Phase change, flowering and postharvest characteristics of *Metrosideros excelsa* (Myrtaceae)

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Abstract

The development of *Metrosideros excelsa* (pohutukawa) as an ornamental crop has been limited by a lack of knowledge on the cultural requirements and underlying physiological processes associated with: (a) vegetative phase change (maturation) following micropropagation, (b) the environmental control of flowering, and (c) the postharvest characteristics of the cut-flower. These three concerns were addressed in this thesis.

First, plantlets of *M. excelsa* that had undergone rejuvenation following micropropagation, were subjected to shoot and root restriction treatments to accelerate vegetative phase change. Leaves of shoot-restricted, single-stemmed plants became progressively more adult with increasing node position, whereas root restriction reduced root growth but did not accelerate vegetative phase change. In single-stemmed plants, light saturated maximum rate of photosynthesis and leaf carbon isotope discrimination decreased within increasing node position. However, carbon isotope composition in leaves of these plants diverged away from those exhibited by leaves of adult plants, possibly reflecting physiological changes resulting from altered source/sink relations.

Second, the effects of photoperiod, temperature and irradiance on floral initiation and development were examined in *M. excelsa* by manipulating these parameters in controlled and greenhouse environments. *M. excelsa* responded as a facultative short-day plant with maximum flowering occurring following a 15 weeks cool (mean 15°C) short-day (10 h) inductive treatment. An irradiance of 567 μmol m^{-2} s^{-1} during induction provided the optimal conditions for floral primordial growth and subsequent flower development. Buds initially 2.0-3.0 mm in diameter had the highest probability of becoming floral, whilst those less than 2.0 mm in diameter were more likely to remain vegetative or to not break.

Finally, the postharvest characteristics of *M. excelsa* as a cut flower were assessed. Generally, holding solution treatments containing sucrose extended vase life, whereas those containing HQC (applied alone or as a pulse) were detrimental. Cut flowers were sensitive to exogenous ethylene and pre-treatment with inhibitors of ethylene action (STS and 1-MCP) conferred significant protection.

This thesis has contributed significantly to furthering our understanding and knowledge of cultural and physiological factors that underlie vegetative phase change, flowering and vase life characteristics in flowers of *M. excelsa.*

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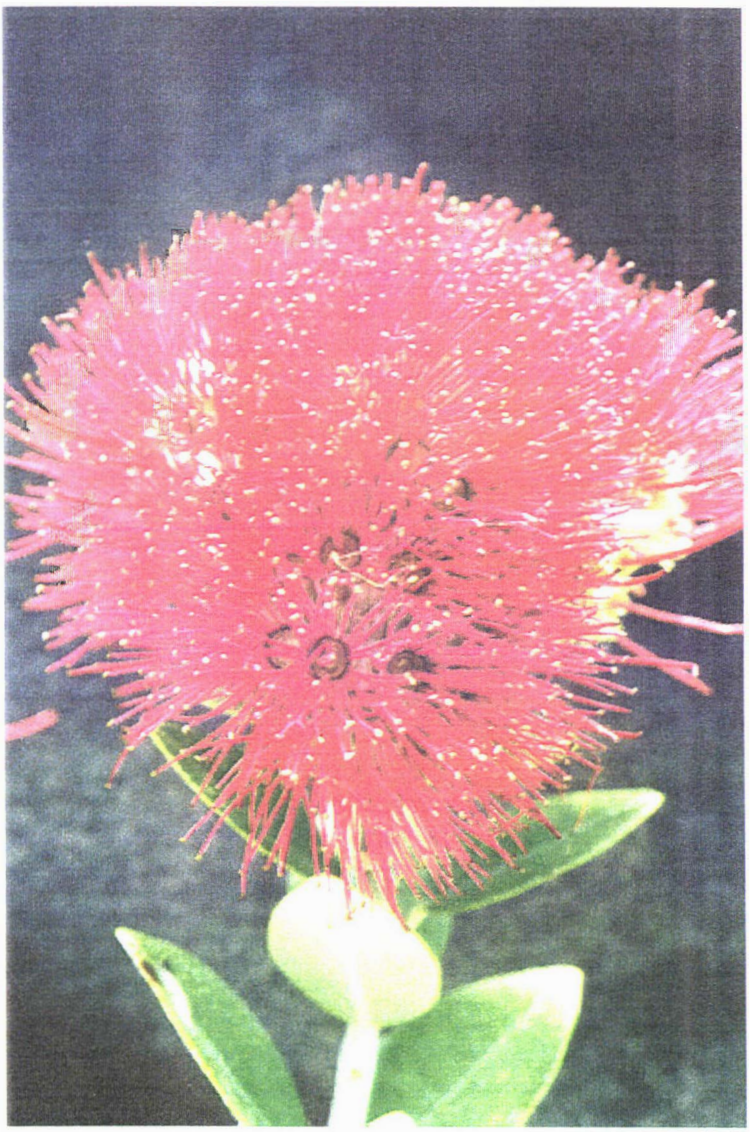
List of Abbreviations

ABA	abscisic acid
ACC	1-aminocyclopropane-1carboxylic acid
A/Ci	CO_2 assimilation over intercellular CO_2 concentration
AOA	aminooxyacetic acid
AVG	aminoethoxyvinylgycine
φ_{app}	apparent (CO_2-limited) photon yield
Δ	carbon isotope discrimination
CK	cytokinin
DL	daylength
DNA	deoxyribonucleic acid
DMF	dimethylformamide
GA	gibberellin
IAA	indole-3-acetic acid
FAA	90% formalin, 5% acidic acid and 5% alcohol fixative solution
GENMOD	generalised linear model using maximised likelihood estimations
HQC	8-hydroxyquinoline citrate
HQS	8-hydroxyquinoline sulfate
J_{max}	maximum electron transport rate
LD	long-day
1-MCP	1-methylcyclopropene
NBD	2,5-norbornadiene
PPF	photosynthetic photon flux
P_{max}	maximum photosynthetic photon flux at 99% light saturation
PPF_{sat}	light saturated maximum rate of photosynthesis
PP333	paclobutrazol
RH	relative humidity
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase
SD	short-day
STS	silver thiosulfate
V_{cmax}	maximum rubisco carboxylation rate
WUE	water-use efficiency



Metrosideros excelsa 'Vibrance'

Chapter 1

Introduction

1.0 Introduction

The worldwide consumption for ornamental flowers is growing rapidly and the demand for novel flowering crops is increasing accordingly. In 1995, the world flower use was estimated at around US $31 billion dollars, with the three most important markets for floral products being the European Union, North America and Japan (Anon. 2001a). New Zealand, although only being a small player in this industry, has a relatively high level of horticultural technology and a well-established container and cut flower industry which is oriented towards the export market. Export earnings for flower products for the year ended March 2001 were approximately NZ $46.2 million, and this estimate is anticipated to rise in the coming years (Anon. 2000, Anon. 2001b). Currently, a number of native genera exported annually, often as liner plants, include live plants of *Hebe*, *Leptospermum*, *Metrosideros*, *Pittosporum*, *Sophora* and *Uncinia*, and smaller volumes of cut native material such as leaves of *Leptospermum* and *Phormium*.

New Zealand has a unique and diverse range of flora with many indigenous shrubs and trees that are not well known worldwide. Thus, many woody plants native to New Zealand have excellent ornamental potential as either container plants or as cut flowers. However, their development has been limited by a lack of knowledge of their cultural requirements, particularly with regards to the hastening of flowering in plants that have lost the ability to flower following micropropagation, and a lack of understanding of environmental factors that control flowering. Moreover, the paucity of research into the postharvest characteristics of native cut flowers has been a limiting factor for further expansion of novel flowers into the international floricultural trade. In light of this, the Native Ornamental Plants Program funded by the Public Good Science Fund (PGSF) was established through the New Zealand Institute for Crop & Food Research (Crop & Food Research). This program sought to address these problems by conducting scientific research into selected native species with potentially high ornamental value. The results would enable the production and rapid technological development of native

genera for expansion to both the New Zealand and export markets, while contributing invaluable scientific knowledge on the flowering biology of the native New Zealand flora. Massey University was sub-contracted by Crop & Food Research to achieve specific objectives in relation to *Metrosideros excelsa*. This formed the basis for the doctoral study described in this thesis.

Metrosideros excelsa (Myrtaceae), also known as the pohutukawa or New Zealand Christmas tree (Hewett 1993), is a native New Zealand tree species considered by the PGSF to have considerable potential as an ornamental flowering crop. In the wild, this species is typically found along the coastal regions of the North Island. Flowering displays that are exhibited by *M. excelsa* under natural conditions during the summer months from December through to January, have been described as a "brilliant spectacle", hence, the popularity of many cultivars among New Zealand growers (Salmon 1980). The flowers in this genus have conspicuous long stamens, and are often brightly coloured making it particularly attractive as an ornamental plant within the floricultural industry.

Development of *M. excelsa* has been limited by several factors associated with flowering. Firstly, seedling-grown plants of *M. excelsa* typically reach an adult reproductive phase within five to ten years, whereas cuttings from adult foliage are capable of flowering within one year (Oliphant *et al.* 1992). However, it has been reported to be difficult to root cuttings from adult trees. The preferred method of propagation for obtaining mass clonal material has, therefore, been through the use of micropropagation (Oliphant 1990). However, during micropropagation, rejuvenation of parent material occurs, and can result in a delay of three or more years before plants are able to bear flowers. Thus, an effective method was required to accelerate vegetative phase change. Secondly, once plants of *M. excelsa* have become reproductively competent, there is a further need to understand what environmental factors are involved in the flowering process. This not only entails an understanding of factors that promote, inhibit or accelerate flowering, but also an understanding of factor(s) intrinsic to the plant that are indicative of flowering ability. Finally, production of an ornamental crop would not be complete without an assessment and evaluation of its postharvest characteristics. This would include research into methods for extending the vase-life and flower quality of cut material.

In this thesis, all three aspects discussed above for advancing *M. excelsa* as a flowering crop were investigated. The literature reviewed in Chapter 1 on phase change, environmental control of flowering and postharvest characteristics in woody plants provides a background from which appropriate hypotheses are advanced. In Chapter 2, several cultural methods for accelerating vegetative phase change in micropropagated plants were tested and evaluation of ontogenetic status was examined using image analysis methods. The physiological changes occurring during vegetative phase change are characterised in Chapter 3, with specific emphasis on leaf gas-exchange and carbon isotope discrimination properties. In Chapter 4, the effect of photoperiod, temperature and bud size on floral induction and subsequent development were investigated using reproductively competent adult plants of *M. excelsa*. The effect of irradiance on flower induction and subsequent development was examined in Chapter 5, including a microscopic investigation of the changes occurring during the initial stages of floral organogenesis, and their relation to irradiance, bud size and timing of development. In Chapter 6, the postharvest characteristics of *M. excelsa* as a cut flower were examined, including a quantitative and qualitative assessment of the effects of holding solution, endogenous ethylene emanation and exogenous ethylene applications on flower quality and duration of vase-life. The effectiveness of treatments designed to inhibit the effects of ethylene-mediated damage was also investigated. Finally, a general discussion and conclusion of the experimental results are provided in Chapter 7 of this thesis.

1.1 Vegetative Phase Change in Woody Plants

1.1.1 Introduction

The transition through a series of developmental phases during growth is inherent in all living organisms. In higher animals, developmental phases represent different episodes in the life of an organism which can encompass changes throughout the entire body plan. Higher plants also exhibit distinct developmental phases during growth, although these changes are manifested within the plant in the developing shoot apical meristem, which are subsequently expressed in parts of the plant derived from this region. The shoot apical meristem in higher plants passes through several, usually three, defined

post-embryonic states of development, which comprise a juvenile vegetative phase, an adult vegetative phase, and a reproductively competent phase (Poethig 1990). The transition between the developmental states in the life of a plant is commonly referred to as maturation or phase change (Hackett 1985, Poethig 1990, Greenwood 1995). An understanding of the mechanisms that underlie phase change is not only scientifically interesting in its own right but also important from a commercial perspective, given the need to produce crops (either flowers, seeds or fruiting bodies) from reproductively competent plants as rapidly as possible. In this section, the current literature on vegetative phase change in woody plants is reviewed. Particular emphasis is placed on the physiological, biochemical and molecular processes occurring in woody angiosperms during phase change, and during specific life-stages with the intent of defining and establishing future directions in this field of research. It should be noted that the study of phase change *per se* is not limited to woody angiosperms, and when appropriate relevant and important findings in other studies of model plants from different groups (e.g. herbaceous angiosperms and gymnosperms) are referred to and discussed.

1.1.2 Applications

Methods for controlling or accelerating the timing of phase change would have significant economic benefits from both an agricultural and horticultural perspective. This is because the breeding efficiency of woody perennials and the selection of improved cultivars is greatly improved by reducing the length of the juvenile period (Hackett and Murray 1992). Similarly, a reduction in the length of the juvenile period can also advance the time to when plants are reproductively competent, hence shortening the time to flower or fruit production. This is in light of the fact that most plants exhibiting juvenile vegetative characteristics can rarely be induced to flower. The same philosophy also applies to the forestry industry given that the quantity and quality of productivity of a forest tree species is directly related to its degree of maturity (Hackett and Murray 1992). With regards to propagation technology, the ease of cutting propagation and *in vitro* organogenesis and somatic embryogenesis for all types of woody perennials is strongly affected by ontogenetic age (Hackett and Murray 1992).

1.1.3 Ontogenetic development and terminology

The three primary phases of development following post-embryonic development that have been recognised include a juvenile vegetative phase, an adult vegetative phase and a reproductively competent phase. The juvenile phase of development starts with the initiation of a stem, true leaves and axillary buds and may last from a few days to several years depending on the species and conditions (Poethig 1990). This phase is often characterised by a number of distinguishable and unique vegetative traits (e.g. leaf morphology) and is, in almost all cases, exempt from producing reproductive organs. The juvenile phase is followed by the adult vegetative phase, which is characterised by a different suite of vegetative traits. The onset of the reproductively competent phase entails the potential for the transformation of the vegetative shoot apex into a reproductive structure where either flowers, inflorescences or cones are formed. Rarely, vegetatively juvenile plants can be reproductively competent and bear flowers on branches bearing juvenile foliage, such as in certain *Eucalyptus* species (Wiltshire *et al.* 1991).

The transition between each of these different phases has been referred to as ontogenetic ageing by Fortanier and Jonkers (1976), meristem ageing (cyclophysis) by Oleson (1978), maturation by Wareing (1959) and phase change by Brink (1962). This is different to physiological ageing as described by Wareing (1959), which is often associated with a reduced growth rate or vigour as a result of increasing size and complexity of the plant. Phase change as currently referred to by several authors (Poethig 1990, Greenwood 1995, Hackett and Murray 1997), can be characterised by progressive changes in a number of species-specific morphological and developmental attributes such as leaf shape, phyllotaxy, thorniness, shoot orientation, shoot growth vigour, anthocyanin pigmentation and by the ability to form adventitious roots (Hackett 1985, Hackett and Murray 1992). Changes in these characteristics during development, which are permanently recorded as variation along a shoot axis, have been referred to as heteroblasty (Kerstetter and Poethig 1998). This term was originally coined by Goebel (1900) to describe the ontogenetic sequence of morphological features of shoots, although more recently, usage of this definition has been extended to include not only the changes in anatomical/morphological traits, but also physiological traits (e.g. photosynthetic rate) during ontogenetic development (Kerstetter and Poethig 1998).

The abruptness with which these differences are expressed between ontogenetic phases can be further expressed as either heteroblastic or homoblastic development. Plants that exhibit gradual changes in leaf morphologies between juvenile and adult vegetative phases have been described as undergo homoblastic development (Goebel 1900, Godley 1985). Examples of homoblastic development are seen in New Zealand species of *Avicennia*, *Corynocarpus*, *Griselinia*, *Macropiper*, *Meryta, Metrosideros*, *Myosotidium*, and *Planchonella* (Godley 1985). This is in contrast to heteroblastic species that exhibit a dramatic transition between strikingly different juvenile and adult leaf morphologies (Goebel 1900), a feature typified by more than 200 New Zealand plant species, including *Pseudopanax crassifolius* (lancewood) *Knightia excelsa* (Cockayne 1928, Godley 1985) and *Elaeocarpus hookerianus* (Day *et al.* 1997). Several authors who have recently characterised phase change in *Eucalyptus globulus* assert that the progressive changes in leaf characters during phase change follows heteroblastic behaviour, although they describe the phenomenon as occurring in a "gradual" manner (James and Bell 2001). Based on the definition proposed by Goebel (1900) and by the subsequent use of the term (e.g. Godley 1985), *E. globulus* would be better described as a homoblastic rather than a heteroblastic species.

According to Jones (1999), part of the problem associated with the confusion in the literature on phase change has been not only the lack of clearly defined terminology but also the inconsistent use of specific terms to describe a particular feature or phenomenon. The terms *mature* and *adult* have both been used widely to refer to the same phase(s) of development, although preference for a particular term has often been author(s) specific (e.g. Poethig 1990, Hackett and Murray 1997). In order to maintain consistency throughout this thesis, the term *adult* has been applied to the ontogenetic phases occurring after the juvenile vegetative phase. This term (as opposed to the term *mature*) allowed for a distinction to be made between either the adult vegetative phase or the adult reproductively competent phase. Similarly, the phrase *phase change* as defined by Poethig (1990) and applied in this thesis, refers to the transition between all ontogenetic phases of development. This is unlike the term *maturation* which only implies a transition to when an organism may become reproductively competent.

1.1.4 Models of phase change

Several models have been proposed to account for the changes occurring during phase change. To account for intermediate leaf forms during phase change, Poethig (1990) proposed the combinatorial model (Figure 1.1 A) based on the model plant *Zea mays*. According to this model, shoot development can be described as a series of overlapping programs (juvenile, adult, reproductive), which are independently regulated and are modulated by expression of a common set of developmental processes rather than a series of mutually exclusive developmental programs. Therefore, during the transition from juvenile to adult leaves, different developmental programs can be expressed on the same leaf. A classic example of this is found in *Acacia heterophylla* where intermediate leaves express both the juvenile (pinnately compound leaf) and adult (phyllodes) leaf morphology on the same leaf, with the juvenile traits expressed at the distal part of the leaf (Taiz and Zeiger 1998). Similar patterns of growth are also exhibited in woody plants, such as *Eucalyptus* and *Hedera helix* (Hackett and Murray 1992), and herbaceous plants, such as maize (Lawson and Poethig 1995). Thus, cells at the base of the leaf are committed to an adult program whereas those at the tip are committed to the juvenile program (Poethig 1990). Kester (1976) had previously proposed a similar model based on the behaviour observed in woody plants (Figure 1.1 B). According to his model, juvenile and adult characteristics can coexist but are expressed at different locations on the plant during growth and development of the main shoot axis and axillary branches. Thus, juvenile traits are expressed at the lower portions of the stem. During developmental growth, intermediate structures are formed which are subsequently followed by the expression of adult traits that eventually reside on the upper portions and periphery of the plant body.

