench.

3.4.2 Experiment 6: Application of gaseous ethylene to cut cymules of *Metrosideros collina* 'Tahiti'.

Flower materials were collected and prepared as described above (Section 3.4.1). Following placement in beakers, cymules were placed in the vase life room for a 24 h period to allow for dissipation of wound ethylene, and for flowers of each cymule to open fully. Ethylene gas was as a 1000 ppm stock, prepared by diluting 1 ml pure ethylene gas in 1000 ml of air held in a glass jar. Cymule with holding solution and beakers were sealed into the glass jars, the ethylene stock (1000 ppm) was used to give five concentrations of ethylene by injecting appropriate volumes into the glass jars. Ethylene treatments applied were: 5, 0.5, 0.1 and 0 ppm (control). In addition, 5g 'Purafil' ($KMnO_4$ impregnant, Papworth Engineering, Cambridge, NZ Limited) was added to a second control (0 ppm). Treatment concentrations were confirmed by gas chromatography (Section 3.4.1) at the start and end of the experiment. Stamens and petals abscission for each replication was recorded at 24 h intervals over five days.

Statistical analysis: The experiment was laid out in a randomised block design. The model of statistical analysis was as described in Experiment 1.

3.4.3 Experiment 7: Ethylene production by cut cymules of *Metrosideros collina* 'Tahiti'.

Plant materials were collected and prepared as described above (Section 3.4.1), and individually weighed cymules held in the standard solution were sealed in 1L glass jars. There were ten replicate jars for greenhouse flowers, and seven replicate jars for flowers grown outside. A 24 h period was allowed for dissipation of wound ethylene. No

exogenous ethylene treatments were applied. Initially 1 ml samples of gas from the headspace were analysed using the gas chromatograph. Four further 1 ml samples of gas were removed from the headspace for analysis over a period of seven days. Three replicate gas samples were withdrawn from each replicate glass jar for every time of gas analysis. Stamen and petal abscission for each replicate was recorded at 24 h intervals over six days.

Table 3.1 Stages of floral development in attached flowers of *Eucalyptus ficifolia* and *Metrosideros collina* 'Tahiti'.

Stage	*Eucalyptus ficifolia*	*Metrosideros collina* 'Tahiti'
1	No difference in colour between flower bud cap (operculum) and cup, both light-dark green.	Cymule showing 3 individual flower buds completely separated, red petals visible in center of flower buds (Fig. 3.1A).
2	Operculum different colour from bud cup, the operculum typically redder than the paler/green cup.	Red petals visible in 3 flower buds of each cymule, red petas just opening on the top of flower buds. Stamens visible, but no straight (Fig. 3.1B).
3	Bud enlarged compared to those at Stage 2, sometimes can find buds in which the abscission zone between bud cap and cup clearly visible (Fig. 3.2a).	Red petals full opening, stamens become straight. Pollen not shed, style become straight (Fig. 3.1C).
4	Stamens visible between operculum starting to separate from the cup (Fig. 3.2b).	Pollen shed, but keeping fresh, stamens full straight (Fig. 3.1D).
5	Stamens expanding ('flower opening') but still relatively bundled; diameter of circle made by stamen tips approximately 2x that of the flower cup. Pollen white, and being shed. Style relatively short, approximately equal length within the cup as protruding above the rim of the cup (Fig. 3.2c).	Stamens begin abscission (Fig. 3.1E).
6	Stamens fully expanded (flower fully open); stamens not being shed: diameter of circle made by stamen tips approximately 3x that of the flower cup. Pollen dry. Style elongated, protruding approximately twice as far above the cup as within the cup (Fig. 3.2d).	Stamens abscission and discolour, petals abscission and dry (Fig. 3.1F).
7	Stamens beginning to crinkle and be shed: no longer bright in colour. Length of style approximately three times longer above the cup as within the cup, usually coloured and relatively thickened compared with earlier stages. Stigma is brown or discoloured (Fig. 3.2e).	Stamens and petals full abscission, the bottom of style is discoloured (Fig. 3.1G).
8	Stamens abscising and becoming discoloured (Fig. 3.2f).	

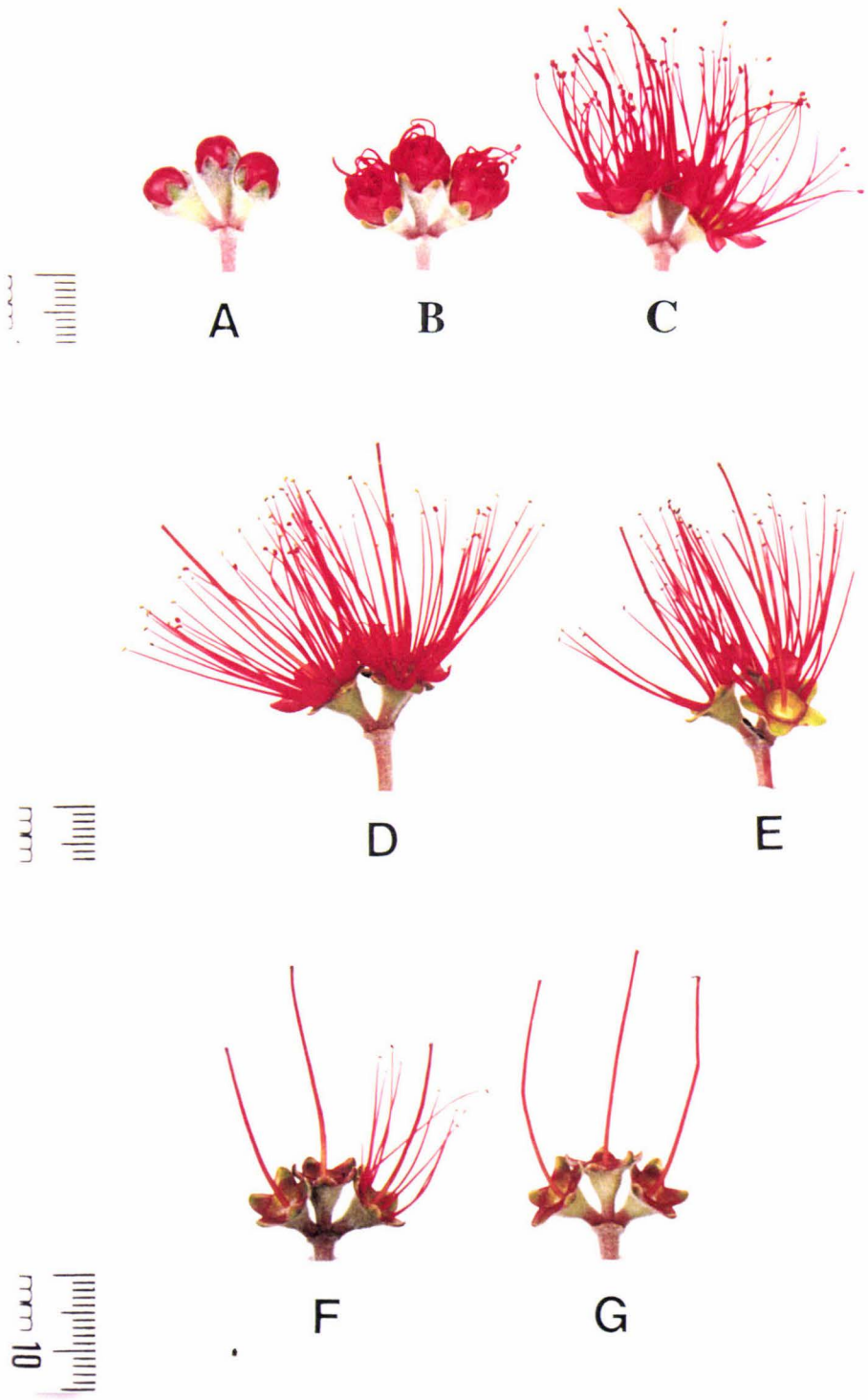

Figure 3.1 Stages of inflorescence development in *Metrosideros collina* 'Tahiti' from bud separation (A=Stage 1) until senescence (G=Stage 7). Refer to Table 3.1 for full description of stages.

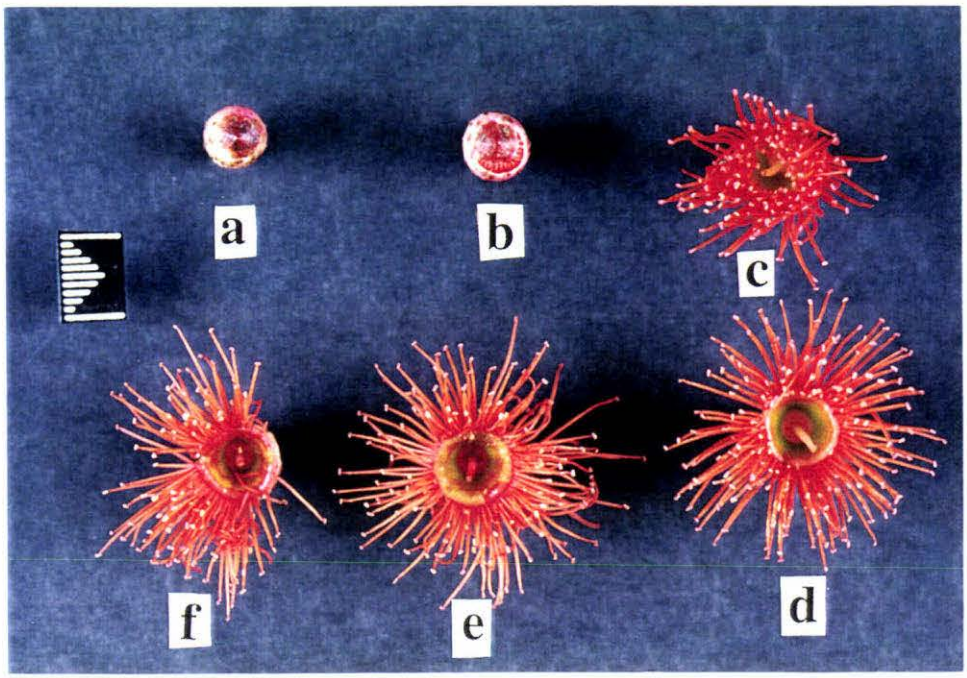

Figure 3.2 Stages of inflorescence development in *Eucalyptus ficifolia* from tight bud (a=Stage 3) until senescence (f=Stage 8). Refer to Table 3.1 for full description of stages.

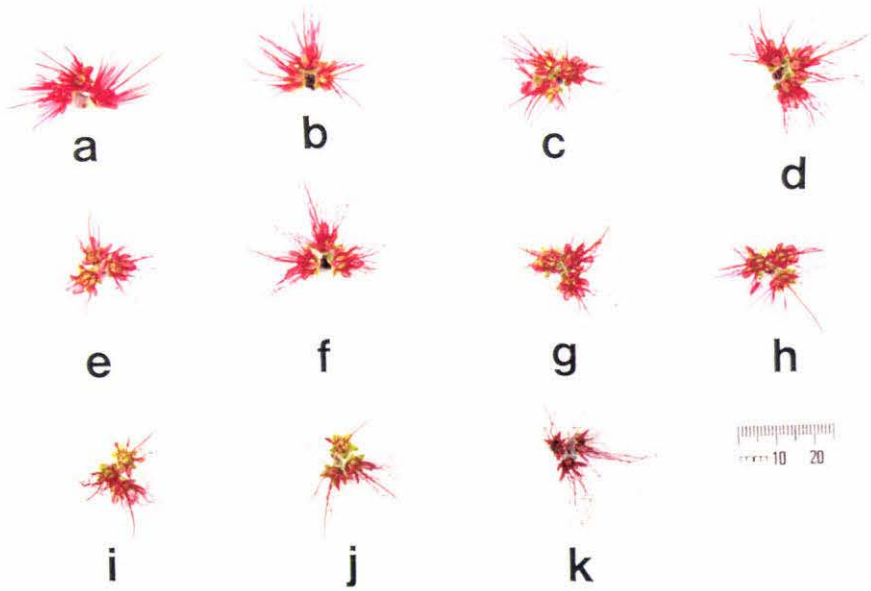

Figure 3.3 Degrees of stamen wilting in cut cymule of *Metrosideros collina* 'Tahiti'. a=0 (no stamen wilting); b=10%; c=20%; d=30%; e=40%; f=50%; g=60%; h=70%; i=80%; j=90%; k=100%.

Chapter Four
Results

4.1 Holding solution treatments for cut inflorescences of Eucalyptus and Metrosideros

4.1.1 Experiment 1: Effect of sucrose and hydroxyquinoline citrate (HQC) on cut flowers of *Eucalyptus ficifolia*

Water relations

Mean water uptake per flower averaged across all treatments decreased from approximately 0.33 to 0.12 $g \cdot day^{-1}$ over the experimental period (Fig. 4.1A), whereas mean transpiration per flower gradually decreased from 0.35 to 0.18 $g \cdot day^{-1}$ (Fig. 4.1B). Mean flower mass decreased from 0.52 to 0.28 $g \cdot day^{-1}$ (7.7% of flower mass per day) (Fig. 4.1C).

Averaged over the whole experimental period, there was a trend of decreasing water uptake with increasing sucrose concentration of the holding solution (Fig. 4.2A). Treatments had a significant effect on water uptake for each day. Water uptake was highest for the water control after Day 3 (Table 4.1).

Treatments had no significant effect on flower mass averaged over the whole experimental period. However, there were significant effects on Days 5 to 6, with the water control having the highest water uptake (Table 4.1).

There was a trend of decreasing transpiration with increasing sucrose concentration of the holding solution (Fig. 4.2B). Transpiration from flowers was significantly affected by treatments on Days 1, 2, 3 and 5. In general, on Days 1-3, transpiration using a holding solution of water or of 200 ppm HQC was significantly higher than that from holding solutions containing sucrose (Table 4.1).

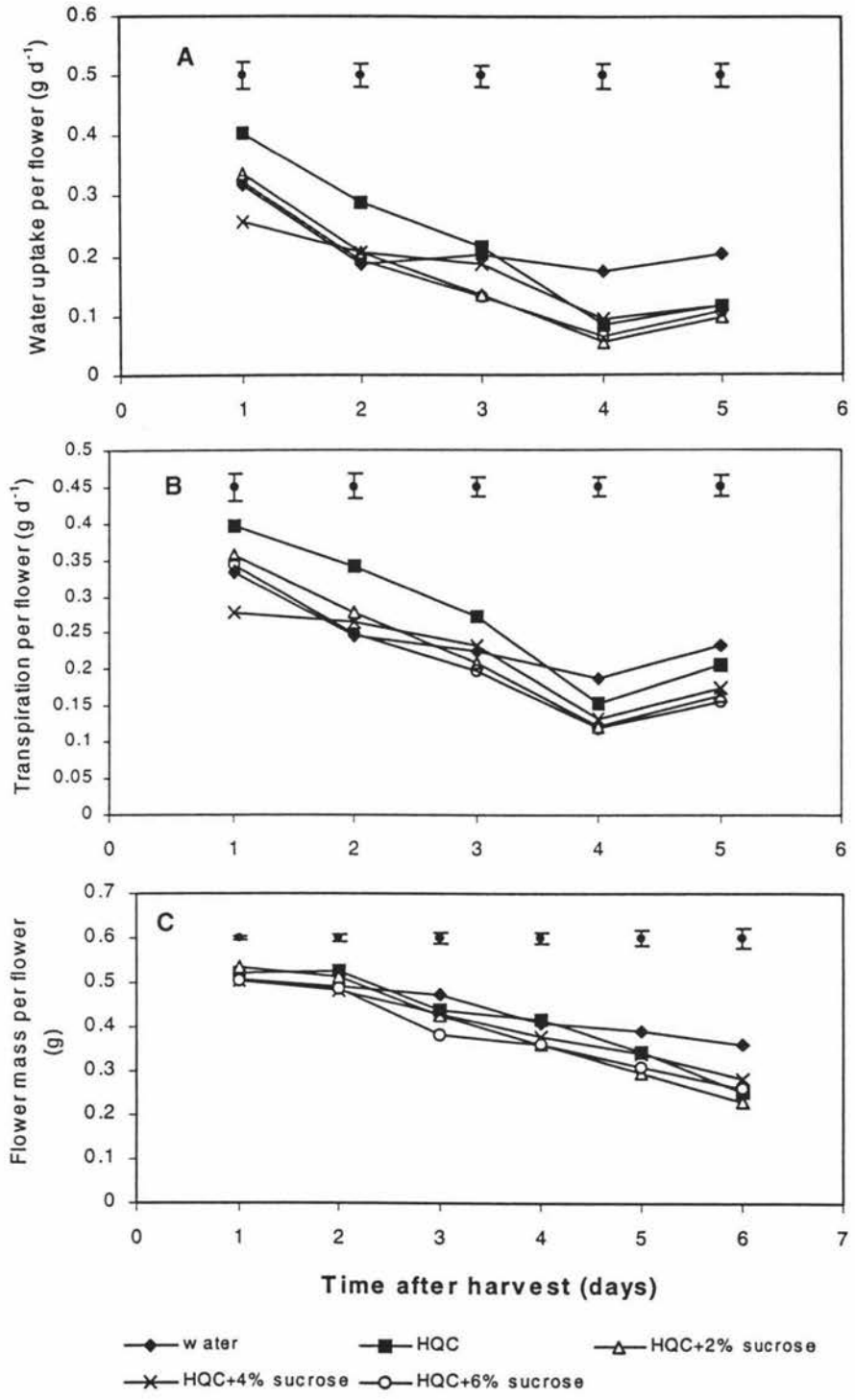

Figure 4.1 Effects of holding solution treatments on water uptake (A), transpiration (B) and flower mass (C) in cut flowers of *E. ficifolia*. Vertical bars represent standard errors of means

Table 4.1 Effect of holding solution treatments on water uptake, flower mass and transpiration in cut flower of *E. ficifolia* (means of ten flowers).