More recently, Hackett and Murray (1997) proposed a set of alternate models for phase change (Figure 1.2). According to their models, five proposed pathways in which phase change can be regulated include a set of phenotypic characters (A′-D′) controlled by either a single-switch, a parallel set of switches or a series of switches. The latter two regulatory pathways can be further classified into switching programs that control groups of characters. Thus far, support for a single-switch event for controlling phase change appears to be lacking. Evidence for this has been based on observations of the timing of expression of particular traits during phase change. In *Picea sitchensis* (sitka

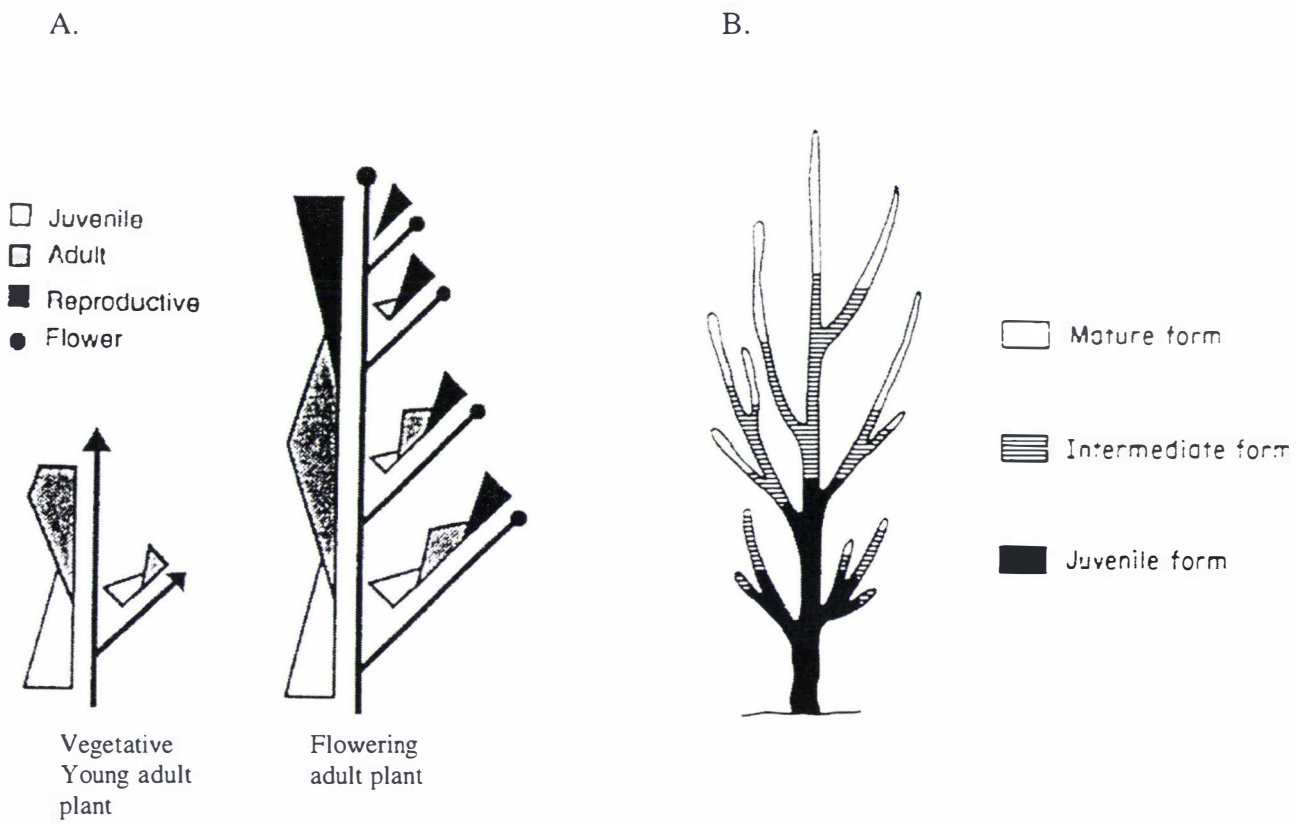


Figure 1.1 Models of phase change described by Poethig (A) and Kester (B). A: Schematic representation of the expression of juvenile vegetative, adult vegetative, and reproductive traits in the shoot of an annual plant (Poethig 1990). B: Schematic representation of the localisation of different ontogenetic states on an adult (mature), woody, seedling-grown plant (Kester 1976).

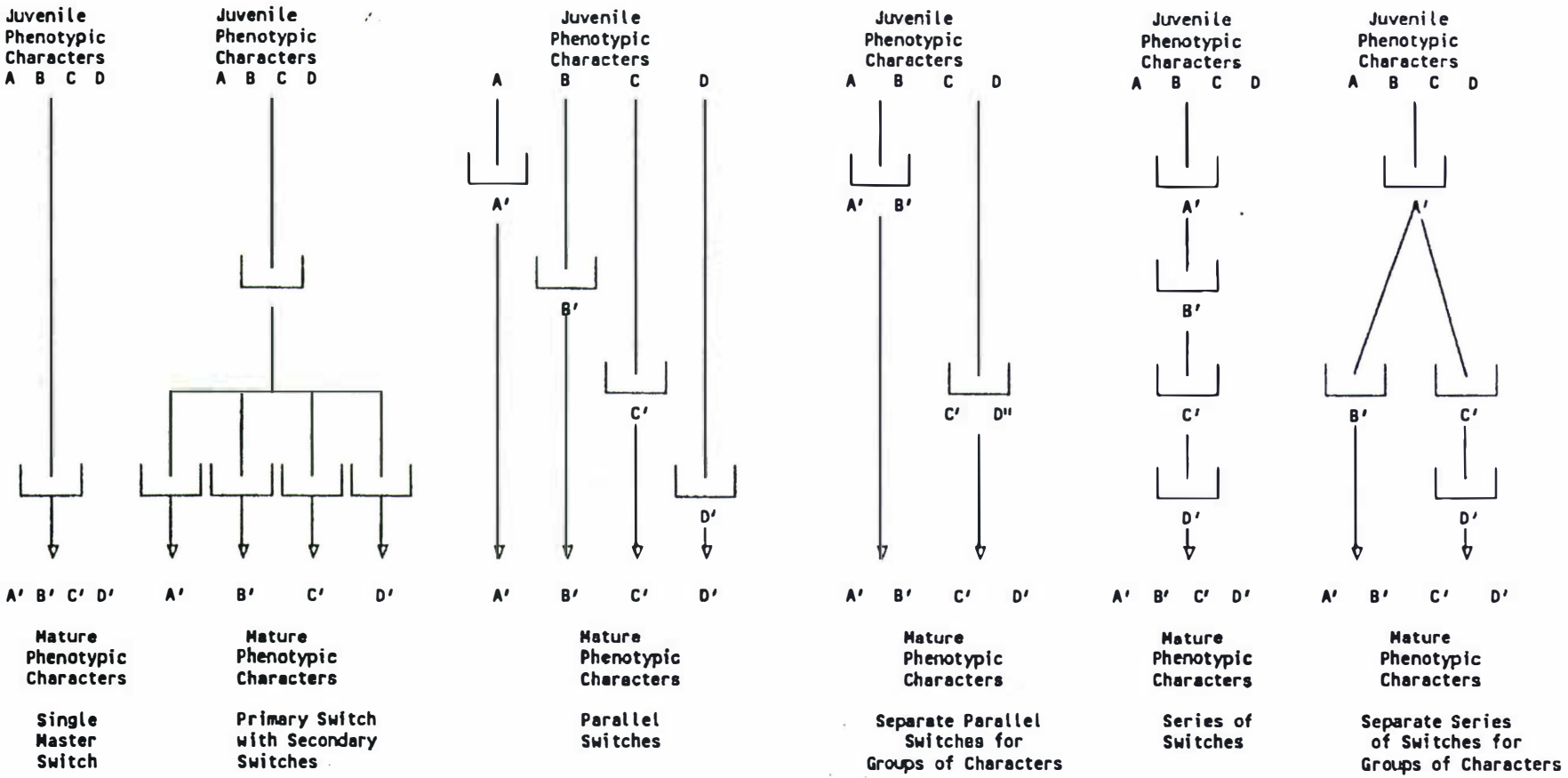


Figure 1.2 Alternate models of phase change or maturation (Hackett and Murray 1997). Note: "mature" refers to adult plants.

spruce), for example, various needle morphological features (e.g. leaf dimension and mass characteristics) were characterised from tree stands of different ages (Steele *et al.* 1989). Consequently, most needle characters changed at different rates with age, suggesting that a multiple set of regulatory processes were involved during phase change. Similarly, the transition from the juvenile to adult leaf form of *Eucalyptus globulus* is also accompanied by different temporal expression in leaf characters (James and Bell 2001). Moreover, further evidence of a multiple regulatory system comes from rejuvenation experiments. Application of varying concentrations of GA_3 to adult plants of *Hedera helix* caused rejuvenation and resulted in differential sensitivity of various physiological and morphological phase-related characteristics (Rogler and Hackett 1975). Low doses of GA_3 only suppressed flowering ability, whereas higher doses inhibited not only flowering ability but also expression of various other adult characters, such as rooting ability or absence of anthocyanin. Saturating GA_3 concentrations appeared to repress expression of all characters associated with the adult form (Rogler and Hackett 1975). The results of these and other studies certainly suggest the role of a multiple switch mechanism and may indicate that a change in one character is required before others are able to be switched (Hackett and Murray 1992). Differential and independent expression of phase-specific traits is also supported by genetic studies. Lawson and Poethig (1995) contend that pathways involved in phase change are likely to be complex with many branching and interacting pathways.

Based on developmental studies of woody species, Greenwood (1995) proposed several mechanisms that ought to be considered in models that account for phase change. First, the onset of the adult phase is usually gradual but in certain cases (e.g. branching frequency) can occur abruptly. Second, a wide variety of morphological, physiological and biochemical traits are involved in phase change and occur independently of one another. Thirdly, maturational traits are often persistent, and their maintenance is not always a function of plant size or root proximity. Next, cells in the apical meristem in some woody plants appear to become determined. Finally, the process of rejuvenation results in a gradual reversion to the juvenile condition. Based on these criteria, Greenwood (1995) contended that a simple model involving only one switch would account for the complex phenomenon of phase change. Greenwood proposes that the switch would reside in individual cells within the apical meristem. Upon activation of the switch, juvenile cells in the meristem would become adult so that over time the

apical meristem would resemble a "mosaic" with an ever-increasing proportion of adult cells. Thus, the number of adult characters at any given time would be a function of the ratio of juvenile to adult cells in the meristem. This hypothesis may explain why fully expanded leaves at intermediate stages of phase change possess phenotypic characters (or cell types) typical of both juvenile and adult phases as exhibited in many woody species (James and Bell 2001). With regards to rejuvenation, Greenwood (1995) hypothesised that this process could be explained by exposure to conditions that favour rapid multiplication of juvenile cells in the apex (Greenwood 1995).

1.1.5 Apical meristems: location of phase-related expression.

1.1.5.1 Role of apical meristem

Research conducted on phase change has often focused on the role of the apical meristem during this process (Poethig 1990, Greenwood 1995, Hackett and Murray 1997). There is strong evidence to suggest that the apical meristem is the site or source of phase change activity. However, it is yet unclear whether the apical meristem behaves autonomously, meaning it acts independently of the rest of the plant, or whether extrinsic factors in other parts of the plant are involved (Sussex 1976, Hackett 1985). This may involve chemical signalling between the roots and/or leaves to the apical meristem, subsequently initiating a developmental phase change response.

Evidence to suggest that the apical meristem behaves as an autonomous unit as opposed to an apical system reliant of extrinsic signals has been obtained through grafting experiments. In order to determine whether the development of grafted juvenile shoots is influenced by an adult rootstock, a number of workers have grafted seedling scions onto reproductively competent adult trees. The formation of reproductive structures has usually been a reliable indicator as to whether phase change has occurred (Poethig 1990). Thus, many of the studies on woody species examining the effect of grafting on phase change have used flowering or cone formation as this indicator. Robinson and Wareing (1969) grafted juvenile scions of *Larix leptolepis* and *L. decidua* onto reproductively competent trees and found that less than two percent of the scions produced cones after one year. The plants from which scions were derived did not form cones during that time period. Conversely, grafting adult scions onto juvenile rootstocks has resulted in a rejuvenation response in some species. For example,

experiments where adult apices of *Citrus* or *Sequoiadendron* with one to three leaf primordia were grafted onto juvenile rootstock resulted in plants that grew out to have adult vegetative characteristics (Navarro *et al.* 1975, Monteuuis 1991). It would appear that upon reaching the adult phase, the rootstock has little effect on the scion status, such as in causing rejuvenation. It is possible that cells in the apical meristem are comprised predominantly of adult rather than juvenile cells (Greenwood 1995) and/or have become determined and remain ontogenetically stable (Hackett and Murray 1997).

In contrast to the above findings, there is also evidence to suggest that factors extrinsic to the meristem can influence the ontogenetic status of the plant. Visser (1973) found that apple seedlings grafted onto dwarfing rootstock precociously flowered compared to those grafted on an "invigorating" rootstock. With regards to vegetative phase change, Brand and Lineberger (1992) grafted adult scions of *Betula* onto six-month old seedling rootstocks. They found partial rejuvenation evidenced by the occurrence of leaves with juvenile characteristics, although this occurred for a transitory period. There has also been some anecdotal evidence that adult characteristics in cuttings of adult plants of *Populus* and *Eucalyptus* disappear rapidly after being grafted or rooted (Greenwood 1995). These studies suggest that other plant organs, such as the roots, can affect some phase-dependent characteristics within the plant. Grafting studies, however, have limitations since the process of grafting itself affects the scion by initiating stress when cutting, providing contact with endogenous substances in the adult tree, and by subjecting the scion and rootstock to competition for assimilates by other branches (Snowball 1989).

The transition from the juvenile through to the adult vegetative phase may require some level of cellular determination (Hackett 1985, Greenwood and Hutchinson 1993). Poethig (1990) argues that both intrinsic and extrinsic factors regulate phase change at the cellular level in the apical meristem *per se*, but that the onset of adult features is initiated by factors extrinsic to the apical meristem. Thus, changes in the apical meristem and changed inputs into that meristem (e.g. carbohydrates, hormones and water) function in determining its expression, and these factors are likely to be modulated by increasing size and complexity of the plant (Hackett 1985, Poethig 1990).

1.1.5.2 Apical meristem: time keeper for phase change?

The timing of phase change transition may, at least in part, be intrinsic to the meristematic cellular tissue. The shoot meristem undergoes a number of morphological and physiological changes during phase change (Poethig 1990, 1997). For example, the apical meristems of adult plants in comparison to those in juvenile plants, are generally larger, cells are smaller and have slower rates of subapical division (Stein and Foskett 1969). Early studies by Brink *et al.* (1968) and Robinson and Wareing (1969) suggest that cells in the apical meristem can act as a time-measuring system whereby information can be maintained on the number of cell divisions. Clearly, phase change can be accelerated by placing plants in conditions that promote rapid growth (Brink *et al.* 1968, Robinson and Wareing 1969, Hackett 1985, Snowball 1989, Snowball *et al.* 1994). Under these favourable growth conditions, Sussex (1976) proposed that cells at the apical meristem would be undergoing a rapid cyclic turnover, which also coincides with plants reaching a minimum size, and thus, attainment of a minimum number of cells divisions would be achieved before phase change occurs.

1.1.6 Timing of phase change

Recent quantitative genetic studies on *Eucalyptus* have focused particular attention on the timing or rate of developmental processses (heterochrony) (Wiltshire *et al.* 1998, Jordan *et al.* 1999). Jordan *et al.* (1999), using the onset of leaf morphological changes and time to first flowering as markers of phase change in *E. globulus* trees, demonstrated in open-pollinated and controlled-cross field experiments that the onset of these events was independently regulated and were under strong genetic control. *E. globulus* occuring in numerous populations throughout south-eastern Australia is known to have a high level of genetic differentiation between populations with regards to the time to phase change (Dutkowski and Potts 1999). Thus, by the comparing ontogenetic development of *E. globulus* grown under a common environment, using progeny from individual populations and crosses from different populations, Jordan *et al.* (1999) were able to establish that the onset of phase change in this taxon was under moderate to strong additive genetic control. However, there was no quantitative genetic association between the timing of phase change and first flowering. In other words, vegetative phase change was not conditional for flowering, since flowers can coincide in plant

parts having both adult and/or juvenile foliage (Potts and Wiltshire 1997). Their findings support previous studies suggesting that many *Eucalyptus* species may have undergone rapid heterochronic evolution of morphologically and ecologically distinct groups (Potts and Wiltshire 1997). The timing of phase change, at least in *Eucalyptus*, is therefore suggested to be under strong genetic control.

1.1.7 Morphological and anatomical features

Characterisation of developmental phases in plants has been achieved through quantitative examination of numerous morphological and anatomical features (Day *et al.* 1997). Leaf shape and size present some of the most recognisable and conspicuous morphological traits that have been used as markers of phase change (Frydman and Wareing 1973a, Hackett 1976, Day *et al.* 1997). Juvenile leaves produced immediately after germination are usually smaller, simpler and anatomically different from leaves produced during the adult phase (Poethig 1997). Juvenile leaves of English ivy (*Hedera helix*), for example, typically have three-to five-lobed palmate leaves arranged in alternate phyllotaxy, while adult leaves are entire and ovate, developing in a spiral arrangement (Frydman and Wareing 1973a). Under natural conditions, the length of this juvenile period can last between five to ten years (Hackett 1985). This is not atypical, considering that the duration of the juvenile period can be as short as 20 to 30 days in perennial *Rosa*, and up to 30 to 40 years in *Fagus sylvatica* (Hackett 1985).

Many New Zealand woody species which include around 200 species from 37 families (Cockayne 1911) exhibit heteroblastic leaf characteristics. Clearwater and Gould (1993) characterised leaf development in *Pseudopanax crassifolius*. Juvenile *P. crassifolius* produce long and narrow (25-100 cm long, 0.5-1.5 cm wide) sharply toothed leaves in contrast to the short and broad, (10 –20 cm long, 1.5 –3.0 cm wide) dentate leaves found in the adult phase (Clearwater and Gould 1993). One New Zealand species, *Elaeocarpus hookerianus*, is known to exhibit three distinct ontogenetic leaf morphological and anatomical phases, comprising a juvenile, an adolescent and an adult phase (Day *et al.* 1997). Each of these phases is characterised by having distinct leaf shapes, being larger in terms of length, width and area in the adult form in comparison to leaves from the adolescent and juvenile phases. Also, adult leaves are typically obcordate, oval or elliptic in shape and are longer (lanceolate) and

wider compared to the non-adult phases (Day *et al.* 1997). The variation in leaf shape or shoot architecture characteristics between ontogenetic phases has been hypothesised to be an environmental adaptation response, whereby these phase-specific modifications provide efficient light capture under forest canopies (Day and Gould 1997, Day *et al.* 1997) and/or tolerance mechanisms against environmental stresses (James and Bell 2001).

Differences in other leaf properties have also been described for a number of model woody plants. In *H. helix* and *Solanum aviculare* leaf thickness can vary between ontogenetic phases, being significantly larger and having a greater leaf mass during the adult phase (Bauer and Bauer 1980, James and Mantell 1994). These ontogenetic differences have been attributed to a more weakly differentiated palisade mesophyll layer in juvenile tissue, and not to differences in the adaxial or abaxial epidermal layers between phases (Day *et al.* 1997, James and Bell 2001). Additionally, environmental factors can also influence leaf anatomy (Poethig 1997). Day *et al.* (1997) showed that *Elaeocarpus hookerianus* plants, irrespective of their growth phase, produced fewer leaf cell layers and palisade was less well developed when grown under shadier conditions.

Within the family Myrtaceae, several studies have characterised the changes in leaf morphology between ontogenetic phases (Ito and Suzaki 1990, Chalmers 1992) and during phase change (James and Bell 2001). Most workers have focused particular attention on *Eucalyptus*, considering that it is a large genus comprising around 700 species (Williams and Brooker 1997) with many species that exhibit homoblastic leaf development. Leaves of juvenile *Eucalyptus* are typically broader and thinner than their adult counterparts. Juvenile leaves can also undergo a transition in stomatal placement, developing on both adaxial and abaxial surfaces (amphistomy) during the adult phase (James and Bell 2001). Many leaf characteristics in this genus, however, may not always be reliable markers of reproductive phase change, since some species are capable of producing flowers deriving from juvenile shoots (Potts and Wiltshire 1997). Studies of phase-related changes in leaf morphology in other genera within the Myrtaceae would provide interesting developmental comparisons.

Studies conducted on several plant species have suggested that the transition between juvenile and adult morphological characters is also correlated with an increase in the

size of the shoot apical meristem (Hackett 1985 and references within). This is in consideration of the fact that the apical and subapical meristem of the shoot apex is the site where different paths of cellular, tissue and organ determination and differentiation originate (Hackett and Murray 1992). Therefore, it was logical to conclude that these are the regions that are involved in the origin of ontogenetically-related changes in development (Poethig 1990, Hackett and Murray 1992). In juvenile plants of *Hedera helix*, for instance, meristematic regions in the apices are smaller, although they contain larger apical cells than those found in adult plants. Cell division rates differ between these ontogenetic phases, and a decrease in cell division in the subapical region of adult plants is correlated with a shorter internode distance (Stein and Fosket 1969). Many of these and other differing phase-dependent features described above have also been reported for a various other woody plant genera such as *Betula* (Brand and Lineberger 1992), *Solanum* (James and Mantell 1994), *Eucalyptus* (James and Bell 2001) and in various crop plants including blueberry (Lyrene 1981) and elderberry (Hrib *et al.* 1980). An example of various features that distinguish the juvenile and adult phases in model plants of the woody English ivy and the herbaceous maize can be seen in Table 1.1

1.1.8 Plant size in relation to phase change

The size of the developing plant may be important in affecting the ontogenetic status. Various studies have shown that attainment of a minimum size is correlated with the transition to an adult condition, as indicated by the attainment of reproductive structures (Hackett 1985). Generally, 'size' refers to the distance from the apical mersitem to the base of the plant (distance to the roots) but it can also be attributed to the mean stem diameter (Visser 1964) or number of nodes (Snowball *et al.* 1994). Visser (1964) for example, showed that the stem diameter of eight- to twelve-year old apple and pear seedlings was inversely correlated with the length of the juvenile period. Similarly, the attainment of a minimum size (height) in *Ribes nigrum* seedlings was strongly correlated with the capability of the plant to respond to short-day inductive cues for flowering (Robinson and Wareing 1969). Further, conditions that promoted rapid growth were negatively associated with the length of the juvenile period (Visser 1964, Robinson and Wareing 1969). How plant size affects the phase change process remains unclear, although several hypotheses have been proposed.

Table 1.1. Features that distinguish juvenile and adult phases of English ivy (*Hedera helix*) and maize (*Zea mays*) (from Poethig 1990).

Traits	Juvenile	Adult
	Hedera helix	
Leaf shape	Entire	Lobed
Leaf thickness	230 μm	330 μm
Phyllotaxy	Alternate	Spiral
Plastochron	1 week	2 weeks
Growth habit	Plagiotropic	Orthotropic
Anthocyanin	Present	Absent
Aerial roots	Present	Absent
Rooting ability	Good	Poor
Flowers	Absent	Present
	Zea mays	
Cuticle thickness	1 μm	3 μm
Epidermal cell shape	Circular	Rectangular
Epicuticular wax	Present	Absent
Aerial roots	Present	Absent
Epidermal hairs	Absent	Present
Bulliform cells	Absent	Present
Lateral buds	Tiller-like	Ears or absent
Anthracnose resistance	Poor	Good

Attainment of a particular size may not, in itself, cause phase change but rather serve as an indicator of the ability of the plant to undergo phase change. Hackett (1985) outlined three hypotheses to account for this, suggesting firstly that size is likely to act as an indicator of the number of cell divisions undertaken in the apical meristem. Secondly, he hypothesised that size can also reflect the position of apical meristems in relation to the other parts of the plant (e.g. roots) and with the corresponding distances between these parts, particularly with respect to translocation of assimilates, water and hormones. Assimilate accumulation and redistribution to shoot meristems might, therefore, be dependent on the complexity of the plant (tree). Thirdly, size can serve as an indicator of relative cumulative sizes and activities of various meristems, both in the shoot and root system. The requirements for growth of these meristems on the plant system as a whole would also influence the competition for assimilates such as photosynthates, water and hormones (Hackett 1985).

1.1.9 Environmental factors

1.1.9.1 Light and temperature

Environments that favour rapid or vigorous growth are also associated with accelerated phase change (Hackett 1985, Snowball *et al.* 1988, 1994). These environmental conditions are usually 'warm' temperature and 'long' day-lengths. This was demonstrated in early studies by Jonkers (1971) who showed that young apple seedlings planted in fields at various latitudes across Europe (Finland to Italy) responded to relatively warmer and longer daylengths in the southern locations by growing more rapidly and reaching reproductive maturity sooner. More recently, several studies growing plants under controlled conditions have been able to quantify the effects of different environments on phase change. James and Bell (2000a) grew juvenile plants of *Eucalyptus* under varying degrees of shade (100, 50 and 10% sunlight). Plants exposed to low light conditions not only showed reduced growth, biomass and leaf area, but also an increased retention of the juvenile phase. Thus, the time taken for initiation of vegetative phase change increased with decreasing light availability despite the fact that the position of phase change on the main shoot remained the same (James and Bell 2000a). Similarly, other experiments on *Citrus* seedlings have also shown that environments that enhance growth and promote attainment of a critical size (critical node number) resulted in a shortening of the juvenile phase (Snowball *et al.* 1988, 1994). The prolonging of the juvenile phase or rejuvenation caused by low light intensity and high temperature are likely due to conditions that reduce the availability of carbohydrates necessary for growth and development towards phase change (Hackett 1985, Tsai *et al.* 1997).

1.1.9.2 Water stress

Little information is available on the effects of various environmental stresses on phase change. Most reports, however, have emphasised the role of water stress in affecting the transition from adult vegetative plants to those that become competent to flower and few on the transition between juvenile and adult vegetative characteristics. There is some evidence, although mostly anecdotal, that water stress in particular can play an important role in the timing into the reproductive ontogenetic phase. R.P. Pharis, in personal communication with Hackett (1985), observed that *Pseudotsuga menziesii*

plants growing on South Vancouver Island in a low rainfall area flowered within six to ten years. In comparison, the same species growing 50 miles away in a cool wet region did not flower until 15 to 20 years in age. These observations were clearly confounded by environment, and as noted by Hackett (1985), it would, therefore, be difficult to distinguish the effect of water stress on phase change from those conditions that promote induction of flowering.

1.1.10 Carbon isotope discrimination studies

Assessment of plant physiological performance has been evaluated using stable isotopes obtained from plant tissue (Farquhar *et al.* 1989, Donovan and Ehleringer 1991, 1994; Poss *et al.* 2000). Discrimination against the naturally occurring stable ^{13}C occurs along the CO_2 diffusion pathway and during photosynthetic CO_2 fixation in plants. The extent to which discrimination occurs and the composition of the carbon isotopes in leaf tissue of C_3 plants are primarily determined by the ratio of intercellular to atmospheric partial pressure of CO_2 during assimilation and by the isotopic composition of the source (Farquhar *et al.* 1982). Both CO_2 assimilation and stomatal conductance directly affect the ratio of intercellular to atmospheric partial pressure of CO_2. The ratio of CO_2 assimilation to transpiration also provides a long-term measure of the intrinsic water-use efficiency (carbon gained per unit of water lost) of the plant, a variable that is highly and consistently correlated with carbon isotope discrimination itself (Farquhar and Richards 1984, Farquhar *et al.* 1989). Thus, plants that are highly water-use efficient tend to have tissue with relatively lower carbon isotope discrimination (Δ) values. Carbon isotope discrimination has also been correlated with a diversity of gas exchange components, including leaf conductance, transpiration, and photosynthetic capacity (Farquhar *et al.* 1989, Ehleringer *et al.* 1993, Sparks and Ehleringer 1997) including properties intrinsic to the leaf, such as leaf mass per unit area (Osorio and Pereira 1994), leaf thickness (Hanba *et al.* 1999) and mineral status (Sparks and Ehleringer 1997, Poss *et al.* 2000).

Inter- and intraspecific variation in ^{13}C discrimination in woody plant species has been reported in numerous plant ecological and physiological studies (e.g. Donovan and Ehleringer 1991, Sparks and Ehleringer 1997, Bonal *et al.* 2000). Considerable attention has been given to the level of Δ, and its relationship to photosynthetic

pathways (Farquhar *et a*l. 1989), water use and gas exchange at a single leaf (Robinson *et al.* 1993) and whole plant canopy scale (Donovan and Ehleringer 1991, Stewart *et al.* 1995, Bonal *et al.* 2000). Reports on the intraspecific variation in Δ between woody plants at different ontogenetic states has been limited primarily to ecophysiological studies of plants from desert assemblages (Sandquist *et al.* 1993, Donovan and Ehleringer 1994) and *Acacia* and *Metrosideros* populations found on the Hawaiian islands (Hansen 1996, Cordell *et al.* 1998). Discrimination is often reported to be higher for juvenile plants than for adult plants, and is associated with higher photosynthetic rates and decreased water-use efficiency during the juvenile phase. This phenomenon is often reported for successful establishment of juvenile plants in environments where water is often a limiting factor (Donovan and Ehleringer 1991, Bond 2000). Therefore, differences in physiological responses between plants of different ontogenetic states may be confounded when examined under variable field conditions. As noted by Donovan and Ehleringer (1994), juvenile and adult plants are potentially under different selective pressures, and variation in ecophysiological characters (e.g. water access as function of root depth) may affect growth and survival. In this thesis, woody plants of *Metrosideros excelsa* were grown under controlled conditions and in the absence of water-stress. This would provide a more accurate evaluation of plant ontogenetic state during the transition between phases. Consequently, these results would make an interesting comparison with the results of many of the ecophysiological studies, where findings may have been confounded by the prevailing environmental conditions.

1.1.11 Photosynthetic characteristics and chlorophyll concentration

Differences in photosynthetic characteristic have been attributed to difference in ontogenetic states. A variety of studies generally indicates that both photosynthesis and stomatal conductance decrease with increasing age or ontogenetic state, although this pattern is not consistent for all species (Bond 2000). For example, decreased net photosynthetic rates with increasing ontogenetic development have been reported in *Picea abies* (Kull and Koppel 1987), *Sequoiadendron giganteum* (Grulke and Miller 1994) and *Pinus ponderosa* (Kolb and Stone 2000), whereas other woody species such as *Quercus rubra* (Boardman 1977) show the reverse trend.

With regards to chlorophyll content, juvenile leaves of a number of species, such as *Solanum aviculare* (James and Mantell 1994) have been reported to have higher total chlorophyll concentrations than those of adult leaves, whereas adult leaves of *Larix* have significantly higher levels of total chlorophyll (Greenwood *et al.* 1989). Variations in the different types of chlorophyll can be confounded by season. In *Solanum aviculare*, the chlorophyll *a* and *b* ratio can be higher in adult leaves during the summer, and reversed in the winter. Considering that differences in chlorophyll content or ratio can vary spatially within a plant and between different seasons or environments (James and Mantell 1994, Bond 2000), these parameters are generally not considered reliable markers of ontogenetic status.

1.1.12 Role of Carbohydrates

The role of carbohydrates in affecting shoot development has often been neglected in studies involving phase change, particularly during vegetative phase change. Most studies focusing on reproductive phase change have shown strong correlations between accumulation of carbohydrate levels and the tendency to flower (reviewed in Bernier *et al.* 1981a). A reduction in carbohydrates can be directly affected by exogenous factors. Environmental treatments that reduce carbohydrate levels such as extreme temperature conditions and low light intensities subsequently delay vegetative phase change (Njoku 1958, Hackett 1976, Schwabe 1976, Hackett 1985). Low light conditions, for example, can delay changes in juvenile and adult leaf shapes in *Ipomoea caerulea* (Njoku 1958). Treatments that promote carbohydrate accumulation in various parts of the plant can also affect the transport and accumulation of other substances (e.g. hormones), and thus, complicate the interpretation of the effects of carbohydrates on development. With the recent advance of molecular studies, however, researchers have been able to clearly elucidate the role of photosynthates in vegetative phase change (Tsai *et al.* 1997). Tsai *et al.* (1997) were interested in determining what effect source strength (carbohydrate production) would have on shoot development. Using an antisense mutant of *Nicotiana tabacum* that had reduced amounts of Rubisco in comparison with a wild-type tobacco plant, Tsai *et al.* (1997) were able to make comparisons of differential shoot development in juvenile plants. Both types of plants displayed distinct phases of shoot development as evidenced by differences in shoot elongation rates, internode distances, plastochron indices, leaf sizes and leaf morphologies. The juvenile phase of the

antisense plants showed a delay in transition to an adult phase. This was suggested to occur in response to the attainment of a threshold source strength, which was delayed in the mutant plants (Tsai *et al.* 1997). Their study clearly emphasised the important role of source strength in the timing of phase change. However, since the delay in phase change in antisense plants was only temporary, this strongly supports the hypothesis that several factors, other than carbohydrate levels, are involved in phase change.

1.1.13 Plant hormones

In addition to nutritional factors, there is convincing evidence to suggest that phytohormones are also intricately involved in phase change. In particular, the ratios of different hormones such as abscisic acid (ABA), gibberellins (GAs), auxin (IAA) and cytokinins (CKs), rather than their absolute concentrations, are suggested to play an important role in promoting phase change (Greenwood 1995). It is well established that hormones such as cytokinins, auxins and gibberellins create metabolic sinks which mobilise assimilates (Sachs 1977). These hormones may serve to create competition between sinks for assimilates (Hackett 1976, Hackett 1985) and are likely to be involved in the partitioning of assimilates in the apical region where phase change occurs (Hackett 1985).

1.1.13.1 Gibberellins and abscisic acid.

Numerous classical studies have closely linked the role of GAs and ABA with phase change (Robbins 1957, Frydman and Wareing 1973a, 1973b, Frydman and Wareing 1974). The suggestion of Robbins (1957) that GAs are important in phase change was first prompted by the finding that exogenously applied GA_3 caused rejuvenation in *Hedera helix*. Subsequent studies showed that "gibberellin-like substances", which can be synthesised in both roots and immature leaves and buds of *H. helix*, could act in the regulation of phase change in the apical region (Frydman and Wareing 1973a, 1973b). High levels of endogenous GAs appear to be associated with juvenility (Wareing and Frydman 1976), and the application of exogenous GA_3 to adult plants induced the development of juvenile characteristics (Rogler and Hackett 1975), a feature described as causing "complete rejuvenation" (Frydman and Wareing 1974). The application of suggested antagonists (e.g. chlormequat, ancymidol and daminozide) or inhibitors (e.g.

ABA) of endogenous gibberellins appear to stabilise the adult phase in *H. helix*. Plants treated with 5 nM of GA_3 and 5 mM of ABA prevented rejuvenation, but when GA_3 was increased to five-fold with the same levels of ABA, reversion did occur. They suggested that the relative rather than the absolute amount of each hormone used was important in controlling the growth form. Although stabilisation of the adult phase may be maintained with these treatments, the application of similar treatments (chlormequat and ABA) has not led to the acceleration of phase change (Frydman and Wareing 1974).

More recent work by Horrell *et al.* (1990) showed that exogenous applications of GA_3 to adult apices of *H. helix* resulted in rejuvenation, but only partially as opposed to complete reversion as previously suggested (Frydman and Wareing 1974). This included the incomplete reversion of characteristics such as leaf lobing and anthocyanin pigmentation that typify the juvenile form. Application of paclobutrazol (PP333) (an inhibitor of endogenous gibberellin biosynthesis) and ABA treatments were ineffective in promoting phase change in juvenile *H. helix* (Horrell *et al.* 1989b, 1990). Contrary to expectations, PP333 promoted shoot extension in juvenile plants, a response hypothesised to be the result of gibberellin production in aerial roots (Horrell *et al.* 1989b). Considering the atypical response of this species to PP333 compared with other heteroblastic species, Horrell *et al.* (1990) proposed that *H. helix* was not an ideal model for studies of phase change.

The effects of plant growth regulators have been reported for various New Zealand plant species. Similar to *H. helix*, application of GA_3 to adult plants of *Carpodetus serratus*, *Pennantia corymbosa* and *Parsonsia heterophylla* caused only partial reversion to a juvenile phase (Horrell *et al.* 1989a). Applications of PP333 or ABA to juvenile plants of these New Zealand species and others (e.g. *Pseudopanax crassifolius*) did not promote phase change but, unlike in *H. helix*, shoot elongation was inhibited or otherwise plants died (Jameson and Horrell 1988, Horrell *et al.* 1989a). Endogenous GAs are, therefore, implicated in the retention of the juvenile phase, although the role of GAs in phase change is, as yet, unclear. The results from these and other studies involving application of anti-gibberellin treatments suggest that plant responses to factors other than a decrease in GAs are likely to be involved in phase change from the juvenile to adult state.

1.1.13.2 Cytokinins

Most physiological studies examining phase change have focused on GAs, although there is some evidence suggesting a role played by cytokinins (Ross and Pharis 1976, Imbault *et al.* 1988, Day *et al.* 1995, Diekman 1997). Cytokinins (CKs), which are predominately root-produced hormones, have been implicated in a host of plant hormone interactions and developmental processes (Voesenek and Blom 1996). Among these has been their role in vegetative to reproductive development. Zaerr and Bonnet-Masimbert (1987), for instance, reported that a reduction in endogenous CKs in the shoot of Douglas fir was associated with treatments that promoted this transition. Similarly, exogenous applications of CKs have also been demonstrated to depress gibberellin-induced reproductive bud production in *Picea mariana* (black spruce) (Smith and Greenwood 1995). It has been hypothesised that CKs may regulate but not promote flowering, considering that juvenile plants do not flower even in the presence of exogenous cytokinin (Imbault *et al.* 1988).

A few studies have compared the relative levels of cytokinins between different phases of vegetative development (Carswell *et al.* 1996, Mercier and Endres 1999). Seedlings of the woody angiosperms *Sophora microphylla* and *S. prostrata* were treated with 6N-benzyladenine (BA) (Carswell *et al.* 1996). In *S. prostrata*, BA appeared to have no effect on growth, whereas in *S. microphylla* the divaricating shoot growth typical of juvenile plants was enhanced. This was exhibited by an increase in the number of outgrowing branches and by a widening of branch angles in comparison with water-treated control plants. Examination of assays suggested the ratio of active to storage CKs was greater in divaricating than non-divaricating forms. Similar reports suggesting that higher concentrations of CKs occur in juvenile tissue have been supported by investigations in *Elaeocarpus hookerianus* (Day *et al.* 1995), *Tillandsia recurvata*, an epiphytic bromeliad (Mercier and Endres 1999) and in the rubber-tree *Hevea brasiliensis* (Perrin *et al.* 1997). In *E. hookerianus*, treatment of seedlings, juvenile and adult plants with exogenous CK resulted in differential responses (Day *et al.* 1998). Juvenile plants showed greater variation in leaf morphology (leaf length, proportion of linear leaves and leaf angle) and growth habit (height and internode length) than seedling or adult plants. However, application of exogenous CK did not induce any substantial ontogenetic development of juvenile plants, nor did it cause rejuvenation in

adult plants. A recent examination of endogenous CKs in juvenile and adult *Pinus radiata* has revealed several novel forms of CK glucosides that are differentially expressed, in terms of concentration, in juvenile and adult buds (Zhang 1998, Zhang *et al.* 2001).

Recent genetic studies are advancing our understanding of the role played by CKs in plant development and phase change, although much progress is still needed. The *Arabidopsis* mutant, *altered meristem program 1* (*amp1*), that produces elevated levels of CK, has provided key insights into the role of CKs during phase change (Diekman 1997). Observations of phenotypes in *amp1* in comparison with the wild-type indicate that heightened levels of CK prolong the juvenile phase. This may be because of the increased number of cotyledons and greater number of rosette leaves expressed in the *amp1*. Another mutant, *cyr1*, produces under-developed cotyledons and true leaves, and is insensitive to CKs (Diekman and Ulrich 1995), suggestive of a disruption in CK perception or signal transduction (Diekman 1997). This may explain why *cyr1* has a relatively short juvenile period. These studies imply that CK is required for the expression of the juvenile phase. The repression of genes involved in flower formation by CK would also support this conclusion (Venglat and Sawhney 1996).

1.1.14 Acceleration of phase change

A number of cultural practices have been employed in order to accelerate phase change. The most successful method of shortening the juvenile phase in woody species has been to grow plants under environmental conditions that promote rapid or vigorous growth (see Section 1.1.9) (Poethig 1990). This usually equates to relatively warm and high light environments. Moreover, this method is often coupled with various cultural practices that physically alter the growth pattern of a plant and accelerate phase change. For example, Snowball *et al.* (1994) grew various citrus species trained to a single-stem (lateral buds removed) in either a warm greenhouse (min/max: 15-30°C), in an outdoor shade-house (50% shade) or under ambient year-round outdoor conditions. Growth rates (number of nodes attained) were significantly higher for citrus plants grown under greenhouse conditions, and generally those that flowered were obtained only from this environment. The practice of growing plants as a single-stem has also been successful in accelerating phase change (reduction to the time to flower) in kiwifruit (Davis 1991)

and various other woody plants (reviewed in Snowball 1989). This practice of growing plants as a single-stem, as opposed to allowing plants to branch freely, is suggested to encourage growth to a minimum size required for phase change to occur, since this feature is often inversely related to the length of the juvenile period (Hackett 1976, 1985). In contrast, most practices that tend to reduce growth or promote flowering in adult plants, such as girdling, scoring, bark inversions, grafting onto dwarfing stocks and application of plant growth regulators, generally have little to no effect on the length of the juvenile period (Zimmerman 1972, Hackett 1976).

The timing of phase change, at least in some species, can be under strong genetic control (Wiltshire *et al.* 1998). Selective breeding of desired genotypes can be a method for obtaining plants with short juvenile phases. For example, by selecting offspring of *Betula pendula* with precocious flowering, Von Stern (1961) was able to reduce the juvenile period from five to 10 years down to two years. This has also been conducted in various other woody crops including *Pinus* species (Heimburger and Fowler 1969), apple (Way 1971) and pear (Zimmerman 1977).

There has been some speculation that root restriction (small pot size) can accelerate phase change (Zimmerman 1972, Oliphant *et al.* 1992). Various reports have claimed that potted trees reach reproductive maturity sooner when grown in environments that promote vigorous growth (reviewed in Zimmerman 1972). However, in many of these and other studies, attainment of the adult phase has been assessed using flowering as an indicator of maturity and, therefore, no quantitative distinction has been made of the progressive changes in vegetative characters and the onset of flowering.

1.1.15 Markers of phase change

The search for reliable markers specific to ontogenetic phases of development has been an ongoing pursuit in studies of phase change. An outcome of such studies would allow assessment of the progression of phase change or the degree of stability in phase-related characteristics (Hackett and Murray 1997). This would provide a basis for understanding the mechanisms associated with stability, as well as devising methods or protocols for manipulating phase change. Several promising biochemical markers indicative of a developmental phase have been described. Anthocyanin pigments,

which are flavonoid glycosides, have been found to accumulate in stems and leaf petioles of juvenile *Hedera helix*, although not in adult tissue (Hackett and Murray 1997). Murray and Hackett (1991) found that the lack of activity in dihydroflavonol reductase (DFR), an enzyme involved in the anthocyanin biosynthetic pathway, limited the accumulation of anthocyanin in adult tissues. Thus, they concluded that DFR activity could be a definitive marker for the juvenile phase in *H. helix*. Various other proteins have been also been identified that are either unique to juvenile shoots or show a higher activity in juvenile than adult shoots. For example, catalase isozymes in *Cupressus sempervirens* show phase-specific differences that may be suitable as biochemical markers for rooting ability (Racchi *et al.* 1996). Other potential biochemical markers have also been described for *Sequoia sempervirens* (Berthon *et al.* 1987), *Prunus avium* (Hand *et al.* 1996) and citrus crops (Snowball *et al.* 1991). However, the relative differential expression (between species) and species-specificity of a number of endogenous substances, such as proteins, appears to be the primary disadvantage for the use of these biochemical markers.