	Treatment	Days					
		1	2	3	4	5	6
Water uptake ($g\ d^{-1}$)	Water (Control)	0.32ab	0.19b	0.20a	0.17a	0.20a	
	HQC (200ppm)	0.40a	0.29a	0.21a	0.08b	0.12b	
	HQC+2% Sucrose	0.34ab	0.20b	0.13b	0.09b	0.10b	
	HQC+4% Sucrose	0.26b	0.21b	0.19a	0.09b	0.12b	
	HQC+6% Sucrose	0.33ab	0.19b	0.13b	0.06b	0.11b	
Flower mass (g)	Water (Control)	NS	NS	NS	NS	0.39a	0.36a
	HQC (200ppm)	.	.	.	.	0.34ab	0.25b
	HQC+2% Sucrose	.	.	.	.	0.30b	0.23b
	HQC+4% Sucrose	.	.	.	.	0.34ab	0.28b
	HQC+6% Sucrose	.	.	.	.	0.31b	0.26b
Transpiration ($g\ d^{-1}$)	Water (Control)	0.33ab	0.25b	0.23ab	NS	0.23a	
	HQC (200ppm)	0.40a	0.34a	0.27a	.	0.21ab	
	HQC+2% Sucrose	0.36ab	0.28b	0.21b	.	0.16c	
	HQC+4% Sucrose	0.28ab	0.27b	0.23ab	.	0.18bc	
	HQC+6% Sucrose	0.34b	0.25b	0.20b	.	0.16c	

Mean separation in columns by Duncan's multiple range test, 5% level.

Stamen wilting

Mean stamen wilting percentage increased from 0 to approximately 50% over the experimental period (Fig. 4.3A). However, the solution with 6% sucrose caused a numerically higher stamen wilting (32%) than that of other treatments (Fig. 4.3B). There were no significant effects of treatments on stamen wilting on individual days.

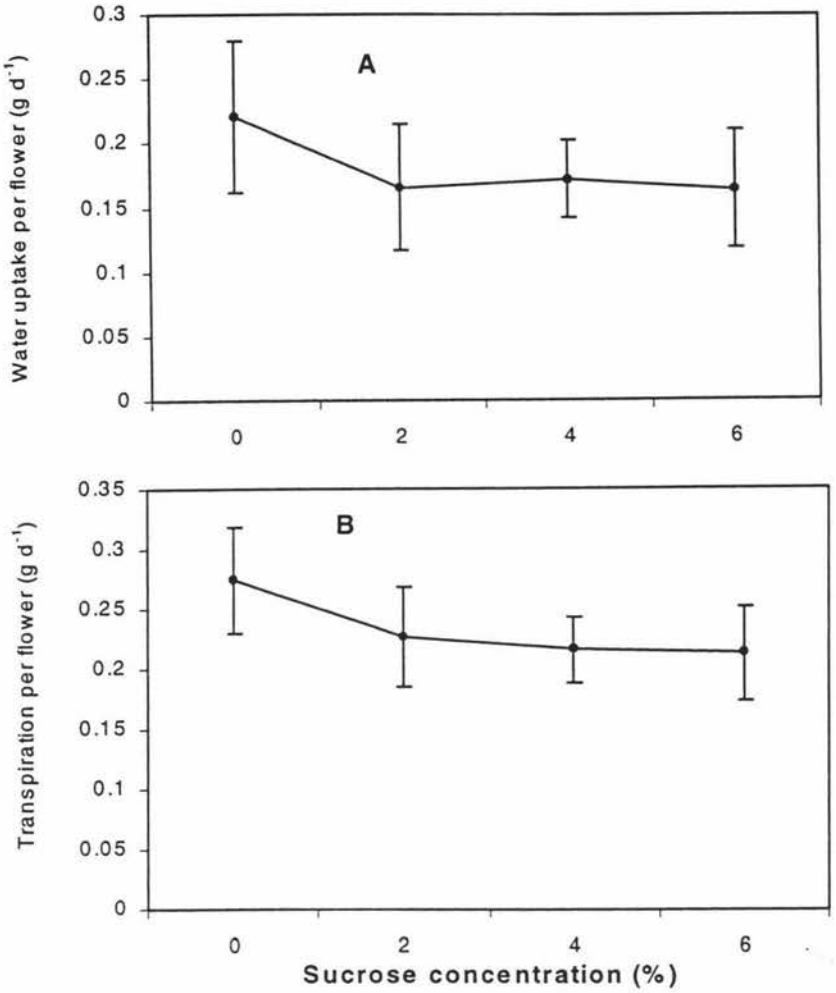

Figure 4.2 Effects of holding solution sucrose concentration on water uptake (A), and transpiration (B) averaged over the whole experimental period. Vertical bars represent standard errors of means.

Buds opening

Not all flowers progressed from Stage 4 (tight bud) to Stage 5 (flower opening). Solutions containing 200 ppm HQC or 200 ppm HQC with 4% sucrose had higher percentage of flower opening, and controls had lower percentage of flower opening than others after five days (Table 4.2). In controls, water uptake did not differ between flowers that remained at Stage 4 and those that progressed to Stage 5. However, the water uptake of open flowers was greater than that of flowers in bud for solutions containing 200 ppm HQC with 0, 2, 4 and 6% sucrose, especially on the first 1-3 days (Fig. 4.4).

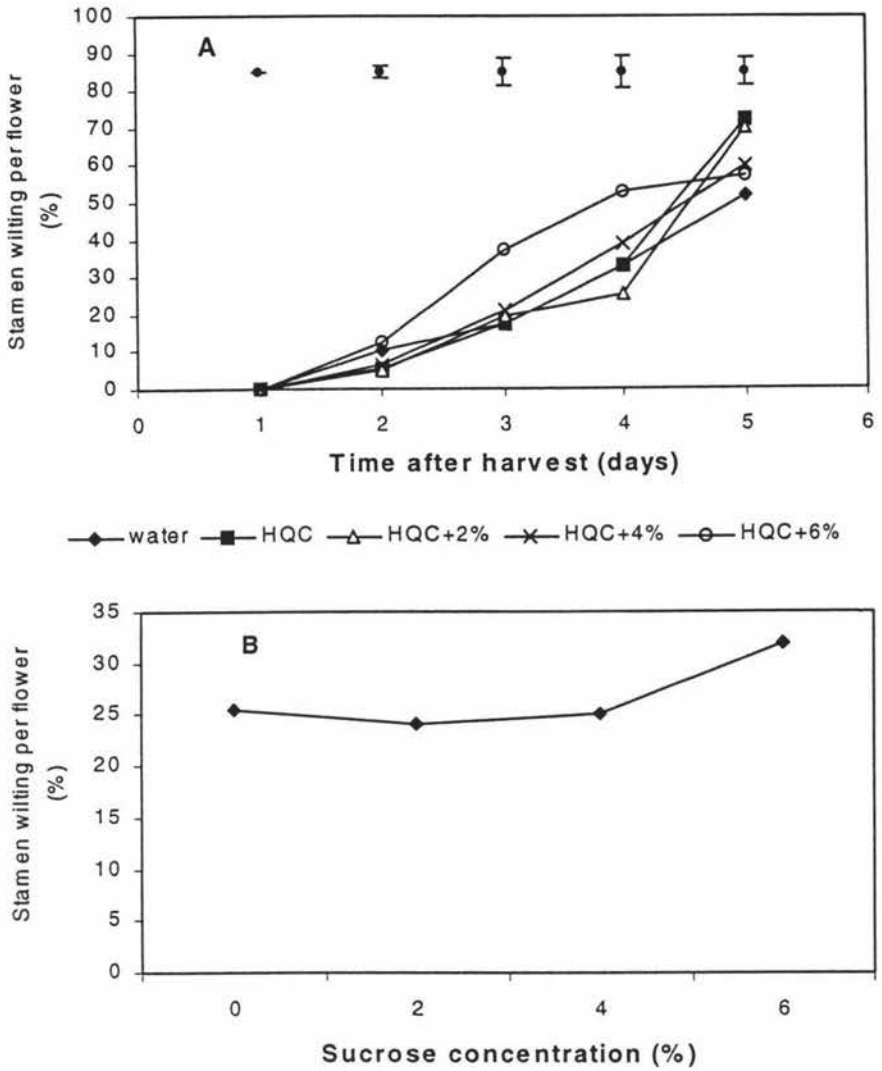

Figure 4.3 Effects of all treatments (A) and holding solution sucrose concentration (B) on stamen wilting. Vertical bars represent standard errors of means for (A).

Table 4.2 Percentage flower opening of different treatment after 5 days in cut flowers of *E. ficifolia.*

Treatment	% flowers at Stage 4 (tight bud) after 5 days	% flowers at Stage 5 (flower opening) after 5 days
Water (Control)	40%	60%
HQC (200 ppm)	20%	80%
HQC+2% Sucrose	30%	70%
HQC+4% Sucrose	20%	80%
HQC+6% Sucrose	30%	70%

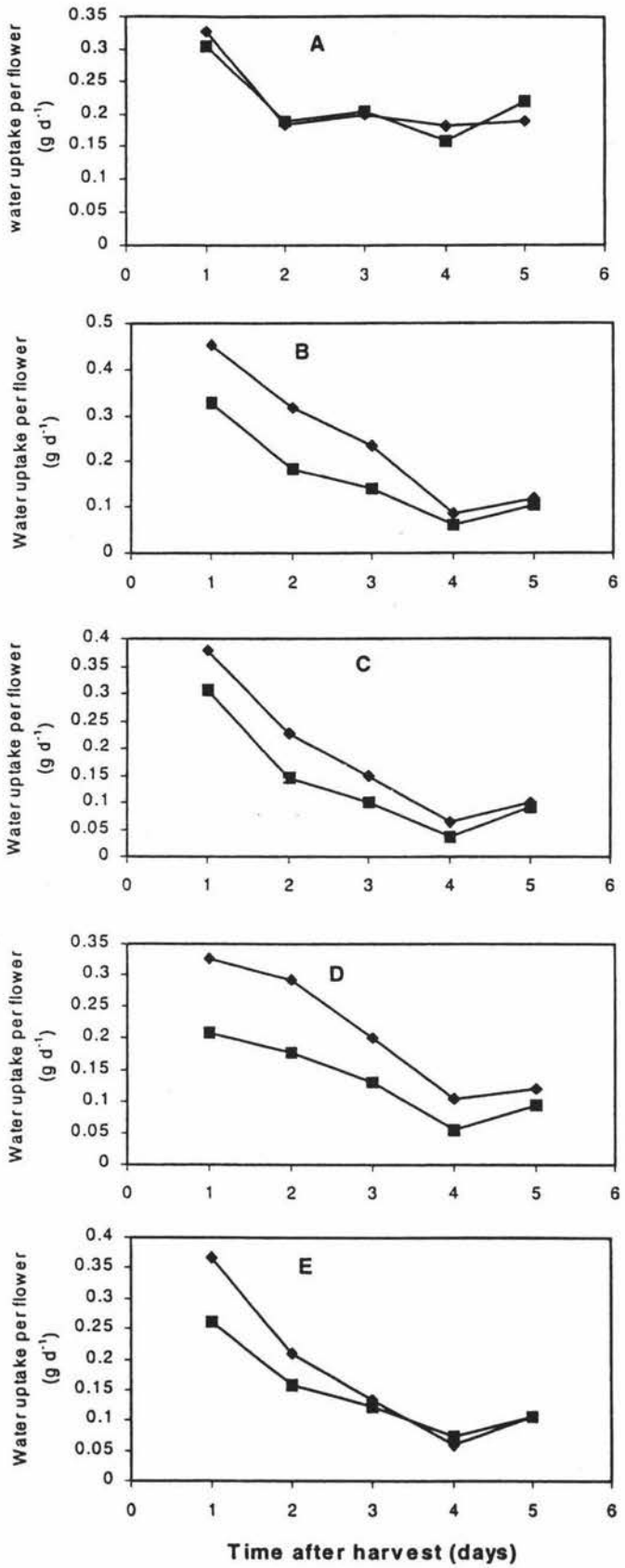

Figure 4.4 Effects of holding solution treatments [water control (A); HQC-200 ppm (B); HQC+2% sucrose (C); HQC+4% sucrose (D) and HQC+6% sucrose (E)] on water uptake of buds opening. Flowers at Stage 5 (◆ "flowers opening") and Stage 4 (■ "tight bud").

4.1.2 Experiment 2: Effect of sucrose, HQC and pH of HQC solution (controlled by citric acid-sodium citrate buffer) on vase life characteristics of *Eucalyptus ficifolia*

Water relations

Changes in water uptake over the four days followed a similar pattern for all treatments, declining significantly ($p<0.0001$) each day for the first two days. Mean water uptake per flower decreased from approximately 0.65 to 0.2 $g \cdot day^{-1}$ over the experimental period (Fig. 4.5A), whereas mean transpiration per flower decreased from approximately 0.75 to 0.3 $g \cdot day^{-1}$ for the first three days (Fig. 4.5B). Mean flower mass decreased from approximately 1.16 to 0.9$g \cdot day^{-1}$ (Fig. 4.5C).

There was a trend of increasing water uptake with increasing sucrose concentration and HQC concentration (Fig. 4.6A and 4.6B). The 200 ppm HQC solution with pH adjusted are significantly better than those with no adjustment from Days 2 to 4 (Table 4.3).

The solution with 2% sucrose was significantly better for water uptake than solution with 0.5% and 0.1% sucrose on Days 3 and 4. On Day 3, 400 ppm HQC solution was significantly better than 200 and 100 ppm HQC solutions (Table 4.3).