Morphologically, the presence of floral structures has readily been used as a marker to distinguish between the vegetative adult and reproductively competent adult phase, and/or signifying the end of the juvenile phase (Hackett 1985, Poethig 1990). However, this method is not always a consistent indicator given that some species can produce reproductive structures arising from branches containing distinctly juvenile foliage (e.g. some *Eucalyptus* species) (Wiltshire *et al.* 1991). An approach to characterise different phases of vegetative growth has been the use of leaf morphological markers. This has been particularly applicable for heteroblastic species where differences between different phases of ontogenetic development are visibly apparent and often represented by striking differences in morphological features (Day *et al.* 1997, James and Bell 2001). Characterisation of homoblastic species such as *Metrosideros excelsa* presents more of a challenge given the subtleties in expression of phenotypic characters during development. However, once the attainment of leaves characteristic of an adult vegetative phase has been reached, further inductive treatments are usually required to confirm or dismiss reproductive competence.

1.2 Floral Induction and Development in Ornamental Woody Plants

1.2.1 Introduction

An intimate understanding of processes that govern flowering has posed a formidable challenge for plant biologists, despite the large volume of literature investigating this phenomenon (References within: Kinet 1993, Meilan 1997, Reeves and Coupland 2000). This is because floral development, *per se*, is a multistage process entailing often a complex series of events that are spatially and temporally related (Bernier *et al.* 1993). First, plants must generally undergo a period of reproductive incompetence generally during the juvenile and adult vegetative states (Hackett 1985). Second, following a period of suitable environmental conditions for induction can the flowering process commence. Each developmental event, starting from floral induction through to flower senescence and subsequent fruit and seed development, is dependent upon specific requirements and is affected differently by environmental and chemical parameters (Kinet 1993). In many species, environmental factors act as cues for the timing of flowering, by providing communication on the time of year and conditions suitable for sexual reproductive growth (King *et al.* 1995).

Numerous fundamental studies have provided us with a broad understanding of the physiological and molecular mechanisms involved in flowering (Bernier *et al.* 1993, Levy and Dean 1998). Additionally, applied studies have sought to determine the optimum combinations of environmental and/or chemical factors affecting the flowering process (Jackson and Sweet 1972, Moncur 1992, Myster 1999). A large range of reviews has been published on flowering, including a six-volume *Handbook of Flowering* edited by Halevy (1985, 1986, 1989) and a three-volume monograph on the physiology of flowering (Bernier *et al.* 1981a, 1981b, Kinet *et al.* 1985). Some reviews have focused on the molecular (Amasino 1996, Levy and Dean 1998, Reeves and Coupland 2000) and physiological (Bernier *et al.* 1993, Srivastava and Iqbal 1994) mechanisms involved in the flowering process, whereas other have directed their attention to specific plant groups, e.g. coniferous trees (Bonnet-Masimbert 1987), woody angiosperm trees (Meilan 1997), and particular ornamental crops (Criley 1998). There have been no published reviews, at least to the author's knowledge, on the factors

affecting flowering specifically in woody angiosperms that have floricultural significance. In this review, both the applied and fundamental studies addressing the role of environmental and chemical factors regulating the flowering process in woody ornamental flowering plants are investigated, although when relevant, reference to other crop and/or model plant systems will be discussed.

1.2.2 Terminology

The term *flowering*, which is often broadly or ill-defined, is referred to in this thesis as the reproductive period including flower opening (King *et al.* 1992). Specific terminology describing the early stages preceding the development of a floral reproductive structure follow those specified by Evans (1969). Evans (1969) made the distinction between *induction* which refers to the events in the leaf which commit plants to the flowering process, and *evocation* which refers to the events that occur at the shoot apex following the arrival there of a floral stimulus, but which precedes the onset of differentiation of the floral organs.

Subsequent stages in the flowering process are defined according to Kinet (1993) who subdivided it into three stages: (1) *floral initiation*, which refers to the reactions required for the initiation of flowers and, given the appropriate conditions, leads to the "irreversible commitment" of the shoot apical meristem towards inflorescence and/or flower primordia production; (2) *floral morphogenesis*, which pertains to the formation of recognisable features of the meristem, including floral primordia and other floral organs; and (3) *floral development*, which refers to the development of reproductive structures through to anthesis.

1.2.3 Flower induction models

Over the past decades, scientists have sought to explain by way of models the mechanism by which flowering is induced and maintained. One of the first physiological hypotheses suggesting the role of a stimulus (florigen) for inducing flowering was proposed in the 1930's by Mikhail Chailakhyan (reviewed in Salisbury and Ross 1992). This model proposed that a key substance (hormone) signalled the onset of flowering. The concept of florigen was based on the transmissibility of

substances or signals across graft unions, as was evident when a reproductively competent shoot was grafted with a vegetative recipient (Levy and Dean 1998). It was suggested that florigen was synthesised in leaves exposed to favourable photoperiods, and was subsequently transported to the shoot apex via the phloem, where it acted as a floral-promoter. A contrasting view sought to explain the onset of flowering based on an antiflorigen concept, which was graft-transmissible, yet acted as a floral inhibitor. Many unsuccessful research years, however, have been spent searching for the illusive key florigen or antiflorigen substance within the phloem.

Due to the inability to identify a single florigenic compound, a second model, the nutrient diversion hypothesis, was introduced by Sachs and Hackett (1983). The nutrient diversion hypothesis proposed that inductive conditions modified the source/sink relationship in the plant, subsequently leading to an increase in the level of assimilates directed to the shoot apical meristem for reproductive growth. However, this model did not account for either the diversity of flowering responses observed in different species, or the influence of different environmental cues, or the concept of inhibitive or promotive role of endogenous substances.

With the concept that flowering was not solely induced by assimilates, Bernier (1988) proposed the multifactorial control model which not only accounted for assimilates but other factors such as hormones, which acted as promotors and inhibitors controlling the timing of flowering. Thus, this model maintained that flowering could occur only if the limiting factors present at the shoot apical meristem were present at the correct times and in the appropriate concentrations. This multifactorial control model is currently the most accepted model for explaining the role of environmental and chemical factors regulating flowering and this hypothesis is supported by findings in various model plants such as pea, cereals and *Arabidopsis*.

1.2.4 The molecular frontier

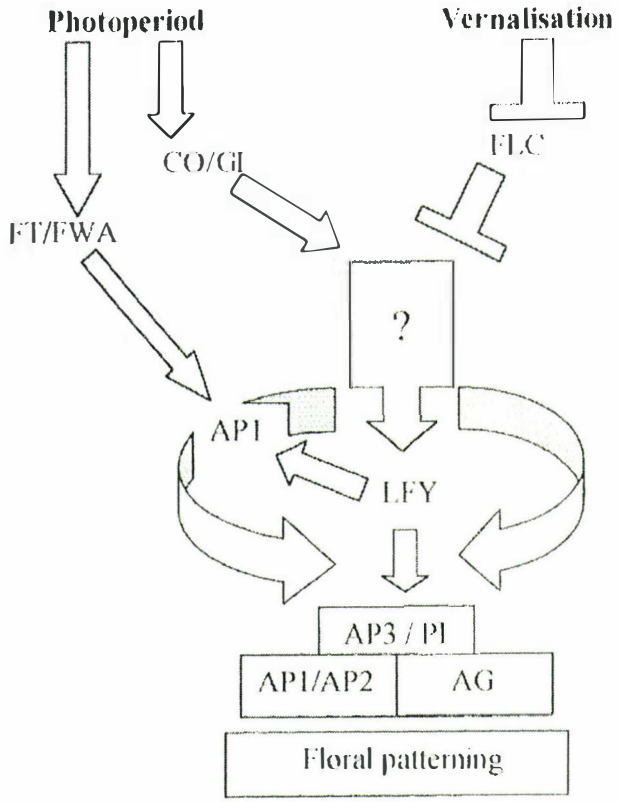
Whatever factors are involved in the flower transition, ultimately control will be exerted at the genetic level. Molecular research has generally been undertaken using herbaceous model plants, in particular *Arabidopsis*, *Antirrhinum* and *Pisum*. This is primarily because woody species are not ideal model systems due to maintenance of a

long juvenile period, the accumulation of secondary metabolites and subsequent difficulty with transformations and regeneration (Meilan 1997). However, much progress has been made using these herbaceous models.

The rosette plant *Arabidopsis* has been an important model in investigations in the timing of flowering and floral development. Recent research into the role of light in controlling flowering has been the focus of photoreceptors, being light-sensitive molecules involved in light perception located in the leaf organs (Thomas and Vince-Prue 1997). Photoreceptors such as phytochrome (PHYA) and cryptochrome (CRY2) initiate signals that interact with the circadian clock and entrain the circadian rhythm (Levy and Dean 1998). The signalling mechanism possibly provides information on light/dark transitions to the circadian clock, since both PHYA and CRY2 protein levels have been found to drop rapidly with exposure to light (Thomas and Vince-Prue 1997). Once daylength is perceived and somehow measured by these photoreceptors (or the length of the dark period has decreased below a critical threshold), 'flowering time' genes such as *CONSTANS* (*CO*) and *GIGANTEA* (*GI*) in *Arabidopsis* are activated, leading to the upregulation of floral meristem identity genes (Amasino 1996, Weigel 1998, Reeves and Coupland 2000) (Figure 1.3 A). Products of the *FLOWERING LOCUS C* (*FLC*) gene inhibit the transition from vegetative growth to flowering, although low temperature can depress the abundance of *FLC* mRNA suggesting an important role of this gene in the vernalisation pathway (Figure 1.3 B). The *FRIGIDA* (*FRI*) gene is involved in the autonomous pathway and promotes *FLC* expression, whereas other genes such as *LD*, *FCA* and *VRN2*, play an inhibitory role in its expression (Reeves and Coupland 2000).

Two classes of genes have been identified that regulate floral development, which comprise meristem identity genes and organ identity genes. The first class of genes contains positive regulators of the floral meristem identity and includes several important genes described in *Arabidopsis*, such as *LEAFY* (*LFY*) and *APETALA1* (*AP1*) (Weigel *et al.* 1992, Bowman *et al.* 1993). Both genes act as developmental switches activating the entire genetic program for the formation of the floral meristem (Weigel and Nilsson 1995). These genes are also positive regulators of a set of genes known as the floral organ identity genes. In *Arabidopsis*, *APETALA2* (*AP2*), *APETALA3* (*AP3*), *PISTILLATA* (*PI*) and *AGAMOUS* (*AG*) are floral organ identity genes that are involved

A.



B.

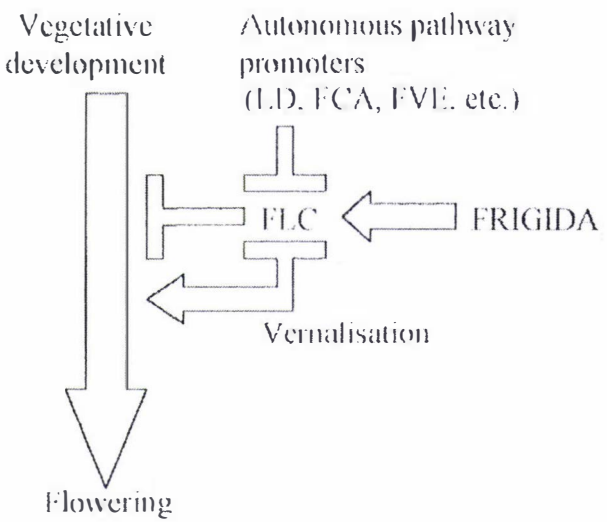


Figure 1.3 General models of the multiple flowering pathways in *Arabidopsis thaliana*. A: Molecular and genetic interactions in the photoperiodic pathway and vernalisation. Long-day photoperiod leads to upregulation of circadian clock and light receptor mediated genes (CO, GI) in addition to the upregulation of other photoperiodic responsive genes (FT, FWA). The autonomous and photoperiodic pathways act additively to promote flowering and lead the upregulation of floral meristem identity genes (e.g. AP1, LFY), although the stage at which conversion of these pathways (?) occur is unknown (modified from Reeves and Coupland 2000). B: Molecular and genetic interactions involved in the autonomous pathway. The FRIGIDA (FRI) gene upregulates FLC despite the inhibitory effect of the autonomous pathway genes.

in specifying the identity of the organ in each whorl (Weigel and Meyerowitz 1994). Coen and Meyerowitz (1991) proposed the ABC model, in which three unique combinations of activities (A, B and C) of these homeotic genes determine the organ identity within each of the whorls. In this model, they proposed that the activity of type A genes controls organ identity in the first and second whorls. Type B genes determine floral organs in the second and third whorls. And finally, type C activity is involved in organ identity in the third and fourth whorls of the flower (Coen and Meyerowitz 1991, Weigel and Meyerowitz 1994). The pattern of floral organ formation in the *Arabidopsis* wild type and most of the mutant phenotypes are supported by this model, although extensions to the model have been proposed (e.g. *SEPALLATA*) (Pelaz *et al.* 2000).

To date, many of the floral genes isolated from model species are highly conserved and function in heterologous systems. Genes cloned from the herbaceous model systems can, therefore, be used as probes to find orthologues in woody plant species. Orthologues of several meristem identity genes, such as *LEAFY* have already been reported in *Eucalyptus* (Southerton *et al.* 1998), *Populus* (poplar) (Rottmann *et al.* 2000) and in *Malus pumila* (apple) (Kotoda *et al.* 2000), including *APETALA1* in the latter species. Recently, partial orthologues of *LEAFY* (McKenzie *et al.* 1997) and *APETALA1* genes have been cloned from *Metrosideros excelsa*, in addition to the isolation of the partial orthologue of the *TERMINAL FLOWER 1* gene (L. Sreekantan, pers. comm.).

Thus far, several candidate woody species look promising as models for molecular research. *Eucalyptus*, cranberry, and poplar are capable of being transformed and regenerated with ease and are capable of floral induction through chemical applications (Serres and McCown 1993, Griffin *et al.* 1993, Meilan 1997). Moreover, flowering can be induced in transgenic poplar *in vitro* (Meilan 1997). The New Zealand woody species, *Metrosideros excelsa*, also looks to be an attractive model system for investigating the molecular and physiological processes associated with flowering. Further studies examining the role of environmental factors in promoting and regulating flowering in this species are investigated in this thesis, and complement existing research to date (Sreekantan *et al.* 2001).

1.2.5 Floral and vegetative growth relationships

Woody angiosperm plants often undergo cyclic and repetitive patterns of vegetative growth with intermittent periods of flowering in response to changing seasons (Srivastava and Iqbal 1994). During these periods, reductions in vegetative growth coincide with the production of flowers (Jackson and Sweet 1972). Treatments that manipulate environmental conditions and/or applications of growth retardants, have been shown to both reduce vegetative growth and subsequently enhance flower number (Jackson and Sweet 1972, Heller *et al.* 1994). These treatments probably cause the diversion of nutrients and carbohydrates from vegetative shoots to floral meristems (Sachs and Hackett 1983). In *Lolium temulentum*, for example, an increase in sucrose concentrations at the apex occurs after evocation and enhances inflorescence development (King and Evans 1991). However, this phenomenon of antagonism between vegetative growth and flowering is not always observed. In many cases growth inhibition is often a secondary event that can occur at advanced stages in the floral transition. In the case of *Bougainvillea* (Hackett and Sachs 1966), *Boronia* (Roberts and Menary 1989) and *Hypocalymma* (Day *et al.* 1994a), both cycles are not mutually antagonistic and a reduction in vegetative growth is not necessary for flowering to occur. Therefore, a reduction in vegetative growth may enhance flowering although it does not appear to be part of the same mechanism controlling floral evocation.

1.2.6 Environmental factors

For many plant species, environmental factors play an important role for signalling the initiation of reproductive structures (Kinet 1993). Manipulation of photoperiod, light quality, light intensity, temperature, and the availability of nutrients and water can serve as signals for the timing and regulation of flowering (Levy and Dean 1998). The effects of each of these factors on the flowering process can function independently or in combination with other factors, and promote flowering based on ecologically optimal limits (King *et al.* 1995). These environmental factors are, however, discussed independently in order to ascertain the effect each factor predominantly has on the flowering process.

1.2.6.1 Light: duration

Light is one of the major environmental factors regulating floral development. Photoperiodism, defined as the ability of an organism to detect the length of a day, was first observed in plants in classic experiments conducted by Garner and Allard (1920). They demonstrated the effect of daylength on the flowering response using a mutant tobacco plant, Maryland Mammoth, which they noticed flowered in the greenhouse only during winter. Garner and Allard (1920) artificially shortened the daylength during the long summer daylengths by enclosing the tobacco plants in light-tight tents, simulating winter day-length conditions. The tobacco plants subsequently flowered, prompting the hypothesis that plants had the ability to measure varying daylengths throughout the seasons, which subsequently signals the time to flower under optimal daylengths. Their hypothesis was confirmed using various other plant species (including soybean and spinach) and under various conditions. This work laid the foundation for subsequent and extensive work on photoperiodic responses. To date, it is known that photoperiodic responses in plants are not limited to just flower initiation but to numerous other *in vivo* responses such as asexual reproduction, storage organ formation and the onset of dormancy (Vince-Prue 1985).

Classification of plants based on their preference for an optimum daylength for flowering can vary widely between species. Flowering in short-day (SD) plants is promoted only when the daylength is less than (or the night length exceeds) a critical threshold in every 24 h cycle. A number of temperate woody species, such as *Chamelaucium uncinatum* (Shillo *et al.* 1985, Dawson and King 1993), *Coleonema aspalathoides* (Heller *et al.* 1994), and *Leptospermum scoparium* (Zieslin 1985, Zieslin and Gottesman 1986) have been classified as SD plants. Opposite to SD plants, flowering in long-day (LD) plants is promoted only when the day length exceeds (or the night length is less than) a critical duration. *Eucalyptus occidentalis* (Bolotin 1975) and *Hamelia patens* (Armitage 1995) are but a few species reported to be LD plants.

In contrast to both SD and LD plants, intermediate-day plants, flower only when exposed to a narrow margin of a day length (e.g. 12 – 14 h photoperiods). Contrastingly, in day-neutral or autonomous flowering plants, a specified daylength or low temperature is not an inductive requirement for undergoing reproductive

development, and flowering is facilitated with time and under favourable growth conditions (Kinet 1993). Several species such as *Eucalytpus landsdowneana* (Moncur 1992), *Pimelea rosea* (King e*t al.* 1992), *Boronia megastigma* and *Hypocalymma angustifolium* (Day *et al.* 1994a) have been described as day-neutral plants. In summary, plants can therefore respond (or not respond) to a specific daylength requirement for flowering, and only by examining a range of photoperiods can a suitable photoperiodic classification be established.

In many horticultural systems, manipulation of daylength has been accomplished within growth rooms, greenhouses or under outdoor ambient conditions through the use of artificially supplied lighting systems. These can include incandescent light-extensions that provide additional daylight hours (Bredmose 1993), night-break interruptions (Langton 1977), and/or through the utilisation of light-covers preventing light penetration at required times (Shillo *et al.* 1997). For a few woody crops of horticultural importance, flowering can be controlled solely by the manipulation of photoperiod, although other factors such as temperature can have an interacting effect. In the case of the ornamentally-valued *Chamelaucium uncinatum* (family Myrtaceae), an evergreen shrub native to the Mediterranean climate of Western Australia, plants were treated to two photoperiod treatments at various inductive intervals in order to determine their flowering response (Shillo *et al.* 1981, 1985). A minimum of four weeks of SD (8 h) before transference to LD (13-14 h) conditions was sufficient for all *C. uncinatum* plants to produce flowers (Shillo *et al.* 1985). Moreover, applying SD treatments for longer than four weeks served only to enhance flowering by further increasing the number of flowers and flowering shoots per branch (Shillo *et al.* 1985).

In some woody species, the inductive daylengths can affect flower initiation and/or development. Armitage (1995) reported that both floral initiation and development occurred in *Hamelia patens* in response to LD treatments. While flower bud initiation occurred in approximately two-thirds of the plants treated to 8 and 12 h photoperiods, flower development was totally arrested in the 8 h photoperiod and only 20 percent of plants under the 12 h photoperiod reached anthesis. Under 16 h photoperiods, all *H. patens* plants reached anthesis. Armitage (1995), therefore, suggested that floral development in this species was more sensitive to unfavourable photoperiods than was floral initiation.

Flowering responses at different stages in development can also be mediated by factors other than photoperiod. In *Hardenbergia violacea*, King (1998) reported that there was an obligate requirement for both short days (<12.5 h) and sustained cool temperatures (15-18°C) for inflorescence initiation. Plants rarely initiated inflorescences under long days, particularly under warm conditions. However, maintenance of inflorescences beyond an intermediate stage in floral development (carpel and anther primordia microscopically evident) required a narrow range of temperatures (15-16°C) in order to prevent the rapid (<30 days) and complete abortion of all inflorescences. Therefore, the flowering response in *H. violacea* was more sensitive to daylength early during floral initiation, and to temperature during floral development (King 1998).

Photoperiodic responsive plants, other than *Hardenbergia violacea*, can also be sensitive to temperature during floral development. Photoperiod appears to be the main factor affecting floral initiation in *Chamelaucium uncinatum*, while temperature was the primary factor regulating flower development (Shillo *et al.* 1985, Dawson and King 1993). By experimenting with both temperature and photoperiod treatments applied in factorial combination for up to nine weeks, Shillo *et al.* (1985) showed that plants formed flowers after exposure to low day/night temperatures (20/14°C), in both SD (8 h) and LD (13-14h). However, at high temperatures (24/18°C), only plants exposed to SD flowered, whereas none flowered under LD conditions. The number of flowers per shoot that formed was also positively related to the number of days that plants were placed under inductive conditions. Shillo *et al.* (1985) subsequently classified *C. uncinatum* as a facultative SD plant.

Although flowering in *C. uncinatum* occurred after exposure to SD with both high and low temperatures, and LD at only low temperatures, the number of flowers produced between treatments was significantly different. Shillo *et al.* (1985) observed that approximately twice as many flowers per branch were formed in those plants held under SD rather than LD conditions. Thus, the application of moderately cool temperatures can overcome the effects of a LD treatment in *C. uncinatum*, despite a reduction in flower number. Evidence that decreasing temperatures can progressively nullify the SD or LD photoperiod requirements for flowering has been well documented, as in the case

with *Bougainvillea* (Hackett and Sachs 1966) and in various herbaceous species (Kinet *et al.* 1985). Therefore, daylength can interact with other environmental variables to significantly affect the final number of flowers produced.

Unfavourable photoperiods during floral initiation or development can cause a decline in flower quality (decreased flower number or flower malformations), an issue particularly important in horticultural crop production. Shillo *et al.* (1984) reported that flowering in *C. uncinatum* plants ceased after the first flowering flush when plants were maintained continuously under inductive SD conditions. They attributed this outcome to the formation of developing flowers that inhibited subsequent flower initiation. Only removal of the flower buds at an early stage enabled the continuous formation of flowers under SD conditions (Shillo *et al.* 1984). Unfavourable photoperiods during flower formation are also associated with malformations in the inflorescences/flower (Kinet *et al.* 1985). These abnormalities may affect bract, flower or floral primordium development indicating that any given floral primordium during development is not committed during initiation and their developmental path may remain optional (Kinet *et al.* 1985). In *Impatiens balsamina*, for example, vegetative growth occurs in undifferentiated floral organs when exposed to unfavourable photoperiods (Krishnamoorthy and Nanda 1968). Floral buds have also been reported to abort early in development in response to unfavourable photoperiods, as in the case of *Boronia megastigma* when treated to constant LD conditions (Roberts and Menary 1989). In summary, exposure to inappropriate photoperiods can clearly have deleterious effects on flowering. However, the responses exhibited can be species-specific.

1.2.6.2 Light: spectral quality

The quality of light is an important aspect in horticultural crop production, particularly under controlled environmental conditions. Relatively few studies, however, have been conducted on flowering responses in woody species to different wavelength ratios. In *Pinus radiata*, Morgan *et al.* (1983) described an increase in shoot elongation rates, internode length and stem weight in adult and juvenile plants as the proportion of far-red (FR) to red (R) light increased. In kiwifruit and grape, exposure to light with a high FR:R ratio coincided with an increase in petiole elongation or internode length, respectively, and with a declining trend in the number of flowering nodes/clusters per

shoot (Morgan *et al.* 1985). Perception and translation of the light quality are linked to the involvement of phytochrome photoreceptors for controlling vegetative and flowering growth (Thomas and Vince-Prue 1997).

The effects of different light qualities (spectral distribution) can also affect growth. Maas and Bakx (1997) examined the effect of different spectral qualities applied continuously at a PAR of 130 μmol m^{-2} s^{-1} on vegetative growth in the rose hybrid 'Mercedes'. After six weeks, they found that shoots in plants grown under amber (85% blue light filtered out) and orange-light (100% blue light filtered out) were considerably longer (increase of 10-16 cm) than those grown under control white light (containing 400-500 nm blue light). Plants held in amber and orange light also partitioned relatively more dry weight into stem than into leaves in comparison with white-light treated plants. None of the different spectral treatments, however, had an effect on the number of flowering shoots per plant. Mor and Halevy (1984) suggested that other factors such as the ratio of R to FR affected the number of flower buds sprouting and that irradiance affected subsequent flower development.

1.2.6.3 Light: intensity

Although the role of light intensity on flower formation has been less studied than that of photoperiod, its involvement in the flowering process is equally important for many species. Irradiance can play an important role in the flowering process for many autonomously flowering species. For instance, floral initiation in *Leucospermum*, an ornamentally-valued day-neutral plant, is induced under high light intensities (0 - 30 percent shading), although this requirement occurs only in conjunction with a cessation of shoot growth and through the release of apical dominance (e.g. removal of the primary inflorescence) (Jacobs 1985). Other day-neutral species such as various species of *Rosa* and grape (e.g. *Vitis vinifera*) are also sensitive to irradiance. For example, transference of *V. vinifera* plants from a low (220 μmol m^{-2} s^{-1}) to a high irradiance (600 μmol m^{-2} s^{-1}) environment, or continuous maintenance (>50 days) under a high irradiance, produced the highest yield of flowers per shoot (Morgan *et al.* 1985). The enhancement effect of high irradiance levels on the number of flowers or floral buds produced has been reported in numerous other woody species, including ornamentals

such as *Leucospermum* (Zieslin 1985), *Begonia x hiemalis* (Myster 1999), *Acacia pycnantha* (Sedgley 1985), *Pimelea ciliata* (Slater *et al.* 1994) and *Boronia megastigma* (Roberts and Menary 1989).

The intensity of light is often important for flower formation considering that relatively low or high levels can have deleterious effects (Sedgley 1985). Low light during induction can result in abnormalities in reproductive structures. For instance, a decrease in light intensity after flower initiation in *Kalanchoë blossfeldiana* has been shown to cause the formation of inflorescences with vegetative parts (Kinet *et al.* 1985). Low light conditions can also lead to abortion of reproductive structures. In *Boronia* and *Rosa*, shady conditions are correlated with a decline in the number of flowers and this is suggested to be due, at least in part, to an increase in flower bud abortion (Zieslin and Mor 1990, Plummer *et al.* 1998). Finally, flowering time can also be affected by irradiance. For example, low light significantly delayed the time to anthesis in *Coleonema aspalathoides* (Heller *et al.* 1994).

Relatively few studies have attempted to document the critical stages during floral development that are most affected by irradiance (Sedgley 1985, Roberts and Menary 1989). Of the many studies investigating the effects of irradiance on flowering in woody species, Sedgley (1985) is one among the few investigators who has employed the use of microscopic examinations to detail the independent effects of irradiance (and temperature) on floral primordium development. Sedgley (1985) found that floral initiation in *Acacia pycnantha* ceased at a very early stage of development when plants were grown under low light conditions. Differentiation did not progress beyond the initiation of the individual primordia. Temperature also had an effect on floral development but at a later stage in development. Floral development in plants grown continuously in a heated greenhouse as opposed to those grown outside proceeded normally up to the stage of megasporogenesis and microsporogensis before floral buds aborted (Sedgley 1985). Thus, both optimum irradiance and temperature levels were important in floral development in *A. pycnantha*, and extremes in both factors blocked different stages in floral development.

In some circumstances, light intensity may have a deleterious effect on inflorescences late in development. Transferring *Pimelea ferruginea* plants to low light conditions

($\leq$113 µmol m^{-2} s^{-1}) late in development (4 days before anthesis) resulted in 30% of inflorescences failing to open (King *et al.* 1992), whilst in two cultivars of *Azalea* low light conditions delayed anthesis (Bodson 1983). In *Leucospermum*, reductions in light intensity of up to 50% had no effect on the rate of flower development (Jacobs and Minnaar 1980). However, flower quality decreased significantly as determined by a decline in the number of styles per flower head, decreased receptacle length and diameter, and decreased inflorescence dry weight (Jacobs and Minnaar 1980).

The effect of different irradiance levels on flowering can also be affected by other factors. In particular, irradiance can interact strongly with daylength in photoperiodically responsive species. Applications of a high light intensity during LD's can override the photoperiodic signal in the SD *Bougainvillea* plant, resulting in flowering (Kinet 1993). In other instances, the effect of irradiance on flower development can be modified by other environmental factors. Dawson and King (1993) tested the effects of different levels of irradiance, CO_2 and daylength on flowering in *Chamelaucium uncinatum*. Their findings showed that the number of flowers present was positively correlated with irradiance level with the highest yields occurring at 800 µmol m^{-2} s^{-1}. A further increase in flower number was observed when CO_2 was doubled at moderate and high irradiances. The high light intensity and CO_2 effects, however, were clearly secondary to a need for an exposure to short days (10 h).

Photosynthetic assimilates appear to play a predominant role in controlling flower development, and a decrease in their production within the plant appears to inhibit inflorescence development and can induce bud abortion (Kinet *et al.* 1985, Halevy 1987). Therefore, treatments that reduce photosynthetic output such as reduced irradiance or low ambient CO_2 concentrations, photosynthetic inhibitor treatments, and/or increases in plant density, can subsequently have an impact on floral development (Kinet 1993).

1.2.6.4 Temperature

Maintenance of low temperatures, varying from a few weeks to several months, plays an important role for many species in order to achieve reproductive development. The promotion of flowering by exposure to this period of low temperature is known as

thermoinduction or vernalisation (Kinet 1993). The site of perception of thermoinduction can be localised to the shoot apical meristem (Thomas and Vince-Prue 1984). Evidence of this has been on excised shoot tips that have been florally induced with low temperatures, an effect that appears to be largely independent of the temperature experienced in the rest of a plant (Taiz and Zeiger 1998). However, in some species, such as mango or lychee, leaves are the sites of induction from which an unidentified floral signal(s) is transferred to the apex (Shu and Sheen 1987, Nunez-Elisea *et al.* 1993). The age of the leaf is also positively related to its competence for floral induction (Nunez-Elisea and Davenport 1995). The effect of low temperature for promoting flowering is possibly related to changes in the pattern of DNA methylation which can alter gene expression. For example, the late-flowering ecotypes of *Arabidopsis* can flower early if vernalised (Burn *et al.* 1993). However, application of a demethylating agent (5-azacytidine) to non-vernalised plants caused them to flower earlier than untreated control plants. Therefore, genes required for early flowering can be inhibited by DNA methylation, at least in late flowering *Arabidopsis* ecotypes (Burn *et al.* 1993).

The response to low temperatures and its duration for floral induction varies according to species. For a number of plants species, cool temperatures are obligatory for a flowering response. Moncur (1992) showed that *Eucalyptus lansdowneana* seedlings were capable of floral induction only when plants were transferred from a greenhouse heated to 24/19°C (day/night) to a cold regime (15/10°C) for five to 10 weeks before returning to the former environment. Several other Australian species also show a similar response to cool temperatures. Both *Boronia megastigma* and *Hypocalymma angustifolium* initiated flowers irrespective of the daylength after transfer to warm conditions (25/17°C) (day/night), following a 10 to 15 week exposure to a cool temperature regime (17/9°C) (Day *et al.* 1994a). Under constant 25/17°C, however, no flowers reached anthesis in either species. An obligate low temperature response has also been described for several other Australian woody species including *Ixodia achillaeoides* (Weiss and Ohana 1996), and the day-neutral *Pimelea ciliata* (Slater *et al.* 1994) and *P. rosea* (King *et al.* 1992).

Several Australasian species also display a facultative response to temperature. For instance, flowering in *Leptospermum scoparium* occurred under both moderate (20/10°C) (day/night) and high temperature (26/20°C) regimes under 8 h short days, although flower number was enhanced in the cooler conditions (Zieslin 1985). Additionally, flowering in the SD plant *Chamelaucium uncinatum* 'Purple Pride' responded favourably in terms of number of flowers when treated to high temperatures (20-25°C), whereas lower temperatures (≤16°C) resulted in low flower number (Dawson and King 1993). In contrast, a *C. uncinatum* x *C. floriferum* hybrid produced a greater number flowers per plant under the cooler (12-15°C) SD regime than did *C. uncinatum* 'Purple Pride', although Dawson and King (1993) did not test this cultivar using temperatures above 15°C (daily mean).

Experimentation on differences in day versus night temperatures (DIF) influence both vegetative and floral developmental characteristics. Generally, increasing positive DIF (higher day than night temperatures) is associated with an increase in internode length, leaf expansion, while negative DIF can decrease plant height (Myster and Moe 1995). DIF effects on internode length and leaf expansion are primarily due to an increase in cellular elongation rather than division (Myster and Moe 1995). With regards to the flowering process, high night temperatures have been reported to be particularly detrimental to flower development compared to high day temperatures (Moe 1990). Increasing positive DIF has been shown to promote flower stalk elongation in some species (Myster and Moe 1995), whereas negative DIF can delay flower development (Mortensen 1994). In the case of *Kalanchoë blossfeldiana*, negative DIF delayed flower opening by two to four days at low CO_2 levels, although the process could be hastened with CO_2 enrichment (Mortensen 1994). Mortensen (1994) attributed the delayed flowering response to a depletion in carbohydrates during times of both high night temperature and high dark respiration rates, whilst CO_2 enrichment was suggested to counteract this effect. High CO_2 concentrations have also been shown to decrease dark respiration rates (e.g. in *Rumex crispus*) which could subsequently improve endogenous carbohydrate levels (Amthor *et al.* 1991).

Application of high temperatures can modify the timing of anthesis by hastening the rate of morphological development of reproductive structures (Kinet *et al.* 1985).

Temperature response curves on growth can delineate the times at which flowers may be expected to reach anthesis. Armitage (1995), for example, demonstrated that growing *Hamelia patens* under a 30°C rather than a 20°C temperature regime accelerated the time to visible flower bud by 30 days. Studies describing the hastening of anthesis have also been reported in a number of other woody ornamental species including *Chamelaucium uncinatum* (Dawson and King 1993), *Heliotropium arborescens* (Park and Pearson 2000), *Pimelea ciliata* (Slater *et al.* 1994) and several cultivars of *Hebe* (Noack *et al.* 1996).

Extremes in temperature can often have unfavourable effects on the flowering process. For many species dependent on low temperatures for flowering, brief or prolonged exposure to high temperatures at any stage in development can induce abortion of reproductive structures. At an early stage in floral development (during meiosis), transferring *Acacia* plants to warm conditions (max/min: 28/16°C) resulted in a cessation of floral development before bud abortion occurred (Sedgley 1985). Similarly, floral buds of *Boronia megastigma* underwent flower reversion or otherwise aborted but at the stage where carpels had initiated (Day *et al.* 1994a). In young buds, exposure to high temperatures resulted in floral reversion evident by the development of flowers with leaf-like sepals at the time of anthesis (Day *et al.* 1994a). *Pimelea ferruginea* also requires cool temperatures for flowering (King *et al.* 1992). However, in this species no flower buds aborted as temperature was increased at any stages of floral development. In summary, high temperatures can have deleterious effects on flower development, although the 'window' of sensitivity, if present, can be species-specific.

The ability to induce flowering at low temperature may be limited by factors intrinsic to the plant. *Eryngium planum*, a perennial herbaceous plant from central Europe, has been used as a cut flower in Israel for over 20 years. However, efforts to hasten and synchronise flowering time have been unsuccessful (Ohana and Weiss 1998). Treatments such as application of growth retardants showed little to no effect. However, an investigation into the effects of low temperatures on the size of the initiated bud on root cuttings was related to flowering time and quantity. Large buds (diameter > 4 mm) on vernalised roots reached anthesis faster and at a higher percentage than smaller buds. Ohana and Weiss (1998) suggested that the ability to

respond to low inductive temperatures related to the size of the apical meristem, which was correlated with bud size. A similar response with regard to bud size was reported in cranberry (*Vaccinium macrocarpon*), showing that small buds (<1 mm) had a lower probability of becoming floral the following summer than did buds >1 mm in diameter (Patten and Wang 1994). There is strong evidence, therefore, to suggest that the size of the bud may be a limiting factor controlling flowering. However, whether this is a common pattern exhibited among other woody plant remains uncertain given the paucity of studies investigating this relationship.

1.2.7 Carbohydrates

Recently, increasing attention has been given to the role of carbohydrates in flower induction (Bernier *et al.* 1993, Jiao and Grodzinski 1998, King and Ben-Tal 2001). From the beginning of the nineteenth century, the role of carbohydrates in controlling flowering had been debated, resulting in the forging of several hypotheses for describi[illegible] Flower induction models; Section 1.2.3). Following the florigen [illegible] growth substances, the role of carboh[illegible] iding mechanism once floral induct[illegible] ence suggests that carbohydrates along [illegible] ative to floral transition (Bernier *et al.* 19[illegible] shown that exposure to either one LD o[illegible] s to increase rapidly and transiently at th[illegible] The increased supply of sucrose to the [illegible] gy-consuming processes in early stag[illegible] n) (Bernier *et al.* 1993). However, Be[illegible] 2001) does not regard sucrose as the on[illegible] he involvement of cytokinins as well (a[illegible] P. Jameson at IPGSA 2001) contends th[illegible] e role in the floral induction process.

Further evid[illegible] crose in the flowering process comes from a floral induction study conducted on *[illegible]achsia hybrida* (King and Ben-Tal 2001). Their study found a strong positive correlation between flower number and sucrose concentration in shoot apices with the level of irradiance applied during induction.

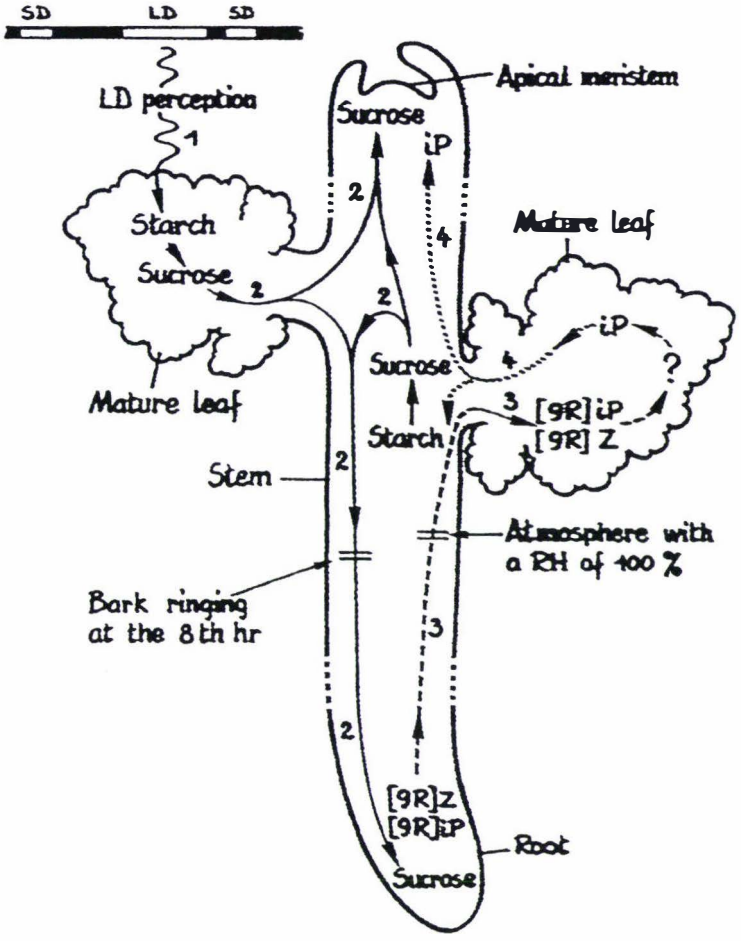


Figure 1.4 Schematic diagram model illustrating the regulatory loop involvement of sucrose and cytokinins in the flowering process in *Sinapis Alba*. LD perception by leaves (1) leads to mobilisation of starch (2) to stems and leaves and sucrose (2) to root and shoot apical regions through the phloem (solid arrow). Zeatin riboside ([9R]Z) and isopentenyladenine riboside ([9R]iP) are transported (dashed arrow) in the xylem from the roots to leaves (3) and is followed by transportation (dotted arrow) of isopentenyladenine (iP) in leaves via the phloem to the apical meristem (4). These hypotheses are supported by experimental manipulation of vascular pathways through bark ringing and reduction in transpiration (RH 100%) and by chemical analysis of sap (Bernier *et al.* 1993).

However, when plants were treated to inductive (2-4 LD) but with low light conditions, flowering was induced although no concomitant increase in shoot apex sucrose content was observed. Similar findings have also been reported in the LD plant *Lolium temulentum* whereby flowering occurred following inductive low light conditions despite there being no changes in apical sucrose content (King and Evans 1991). King and Ben-Tal (2001) conclude that sucrose plays a florgenic role in *F. hybrida*, although it does not act as a stimulus for flowering specific to LD photoperiodic exposures.