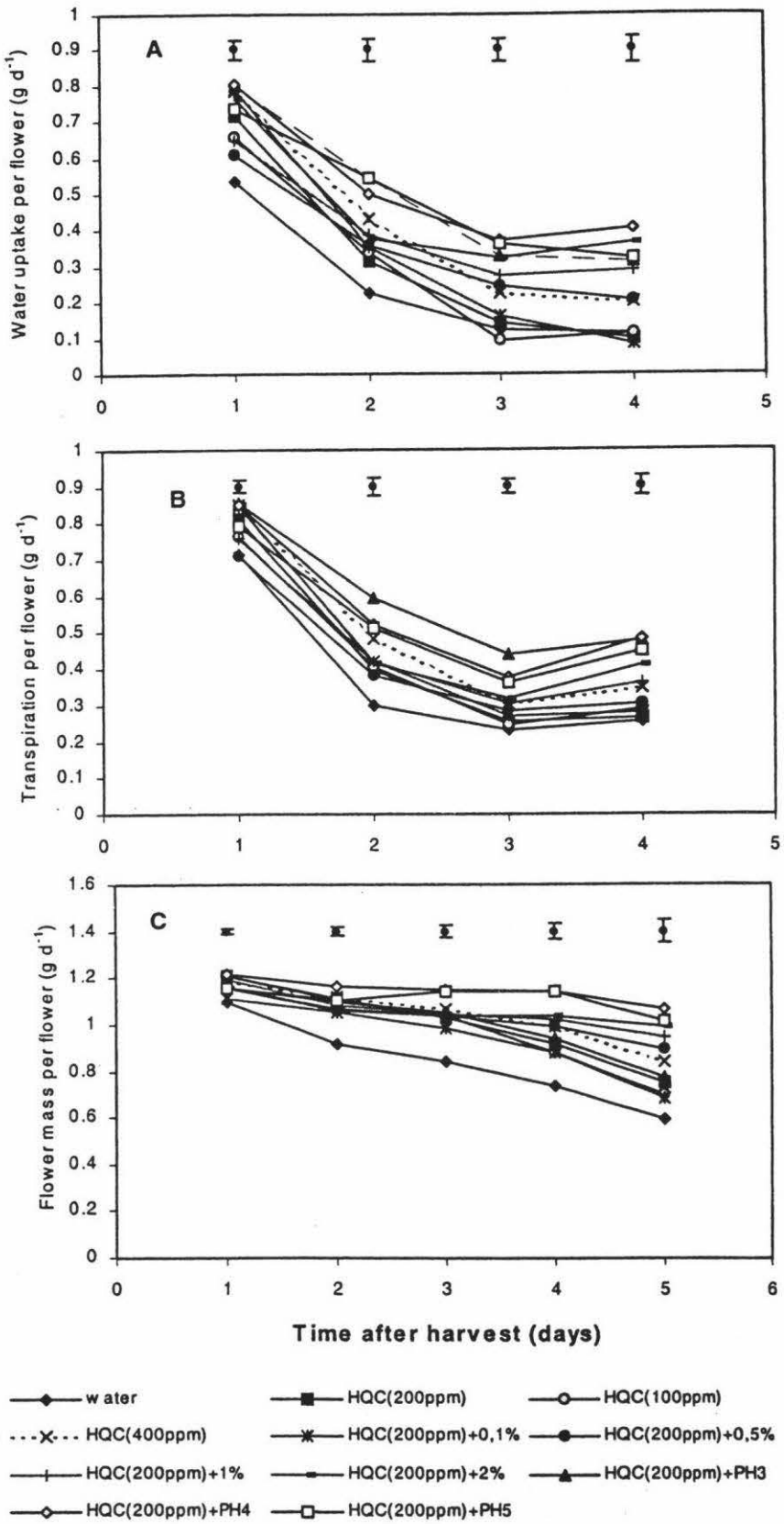

Figure 4.5 Effects of ten holding solutions and water control on water uptake (A), transpiration (B) and flower mass (C) in cut flower of *E. ficifolia*. Vertical bars represent standard errors of means.

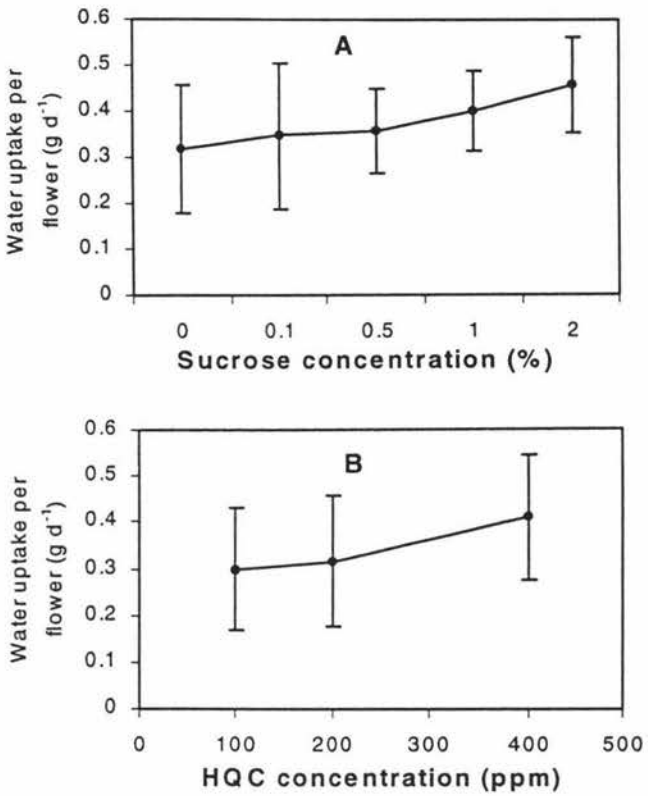

Figure 4.6 Effects of holding solution with sucrose concentration (A), HQC concentration (B) on water uptake. Vertical bars represent standard errors of means.

Table 4.3 Effect of holding solution treatments on water uptake of cut flower of *E. ficifolia*.

Treatment	Days		
	2	3	4
HQC+PH5	0.55a	0.36a	0.32a
HQC+PH3	0.54a	0.33ab	0.31ab
HQC+PH4	0.50ab	0.37a	0.41a
HQC-400ppm	0.43abc	0.23bcd	0.20bc
HQC+1% Sucrose	0.39bc	0.28ab	0.29ab
HQC+2% Sucrose	0.38bcd	0.32ab	0.37a
HQC+0.5% Sucrose	0.36bcd	0.24bc	0.20bc
HQC+0.1% Sucrose	0.35bcd	0.16cde	0.08c
HQC-100ppm	0.34cd	0.09e	0.11c
HQC-200ppm	0.31cd	0.14cde	0.10c
Water (Control)	0.22d	0.12de	0.11c

Mean separation in columns by Duncan's multiple range test at 5% level.

In general, transpiration was unaffected by pH and HQC on Days 2, 3 and 4 (Table 4.4). Transpiration from the solution with 2% sucrose was significantly higher than solution with 0.1% sucrose on Days 3 and 4, and there were trend of increasing transpiration with increasing sucrose concentration of holding solution (Fig. 4.7).

Mean of flower mass was lower for holding solution at pH3 compared to pH 4 and 5 (Fig. 4.8A). There were trend of increasing mean of flower mass with increasing sucrose concentration or HQC concentration of the holding solution (Fig. 4.8B and 4.8C).

Treatment had significant effect ($p<0.001$) on flower mass on Days 3, 4 and 5. Flower mass on Days 4-5 was significantly higher for holding solutions at pH 4 and 5, than at pH 3 (Table 4.5). All treatments except 100 ppm HQC and HQC plus 0.1% sucrose gave significantly higher flower mass than the water control. Flower mass was significantly higher for the 400 ppm HQC solution than for the 100 ppm HQC solution on Day 5. Flower

mass was significantly higher for solutions with 0.5-2% sucrose than for the solution containing 0.1% sucrose (Table 4.5).

Table 4.4 Effect of holding solution treatments on flower transpiration of cut flower of *E. ficifolia.*

Treatments	Days		
	2	3	4
HQC+PH4	0.52ab	0.37a	0.48a
HQC+PH5	0.51ab	0.36a	0.45ab
HQC+PH3	0.59a	0.33ab	0.48a
HQC+2% Sucrose	0.41bc	0.32ab	0.41abc
HQC+1% Sucrose	0.42ce	0.28ab	0.36bcd
HQC+0.5% Sucrose	0.38bc	0.24bc	0.31cd
HQC-400ppm	0.48ab	0.23bcd	0.35bcd
HQC+0.1% Sucrose	0.42bc	0.16cde	0.28d
HQC-200ppm	0.39bc	0.14cde	0.27d
HQC-100ppm	0.41bc	0.09e	0.29d
Water (Control)	0.30c	0.12de	0.26d

Mean separation in columns by Duncan's multiple range test at 5% level.

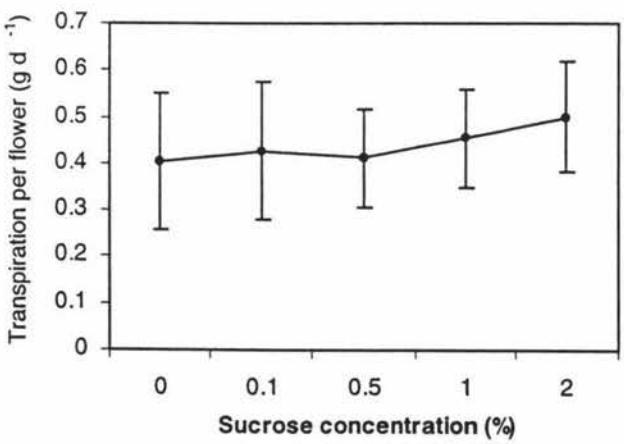

Figure 4.7 Effects of sucrose concentration on transpiration rate. Vertical bars represent standard errors of means.

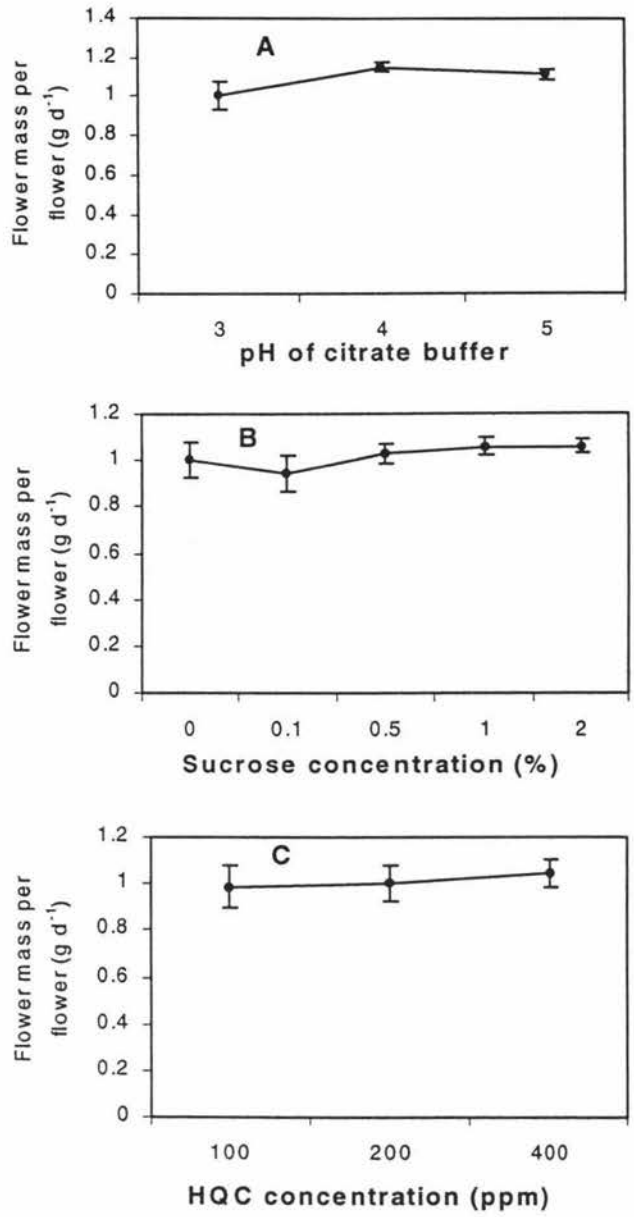

Figure 4.8 Effects of holding solution pH (A), sucrose concentration (B) and HQC concentration (C) on flower mass. Vertical bars represent standard errors of means.

Table 4.5 Effect of holding solution treatments on flower mass of cut flower of *E. ficifolia.*

Treatments	Days		
	3	4	5
HQC+PH4	1.15a	1.14a	1.07a
HQC+PH5	1.14a	1.14a	1.02ab
HQC-400ppm	1.07ab	0.99bc	0.84cde
HQC+PH3	1.05ab	0.94bc	0.77edf
HQC+1% Sucrose	1.05ab	1.02ab	0.95abc
HQC+0.5% Sucrose	1.04ab	0.99bc	0.89bcd
HQC+2% Sucrose	1.03ab	1.04ab	0.99ab
HQC-100ppm	1.03ab	0.88c	0.70fg
HQC-200ppm	1.03ab	0.92bc	0.75ef
HQC+0.1% Sucrose	0.99b	0.88c	0.68fg
Water (Control)	0.84c	0.74d	0.59g

Mean separation in columns by Duncan's multiple range test at 5% level.

Stamen wilting

Mean stamen wilting per flower increased from 2 to 75% over the experiment (Fig. 4.9). Stamen wilting percentage over the whole experimental period was significantly higher ($p<0.0001$) for flowers held in solution at pH 3 than for those held at pH 5 or 4, which were not significantly different (Table 4.6). Flowers held in 400 ppm HQC solution was significantly lower stamen wilting than those held at 200 or 100 ppm solution. Controls had significantly higher wilting than all treatments, except for the solution adjusted to pH 3 (Table 4.6). Solutions with 0 or 0.1% sucrose gave significantly higher on stamen wilting than those with 2, 1 or 0.5% sucrose (Table 4.6).

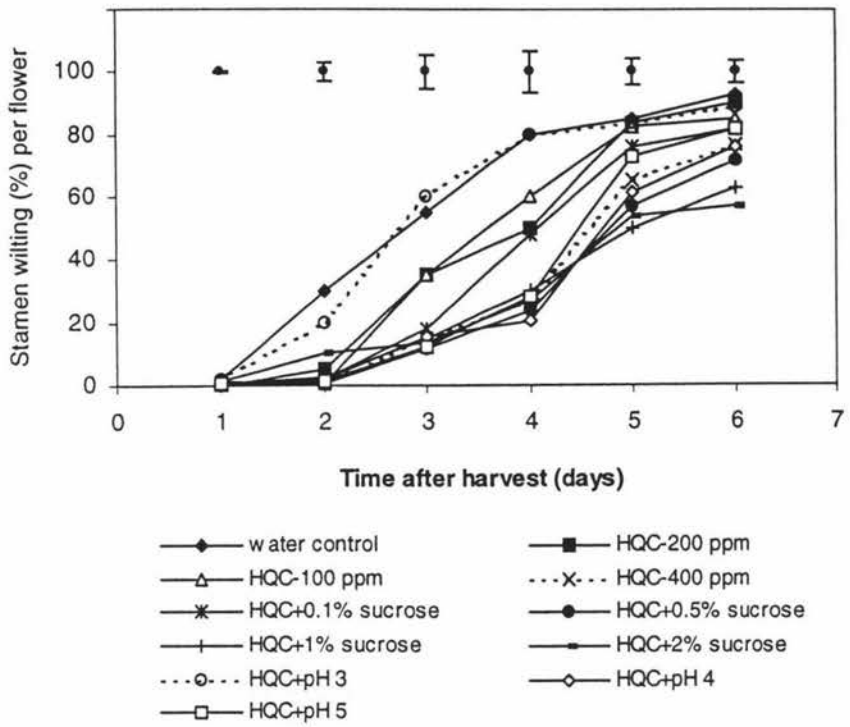

Figure 4.9 Effect of holding solution treatments on stamen wilting in cut flowers of *E. ficifolia*. Vertical bars represent standard errors of means.

Table 4.6 Effect of holding solution treatments on stamen wilting of opening flower over the experiment.

Treatment	Mean
Water (Control)	57.42a
HQC+PH3	55.71a
HQC-200ppm	44.04b
HQC-100ppm	44.00b
HQC+0.1% Sucrose	37.73bc
HQC+PH5	32.77bc
HQC-400ppm	30.65c
HQC+PH4	29.21c
HQC+0.5% Sucrose	27.37c
HQC+2% Sucrose	26.94c
HQC+1% Sucrose	26.75c

Mean separation in columns by Duncan's multiple range test at 5% level.

Stamen abscission

Stamen abscission was observed only for solutions that had pH adjusted (Fig. 4.10). Stamen abscission over the whole experimental period was significantly higher ($p<0.0001$) for flowers held in the solution at pH 3 than for those held at pH 5 or 4, which were not significantly different (Table 4.7).