The mobilisation of starch early during the inductive treatment has been suggested to play an important role in flower induction. The increased supply of sucrose to the shoot apical meristem of induced plants seems to arise from the storage of carbohydrates (possibly starch) from stems and leaves, and not necessarily from increased photosynthesis (Bernier *et al.* 1993). Studies using an *Arabidopsis* starchless mutant (*pgm* TC75) and a starch overproducer mutant (*sop* TC26T) have shown that the transport of starch to the apex can affect flowering (Bernier *et al.* 1993). When grown in continuous lighted conditions, the mutants responded similarly in both growth and flowering time to that of the wild-type. However, as the daylength was decreased, growth was reduced and flowering was delayed in the mutants. This response was suggested to be the result of an inability to mobilise starch, a feature exhibited by both mutants (Bernier *et al.* 1993).

Investigations into the role of carbohydrates in flowering in woody species have centred on the changes occurring between shoot/leaf and shoot meristems under various environmental conditions (Jiao and Grodzinski 1998). In *Rosa* plants, leaves on the flowering shoot act as source leaves even before the presence of a visible bud (Mor and Halevy 1979) and subsequently act in transporting assimilates to the developing flower bud (Mor and Halevy 1979, Jiao *et al.* 1989). Using ^{14}C radioisotope experiments, Jiao and Grodzinski (1998) demonstrated that in *Rosa* various environmental conditions could affect photosynthesis and concurrent export rates from the source leaves to the developing flower. These authors showed that export rates of carbon (sucrose) were greatest at late stages of floral development (e.g. petal colour visible) and this occurred under both ambient and enriched CO_2 levels, being 35 and 90 Pa, respectively (Jiao and Grodzinski 1998). However, at higher temperatures (25-40°C), photosynthesis decreased by 40 percent and carbon (sucrose) export rates dropped by 80 percent.

Irradiance also affected sucrose export. Export rates increased significantly under higher irradiance levels (>1000 μmol m^{-2} s^{-1}) due to heightened stimulation of photosynthesis which caused the mass flow of carbon into sucrose synthesis (Jiao and Grodzinski 1998). Low irradiance levels during induction are generally unfavourable for continued flower development, and can subsequently cause flower bud abortion (Zieslin and Mor 1990, Plummer *et al.* 1998). Plummer *et al.* (1998), for instance, showed that the number of *Boronia heterophylla* flowers that reached anthesis decreased significantly under shaded conditions (less than 39% shade) and this was attributed to early abortion due to a limited assimilate supply. Their findings are also supported by Richards (1985) who suggested that flower abortion in *B. heterophylla* can occur in response to a diversion of photoassimilates away from the flower bud and towards vegetative shoots. In summary, various environmental factors can act independently or in combination to affect flowering through changes in carbohydrate levels and/or fluxes.

1.2.8 Hormonal regulation

In higher plants, plant hormones are intimately involved in the regulation of metabolism, growth and morphogenesis and serve as chemical signals within the plant (Taiz and Zeiger 1998). Plant development is primarily regulated by the five classic types of hormones, being auxins, gibberellins, cytokinins, ethylene and abscisic acid. There is compelling evidence for the addition of a sixth group, these being a steroid family of plant hormones – the brassinosteroids. However, the vast majority of research into the flowering process has specifically focused on the role played by gibberellins, and to a lesser extent by cytokinins.

1.2.8.1 Gibberellins

The effects of applied or endogenous gibberellins (GAs) on flowering responses have been inconsistent across plant groups and between species. Application of exogenous GAs (e.g. $GA_{4/7}$) has been successful in promoting flowering in conifers (Pharis *et al.* 1986), but gibberellins generally appear ineffective in many SD or woody angiosperm species (Shillo *et al.* 1985). In many woody angiosperm plants, responses to growth regulators have generally been two-fold, including reports on their effects on timing and

on the number of flowers initiated. For instance, Day *et al.* (1994b) reported on the effects of PP333 on flowering in *Hypocalymma angustifolium* and *Boronia megastigma.* In comparison with untreated plants (control), PP333 increased the proportion of flowers produced in *H. angustifolium*, but not in *B. megastigma.* However, with regards to days to anthesis, only *B. megastigma* differed significantly from control plants, reaching anthesis five weeks earlier than controls (averaging 31 weeks) (Day *et al.* 1994b). In contrast, applications of PP333 to *Acacia elegans* and *A. vestita* produced significantly fewer inflorescences per plant than the untreated control plants (Parletta and Sedgley 1996). However, plant height and width were decreased in all three species by this growth retardant, presumably through the effect of PP333 on branching frequency and/or a reduction in shoot internode length (Day *et al.* 1994b). Moreover, application of GA_4 or 2,2-dimethyl GA_4 stimulated shoot elongation in micropropagated plants of *Metrosideros collina*, whereas high doses of GA_4 reduced flowering on treated shoots (Clemens *et al.* 1995).

Reductions in plant stature with growth retardant (e.g. PP333, chlormequat chloride) applications are not surprising given that GAs are associated with promoting internode elongation (Oren-Shamir and Nissim-Levi 1999). However, responses that include (but do not exclude) a decline in flower number or differences in flowering time with application of growth retardants implicate gibberellins in the flowering process, at least in the above species and in various other woody angiosperms, such as *Chamelaucium* (Shillo *et al.* 1985) and *Eucalyptus* (Moncur and Hasan 1994).

In summary, the precise role of GAs in the transition to flowering remains unclear although recent molecular approaches to this problem are beginning to unravel the role of gibberellin in flowering. In *Arabidopsis*, GA activates, at least in part, the expression of *LEAFY* (*LFY*), a floral meristem identity gene, and the activity of the *LFY* promoter can be maximised when GA_3 is present with sucrose (Blazquez *et al.* 1998). Therefore, this suggests that in some species GAs stimulate flowering through a pathway regulating the transcription of the *LFY* gene.

1.2.8.2 Cytokinins

Another plant hormone group implicated in the flowering process is that of the cytokinins. Studies on *Sinapis alba* suggest that cytokinins (CKs) are involved in the flowering transition pathway in response to inductive LD photoperiods (Bernier *et al.* 1993) (see Figure 1.4). Experiments in which a ring of living tissue (including phloem) was excised between the root and lowest stems portion (bark-ringing) of induced *S. alba* plants inhibited flowering when applied during hour eight of the LD, but not during or after hour 12. This is suggestive of a flowering signal that is translocated from mature leaves to the root system (Bernier *et al.* 1993). The chemical nature of this signal is not known although sucrose is suggested to be a candidate since sucrose levels increase rapidly (within 1 h) in the roots at the onset of the LD photoperiod (see Figure 1.4). In addition to sucrose, CKs are also suggested to play a signalling role since a bark-ringing treatment at hour eight which inhibited flowering could be reversed by application of CKs to the apical meristem at hour 16 (Bernier *et al.* 1993). From these and other experiments, Bernier *et al.* (1993) hypothesised that the transportation of sucrose to the roots lead to the export of mostly the zeatin riboside form of CK to shoots and leaves, possibly via the xylem. Within the leaves, isopentenyladenine riboside (a CK) was subsequently exported to the shoot apical meristem where increasing levels were detected within 16 h of the inductive treatment (Bernier *et al.* 1993). CK, applied in low doses to apical meristems when under inductive conditions, are known to increase the rate and synchronisation of cell divisions, vacuole splitting and decrease the size of DNA replication units in half (Bernier *et al.* 1993). Bernier (pers. comm. P. Jameson at IPGSA 2001) contends that CKs as well as sucrose play an essential role in the signal transduction process, although these factors alone can not replace a LD signal.

Application of exogenous synthetic CKs (e.g. 6N-benzyladenine) has been trialed on several ornamental woody plants. In *Boronia megastigma*, 6N-benzyladinine (BA) was applied to plants during transference to an environment with marginally inductive temperatures (night/day: 19/11°C). BA treatments reduced the number of days to anthesis to less than half the time (13 weeks) required in untreated plants (control), although comparatively, the number of florally induced buds was significantly depressed by the BA treatment (Day *et al.* 1994b). Day *et al.* (1994b) acknowledged that above-optimal temperatures probably inhibited the effectiveness of BA for

flowering, since *Boronia* grown in cooler optimum conditions showed no inhibitory responses with BA applications (Richards 1985, Day *et al.* 1994b).

Successful floral bud initiation and development has been achieved in *B. megastigma in vitro* under optimal environmental conditions. However, flower bud reversion was observed in media containing high levels of CK whilst media without added CK prevented anthesis from occurring (Roberts *et al.* 1993). Synthetic CKs have been used on the premise that a stimulation of branching, either by pruning and/or with BA applications, can increase the number of growing points for floral initiation (Parletta and Sedgley 1996). In many cases, an increase in branching is usually achieved, although this does not always appear to be associated with an enhancement in final flower number (e.g. King *et al.* 1992, Dawson and King 1993). Therefore, evidence exists that CKs may play a role in the flowering process in woody angiosperm species. However, like GAs, plant responses to exogenous applications have been inconsistent between species and can be modified by other environmental treatments.

1.2.9 Effect of cultural factors

A number of studies have utilised physical methods for controlling flowering. Treatments involving scoring or applying various forms of girdling (e.g. overlapping girdles, wire girdles, scoring) to shoots have been implicated in promoting flowering (reviewed by Meilan 1997). These treatments restrict vascular flow and cause the accumulation of shoot produced metabolites (e.g. ABA, auxin and carbohydrates) above the restriction zone and below that zone for root-produced metabolites (e.g. gibberellins and nitrogen) (Meilan 1997). Those techniques that manipulate possible carbohydrate and/or hormone levels within the plant have been successful in promoting higher crop yields in apple (Veinbrants 1972) and olive (Eris and Barut 1993), cone formation in conifers (Pharis *et al.* 1986) and flowering in lychee (Young 1977) and mango (Reboucas and Jose 1997).

1.3 Postharvest Physiology of Cut Flowers

1.3.1 Introduction

Extensive research has been conducted on the postharvest features of numerous types of cut flowers. In particular, the vast majority of these investigations have focused on the suitability of herbaceous rather than woody perennial crop species for ornamental purposes. Introduction of novel cut flower species with attractive floral displays varying in either colour, shape, and/or prominence of flower parts may have commercial appeal. Many new woody genera on the market meet these standards and have been successfully introduced into the floricultural trade, including *Leptospermum* (Burge *et al.* 1996), *Chamelaucium* (Joyce 1988) and *Verticordia* (Joyce and Poole 1993). The commercial success of any new woody crop, however, has been dependent on an understanding of the flowering biology of the species in question, with particular emphasis on knowledge of postharvest technologies for delaying senescence-related symptoms and abscission of flowers or floral organs. In an exhaustive study of the effects of ethylene on petal senescence conducted on 93 species (23 families), Woltering and van Doorn (1988) showed that most plant families, not sensitive to the ethylene, were characterised by flowers that underwent wilting as the primary symptoms of senescence. Flowers, however, that were sensitive to low exogenous concentrations of ethylene displayed abscission of petals as the initial symptoms of senescence. Thus, the basis for much of the research on postharvest flowering has been on the role of ethylene as well as water relations on senescence and abscission of floral organs.

In this review, the biology of flower senescence and associated floral organs is discussed. Secondly, a description is provided of the effect of various components of vase solutions on vase-life and flower quality with particular reference to woody species. Finally, treatments employed to extend vase-life by delaying senescence via inhibition of ethylene biosynthesis or ethylene action are reviewed. The focus of this review is primarily concerned with the postharvest physiology of cut flowers of woody ornamental plants, although this would be incomplete without reference to the

numerous fundamental and applied studies conducted on herbaceous plant species that have contributed to our understanding in this field.

1.3.2 Flower senescence

Broadly defined, senescence refers to the combination of events that leads to the death of cells, tissues or organs (Reid and Wu 1992). The senescence process involves the occurrence of a series of highly coordinated physiological, biochemical and ultra-structural changes in cells, typified by increases in hydrolytic enyzmatic activity, degradation in starch and chlorophyll, disintegration of cellular compartments, and climacteric surges in respiration (Van Altvorst and Bovy 1995). These events are associated with changes in gene expression and protein synthesis (Van Altvorst and Bovy 1995). In many plant species, ethylene regulates these processes during flower senescence. Generally, flower senescence is independent of flower morphology so that closely related species may exhibit similar senescent symptoms despite their differing floral morphology (Woltering and Van Doorn 1988).

1.3.2.1 Petals

Characteristic changes in petal structure have been described for countless species during the senescence process. Depending on the species and conditions, these responses can include symptoms of petal wilting, inrolling or folding, withering, fading, discoloration (pigment breakdown) and/or organ abscission (Reid and Wu 1992, Van Doorn 1997, Van Doorn and Stead 1997). Symptoms of abscission-related senescence are generally restricted to dicotyledonous plants, whereas petal senescence in monocotyledons are typically displayed by symptoms of wilting or withering (McKenzie and Lovell 1992). Moreover, the similarity in the types of petal senescence responses can be expressed at a family level. For example, flowers featuring the abscission of turgid petals have been reported for many woody species from families such as Fagaceae, Malvaceae, Myrtaceae, Proteaeae, Rosaceae, Rutaceae and Solananceae (Woltering and Van Doorn 1988, Van Doorn and Stead 1997). In some families (e.g. Ericaceae and Primulaceae), turgid petal abscission occurs in some species and petal wilting, either preceding abscission or not at all, in others (Van Doorn and Stead 1997). Abscission of petals can occur following various physiological events. In

some species, petal shedding can occur in response to cell wall dissolution situated at abscission zones, whereas in others, this action can occur as a result of fruit growth (Van Doorn and Stead 1997). Pollination or fertilization can also hasten petal abscission, as observed in *Pelargonium* x *hortorum* (Hilioti *et al.* 2000).

1.3.2.2 Sepals

While petals are typically regarded as the most prominent part of cut flowers, other floral structures such as sepals have ornamental value. This include species in the genera *Ceratopetalum* (Johnson and Ronowicz 2000) and *Eucalyptus* (Sun *et al.* 2001). In some *Eucalyptus* species, the sepals remain united at the top of the flower forming a cap (calyptra), derived from either the sepals, petals or both, which abscises upon flower opening (Moncur and Boland 1989). In most plant families, sepals do not undergo abscission but rather dessicate while remaining attached to the plant, as in the case of species within the Rutaceae and Rosaceae families (Van Doorn and Stead 1997). In a few exceptions, such as species within Scrophulariaceae, sepals grow after fertilisation and eventually cover the ovary (Endress 1994). Sepals may also form part of the fruit during development, as in the case of species within Malvaceae (Endress 1994).

1.3.2.3 Stamens

Relatively little attention has been given to stamen senescence, probably because other floral structures, specifically petals or flowers with fused petals and sepals, are typically the focus of postharvest flower senescence studies. Despite this, several woody plant families (e.g. Myrtaceae and Proteacae) contain species with flowers that contain relatively prominent and attractive stamens with commercial appeal. Specifically, these include several woody myrtaceous plants within the genus *Metrosideros* which are characterised by having flowers (borne in cymules subtended by a pedicel, on a compound inflorescence) that contain numerous (~ 25), unfused stamens (Orlovich *et al.* 1996, Sun *et al.* 2000). Other ornamental genera with prominent stamens include *Verticordia* (Joyce and Poole 1993) and *Syzygium* (Payne 1997). According to Van Doorn and Stead (1997), there are various dicotyledonous families that feature particular forms of stamen senescence. These include: (a) abscission of turgid stamens before petal abscission (e.g. in Balsaminaceae), (b) simultaneous abscission of both

wilted stamens and petals (e.g. in Malvaceae and Solanaceae), (c) abscission of desiccated stamens with or after petal fall (e.g. in Ranunculaeae), and (d) abscission of turgid or partly wilted stamens with or after petal drop (e.g. in Rutaceae and Scrophulariaceae). *Metrosideros* flowers typically follow the latter pattern of abscission with turgid stamens shedding within three to four days of becoming fully expanded (Sun *et al.* 2000).

1.3.2.4 Style

Depending on the species or family, various patterns in senescence of the style can occur. In families such as Rutaceae or Rosaceae, abscission typically occurs when styles are fully turgid. In contrast, style abscission usually occurs after wilting such as in *Eucalyptus* species (Myrtaeae) (Moncur and Boland 1989). In both the Boraginaceae and Onagraceae family, styles desiccate before abscising. It is unclear, however, as to whether style shedding in both these families is due to abscission or to mechanical tearing from the developing fruit (Van Doorn and Stead 1997).

1.3.3 Water relations

The limiting factor regulating the vase-life in many cut flowers is water stress. Intact flowers undergo senescence either by colour changes (Jones *et al.* 1993a), flower closure, petal wilting/in-rolling (Zieslin and Gottesman 1983, Jones *et al.* 1993a) or abscission of floral parts (Joyce 1988, Joyce and Poole 1993). However, when flowers are cut and placed in water, symptoms of senescence, as exhibited in intact flowers, are not always observed but rather those of water stress, such as premature wilting of flower parts or leaves (Burge *et al.* 1996, Wirthensohn *et al.* 1996). At a cellular level, growth of floral organs (e.g. petals) is due to an increase in cell number and cell size. A balanced water status plays a role in maintaining cell turgor, even when petals have fully expanded. However, once petals are fully expanded, the duration that cells remain turgid can, depending on the species, vary from a few hours to several months (Stead and Van Doorn 1994). In flowers where turgid petal abscission is not the first symptom of senescence, partial or full wilting or withering can occur. This is usually preceded by a loss of solutes (e.g. inorganic ions, organic acids, reducing sugars, amino acids and anthocyanins) that result in cell leakiness (Stead and Moore 1983, Van Doorn 1997).

The direct cause of this leakiness is not known but is suggested to be related to a loss of semi-permaneability of the tonoplast and the plasma membrane (Van Doorn 1997). Thus, adverse water relations may inhibit growth and maintenance of turgidity in floral organs and subsequently accelerate premature senescence.

1.3.4 Water uptake

The pattern in water uptake following cutting of flowers can vary depending on the taxa. In many species, the rate of water uptake may initially be high due to a low water potential at cutting (Van Doorn 1997). Subsequently, water uptake may either stabilise or decline rapidly, as in the case of many cut woody species. This includes genera such as *Rosa*, *Anigozanthos* (Kangaroo paw), *Banksia*, *Grevillea*, *Thryptomene*, *Leptospermum*, *Chamelaucium* and *Telopea* (Mayak *et al.* 1974, Faragher 1989). In contrast, water uptake in other species such as *Heliconia* is low even after the stem is freshly cut and placed in water (Ka-Ipo *et al.* 1989). The rate of water uptake can also depend on other factors, other than species type, such as on the transpirational pull of the flower, the temperature and composition of the holding solution, and factors innate to the flower stem that may prevent water uptake.

1.3.5 Transpiration

The extent of water stress within a cut flower is dependent upon water balance, and a water deficit can occur when the rate of water uptake is less than the rate of transpiration. Therefore, water stress can be alleviated or delayed by reducing the rate of transpiration. Stomatal transpiration can be decreased through the removal of leaves since cut (flower and foliage) stems with relatively small leaf areas lose less water per unit stem area and time than those with relatively large leaf areas. Stomata *per se* are usually present in all green epidermal tissues, such as leaves and can also be present in stem and in epidermal tissue of non-green flower parts (e.g. petals). Stomata have also been reported to be present on stamens and nectaries, although in the latter case, they are suggested to function in nectar exudation rather than in gas exchange (Zer and Fahn 1992).

The addition of plant hormones to vase solutions can subsequently affect transpiration. The addition of exogenous abscisic acid (ABA), at concentrations of 10 ppm or more, effectively preserved the water balance in *Chamelaucium uncinatum* and extended vase-life up to 10.6 days compared to 6.4 days for control stems (Joyce and Jones 1992). Similar results have also been obtained in cut roses but only when applied in solution (Halevy and Mayak 1981) and not as a foliage spray (Müller *et al.* 1999). The effectiveness of ABA in extending vase-life is attributed to its role in inducing stomatal closure, thus, inhibiting transpirational water loss (Aspinall 1980). The relatively high cost of ABA, however, may not be conducive for commercial use (Joyce and Jones 1992). Transpiration has been reported to decrease when solutes are added to the vase solution. Van Doorn (1997) suggested that sugars can promote bacterial growth in vascular vessels, which can lead to a water stress response causing stomatal closure.

1.3.6 Water temperature

Water temperature has been of considerable importance particularly for dry-stored stems. In some species, the rate of rehydration is correlated with water temperature, hence, warm water (35-40°C) has been recommended for use with many commercial cut flowers, such as *Freesia*, *Gladiolus*, *Lilium* and *Protea* and *Syringa* (Sacalis 1993). The mode of action is not fully understood but may relate to the decreased viscosity of water with increasing temperature. Cut roses, on the other hand, rehydrate more rapidly in cold (2°C) water in comparison to water at 23°C (Durkin 1979). The increased solubility of gases in cooler water may also contribute to improved water uptake (Van Doorn 1997).

In freshly cut flowers, dipping of stem ends in hot water has been implicated for improving water relations (R. Lill pers. comm. with Burge *et al.* 1996). However, treatments, in which stem ends of *Leptospermum scoparium* were dipped in 60°C water for 1 minute, resulted in little to no effect on water uptake or vase life (Burge *et al.* 1996).

1.3.7 Vascular occlusions

A decrease in water uptake and subsequent turgor may be attributed to vascular occlusions. These occlusions may develop via several means: through (1) gas (air) uptake, (2) a plant response mechanism or (3) microbial growth. The formation of air bubbles in cut stems may impede water uptake. This usually occurs from air that has aspired directly after cutting or from vascular cavitation (Williamson and Milburn 1995). Re-cutting of stems under water is an effective method for preventing air vascular blockages and promoting water uptake (Van Doorn 1997), and is currently a standard practice used in the floricultural industry.

Plant-specific responses to cutting may affect water uptake. Development of vascular occlusions as a result of wounding (cutting) or as a defensive mechanism to wounding can lead to the deposition of material in xylem conduits, such as suberin, lignin, tannin or various gums (Van Doorn 1997). Additionally, cutting may lead to exudation-related blockages (e.g. resin or latex) or to the formation of tyloses (cell outgrowths) that inhibit water flow (Zimmerman 1983). The addition of ethanol (1%) in holding solutions containing detached flowering peach (*Prunus* spp.) stems increased xylem hydraulic conductivity by reducing the number of plugged vessels and delayed a surge in ethylene production (Munoz *et al.* 1982). This may be an effective treatment in plant families where exudate deposition occurs following cutting (e.g. Proteaceae and Rutaceae) (Chattaway 1948).

There is strong evidence that the accumulation of microbes in cut stems can lead to vascular occlusions. Microbial organisms typically found in vase solutions and at the base of stem cuttings include yeast, filamentous fungi, bacteria and their degradation products (Put and Clerkx 1988, Van Doorn 1997). Unless vase water changes are conducted regularly, microbial growth can increase rapidly over time. For example, bacterial counts (cfu ml^{-1}) on vase water containing cut stems of *Thryptomene calycina* increased $3x10^4$ fold over a 72 hour period (Jones *et al.* 1993a). Further, microscopic examinations (SEM) of xylem vessels in cut flowers of *Gerbera* and *Rosa* (at 24 hours vase life) showed the adhesion of microbes primarily on the cut stem surfaces causing blockages of xylem vessels (Put and Clerkx 1988). The extent of microbial infiltration

is also dependent on the number, shape and size of microbes found in the vase water and on the width of xylem vessels. Eventual microbial blockages in the xylem can lead to water stress and to premature wilting of flowers (Put and Clerkx 1988).

1.3.8 Biocides

Various compounds have been developed to suppress microbial growth in vase solutions. Most compounds when used at concentrations that adequately control microbial growth are toxic to cut flowers (Van Doorn 1997). With exception, a considerable number of compounds which have been shown to be non-toxic to cut flowers and have been associated with improved vase-life include salts of various elements, chlorine/chlorinated aromatics and ammonium or quinoline based compounds (Van Doorn 1997). Quinoline based compounds such as 8-hydroxyquinoline citrate (HQC) or 8-hydroxyquinoline sulfate (HQS) have been incorporated into a number of studies examining vase-life of Australian and Pan-Pacific ornamental plants. These include studies on cut foliage from *Eucalyptus* spp. (Joyce *et al.* 1993) and cut flowers from *Leptospermum scoparium* (Burge *et al.* 1996), *Chamelaucium uncinatum* (Joyce and Jones 1992), *Metrosideros collina* (Sun *et al.* 2000) and *Eucalyptus ficifolia* (Sun *et al.* 2001). In cut flowers of *E. ficifolia*, HQC concentration (maximum 400 ppm) was correlated with decreased flower mass, as recorded on day five after harvest (Sun *et al.* 2001). In cut flowers of *L. scoparium*, slightly lower concentrations of quinoline were equally effective. The use of 200 ppm of HQS effectively delayed the gradual decline in water uptake and leaf moisture content and extended the vase life by two days (Burge *et al.* 1996). Similar concentrations have also been reported to be effective in other myrtaceous species, for example, *M. collina* (Sun *et al.* 2000) and *C. uncinatum* (Joyce and Jones 1992).

1.3.9 pH of vase solution

The effectiveness of pH on water uptake has been evaluated in only a few cut flower species. Solutions with a pH below 7 clearly improved water uptake in *Eucalyptus ficifolia*, since flower mass was highest at Day 5 when solutions were maintained at pH 4.3 (Sun *et al.* 2001). In *Metrosideros collina*, the effects of a factorial combination of sucrose and pH (2.7-7.0) of solution were tested on the stamen quality. A sucrose (30-

40 g l^{-1}) and HQC solution (pH 5) produced the least amount of stamen wilting, whereas in treatments with the highest stamen wilting (pH 3), stamen abscission was the lowest (Sun *et al.* 2000). Increased solution acidity is associated with a decline in microbial growth, which may improve water uptake via a reduction in stem blockages (Sacalis 1993, Jones *et al.* 1993a).

1.3.10 Carbohydrates

The carbohydrate content of a vase solution is an important factor for controlling the vase-life of many cut species. Sugars, such as sucrose, provide a source of carbon by substituting for the natural depletion of carbohydrates during the life of a cut flower (Marousky 1972, Van Doorn 1997). The addition of sucrose to vase solutions applied continuously or as a pulse have been effective in extending the vase-life of a number of woody species, such as *Rosa* spp. (Ichimura *et al.* 1999), *Chamelaucium uncinatum* (Joyce 1988), *Leptospermum scoparium* (Burge *et al.* 1996), *Metrosideros collina* (Sun *et al.* 2000), *Grevillea* (Joyce and Beal 1999) and *Eucalyptus* foliage crops (Jones *et al.* 1993b, Wirthensohn *et al.* 1996). In some other species, however, sucrose treatments (0.5-5%) did not enhance the quality or longevity of cut flowers, such as in *Banksia* (Sedgley 1998) or *Eucalyptus tetragona* and *E. youngiana* (Delaporte *et al.* 2000). Additionally, high sucrose concentrations (≥10%) can damage tissue as exhibited by the browning of leaf margins in cut eucalypt foliage (Jones and Sedgley 1993). Generally, sucrose concentrations of 1-2% in vase solutions were effective for extending the vase-life in several other myrtaceous cut flowers, for example in *C. uncinatum* (Joyce 1988), *Thryptomene calycina* (Jones *et al.* 1993a), *M. collina* (Sun *et al.* 2000) and *E. ficifolia* (Sun *et al.* 2001). Typically, biocides are added with sucrose in vase solutions in order to prevent the rapid accumulation of micro-organisms that lead to occlusions and subsequent premature flower senescence (Van Doorn 1997).

1.3.11 Ethylene

Plant growth substances play a critical role in regulating different events in the life cycle of plants. Ethylene is renowned for its role in initiating and regulating the processes that eventually lead to programmed cell death in whole plants or specific organs or tissues (Bleecker and Kende 2000). Increased endogenous production of this gas can

occur in response to physical wounding or physiological stress (e.g. flooding, chilling disease, temperature and drought) and during normal developmental processes, such as in leaf or flower abscission and senescence (Reid and Wu 1992). The role of ethylene in affecting flower abscission or senescence processes has for many years been of considerable interest to postharvest physiologists researching ornamental crops. This is because ethylene, which is not only a potential hazard as a pollutant, is involved in causing damage and premature senescence in both leaf and floral organs (Nell 1994).

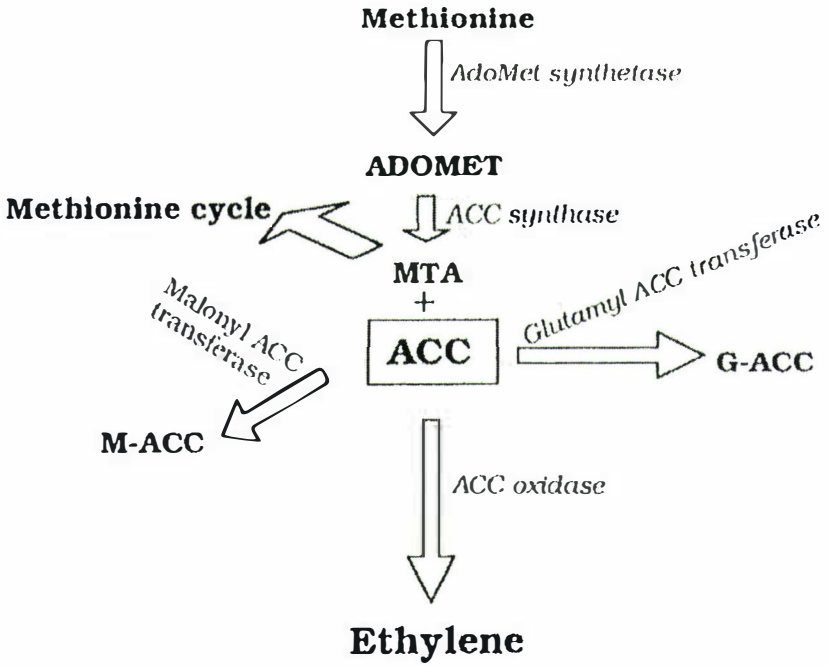
1.3.12 Background on ethylene

To date, a considerable amount of research has been undertaken on ethylene gas and its role in plant growth and development. This has included numerous studies that have investigated the processes incurred during *in vivo* ethylene evolution, production and perception, through to its deleterious effects on plant organs (e.g. flower abscission). From a postharvest perspective, a thorough understanding of ethylene biosynthesis, mode of action and consequential effects on specific ornamental crops are prerequisites for understanding the factors that either inhibit ethylene production and/or action.

1.3.13 Ethylene biosynthetic pathway

Knowledge of the ethylene biosynthetic pathway has been reviewed in various reports (Yang and Dong 1993, Van Altvorst and Bovy 1995, Bleecker and Kende 2000). Here, a summary is presented of the processes that occur during ethylene biosynthesis (Figure 1.5 A). In the first step in the pathway, the enzyme S-adenosylmethionine synthetase catalyses the conversion of methionine and ATP to S-adenosylmethionine (AdoMet). AdoMet is subsequently converted by 1-amino cyclopropane-1-carboxylic acid synthase (ACC synthase) to form 5′-methylthioadenosine (MTA) and 1-amino cyclopropane-1-carboxylic acid (ACC), the immediate precursor to ethylene. Recycling of MTA to methionine occurs, thus allowing high production rates of ethylene even if methionine concentrations are low. Oxidation of ACC by the enzyme ACC oxidase results in the production of CO_2, HCN and ethylene (Bleecker and Kende 2000). It is suggested that hydrogen cyanide detoxification occurs through conversion of β-cyanoalanine by the enzyme β-cyanoalanine synthase (Van Altvorst and Bovy 1995).

A.



B.

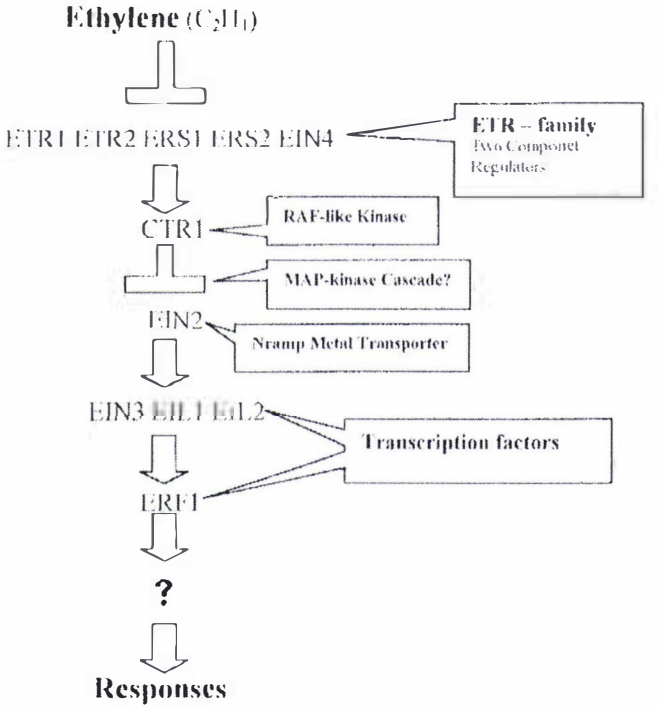


Figure 1.5 Models of ethylene biosynthesis (A) and ethylene signal transduction pathway (B). A: Schematic representation of the major pathway of ethylene biosynthesis in higher plants and the enzymes involved. Shown also are the paths to the methionine cycle and conjugation reactions that inhibit ACC from being directly available for ethylene production (Fluhr and Mattoo 1996). B: Schematic representation of the genetic interactions and biochemical identities of components in the ethylene signal transduction pathway. Negative regulation of the ETR-family of membrane-associated receptors by ethylene interacts with the Raf-like kinase CTR1 regulatory domain, which in turn negatively regulates the EIN2 membrane protein. The cytoplasmic EIN2 domain positively signals down-stream to the EIN3 family (nucleus) which subsequently promotes the ERF1 gene followed by a down-stream activation of a subset of ethylene responses (modified from Bleecker and Kende 2000).

1.3.13 Signal transduction

An ethylene response is suggested to occur following the binding of ethylene to a specific ethylene receptor. An activation signal is subsequently transcribed along a signal transduction pathway. Most of the recent research has been based on studies using etiolated mutants of *Arabidopsis thaliana* that do not display the 'triple response' characteristics. Thus far, three types of mutants have been identified that (1) are insensitive to ethylene, (2) display an ethylene response in the absence of the hormone or (3) are affected only in specific tissues (Van Altvorst and Bovy 1995).

To date, a thorough understanding of ethylene signalling in Arabidopsis is still incomplete, although a conceptual model of the interactions is starting to emerge (Bleecker and Kende 2000, Hall *et al.* 2000, Hirayama and Alonso 2000) (Figure 1.5 B). Based on these recent publications, a summary of the events occurring along the ethylene signal transduction pathway is described. The binding of ethylene occurs at a receptor site known as a two-component-receptor that is membrane-localised. In the two-component-receptor, the N-terminal domain is responsible for binding with ethylene (sensor) while the intracellular portion of the protein receptor is a protein kinase (response regulator) which is activated upon the binding of ethylene. This is includes a family of five membrane associated receptors (ETR1, ETR2, ERS1, ERS2 and EIN4). The protein kinase transmitter domains interact with the regulatory domain of Raf-like kinase CTR1 which, in turn, negatively regulates EIN2, a membrane protein. Downstream positive signalling by the cytoplasmic C-terminal domain of EIN2 occurs on the nucleus bound family of transcription factors (e.g. EIN3). A member of a second family of transcription factors, ERF1, can subsequently activate a subset of ethylene responses. This can entail an increase in mRNA transcript levels of numerous genes including those that encode for cellulase, chitase, β-1,3-glucanase, peroxidase, chalcone synthase, pathogenesis-related protein, in addition to ripening-related genes and ethylene biosynthesis genes (Bleecker and Kende 2000).

1.3.14 Physiology of abscission zones and ethylene

Prior to abscission of floral organs, abscission zones are reported to undergo various anatomical and physiological changes. Anatomically, variations in the size of cells

located at abscission zones have been observed. For instance, Van Doorn and Stead (1997) classified the size of abscission-zone cells as either those that were (1) smaller than adjacent cells but mostly isodiametric, (2) smaller than adjacent cells but mostly oblong, or otherwise, (3) of similar size to adjacent cells. The role of changing cell size with respect to the abscission process, however, remains unclear, although it has been suggested to assist in breaking of vascular strands (Sexton and Roberts 1982). Additionally, the process of cell swelling at the abscission zone is apparently not present in some species, such as in tobacco, tomato and *Phaseolus* flowers (Jensen and Valdovinos 1968, Webster and Chiu 1975).

More consistent reports on the abscission process refer to an increase in intracellular activities of cells at abscission zones (Oberholster *et al.* 1991, Evensen *et al.* 1993). Ultrastructural data suggest an increase in protein synthesis and high secretory activity of material towards cell walls in abscission-zone cells, indicative of increased enzymatic activity required for cell wall separation (Van Doorn and Stead 1997). This is evidenced by the appearance of numerous rough endoplasmatic reticulum profiles and ribosomes occurring at an early stage of the abscission process. In *Pelargonium* x *hortorum*, for example, protein levels doubled before the occurrence of petal abscission in conjunction with newly synthesised proteins (Evensen *et al.* 1993). The enzymes most commonly associated with the abscission process include cellulases and pectinases, and their regulation, at least in part, is suggested to be the result of ethylene (Brown 1997). Ethylene regulation is through the transcription of genes that encode these enzymes. Subsequently, abscission zone cells undergo enzymatic solubilisation of their middle lamella and degradation of the primary cell wall, which is often followed by the partial or complete autolysis of cell contents (Van Doorn and Stead 1997).

1.3.15 Ethylene and flower abscission

The role of ethylene in plant organ abscission is well recognised. Exposure to exogenous ethylene has been shown to hasten inflorescence or floral organ abscission in a number of woody ornamental plants. These include a number of species within the family Myrtaceae, such as *Verticordia nitens* (Joyce and Poole 1993), *Chamelaucium uncinatum* (Joyce 1989), *Metrosideros collina* (Sun *et al.* 2000), *Leptospermum petersonii* and various other Australian cut flowers (Macnish *et al.* 2000). Other woody

ornamental genera also include *Grevillea* (Joyce *et al.* 1995) and *Camellia* (Woolf *et al.* 1995, 1999).

The abscission of floral organs via exogenous ethylene applications appears to be regulated by various factors, including the concentration and duration of ethylene exposure, the timing of endogenous production, the sensitivity at various developmental stages (Zieslin and Gottesman 1983, Mor 1987, Wu *et al.* 1991) and species lineage (e.g. family level) (Woltering and Van Doorn 1988). *Metrosideros collina* exemplifies that the abscission response of ethylene-sensitive flowers can depend on the ethylene concentration used and on the duration of the exposure. Petal and stamen abscission in this species occurred over a seven-day period following exposure to low levels (0.1 $\mu l\ l^{-1}$) of exogenously applied ethylene. Concentrations $\geq 0.5\ \mu l\ l^{-1}$ of ethylene, however, resulted in abscission of greater than 80 percent of floral buds (receptacles) by day five (Sun *et al.* 2000). In another myrtaceous species, *Leptospermum scoparium,* the concentration of exogenous ethylene treatments was positively related to the proportion of flower abscissions (Zieslin and Gottesman 1983). A 30 h exposure to 100 ppm ethylene was required to cause half of all floral abscissions on shoots of *L. scoparium*, whereas a further 18 hour exposure increased the proportion of abscissions to 80 percent (Zieslin and Gottesman 1983).

1.3.16 Ethylene production

The rate of endogenous ethylene production usually increases before flower abscission (Reid and Wu 1992). The time course for ethylene production as observed in carnation, rose and other flowers typically follows a profile composed of three distinct phases, including (1) a low steady state, (2) an increased rise to a maximum and (3) a decline in ethylene production (Mor 1987). Each phase can be associated with various events during senescence. The onset of the second phase, for instance, is usually associated with terminal stage of senescence while visual symptoms of the effects of ethylene (e.g. flower abscission) appear at the end of that phase (Mor 1987, Van Doorn and Stead 1997). For example, Mayak *et al.* (1972) reported that endogenous ethylene production by rose flowers peaked two days before the onset of petal abscission.

The start of the second phase in ethylene production can also be associated with a rise in respiration (known as climacteric) and subsequent CO_2 evolution (Taiz and Zeiger 1998). The climacteric pattern of ethylene production during senescence has been particularly well described for carnation (Wu *et al.* 1991) and *Petunia* (Borochov *et al.* 1997). In the carnation cultivar 'Crowley Pink', the increasing ethylene production during the senescence process also serves in accelerating the rate of senescence itself (Wu *et al.* 1991). This can be expressed as an autocatalytic response, whereby the triggering of tissue to produce ethylene is catalysed by previously produced ethylene. In non-climacteric species, such as in excised *Grevillea* flowers, ethylene production increases over time, peaking at day five although respiration levels remained relatively constant. Other non-climacteric flowers include Geraldton waxflower (Joyce *et al.* 1993, Olley *et al.* 1996) and ethylene-insensitive species, such as *Sandersonia* (Eason and DeVre 1995) and daylily (Bieleski and Reid 1992).

The source of ethylene production within a flower appears to be initiated in specific floral organs. Pollination in *Lathyrus odoratus* (sweet pea) flowers serves as a catalyst for ethylene production, although the level of this production varies between floral organs. Combined, both the style and stigma produce more ethylene than the petals, ovary and receptacle collectively, thus indicating that ethylene production first increases in the stigma and style before extending to various other flower parts (Singh and Moore 1994). More recent findings support this hypothesis. Hilioti *et al.* (2000) proposed that rapid petal abscission occurred in *Pelargonium* x *hortorum* during increased post-pollination ethylene production via the activation of ACC synthase primarily in the stigma and style. Applications of aminoethoxyvinylglycine (AVG), an inhibitor of ACC synthase activity, to the stigma effectively prevented pollination-induced ethylene production and petal abscission (Hilioti *et al.* 2000). Post-transcriptional regulation of ACC synthase is suggested to occur in response to other factors besides pollination, including various types of stress (Felix *et al.* 1991, Mathooko *et al.* 1998) and after cytokinin treatment (Vogel *et al.* 1998).