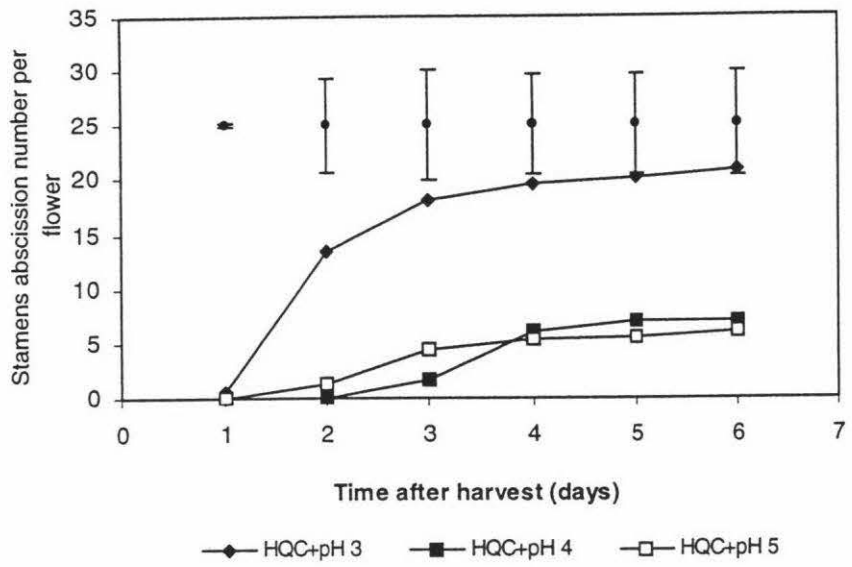

Figure 4.10 Effect of holding solutions and control treated cut flower of *E. ficifolia* on stamen abscission. Vertical bars represent standard errors of means.

Table 4.7 Effect of holding solutions pH on stamen abscission of opening flowers over the experiment.

Holding solution HQC-200 ppm With pH adjusted	Mean
HQC+PH3	15.38a
HQC+PH4	3.63b
HQC+PH5	3.77b

Mean separation in columns by Duncan's multiple range test at 5% level.

4.1.3 Experiment 3: Effect of sucrose and pH of HQC solution (controlled by citric acid-sodium citrate buffer) on water use and vase life characteristics in cut cymules of *Metrosideros collina* 'Tahiti'

Water relations

Mean water uptake per cymule averaged across all treatments stayed relatively constant at approximately 0.5g·day^{-1} over the experimental period, whereas mean transpiration per cymule gradually increased from approximately 0.4 to 0.5g·day^{-1}. Mean cymule mass increased in the initial 1-2 days after harvest before declining steadily at a rate of approximately 0.01g·day^{-1} (3% of cymule mass per day) (Fig. 4.11).

There was a trend of increasing water uptake with increasing pH of the holding solution (Fig. 4.12A). Although pH did not have a significant effect over the whole experimental period, the effect of pH approached significance (p = 0.05-0.07) on Days 5-8. Comparison of means at these times using Duncan's Multiple Range Test indicated that water uptake from the holding solution at pH 5 was consistently better than that at either pH 3 or pH 4; the latter were not significantly different from each other.

There was also a trend of increasing water uptake with increasing sucrose concentration of the holding solution (Fig. 4.12B). However, this effect did not achieve significance tested over the whole experimental period, nor did the effect of sucrose concentration for each day approach significance (p > 0.09). The interaction between sucrose concentration and pH was not significant.

When data for water uptake for the 12 factorial treatment combinations and for the water control were analysed, there were no significant effects.

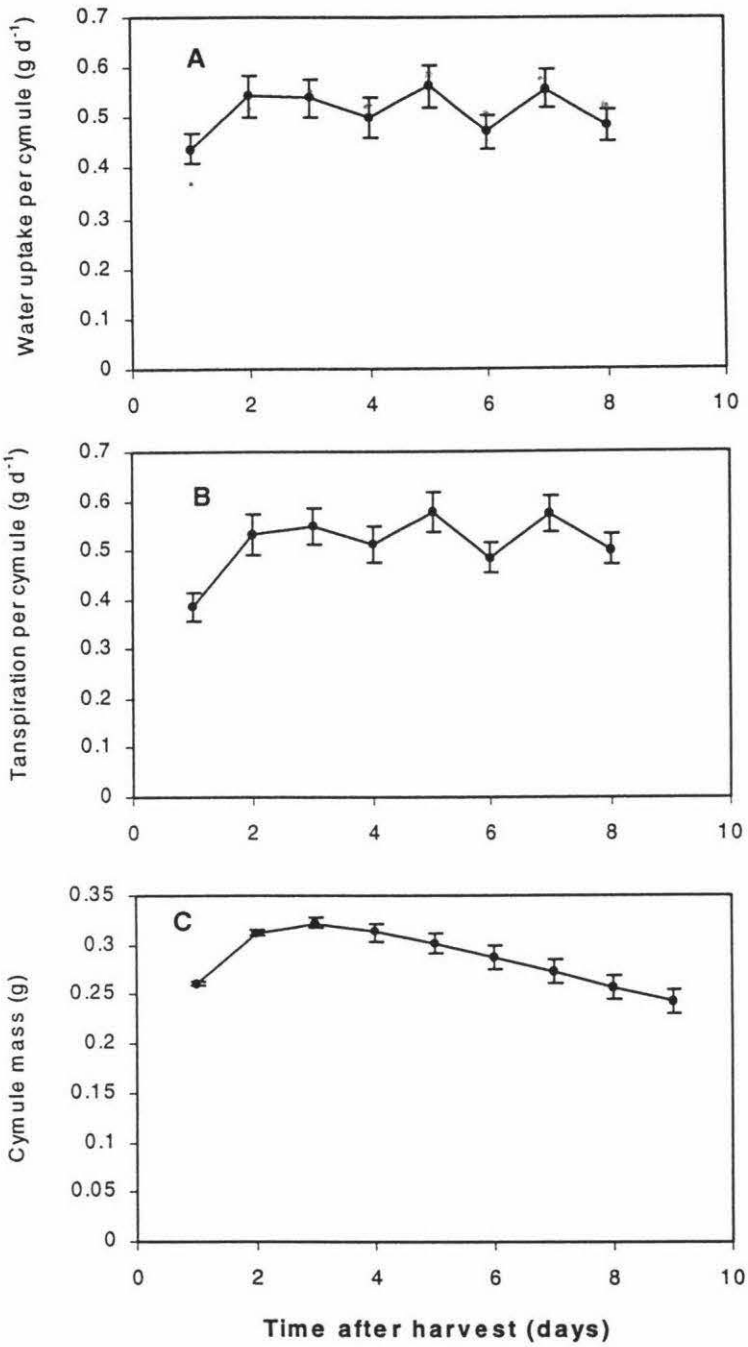

Figure 4.11 Effects of 13 treatments (average data) for water uptake (A), transpiration (B) and cymule mass (C) in cut cymules of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

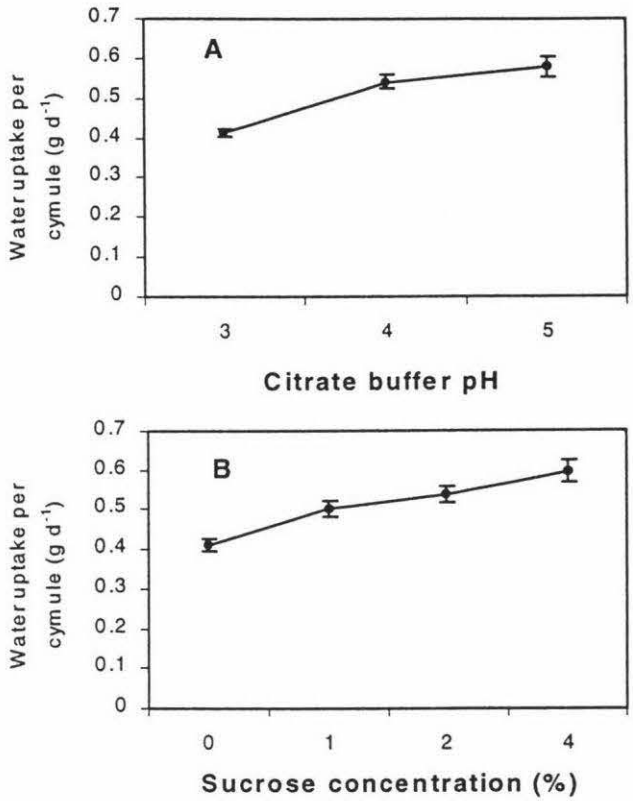

Figure 4.12 Effects of holding solution with pH adjusted (A) and sucrose concentration (B) on water uptake by cymules of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

In general, transpiration from cymules was unaffected by pH and sucrose treatments, and the interaction between these factors was not significant. However, on Days 7 and 8, transpiration using a holding solution at pH 5 was significantly higher ($p < 0.05$) than that from one at pH 3 (Table 4.8). There was a trend of increasing transpiration with increasing pH of the holding solution (Fig. 4.13). There were no significant effects on transpiration when the 12 factorial treatment combinations and the water control were analysed.

There was a trend of increasing cymule mass with increasing pH (Fig. 4.14A). From Day 4 until the end of the experiment, cymule mass was significantly lower ($p < 0.0001$) for holding solutions at pH 3 compared to pH 4 or 5 (Table 4.9).

Sucrose concentration had no effect on cymule mass in analyses for the whole experimental period. However, the decline in cymule mass over time was less marked for holding solutions containing increasing concentration of sucrose (Fig. 4.14B), and on Days 8 and 9 there were significant effects of sucrose concentration on cymule mass. Cymules receiving 4% sucrose had higher masses than those receiving less sucrose (Table 4.9).

Including the water control, analyses of variance for the 13 treatments revealed significant effects of treatments for Days 4–9 ($P < 0.012$). In general, solutions with pH 5 and 2% or 4% sucrose gave rise to significantly higher cymule mass than other solutions with pH 3 and sucrose (Table 4.10). However, no solution gave a higher cymule mass than the water control.

Table 4.8 Effect of holding solution with pH adjusted treatments on transpiration in cut cymule of *M. collina* 'Tahiti'.

pH	Day 7	Day 8
3	0.45b	0.40b
4	0.58ab	0.51ab
5	0.66a	0.57a

Mean separation within columns by Duncan's Multiple Range Test at $P<0.05$

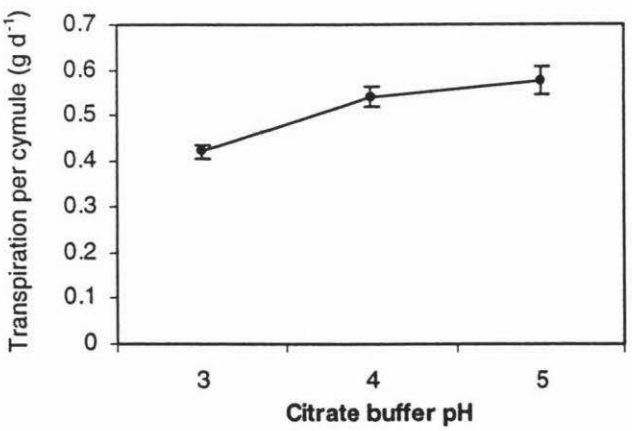

Figure 4.13 Effects of holding solution pH on transpiration of cymules of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

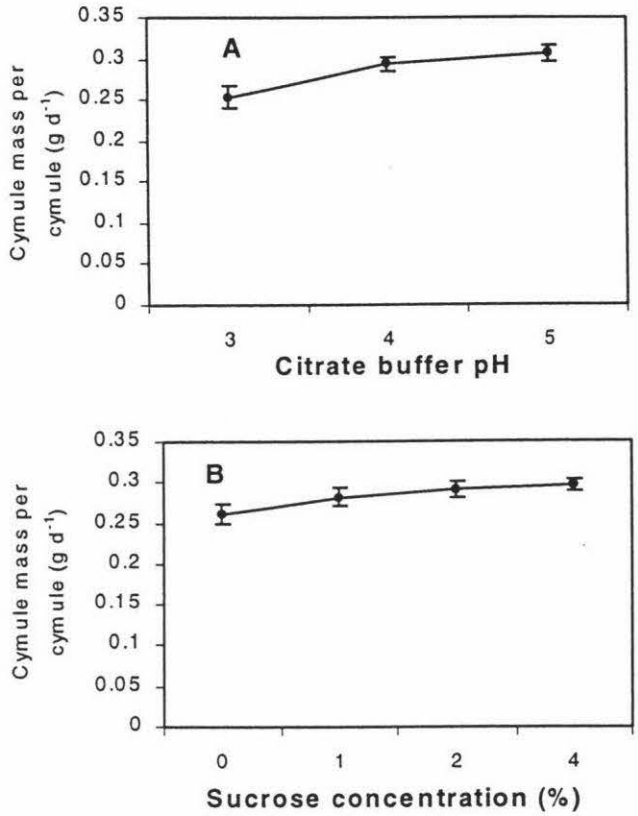

Figure 4.14 Effects of holding solution pH (A) and sucrose concentration (B) on cymule mass of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

Table 4.9 Effect of holding solution with pH adjusted and sucrose concentration treatments on cymule mass in cut cymule of *M. collina* 'Tahiti'.

		Days					
		4	5	6	7	8	9
Holding solution with pH adjusted	3	0.27b	0.25b	0.23b	0.21b	0.20b	0.20b
	4	0.32a	0.31a	0.30a	0.28a	0.27a	0.26a
	5	0.34a	0.33a	0.31a	0.30a	0.29a	0.27a
Holding solution with sucrose concentration (%)	0					0.22b	0.21b
	1					0.25b	0.23ab
	2					0.26ab	0.25ab
	4					0.26a	0.27a

Mean separation within columns by Duncan's Multiple Range Test at P<0.05

Table 4.10 Effect of 13 holding solution treatments on cymule mass in cut cymule of *M. collina* 'Tahiti'.

Treatment	Days					
	4	5	6	7	8	9
PH5+HQC+2% Sucrose	0.35a	0.35a	0.34a	0.33a	0.31ab	0.29a
PH 5+HQC+4% Sucrose	0.35a	0.35a	0.34ab	0.33a	0.32a	0.31a
PH 5+HQC+1% Sucrose	0.34a	0.33ab	0.31abc	0.28abcd	0.26abcd	0.24ab
PH 4+HQC+1% Sucrose	0.33ab	0.33ab	0.31abc	0.29abc	0.27abcd	0.25ab
Water (control)	0.33ab	0.32ab	0.33ab	0.32ab	0.28abc	0.26ab
PH 4+HQC+2% Sucrose	0.32ab	0.31abcd	0.30abc	0.29abc	0.27abc	0 .26ab
PH 4+HQC	0.32ab	0.30abcde	0.27bcde	0.24bcd	0.22cd	0.21b
PH 5+HQC	0.32ab	0.31abcd	0.29abcd	0.27abcd	0.26abcd	0.24ab
PH 4+HQC+4% Sucrose	0.31abc	0.32abc	0.32abc	0.32ab	0.32a	0.31a
PH 3+HQC+4% Sucrose	0.29abc	0.27bcde	0.25cde	0.24bcd	0.23bcd	0.20b
PH 3+HQC	0.28bc	0.25de	0.23de	0.21d	0.19d	0.18b
PH 3+HQC+2% Sucrose	0.27bc	0.24e	0.22e	0.21d	0.20cd	0.21b
PH 3+HQC+1% Sucrose	0.25c	0.26cde	0.23de	0.22cd	0.21cd	0.20b

Mean separation within columns by Duncan's Multiple Range Test at P<0.05

Stamen wilting

Mean stamen wilting per cymule increased from 0 to approximately 30% over the experimental period (Fig. 4.15A). There was a trend of increasing stamen wilting rate with decreasing pH values for holding solutions (Fig. 4.16). The effect of pH on stamen wilting became significant ($p<0.0001$) 3-8 days after harvest. Cymules held in solutions at pH 3 exhibited 10% wilting after three days, rising to a mean of 49% wilting at Day 8. In contrast, stamens of cymules held at pH 4 or 5 exhibited only 6-17% wilting on Day 8 (Table 4.11).

Sucrose concentration had no effect on stamen wilting, or significant interaction with pH when data for the whole experimental period were analyzed. However, stamen wilting was significantly lower on Day 8 using a 4% sucrose solution (12% wilting) compared to a 0 or 2% sucrose solution (32% and 27% wilting, respectively) (Table 4.11). There was also a highly significant interaction ($p<0.001$) between sucrose concentration

and pH on Days 3 and 4. Solution with pH 3 and 2% sucrose had significantly higher wilting percentage than other solutions (Table 4.12).

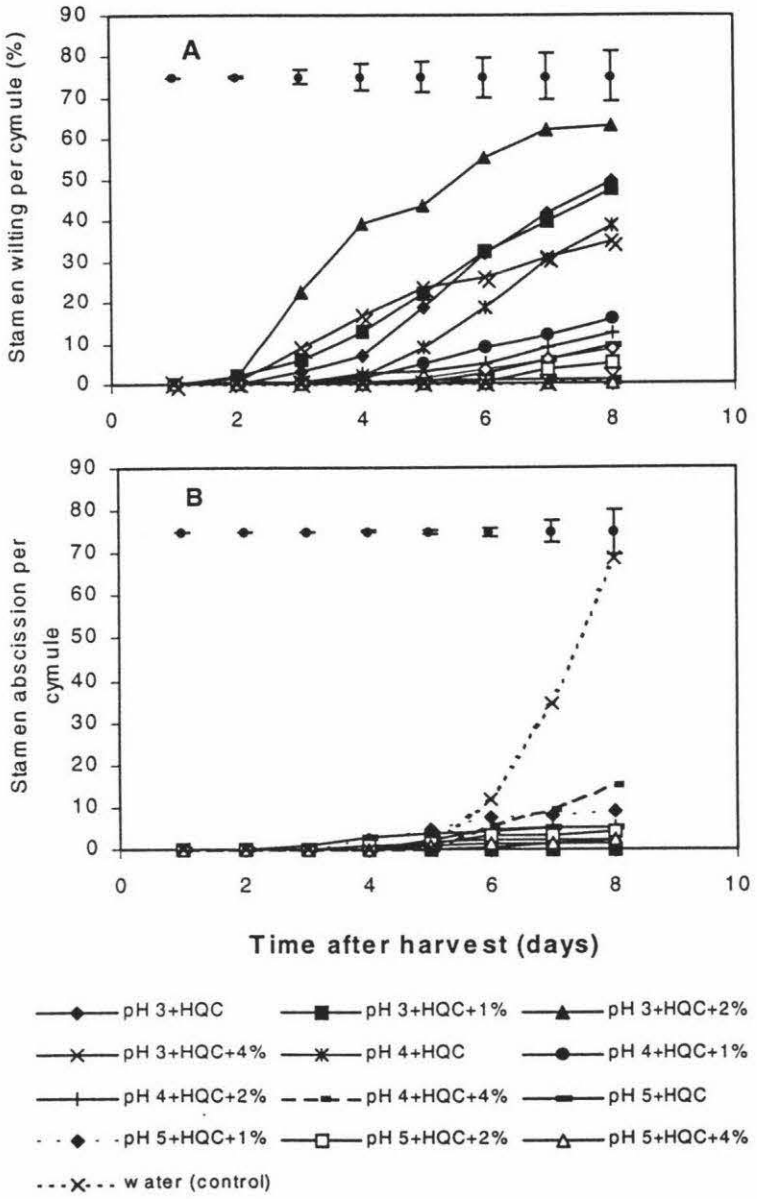

Figure 4.15 Effects of thirteen treatments (holding solutions) on stamen wilting percentage (A) and stamen abscission (B) in cymules of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

Including the water control, analyses of variance for the 13 treatments revealed significant effects of treatment for Days 3-8. In general, solutions with pH 3 and 0% or 2% sucrose gave rise to significantly higher wilting percentages than other treatments. The water control exhibited the same stamen wilting as pH 5 and pH 4 treatments (Table 4.13). Stamen wilting over whole experiment for 13 treatments was highest in cymules that had the least favorable water balance (i.e. low cymule mass) (Fig. 4.17).

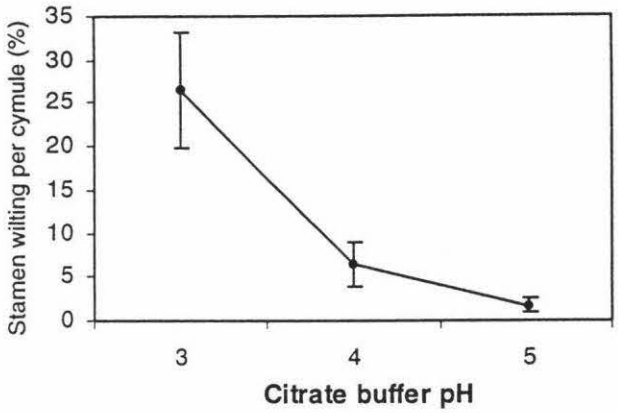

Figure 4.16 Effects of holding solution pH on stamen wilting percentage of cut cymules of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

Table 4.11 Effects of holding solution with pH adjusted and sucrose concentration on stamen wilting in cut cymules of *M. collina* 'Tahiti'.

		Day					
		3	4	5	6	7	8
pH	3	10.00a	18.75a	26.92a	36.42a	43.42a	48.75a
	4	0.412b	1.58b	4.50b	8.33b	13.00b	6.83b
	5	0.00b	0.08b	0.58b	1.67b	3.75b	5.67b
Sucrose concentration (%)	0						32.44a
	1						23.89ab
	2						26.78a
	4						11.89b

Mean separation within columns by Duncan's Multiple Range Test at P<0.05

Table 4.12 Effect of interaction between pH and sucrose concentration in cut cymules of *M. collina* 'Tahiti'.

Sucrose (%)	pH	Day 3 (Lsmean)	Day 4 (Lsmean)
0	3	3.00a	6.67a
0	4	0.67a	2.00a
0	5	0.00a	0.33a
1	3	5.67a	12.67a
1	4	0.00a	1.67a
1	5	0.00a	0.00a
2	3	22.33b	39.00b
2	4	0.67a	2.33a
2	5	0.00a	0.00a
4	3	9.00a	16.67a
4	4	0.33a	0.33a
4	5	0.00a	0.00a

Mean separation within columns by Duncan's Multiple Range Test at P<0.05

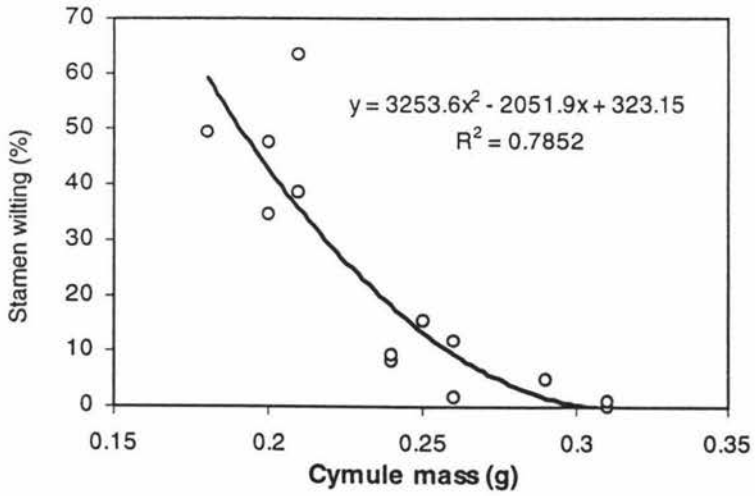

Figure 4.17 Relation between cymule mass and stamen wilting on Day 8 in cut cymules of *M. collina* 'Tahiti'.

Table 4.13 Effect of 13 holding solution treatments on stamen wilting in cut cymules of *M. collina* 'Tahiti'.

Treatment	Days					
	3	4	5	6	7	8
PH3+HQC+2% Sucrose	22.33a	39.00a	43.66a	55.33a	62.00a	63.33a
PH3+HQC+4% Sucrose	9.00b	16.67b	23.33b	26.00bc	30.67bc	34.67bcd
PH3+HQC+1% Sucrose	5.67bc	12.67bc	22.00b	32.33b	39.67b	47.67ab
PH3+HQC	3.00c	6.67bcd	18.67bc	32.00b	41.34ab	49.33ab
PH4+HQC+2% Sucrose	0.67c	2.33cd	3.00cd	4.99d	9.00cd	12.00de
PH4+HQC	0.67c	2.00cd	9.00bcd	18.67bcd	30.33bc	38.67abc
PH4+HQC+4% Sucrose	0.33c	6.33d	1.00d	1.00d	1.00d	1.00e
PH4+HQC+1% Sucrose	0.00c	1.67cd	5.00cd	8.67cd	11.67cd	15.67cde
PH5+HQC	0.00c	0.33d	0.67d	2.67d	5.67d	9.33e
PH5+HQC+1% Sucrose	0.00c	0.00d	1.67d	3.33d	5.67d	8.33e
PH5+HQC+2% Sucrose	0.00c	0.00d	0.00d	0.67d	3.67d	5.00e
PH5+HQC+4% Sucrose	0.00c	0.00d	0.00d	0.00d	0.00d	0.00e
Water (Control)	0.00c	0.00d	0.00d	0.00d	0.00d	1.67e

Mean separation within columns by Duncan's Multiple Range Test at $P<0.05$

Stamen abscission

Mean stamen abscission per cymule gradually increased from 0 to 5, except in the water control which exhibited very high abscission (68.6% at the end of experiment) (Fig. 4.15B).

Stamen abscission, which began three days after harvest, there was a trend of increasing stamen abscission with increasing pH (Fig. 4.18). Abscission for holding solutions at pH 4 and 5 were not significantly different on Days 5–8. Analyses for separate days indicated that this effect was apparent after Day 3 ($p<0.05$) (Table 4.14).

There were significant interactions of sucrose concentration with pH on Days 7 and 8 ($p<0.03$). At the end of the experiment (Days 7 and 8), there was significantly greater abscission at pH 4 than at pH 5 when sucrose was supplied at the highest concentration (4%) (Table 4.15).

Analyses of 13 treatments: On Day 3, the cymules in a holding solution at pH 5 with no added sucrose exhibited a significantly higher abscission than all other treatments. Over the following three days, treatments with solutions at pH 4 and 5 with low or nil concentration of sucrose exhibited significantly higher abscission rates than the other treatments (approximately 3-4 stamens compared to < 1 stamen abscised). However, on Days 6-8, the water control had numerically the highest stamen abscission, and on Days 7 and 8 this was significantly higher than all other treatments (34 and 69 stamens on each day compared to < 10-15 stamens in other treatments) (Table 4.16).

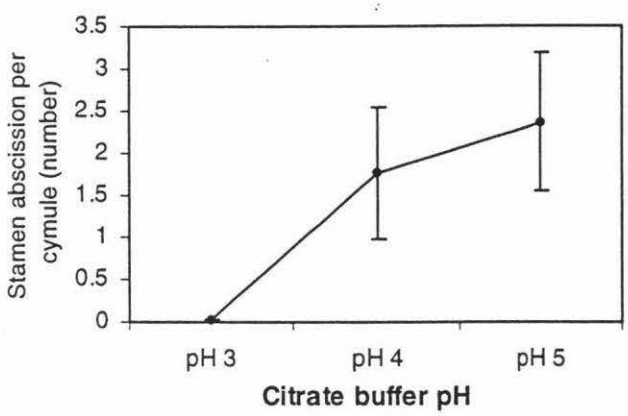

Figure 4.18 Effects of holding solution pH on stamen abscission in cut cymules of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

Table 4.14 Effect of holding solution with pH adjusted on stamen abscission in cut cymules of *M. collina* 'Tahiti'.

pH	Days					
	3	4	5	6	7	8
3	0.00b	0.00b	0.00b	0.00b	0.05b	0.05b
4	0.00b	0.00b	1.05ba	3.30a	4.50a	5.25a
5	0.25a	1.50a	2.60a	4.15a	4.50a	5.95a

Mean separation within columns by Duncan's Multiple Range Test at P<0.05

Table 4.15 Effect of interaction between sucrose concentration and pH on stamen abscission in cut cymules of *M. collina* 'Tahiti'.

Sucrose (%)	pH	Day 7 (Lsmean)	Day 8 (Lsmean)
0	3	0.00a	0.00b
0	4	2.20a	2.20b
0	5	5.00a	5.00ab
1	3	0.00a	0.00b
1	4	1.20a	1.20b
1	5	8.00a	9.20ab
2	3	0.20a	0.20b
2	4	5.20a	5.20ab
2	5	3.40a	4.40ab
4	3	0.00a	0.00a
4	4	9.40a	15.20a
4	5	1.60a	2.40b

Mean separation in columns by Duncan's Multiple Range Test at P<0.05

Table 4.16 Effect of 13 holding solution treatments on stamen abscission in cut cymules of *M. collina* 'Tahiti'.

Treatments	Days					
	3	4	5^{NS}	6^{NS}	7	8
PH5+HQC	0.80a	3.00a	3.80	4.40	5.00b	5.00cd
PH5+HQC+2% Sucrose	0.20b	6.80bc	1.20	3.20	3.40b	4.40cd
PH3+HQC+1% Sucrose	0.00b	0.00c	0.00	0.00	0.00	0.00d
PH3+HQC+4% Sucrose	0.00b	0.00c	0.00	0.00	0.00	0.00d
PH4+HQC	0.00b	0.00c	1.80	2.20	2.20b	2.20cd
PH4+HQC+1% Sucrose	0.00b	0.00c	0.20	0.40	1.20b	1.20d
PH4+HQC+2% Sucrose	0.00b	0.00c	2.20	4.80	5.20b	5.20cd
PH4+HQC+4% Sucrose	0.00b	0.00c	0.00	5.80	9.40b	15.20b
PH3+HQC	0.00b	0.00c	0.00	0.00	0.00	0.00d
PH5+HQC+1% Sucrose	0.00b	0.20ab	4.60	7.40	8.00b	9.20bc
PH3+HQC+2% Sucrose	0.00b	0.00c	0.00	0.00	0.20b	0.20d
PH5+HQC+4% Sucrose	0.00b	0.00c	0.80	1.60	1.60b	2.40cd
Water (Control)	0.00b	0.00c	0.60	11.80	34.40a	68.60a

Mean separation in columns by Duncan's Multiple Range Test at P<0.05

Petal abscission

Petal abscission for the 12 factorial treatments was <1 per cymule on Days 1–8. Only the water control exhibited increasing petal abscission with time, rising to a mean of 7.4 petal per cymule on Day 8 (Fig. 4.19).

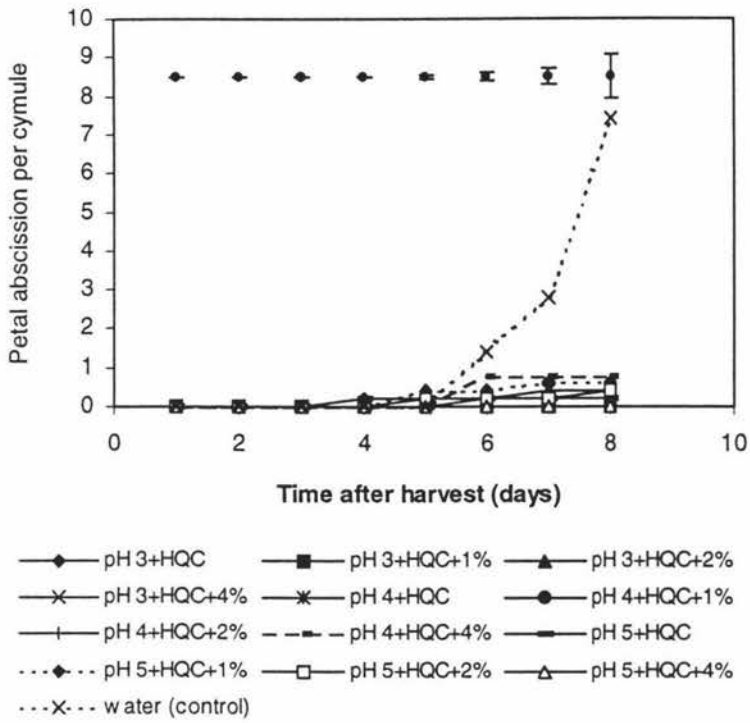

Figure 4.19 Effects of thirteen treatments (holding solutions) on petal abscission in cut cymules of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

4.2 Effect of ethephon and a pre-treatment with silver thiosulphate (STS) on stamen abscission and wilting of Eucalyptus and Metrosideros

4.2.1 Experiment 4: Effects of STS and ethephon on vase life characteristics of *E. ficifolia* flowers

Stamen wilting

Mean stamen wilting percentage per flower averaged across all treatments increased from 3 to 65% over the experimental period (Fig. 4.20). Stamen wilting percentage was significantly affected ($p<0.0001$) by ethephon concentration on Days 3 to 6 after treatment. In general, stamen wilting was increased with increasing ethephon concentration (Fig. 4.21), with ethephon concentrations ≥100 ppm causing higher stamen wilting than controls, There was no significant difference between controls and 10 ppm ethephon on Day 3 to 6 (Table 4.17).

STS concentration had no effect on stamen wilting when data for any day were analysed. There was no significant interaction between STS and ethephon for any day.

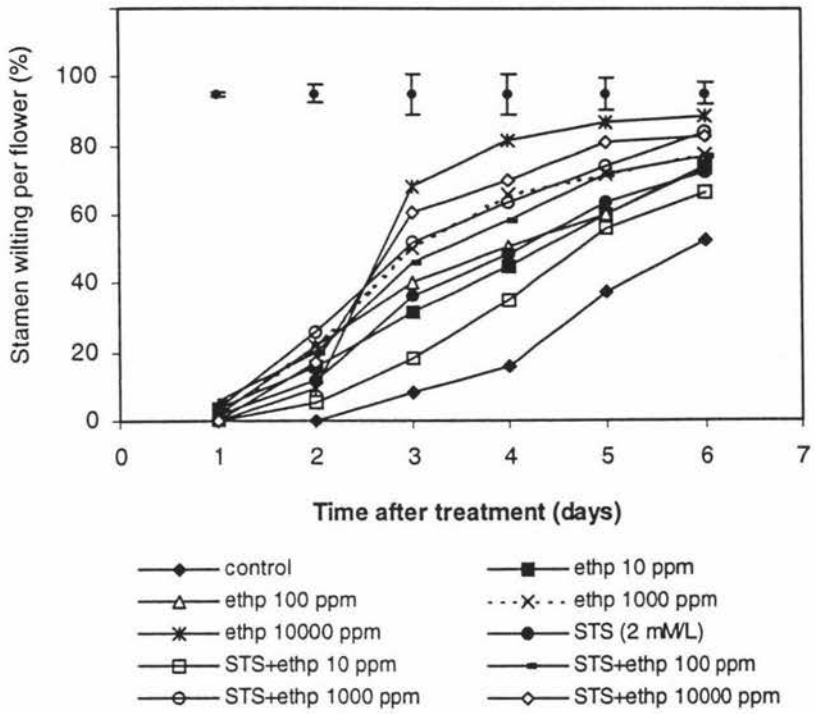

Figure 4.20 Effects of ethephon and STS on stamen wilting in cut flowers of *E. ficifolia*. Vertical bars represent standard errors of means.

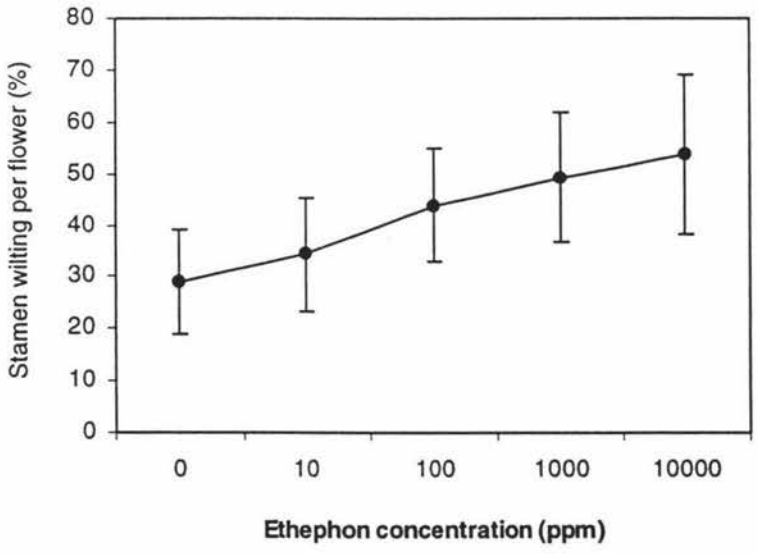

Figure 4.21 Effect of ethephon on stamen wilting in cut flowers of *E. ficifolia*. Verticalbars represent standard errors of means.

Table 4.17 Effects of ethephon on stamen wilting in cut flowers of *E. ficifolia.*

Ethephon Concentration (ppm)	Days			
	3	4	5	6
10,000	64.58a	75.83a	83.75a	85.42a
1,000	50.83ab	64.58ab	72.92ab	80.83ab
100	42.92bc	54.58bc	65.83bc	75.00bc
10	25.00cd	40.00cd	57.92cd	70.42cd
0	22.08d	32.08d	50.42d	62.50d

Mean separation in columns by Duncan's multiple range test at 5% level.

4.2.2 Experiment 5: Effects of STS and ethephon on vase life characteristics of *M. collina* 'Tahiti' cymules

Stamen wilting

Mean stamen wilting per cymule averaged across all treatments increased from 1 to 25% over the experimental period (Fig. 4.22A). The effect of ethephon on stamen wilting was significant ($p<0.05$) on Days 3 to 8 after treatment. In general, stamen wilting increased with increasing ethephon concentration (Fig. 4.23), and there was no significant difference between treatments when ethephon was applied at a concentration $\geq$ 100 ppm. Ethephon applied at 10000 ppm gave significantly higher stamen wilting than at 10 and 0 ppm from Days 5 to 8 (Table 4.18).

STS concentration had no effect ($p>0.05$) on stamen wilting, and no significant interaction with ethephon when data for different days were analysed.

Stamen abscission

Mean stamen abscission gradually increased from approximately 0 to approximately 7 over the experimental period (Fig. 4.22B). Stamen abscission was significantly lower

($p<0.001$) for cymules treated in STS than for controls not pre-treated with STS (Fig. 4.24). This effect was significant from Day 2 to the end of the experiment (Table 4.19).

There was no overall effect of ethephon concentration for and day. However, there were significant interactions of ethephon with STS on Days 1-2, when combination of 1000 ppm ethephon and no STS pre-treatment had significantly higher stamen abscission than others treatments (which all exhibited no abscission) (Table 4.20).

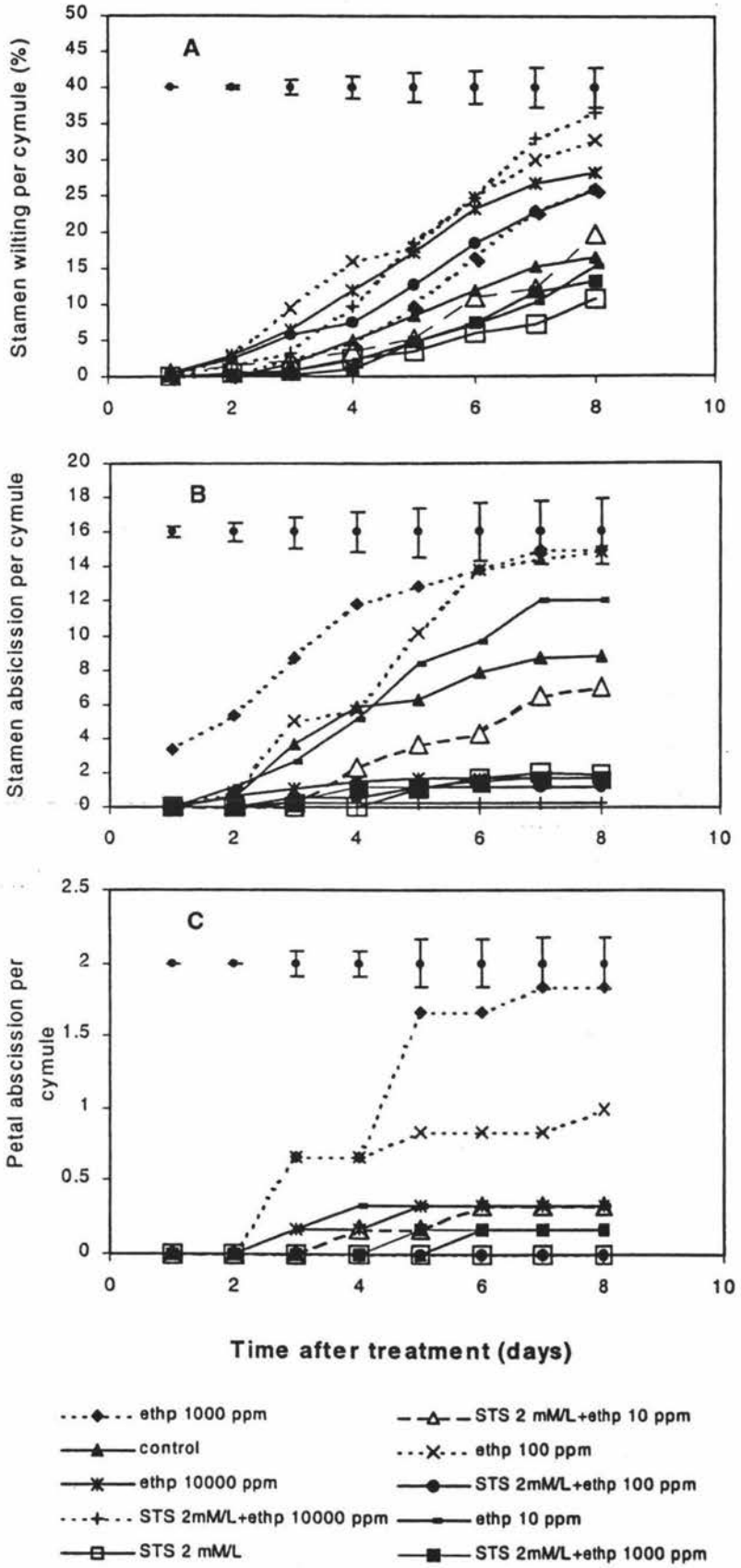

Figure 4.22 Effects of STS pre-treatment and ethephon on stamen wilting rate (A), stamen abscission (B) and petal abscission (C) in cut cymules of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

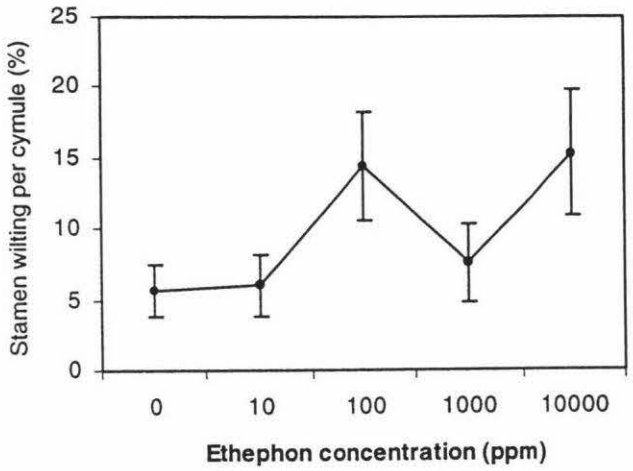

Figure 4.23 Effect of ethephon on stamen wilting in cut cymules of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

Table 4.18 Effect of ethephon on stamen wilting in cut cymules of *M. collina* 'Tahiti'.

Ethephon Concentration (ppm)	Days					
	3	4	5	6	7	8
10,000	4.86ab	10.83a	17.92a	24.03a	29.86a	32.36a
1,000	1.39b	2.78b	7.22bc	11.95bc	17.36ab	19.72abc
100	7.64a	11.81a	15.42ab	21.67ab	26.39a	29.31ab
10	1.53b	2.92b	5.00c	9.17c	11.25b	17.50bc
0	1.25b	3.75b	6.11c	9.03c	11.25b	13.61c

Mean separation in columns by Duncan's multiple range test, 5% level.

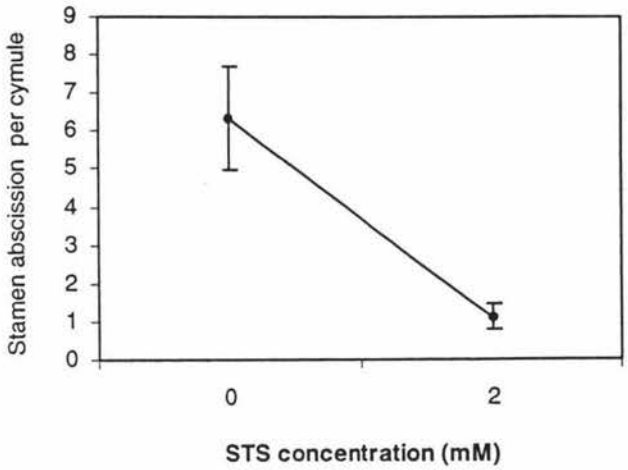

Figure 4.24 Effect of STS pre-treatment on stamen abscission in cut cymules of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

Table 4.20 Effect of interaction between STS pre-treatment and ethephon on stamen abscission in cut cymules of *M. collina* 'Tahiti'.

Ethephon Concentration (ppm)	STS Concentration ($mM \cdot L^{-1}$)	Day 1(Lsmean)	Day 2(Lsmean)
0	0	0.00b	0.50b
0	2	0.00b	0.00b
10	0	0.00b	1.17b
10	2	0.00b	0.00b
100	0	0.00b	0.83b
100	2	0.00b	0.00b
1,000	0	3.33a	5.33a
1,000	2	0.00b	0.00b
10,000	0	0.00b	0.67b
10,000	2	0.00b	0.00b

Mean separation in columns by Duncan's multiple range test, 5% level.

Table 4.19 Effect of STS pre-treatment on stamen abscission in cut cymules of *M. collina* 'Tahiti'.

STS Concentration ($mM \cdot L^{-1}$)	Days						
	2	3	4	5	6	7	8
0	1.70a	4.20a	6.00a	7.87a	9.37a	10.37a	10.47a
2	0.00b	0.20b	0.83b	1.43b	1.77b	2.30b	2.37b

Mean separation in columns by Duncan's multiple range test, 5% level.

Petal abscission

Little petal abscission occurred, except when no STS (0 mM/L) and 1000 ppm or 100 ppm ethephon were applied (Fig. 4.22C). Petal abscission, which began three days after treatment (five days after harvest), was significantly lower for cymules treated with STS (2 mM) than for controls (0 mM) over the whole experiment period (Fig. 4.25). The effect of STS on petal abscission was significant ($p<0.05$) on Days 5 to 8 after treatment (Table 4.21). Ethephon concentration had no effect on petal abscission, and there was no significant interaction with STS.

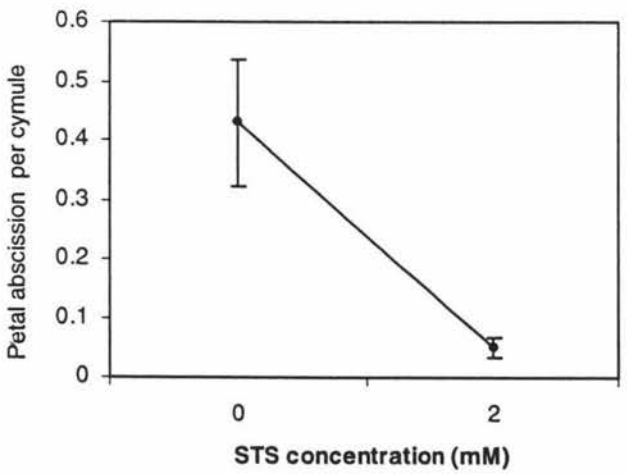

Figure 4.25 Effect of STS pre-treatment on petal abscission in cut cymules of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

Table 4.21 Effect of STS pre-treatment on petal abscission in cut cymules of *M. collina* 'Tahiti'.

STS Concentration ($mM \cdot L^{-1}$)	Days			
	5	6	7	8
0	0.63a	0.67a	0.70a	0.73a
2	0.07b	0.10b	0.10b	0.10b

Mean separation in columns by Duncan's multiple range test, 5% level.

4.3 Ethylene treatment and ethylene production in cut cymules of Metrosideros collina 'Tahiti'

4.3.1 Experiment 6: Effect of exogenous ethylene in cut cymules of *M. collina* 'Tahiti'

Stamen abscission and petal abscission increased with time for all treatments. Mean stamen abscission per cymule averaged across all treatments increased from 0 to 65% over the experimental period (Fig. 4.26A), whereas mean petal abscission rate gradually increased from approximately 0 to 40% (Fig. 4.26B). Concentrations of ethylene were close to those expected for each treatment at the start of the experiment. However, after five days, ethylene concentration had increased by 0.1-0.2 ppm, except in the purafil treatment (Table 4.22).

Stamen abscission

There were significant effects of ethylene treatment on stamen abscission on Days 3-5 ($p<0.0001$). Treatment with 5 ppm ethylene caused the onset of stamen abscission on Day 2 (Fig. 4.26), and the effect that became significantly different from that of all other treatments on Day 3 (Table 4.23A).

On Days 4 and 5, stamen abscission was significantly lower for control and control plus 'Purafil' cymules than for cymules treated with ethylene at any concentration of in the range 0.1-5 ppm (Table 4.23A). Cymules treated with ethylene 0.5 and 5 ppm exhibited complete stamen abscission (75) on Day 5. Cymules treated with no ethylene plus 'Purafil' exhibited a lower stamen abscission than that of controls on Days 4 and 5 (Table 4.23A).

When the two treatments (0.5 and 5 ppm ethylene) for with flower abscission took place (as opposed to stamen abscission) were omitted from the analysis, there were significant effects of ethylene treatment on stamen abscission on Days 3-5 ($p<0.002$). Cymules treated with 0.1 ppm ethylene exhibited a higher stamen abscission than that of the control and control plus Purafil on Days 3-4 (Table 4.23B). However, stamen abscission in controls was not significantly different from that of cymules treated with 0.1 ppm ethylene on Day 5. Controls plus Purafil gave consistently the least stamen abscission.

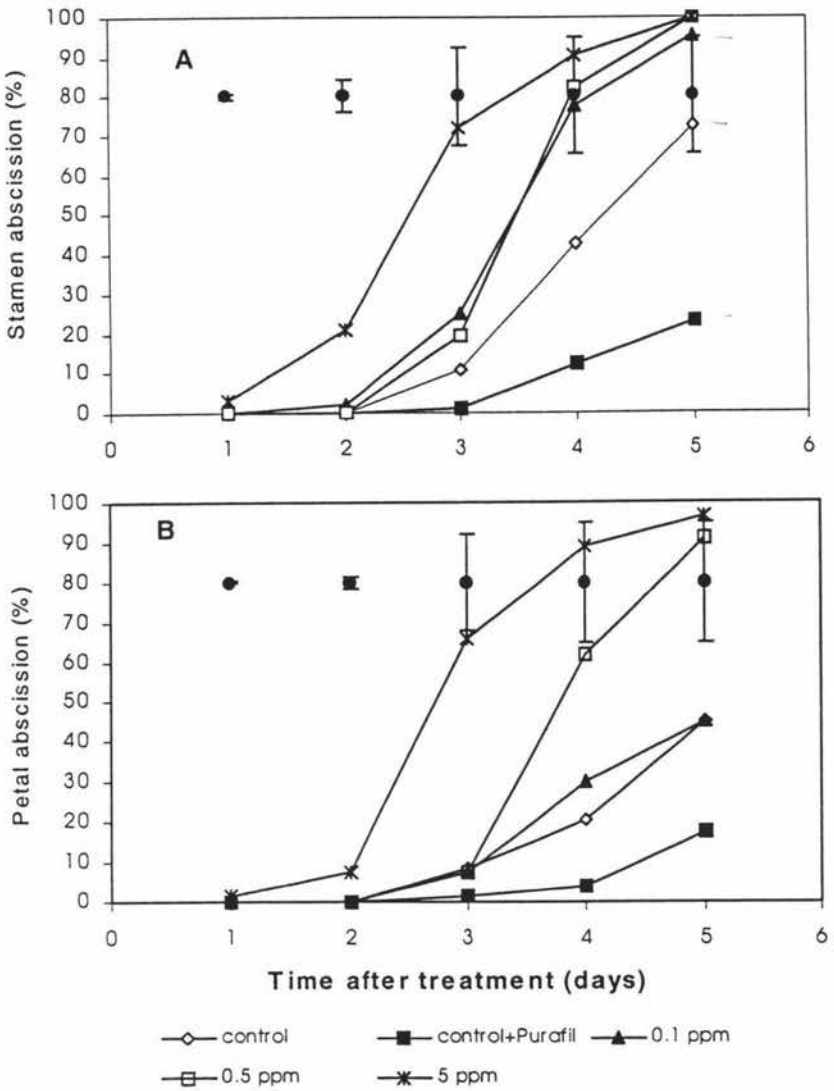

Fig. 4.26 Effects of ethylene treatment on percentage stamen abscission (A) and percentage petal abscission (B) in cut cymules of *M. collina* 'Tahiti'. Vertical bars represent standard errors of means.

Table 4.22 Ethylene concentrations at start and end of the experiment (n=24).

Time (days)	Ethylene concentration (ppm)				
	0 (control)	Control+Purafil	0.1	0.5	5
0 (start of experiment)	0.02	0.00	0.17	0.57	4.84
5 (end of experiment)	0.21	0.01	0.41	0.63	4.95

Table 4.23 Effect of ethylene on stamen abscission in cut cymules of *M. collina* 'Tahiti'.

Ethylene concentration (ppm)	Days		
	3	4	5
A			
5	54.00a	67.75a	75.00a
0.5	14.63b	61.63a	75.00a
0.1	18.50b	58.38a	71.50a
0 (control)	8.13b	32.00b	54.50b
Control +Purafil	0.75b	9.13c	17.00c
B			
0.1	18.50a	58.38a	71.50a
0 (control)	8.13b	32.00b	54.50a
Control+Purafil	0.75c	9.13c	17.00b

Mean separation in columns by Duncan's multiple range test, 5% level.

Petal abscission

The effect of ethylene concentration on petal abscission was significant ($p<0.0001$) on Days 3-5 after treatment. Petal abscission, which began two days after treatment with 5 ppm ethylene, was significantly higher for this treatment than all others treatments on Day 3. Cymules treated with 5 ppm ethylene exhibited a mean of 10 petals abscised on Day 3, rising to a mean of 14.5 (near full abscission) on Day 5. Cymules treated with no ethylene plus Purafil exhibited a petal abscission of <3 on Day 5 (Table 4.24A), whereas controls (0 ppm ethylene, no Purafil) exhibited the same petal abscission as cymules treated with 0.1 ppm ethylene.

When the two treatments (0.5 and 5 ppm ethylene) for which flower abscission occurred were omitted from the analysis, there were significant effects of ethylene treatment on petal abscission on Days 4-5 ($p<0.05$). Cymules treated with 0.1 ppm ethylene exhibited a higher petal abscission than that of control plus Purafil on Days 4-5 (Table 4.24B). However, petal abscission in controls was not significantly different from that of

cymules treated with 0.1 ppm ethylene on Days 4-5. Controls plus Purafil gave consistently the least stamen abscission.

Table 4.24 Effect of ethylene on petal abscission in cut cymules of *M. collina* 'Tahiti'.

Ethylene concentration (ppm)	Days		
	3	4	5
A			
5	9.88a	13.38a	14.5a
0.5	1.13b	9.25b	13.63a
0.1	1.13b	4.50c	6.75b
0 (control)	1.25b	3.13cd	6.75b
Control +Purafil	0.25b	0.63d	2.63c
B			
0.1		4.50a	6.75a
0 (control)		3.13a	6.75a
Control+Purafil		0.63b	2.63b

Mean separation in columns by Duncan's multiple range test, 5% level.

Flower abscission

The higher ethylene concentrations (5 and 0.5 ppm) effected flower abscission from the cymule pedicel. Flower abscission of cymules treated with 0.5 ppm ethylene occurred from Day 4, and with 5 ppm ethylene from Day 3 (Fig. 4.27).

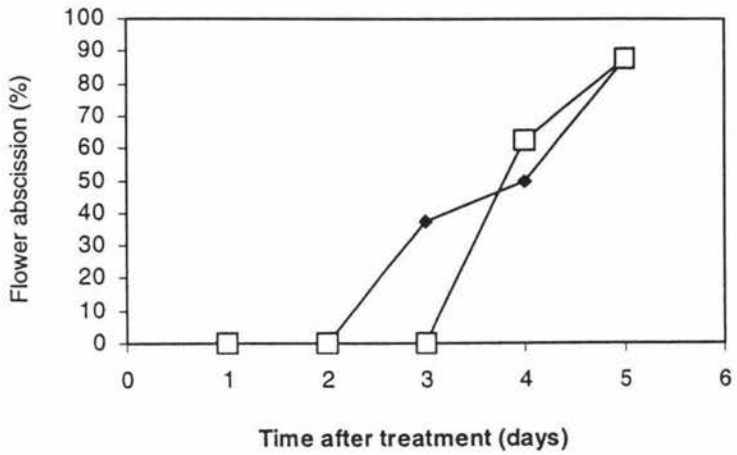

Figure 4.27 Effects of ethylene treatment ◆=5 ppm, ❑=0.5 ppm ethylene on percentage flower abscission in cut cymules of *M. collina* 'Tahiti'.

4.3.2 Experiment 7: Ethylene production in cut cymules of *M. collina* 'Tahiti'

From Days 1 to 6, cymules of greenhouse flowers produced progressively less ethylene than flowers harvested from plants grown outside (Fig. 4.28A). But flowers grown outside had a large fresh weight than flowers grown in the greenhouse, so on a fresh weight basis, greenhouse flowers produced consistently more ethylene than that of flowers grown outside (Fig. 4.28B).

Flowers grown outside exhibited higher abscission of stamens and petals than flowers grown in the greenhouse (Fig. 4.29A and 4.29B).

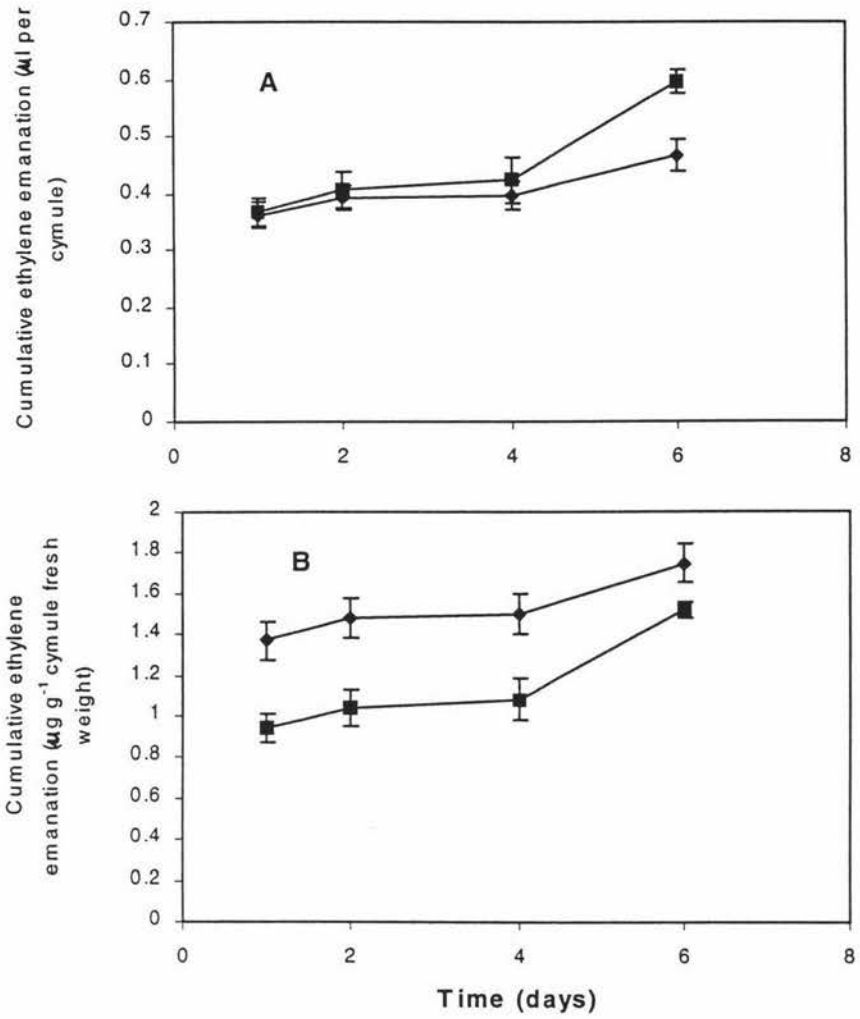

Figure 4.28 Ethylene emanation per cymule (A), and cymule on a fresh weight basis (B) for *M. collina* 'Tahiti' grown in the greenhouse and outside over six days. ◆ = greenhouse flowers, ■ = outside flowers. Bar on each point represents the standard error of mean of ten replicates for greenhouse flowers, and seven replicates for outside flowers.

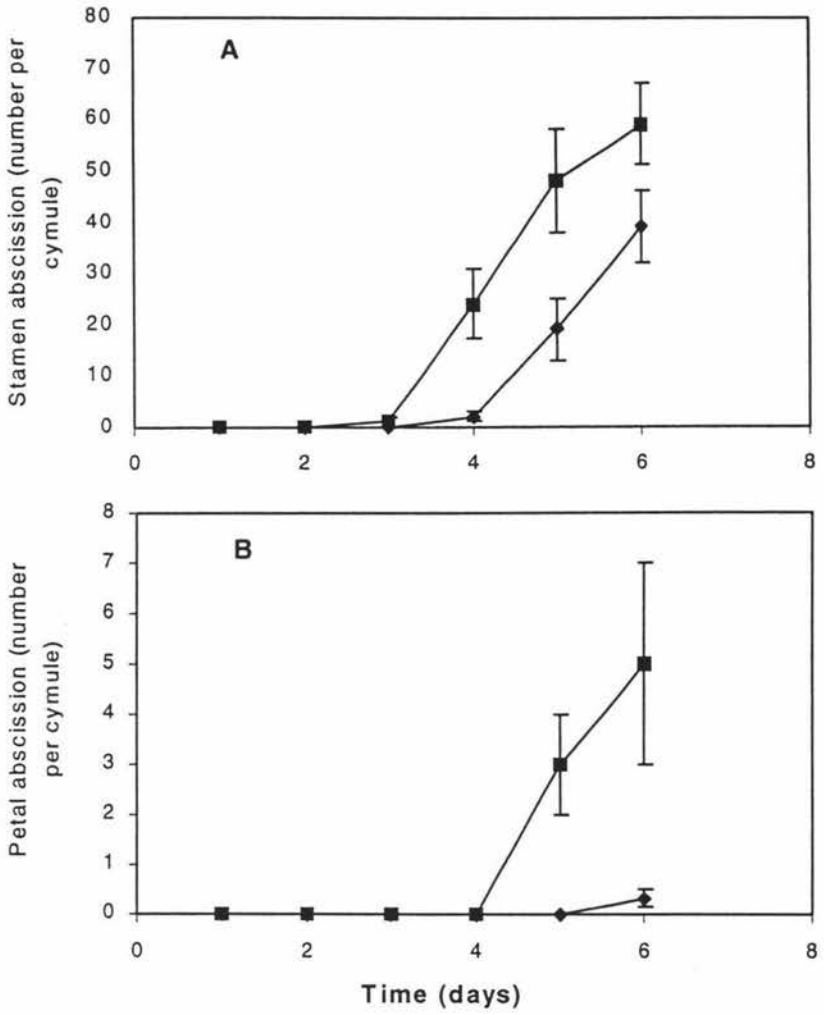

Figure 4.29 Stamen abscission (A) and petal abscission (B) in cut cymules of *M. collina* 'Tahiti' held in closed jars. ◆= Greenhouse flowers, ■= Flowers grown outside. Bar on each point represents the standard error of mean of ten replicates for greenhouse flowers, and seven replicates for flowers grown outside.

Chapter Five
Discussion

The changes in the cut inflorescences of *Eucalyptus ficifolia* or *Metrosideros collina* 'Tahiti' (Myrtaceae) that determined the end of vase life were stamen wilting or abscission. The end of vase life may be caused by one or more of the following changes in the inflorescence of both species: (i) decreased water uptake, an unfavourable water balance and loss of flower mass, (ii) flower or stamen abscission associated with endogenous ethylene production.

Water uptake by the cut flowers of *E. ficifolia* decreased with time, and transpiration also decreased, although it was always slightly greater than water uptake (Fig. 4.1A,B and 4.5A,B). In cut cymules of *M. collina* 'Tahiti', water uptake stayed relatively constant, while transpiration slightly increased with time (Fig. 4.11A,B). However, flower or cymule mass decreased with time in both species (Fig. 4.1C, 4.5C, 4.11C). These results indicate that the water relations characteristics differed between species, possibly because flowers of *E. ficifolia* were harvested from trees grown outside whereas those of *M. collina* 'Tahiti' were harvested from shrubs grown in the greenhouse. A negative water balance and loss of fresh weight of cut flowers or cymules would lead to end of vase life. In this study, it was found that the premature wilting of harvested *E. ficifolia* and *M. collina* 'Tahiti' flowers clearly resulted from a deteriorating water balance. Similarly, Doi and Reid (1996) reported that an unfavourable water balance resulted in the premature wilting of harvested *Camellia japonica* flowers. Van Doorn et al. (1995) suggested that tepal wilting in cut *Iris* flowers occurs within about four days of flower opening. The rate of water uptake decreased prior to tepal wilting and was more reduced than transpiration, resulting in the water balance becoming negative prior to tepal wilting. The reduction in water uptake with time from cutting, may be the result of the development of stem occlusions, which occur in other flower crops, such as *Leptospermum scoparium* (Burge et al. 1996).

Holding solutions with 2% sucrose were significantly better for water uptake than solutions containing sucrose at other concentrations (0.5%, 0.1%, 0%) (Table 4.3). There

was also a trend of increasing water uptake with increasing sucrose concentration of the holding solution up to 2% sucrose (Fig. 4.6A, 4.12B) in Experiments 2 and 3, although in Experiment 1, sucrose in the range 2-6% did not increase water uptake. In all holding solution experiments, solutions containing relatively low concentrations of sucrose delayed stamen wilting, stamen abscission and petal abscission. However, when solutions with 6% sucrose were used stamen wilting increased (Fig. 4.3B). It is suggested that high sucrose concentrations (more than 4%) are not beneficial for water uptake. Burge et al. (1998) pointed out that water uptake was reduced by their highest sucrose concentration (10% sucrose) which was used during the 24 h pulse period and for several days after treatment. Sucrose solutions of 1% and 2% extended the vase life of cut foliage of *Eucalyptus* (Wirthensohn and Sedgley, 1996). In their study, *E. cinerea* had a vase life of 19 days when held in 2% sucrose, but only 13-15 days after pulsing with 5% sucrose and holding in water. Sucrose solutions more concentrated than 5% were not beneficial to the stems due to browning and wilting of the leaves. Our results are in agreement with previous research showing that holding solutions containing up to 2% sucrose are beneficial, whereas a pulse of up to 10% sucrose is not (Joyce, 1988; Forrest, 1992; Jones et al., 1993c). In all experiments, sucrose delayed the decline in flower or cymule mass, and had no effect on transpiration. Overall, these results suggest that the sucrose status of holding solution has an important role in the postharvest senescence of *E. ficifolia* and *M. collina* 'Tahiti'.

Untreated cut flowers of *E. ficifolia* and cymules of *M. collina* 'Tahiti' had a vase life of approximately 4d when held in water. Stamens started to wilt or abscise, and flower buds failed to open. The inclusion of sucrose, with an adequate anti-microbial compound (200 ppm HQC) stimulated bud opening by increasing water uptake (Fig. 4.4). Jones et al. (1993a) reported that the inclusion of 1% sucrose in the vase solution resulted in a significant increase in the opening of flower buds, significantly extending longevity of *Thryptomene*. Sucrose (2%) vase solution treatments increased the number of open flowers of *Limonium* as well as the period over which flowers continued to open (Burge et al. 1998). Sucrose serves as a nutritional source and allows flower buds to open (Marousky, 1972). Thus, addition of sucrose to the vase solution often improves the postharvest quality

of flowers by promoting the opening of immature buds, and delaying senescence of open flowers (Halevy and Mayak, 1979).

A holding solution of 400 ppm 8-hydroxyquinoline citrate (HQC) of holding solution significantly delayed the decline of water uptake on Day 3, and flower mass on Day 5 (Table 4.3, 4.5) in Experiment 2. However, for the whole experimental period, the holding solution containing 400 ppm HQC was not significantly better than that with 200 ppm HQC. Moreover, 200 ppm HQC combined with 2% sucrose was better than other solutions for increasing water uptake and decreasing stamen wilting or abscission. Esters and citrate of 8-hydroxyquinoline are sometimes effective at reducing vascular blockages. As bactericides they help prevent microbial occlusion. However, their ability to reduce vascular blockages may also be because of their ability to inactivate enzyme systems (Marousky, 1972). Burge et al. (1996) showed that water uptake was higher and vaselife longer in cut flowers of *Leptospermum* treated with 200 ppm 8-hydroxyquinoline sulphate (HQS) plus 3% sucrose compared to solutions containing 200 ppm HQS plus 10% or 1% sucrose. A similar result was found for Geraldton wax flowers (Joyce, 1988). 8-hydroxyquinoline citrate is known to reduce microbial growth in vase water (Marousky, 1969), and van Doorn (1998) found the effect of 200 ppm HQC on vase life of roses coincided with a reduction of bacterial counts. Holding solutions containing 2% sucrose and 8-HQC at 200 ppm significantly increased vase life of *Eucalyptus* foliage (Wirthensohn and Sedgley, 1996). Apparently, 8-HQC inhibits stem blockage and allow cut flowers to absorb more water, and thus remain turgid (Marousky, 1972).

Acidic solutions have also been found to facilitate water uptake (Durkin, 1980). When the acid is effectively buffered around pH 3 it results in the inhibition of bacterial growth in rose stems (van Doorn and Perik, 1990). A solution of citric acid at pH 3-3.5 has been suggested for rehydration of cut flowers (Sacalis, 1993). Occlusion, apparently related to the development of bacteria in the vase water, has been overcome by the use of citric acid (250 ppm, pH 3.5) which improved stem hydraulic conductivity, and prevented bacterial proliferation in the vase solution (Jones et al., 1993a).

The vase solution containing citrate buffer at pH 4 was consistently better than others for increasing water uptake and delaying the decline of flower mass of *E. ficifolia*. Citrate buffer at pH 3 led to higher stamen wilting and abscission than pH 4 or 5, which were not significantly different (Table 4.6, 4.7). In cut cymules of *M. collina* 'Tahiti', holding solution containing citrate buffer at pH 5 was better than pH 3 and 4 for increased water uptake (Fig. 4.12A) and delayed the decline of cymule mass (Table 4.9). Vase solutions adjusted to pH 3 had the highest stamen wilting and the lowest stamen abscission. These results indicated that the response to pH differed between species. Longevity of *E. ficifolia* was increased with solution containing citrate buffer at pH 4 (adjusted solution pH 3.76), while longevity of *M. collia* 'Tahiti' was only increased at pH 5 (adjusted solution pH 4.78). Low pH is known to increase the rate of flow in isolated 5-cm stem segments of rose flowers (Durkin, 1979a). Marousky (1971) studied water flow in stems of cut roses held in bottles, containing either a buffer at pH 6 or pH 3, or 8-HQC. After two days, most of the bottles contained no bacteria in the vase water, yet hydraulic conductance was lower in water controls than in the 8-HQC treatment, and lower at pH 6 than at pH 3.

In the results reported in this thesis, HQC combined with citrate buffer pH 4 in the vase solution was better for extending longevity of cut flowers in both species. Similarly, van Doorn (1997) found that bacterial growth was not stopped by keeping the pH at a low level by either adding a phosphate-citrate buffer (pH 3.1) or including HQC in the vase solution (up to 500 ppm). However, the combination of these two treatments was effective in stopping bacterial growth.

Overall, this study showed that the inclusion of 2% sucrose, 200 ppm 8-HQC and citrate buffer at pH 4 in the vase solution resulted in the best increase in the opening of flower buds and extending the vase life of harvested *E. ficifolia* and *M. collia* 'Tahiti'. To our knowledge, this is the first study to investigate the effects of vase solutions on cut flowers or cymules of *E. ficifolia* and *M. collia* 'Tahiti'. It has revealed a solution to the difficult problem of overcoming the poor water relations of harvested *E. ficifolia* and *M. collina* 'Tahiti' (Myrtaceae) flowers.

Faragher (1985) pointed out that there was an increase in ethylene production and a decrease in water content in waratah flowers (*Telopea speciosissima*) before abscission, so these results are consistent with either ethylene or water stress causing abscission. However, in inflorescences which remained on the plant, abscission occurred before there was any visible wilting, suggesting that abscission did not necessarily depend on water stress. In our research, it was found that for inflorescences of both species which remained on the plant, abscission, when it took place, occurred before wilting. In contrast, for cut flowers or cymules of both species, stamen wilting was more common than abscission, suggesting that there are other factors involved in stamen abscission.

2-chloroethylphosphonic acid (ethephon) is an ethylene-releasing compound, which is widely available and relatively simple to apply in a commercial system (Woolf et al., 1995). Previous work has demonstrated that ethephon can induce flower abscission of cut flowers of *Leptospermum* (Zieslin and Gottesman, 1983). In *Begonia* flowers, increasing ethephon concentrations resulted in enhanced flower and flower bud abscission (Moe and Smith-Eriksen, 1986). Stamen wilting increased with increasing ethephon concentration (Fig. 4.21, 4.23). Pre-treatment with STS slightly reduced stamen wilting at lower concentrations of ethephon (Fig. 4.20, 4.22A), although this effect was not significant in either species. A similar result was reported for *Leptospermum* (Burge et al., 1996), when STS pulse treatments slightly reduced the rate of senescence of flowers when these were subsequently placed in water.

Zieslin and Gottesman (1983) reported that no flower abscission occurred on untreated or ethephon treated shoots under low relative humidity, but these conditions induced desiccation in *Leptospermum.* Joyce and Poole (1993) found that cut flowers and leaves of *Verticordia chrysantha* and *V. plumosa* did not abscise when treated with ethephon (500 or 1000 ppm), while no damage was reported when floral buds of potted *Camellia* plants were treated with ethephon (500 or 1000 ppm) (Woolf et al., 1995). However, in this research, stamens were damaged, resulting in wilting when flowers were treated with ethephon (more than 100 ppm) (Fig. 4.21, 4.23). Our results (based on ethephon application) led to the hypothesis that ethylene production as a result of water

stress can cause flower wilting before stamen or petal abscission occurs, or ethephon at the high rates used resulted in such a high release of ethylene that stamens were damaged, resulting in stamen wilting.

In cut cymules of *M. collina* 'Tahiti', pre-treatment with STS (2mM) resulted in significantly lower stamen and petal abscission (Fig. 4.24, 4.25). STS combined with 1000 ppm ethephon had significantly lower stamen abscission with cut cymules of *M. collina* 'Tahiti' than 1000 ppm ethephon with no STS pre-treatment on Days 1 to 2 (Table 4.20). Silver ion, a potent and apparently specific inhibitor of the action of ethylene, inhibits not only ethylene-stimulated abscission, but also abscission caused by other stresses (Reid, 1985). STS is widely used for the reduction of senescence and leaf abscission in cut flowers (Dostal et al., 1991). Moe and Smith-Eriksen (1986) suggested STS spray prevents the abscission of flowers or flower buds of *Begonia* when the STS concentration is high (1.05-6.25 mM). Spraying *Zygocactus* plants with 0.2 mM STS at the stage when buds were starting to show colour completely prevented subsequent flower abscission in response to low concentration of ethylene (Cameron and Reid, 1981). Exposure of Geraldton waxflower to ethylene caused bud and flower abscission, and the most successful preventive treatment at that time was pulsing with STS (Joyce, 1988; 1989).

Ethylene is known to control flower and petal abscission in many species (Reid and Wu, 1992). For example, in Geraldton waxflower, the main postharvest problem is flower and bud abscission. Exogenous ethylene induced flower fall (Joyce, 1988), and endogenous ethylene can cause floral organ abscission. High concentrations of ethylene were not necessary for extensive flower abscission in cut carnation flowers (Kader, 1985). Application of exogenous ethylene caused stamen and petal abscission in cut cymules of *M. collina* 'Tahiti' (Fig. 4.26A, 4.26B). The higher ethylene concentrations (0.5 and 5 ppm) were sufficient to lead to whole flower abscission from the cymule pedicel (Fig. 4.27). The present result clearly demonstrated that cut cymules of *M. collina* 'Tahiti' can be severely injured by levels of exogenous ethylene ranging from 0.1 to 5 ppm. There have been no previous reports of how ethylene affects this species. The deleterious effect of 0.05 ppm ethylene on flower and bud abscission is well documented for *Begonia* (Hoyer, 1985a), and

Streptocarpus suffered from severe bud and flower abscission after exposure to 0.1 ppm ethylene for 24 hours (Rewinkel-Jansen, 1986). Further, substantial abscission in florets of *Pelargonium* × *domesticum* occurred after treatment with 0.5 ppm ethylene for only 1 h (Deneke et al., 1990), and 1.0 ppm ethylene caused 100% corolla abscission in New Guinea Impatiens (Dostal et al. 1991). Woolf et al. (1995) found that 10.5 ppm ethylene promoted abscission of floral buds of *Camellia.*

To avoid interference from endogenous ethylene in this study, some flowers were sealed in glass jars with 'Purafil'. 'Purafil' is an absorbent of ethylene and is used to absorb ethylene generated endogenously. 'Purafil' gave the maximum reduction of leaf abscission among ethylene absorbents as reported by Lemos and Blake (1996). In our work, Purafil, significantly reduced stamen and petal abscission (Table 4.22, 4.23, 4.24). The data also indicate that endogenous ethylene is clearly involved in stamen and petal abscission in *M. collina* 'Tahiti'.

Endogenous ethylene production may regulate abscission in *Pelargonium* florets which exhibit increased ethylene synthesis during development or following pollination (Evensen, 1991). Low levels of ethylene produced by *Annona squamosa* nodal explants in culture (0.1 $\mu l \cdot l^{-1}$ day^{-1}) were found to be enough to produce physiological effects, such as leaf abscission (Lemos and Blake, 1996). In this project, it has been shown that ethylene emanation differed between growth environments (Fig. 4.28A, 4.28B), and that the higher ethylene emanation led to higher stamen and petal abscission (Fig. 4.29A, 4.29B). The low ethylene production (0.06~0.08 $\mu l \cdot l^{-1}$ day^{-1}) (Fig. 4.28A) was sufficient to induce stamen and petal abscission. The result indicated *M. collina* 'Tahiti' is sensitivite to ethylene. The role of ethylene in flower senescence varies greatly depending on plant species. For sensitive species, ethylene-induced senescence and abscission of flowers may result from endogenous production of ethylene or from exposure to exogenous ethylene (Halevy and Mayak, 1979). Differences in tissue response may be due to the interaction of tissue sensitivities and the concentration and duration of ethylene present at the abscission zone (Lang and Martin, 1989).

In this study, application of exogenous ethylene only caused flower, stamen and petal abscission. No stamen wilting occurring over a six day period. Similarly, Zieslin and Gottesman (1983) reported that ethylene caused flower abscission in cut flowers of *Leptospermum*, and the abscised flowers retained turgidity without symptoms of shriveling and in-rolling over a ten day period. We suggest that "stamen wilting" may be a result of unfavourable water balance (uptake vs transpiration), and/or tissue damage (loss of turgor) due to exogenous ethephon.

Chapter Six
Conclusion

This study found that cut flowers or cymules of *E. ficifolia* and *M. collina* 'Tahiti' had a short vase life, because of an unfavourable water balance and severe stamen and petal abscission. An unfavourable balance between water uptake and transpiration was the major cause of wilting in the both species. In addition, due to the effects of both pre-treatment with STS, and the effects of 'Purafil', we propose that ethylene is a key factor in inducing flower, stamen and petal abscission.

A holding solution containing 2% sucrose, 200 ppm 8-HQC and citrate buffer pH 4 was found to be the best for increasing the opening of flower buds and extending vase life of both species.

Ethephon treatments caused stamen wilting, but failed to cause stamen or petal abscission in both species. It is possible that ethephon resulted in such high ethylene production that stamens were damaged, resulting in stamen wilting. This phenomenon warrants further exploration. However, pre-treatment with STS reduced stamen wilting, and significantly reduced stamen or petal abscission in both species.

This research suggests that the flower, stamen and petal abscission of *M. collina* 'Tahiti' is due to it being sensitive to ethylene. The sensitivity of *M. collina* 'Tahiti', which is injured by exogenous or endogenous levels of ethylene between 0.1 and 5 ppm, indicated that for this plant pretreatment with inhibitors of ethylene action and/or ethylene biosynthesis is highly recommended. In further study, it will be beneficial to investigate whether stamen or petal abscission can be inhibited in both species by application of the new ethylene perception inhibitor 1-methylcyclopropene.

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