1.3.17 Ethylene sensitivity

Species-specific differences in responses to ethylene have been broadly examined. Woltering (1987), for instance, conducted a large study to compare the relative effects

of exogenous ethylene on intact flowers in various ornamental pot plants. Leaves on plants marketed as foliage crops exhibited less sensitivity to exogenous ethylene (0-15 $\mu l\ l^{-1}$ for 24-72 hours) than did floral organs on flowering plants. Flower and floral bud abscissions were frequently observed at relatively low ethylene concentrations or during the short exposure time. For example, *Hibiscus* plants, classified as 'extremely sensitive' to ethylene, incurred flower and floral bud abscission following a short (24 hour) exposure to low ($<4\ \mu l\ l^{-1}$) concentrations of exogenous ethylene. The significance of this study was highlighted by the establishment of a classification scheme for a number of popular ornamentals, thus, enabling comparison of plant responses from other studies (e.g. Høyer 1996a, 1996b; Sun *et al.* 2000). This study also broadly described within-plant differential responses of leaf and floral organs to various ethylene treatments. Woolf *et al.* (1999) suggested that the terminology defining the relative effects of ethylene on abscission rates (e.g. time to 50%) required clarification. They proposed that the term "responsiveness" be defined as differences in abscission response to a high ethylene concentration (e.g. $10\ \mu l\ l^{-1}$), whereas differences in abscission response to low ethylene concentrations ($<1\ \mu l\ l^{-1}$) be defined as "sensitivity" differences.

Not only does the concentration of ethylene or exposure time cause a response in flowers, but rather the combination of both these factors and the sensitivity of the plant (or plant part) to ethylene have been considered in a number of reports (Moe and Smith-Eriksen 1986, Evensen 1991, Høyer 1996b). For flowering plants in general, there is a gradual increase in ethylene sensitivity with increasing age, and thus, older flowers usually require relatively lower concentrations of ethylene to induce flower abscission and senescence (Zieslin and Gottesman 1983, Høyer 1996b). This response has been observed in flowers of carnation (Camprubi and Nichols 1978), *Hibiscus* (Woodson *et al.* 1985), *Begonia* x *cheimantha* (Moe and Smith-Eriksen 1986), *Pelargonium* x *domesticum* (Evensen 1991) and *Camellia* (Woolf *et al.* 1995, 1999). Woolf *et al.* (1995), for example, demonstrated that different organs in *Camellia* (leaves, floral and vegetative buds) displayed differential sensitivities to exogenous ethylene, and this effect could be mediated by temperature. Generally, floral buds rather than vegetative tissue were more sensitive to ethylene. Similar responses were also observed following treatment with ethephon, an ethylene-releasing compound (Woolf *et al.* 1992). The

effect of exogenous ethylene on plant organs of *Camellia* was also dependent on the physiological age of that tissue, showing increased sensitivity and responsiveness with age (Woolf *et al.* 1999).

Increased floral sensitivity has been reported following pollination or fertilisation. Porat *et al.* (1994) showed that increased flower sensitivity to ethylene (occurring after four hours) appeared to be independent of endogenous ethylene production, at least following pollination in *Phalaenopsis* hybrids (orchids). Ethylene production was detected only 12 hours after pollination and reached its peak after 30 hours. Similarly, petunia flowers also increased in ethylene sensitivity by at least seven hours after pollination, while ethylene production started a few hours later (Halevy and Whitehead 1989).

The biological explanation to account for differences in ethylene sensitivity in the life-cycle of a flower remains, as yet, unclear. Brown *et al.* (1986), however, suggested that ethylene sensitivity was not related to an increased ethylene binding capacity but rather to synthesis of an ethylene sensitivity factor (Van Altvorst and Bovy 1995). Increases in tissue sensitivity to ethylene have been associated with the accumulation of short-chain saturated fatty acids produced in carnation petals during early stages in senescence. The pollination of carnation styles also coincides with the formation of fatty acid synthesis (Whitehead and Vasiljevic 1993). The increased fatty acid concentrations are hypothesised to increase the ethylene binding affinity by altering the properties of certain membranes (Van Altvorst and Bovy 1995).

1.3.18 Hormone interaction: Auxins

Auxin has been implicated in playing a role as an inhibitor of floral abscission. A number of studies successfully delayed or inhibited the abscission of flowers or floral parts in a number of woody crop species by using exogenously applied synthetic auxins, such as α-naphthaleneacetic acid (NAA) or indole-3-acetic acid (IAA) (Einset 1986, Joyce 1989, Joyce and Beal 1999). Auxin treatments can also inhibit abscission in specific floral parts, such as anthers in *Cleome hassleriana* (Koevenig and Sillix 1973) and styles in *Citrus* (Einset 1986). Experimental work on cut stems of *Rosa*, showed that pedicel abscission following treatments with exogenous auxin was significantly

reduced, even after cut stems were treated with ACC, the precursor to ethylene. Goszczynska and Zieslin (1993) subsequently hypothesised that auxin played a role in decreasing the sensitivity of abscission zone cells to ethylene. The application of auxins to either prevent or delay floral abscission has not always been successful, as recently highlighted in a study where dip applications of IAA ineffectively prevented flower abscission in *Grevillea* (Joyce and Beal 1999). Moreover, excessive quantities of auxin can also induce organ abscission by promoting ethylene production (Abeles *et al.* 1992).

1.3.19 Ethylene inhibitors

Various chemical applications have been successfully employed for preventing ethylene-related damage (e.g. abscission of flowers or floral parts) in flowering ornamental crops. Specifically, two approaches to this problem have been addressed; incorporation of treatments either targeted at interfering with ethylene biosynthesis or aimed at reducing the effects of ethylene by inhibiting or depressing its action. Substances commonly used as inhibitors of ethylene biosynthesis include chemicals such as amino-ethoxyvinylgycine and aminooxyacetic acid, whereas popular inhibitors of ethylene action have primarily been those with silver-based compounds (e.g. silver thiosulfate) (Macnish *et al.* 1999) and/or with the use of the novel gaseous compound, 1-methylcyclopropene (Serek *et al.* 1994, Macnish *et al.* 2000).

1.3.20 Inhibitors of ethylene biosynthesis

Several effective chemical inhibitors of ethylene biosynthesis include amino-ethoxyvinylgycine (AVG) and aminooxyacetic acid (AOA). Specifically, both AVG and AOA are suggested to inhibit the action of the enzyme ACC-synthase that converts S-adenosylmethionine to 1-aminocyclopropane-1carboxylic acid (ACC) (see Figure 1.5 A). In carnation, for example, increased ethylene biosynthesis associated with petal senescence was accompanied by an increase in ACC synthase activity and ACC content. Treatment of climacteric carnation petals with 2,5-norbornadiene (NBD), another ethylene biosynthesis inhibitor, resulted in the subsequent inhibition of ACC synthase and ACC oxidase activities (Woodson 1991). These and other ethylene biosynthesis inhibitors have been effective in delaying ethylene-mediated senescence or abscission in *Strongylodon macrobotrys* (ornamental blue jade vine) (Furutani *et al.*

1989), carnation (Staby *et al.* 1993), *Phalaenopsis* (orchid) (Porat *et al.* 1994) and various other species (Staby *et al.* 1993). However, deterrent factors associated with their use have been attributed to the cost of the products themselves, the possible toxicity or side-effects of the chemicals (Staby *et al.* 1993) and to their lack of effectiveness in delaying or preventing senescence/abscission in some species (e.g. *Digitalis* flowers) (Van Doorn and Woltering 1991). Furthermore, inhibitors of ethylene biosynthesis do not protect flowers and plants from the damaging effects of exogenous ethylene (Van Doorn and Woltering 1991).

1.3.21 Silver thiosulfate (STS)

Extensive research has been directed towards extending the post-harvest life of ornamental crops via treatments that inhibit ethylene action. In particular, the efficacy of silver thiosulfate, coined as the 'the original anti-ethylene compound' (Nell 1994), has been extensively researched on numerous flowering crops since the discovery of its effects in the late 1970's. Originally, Beyer (1976) showed that silver ions, in the form of silver nitrate, effectively inhibited ethylene-mediated processes. However, silver nitrate produced phytotoxic effects when applied as a spray, and was ineffectively translocated in cut flower stems when added to vase solutions. Silver in the form of a silver thiosulfate (STS) complex was subsequently found to be more suitable for protecting flowers from the damaging effects of ethylene. Not only was STS readily translocated in cut flowers, but it was also deemed non-phytotoxic when used at effective concentrations (Staby *et al.* 1993).

The effectiveness of STS lies in its ability to bind at ethylene receptor sites, thus preventing the subsequent binding and action of ethylene molecules (Beyer 1976, Van Meeteren and Proft 1982). Numerous reports have described the effectiveness of STS in preventing floral abscission or delaying the onset of senescence-related symptoms in flowers of woody ornamentals. For instance, the postharvest potential of *Grevillea* spp. as cut flowers is limited by its short vase-life (<10 days) due to abscission of perianth and perianth segments (tepal) and/or whole flowers (Joyce and Beal 1999). Short (≤1 hour) pulse treatments of STS (4 mM l^{-1} Ag^{+}) applied in vase solutions successfully prevented flower abscission and extended vase life by inhibiting the action of endogenously produced ethylene (Joyce and Haynes 1989, Joyce and Beal 1999).

Similar studies on the efficacy of STS in preventing flower and flower bud abscission has been reported in numerous other ornamental woody crops, for example in *Rosa* (Serek 1993), *Boronia heterophylla* (Macnish *et al.* 1999) and *Bougainvillea glabra* (Cameron and Reid 1983).

Limited research has shown that ethylene is an important factor in the postharvest life of a number of species within the family Myrtaceae. Moreover, the efficacy of STS in either preventing ethylene-related abscission or shrivelling/wilting of floral parts has been investigated in *Leptospermum scoparium* (Zieslin and Gottesman 1983), *Chamelaucium uncinatum* (Joyce 1988), *Verticordia* spp. (Joyce and Poole 1993) and *Metrosideros collina* (Sun *et al.* 2000). Pulse pre-treatments of 4 mM of STS increased flower fresh mass and successfully prevented floral abscission in *C. uncinatum* and *V. nitens* against exogenous ethylene (8.6-9.4 $\mu l\ l^{-1}$) treatments applied for 20 to 24 h, although these effects did not differ from controls (no STS or ethylene treatments) (Joyce 1989, Joyce and Poole 1993). Similar findings were observed in *L. scoparium*, although Zieslin and Gottesman (1983) also reported on the level of ethylene emanation and petal abscission in young (at full petal opening) and mature (1-2 weeks after petal opening) flowers exposed to conditions with high (>70%) relative humidity (RH). Under these conditions, petal abscission occurred only in mature flowers, irrespective of STS treatment, while both young and mature flowers emanated significantly higher rates of ethylene (compared to controls) only following STS treatment (Zieslin and Gottesman 1983). Therefore, STS prevented ethylene-induced, but not natural abscission (senescence) of mature flowers. Under 70 percent RH, ethylene evolution occurred in a climacteric pattern, peaking at seven days after harvest (Zieslin and Gottesman 1983). Therefore, applications of STS have been successful in preventing exogenous ethylene-induced damage on perianth flower parts, at least in these species.

While the majority of investigations of STS-related effects in myrtaceous species have focused on perianth abscission, only a few studies has investigated its role in preventing both stamen and petal abscission. Sun *et al.* (2000) described the effects of pre-treatments of STS (dip of 2.0 mM) on cut cymules of *Metrosideros collina* followed by 24 h exogenous ethylene (0-5 $\mu l\ l^{-1}$) treatments. STS treatments conferred considerable protection against exogenous ethylene, since between Day 5-8, the level of abscission from petals and stamens was reduced by 32.4 and 12 percent per floral part,

respectively. In contrast, similar applications of STS on flowers of *Eucalytpus ficifolia* provided no protection from ethephon-induced (an aqueous ethylene-releasing compound) stamen wilting, symptoms that were probably associated with water loss rather than an ethylene-mediated response (Sun *et al.* 2001). STS, therefore, appears to be an effective treatment for preventing exogenous ethylene-related damage in some species within Myrtaceae. However, the protection conferred has not been associated with an improvement in vase-life above that found in ethylene-untreated (control) plants.

1.3.22 1-Methylcyclopropene (1-MCP)

Concern over the toxic effects of silver (STS) on human health and the environment has prompted research into alternative protocols for preventing ethylene-induced damage in cut flowers (Halevy 1994, Nell 1994). Interest in a relatively new gaseous compound, 1-methylcyclopropene (1-MCP), has developed due to its odourless, non-toxic properties and relative ease of use (Serek *et al.* 1994, Newman *et al.* 1998). In a large screening study conducted by Macnish *et al.* (2000), the efficacy of 1-MCP was examined on 14 different native Australian cut flowers treated with exogenously applied ethylene (10 $\mu l\ l^{-1}$) for 13 h at 20°C. Pre-treatments of 1-MCP successfully prevented floral abscission and associated loss in vase life in *Ceratopetalum gummiferum, Chamelaucium uncinatum*, two *Grevillea* hybrids, *Leptospermum petersonii* and *Verticordia nitens*. These results were in accordance with studies using STS-treated cut flowers from *C. uncinatum* (Joyce 1989, Macnish *et al.* 2000), *V. nitens* (Joyce and Poole 1993) and *Grevillea* hybrids (Joyce *et al.* 1993). In particular, 1-MCP improved the vase-life of only one species, *C. gummiferum* (data not shown), while in another species, *Telopea speciosissima*, no significant protection from ethylene was provided. Overall, with the exception of *C. gummiferum*, the vase-life of all 13 species tested was not significantly extended in the absence of exogenous ethylene exposure (Macnish *et al.* 2000).

Similar findings with regards to the level of protection afforded by 1-MCP have been reported in other woody plants. Müller *et al.* (1999) demonstrated that 1-MCP was effective in preventing petal fading in intact flowers of two *Rosa* hybrid varieties after undergoing a simulated transportation experiment designed to induce a stress-related

ethylene production response. Similar to the effects of STS, 1-MCP effectively protected against the deleterious effects of exogenous ethylene but did not extend shelf-life of these *Rosa* hybrids in the absence of ethylene. In some cases, pre-treatments of 1-MCP were more effective than STS in preventing exogenous ethylene-mediated effects. For instance, in *Boronia heterophylla*, 1-MCP further reduced the level of flower wilting, stem fresh weight and extended vase-life (Macnish *et al.* 1999) from STS-treated cut flowers. As in the case with most other studies, the efficacy of 1-MCP and STS was not greater than that found in control plants.

To date, only one study has examined the efficacy 1-MCP in protecting ethylene-induced stamen abscission. As previously discussed, pre-treatments of STS followed by exogenous ethylene exposures effectively reduced stamen and petal abscission in cut cymules of *Metrosideros collina* (Sun *et al.* 2000). The results obtained from STS, however, did not parallel those found for 1-MCP. Initially, stamen abscission was inhibited with 1-MCP (150 nl l^{-1} applied over 6 hours) for a maximum of 24 hours after the ethylene (0.1 µl l^{-1}) treatment. Over the subsequent five days, stamen abscission in control and 1-MCP treatments increased significantly (>87 percent by Day 7) in comparison with the STS treatment (13% by Day 7). Not only was 1-MCP found to be ineffective in preventing ethylene-induced abscission in cut cymules, but it also enhanced endogenous ethylene production, thus corresponding with the increased stamen fall (Sun *et al.* 2000). The short-term protection afforded by 1-MCP was attributed to the incomplete blocking of receptors or the subsequent synthesis of new ethylene receptors (Sisler and Serek 1999). Whether the response exhibited by *M. collina* to 1-MCP is unique within this genus is, as of yet, unknown and thus merits further investigation.

1.4 Summary and Thesis Objectives

The continuing efforts to understand the physiological, biochemical and genetic changes during ontogenetic development show promise. This is in spite of decades spent investigating this phenomenon, given that first studies examining this phenomenon date to the early part of the last century (Goebel 1900). The use of mutants in genetic studies show promising results and are helping to establish the role of different genes and/or

hormones and their interactions during different phases in development. However, current methods to accelerate phase change are not always consistent and/or require high maintenance and extended periods of observation, for example, selection and breeding of genotypes with short juvenile periods. Until such a time that genetic engineering becomes the preferred method for accelerating phase change, reliance on cultural methods and environmental conditions that favour vigorous growth appear favourable. Moreover, a further understanding of vegetative phase change would benefit from studies that examine the physiological changes that occur during this transition as opposed to morphological comparisons between different states of ontogenetic development. These observations should also be considered in light of the currently proposed hypotheses and models so that a conceptual understanding of phase change can be advanced.

Numerous investigations into the environmental and chemical factors that regulate flowering in woody angiosperm species have been reported. These findings are supported by basic research, particularly from model herbaceous plants have contributed to our understanding and knowledge of factors involved in the flowering process. Clearly, environmental and chemical factors underpinned by genetic mechanisms function to determine the timing and subsequent development of reproductive organs. However, even with an enhanced knowledge at the molecular level, there will still be a need to induce flowering, either through environmental manipulation and/or chemical applications. This is particularly important within the floricultural industry. Given the proper knowledge of the flowering requirements of a target crop, the timing, quantity and quality of flowers should then be able to be culturally manipulated.

The postharvest life of cut flowers has been investigated in numerous studies. The role of ethylene in decreasing the vase-life of many woody species is well noted, although treatment applications that either inhibit ethylene biosynthesis or its action on flowers have not been consistent across species. In particular, further studies are needed to examine the role of ethylene and chemical inhibitors in preventing its deleterious effects on flower parts other than sepal/petals, such as stamens. With regards to water relations, most studies concede that treatments with biocides and/or sucrose are important in extending vase-life, at least in a number of woody species. Whether these

plant responses are exhibited in most species that have stamens as their ornamental feature, is not well documented. Additionally, an extensive and comprehensive postharvest knowledge of both water relations and ethylene-related research is lacking for numerous woody genera. Studies that encompass both of these factors would be particularly important in producing a successful flowering crop in the floricultural industry.

A proper evaluation of plant performance would not be complete without experiments conducted on various cultivars of a given species. In this thesis, several cultivars of *Metrosideros excelsa* were examined in order to assess their relative responses to different treatments. However, in certain instances, determination of responses from several cultivars was not possible due to the unavailability of particular cultivars at the start time of a given experiment(s).

In light of this review, several hypotheses with regards to phase change, environmental control of flowering and the postharvest treatment of cut flowers of *Metrosideros excelsa* are advanced and tested in this thesis. It is hypothesised that:

1) The restriction of shoots to a single-stem axis and/or application of a root restriction regime accelerates vegetative phase change in *M. excelsa*, as has been documented for several other woody species (Zimmerman 1972, Davis 1991, Snowball *et al.* 1994) (Chapter 2).

2) Juvenile plants of *M. excelsa* undergoing vegetative phase change exhibit decreasing carbon isotope discrimination (Δ) because of changes in leaf morphology consistent with greater water use efficiency, notably more tomentose and rounder leaves. This may also be associated with a reduction in photosynthetic capacity with increasing node position. This hypothesis would be consistent with the bulk of published ecophysiological reports, in which leaves from adult plants exhibit lower Δ and associated differences in photosynthetic capacities than leaves from juveniles growing in the field (Donovan and Ehleringer 1992, 1994; Hansen 1996, Cordell *et al.* 1998) (Chapter 3).

3) Changes in Δ are not a function of leaf age, but are the result of changes in leaf conductance relative to photosynthetic capacity at the time of leaf expansion (Chapter 3).

4) Both low temperature and/or short day-length are important in promoting floral induction in *M. excelsa*, as reported for a number of closely related genera (Shillo *et al.* 1985, Zieslin 1985, Moncur and Hasan 1994) (Chapter 4).

5) The size of the developing bud at the time of application of an inductive treatment will determine whether or not floral initiation will take place, with larger buds becoming floral more readily than smaller buds (Chapter 4).

6) Irradiance is positively related to the number of flowers produced in *M. excelsa* when applied in conjunction with treatments inductive for flowering (Chapter 5).

7) The vase life of *M. excelsa* flowers is limited by adverse water relations and by the ethylene-related abscission of whole flower and floral organs. Appropriate holding solutions and inhibitors of ethylene action may, therefore, provide effective treatments for extending the vase life and minimising ethylene-related damage, as demonstrated for closely related taxa (Burge *et al.* 1996, Macnish *et al.* 2000, Sun *et al.* 2000, 2001) (Chapter 6).

Chapter 2

Effect of shoot and root restriction on vegetative phase change in *Metrosideros excelsa*

2.1 Introduction

Early developmental phase change in higher plants is characterised by a transition from a juvenile through to an adult vegetative phase. During this transition there are a number of accompanying changes in vegetative characteristics such as in leaf morphology, phyllotaxy, thorniness or leaf 'hairiness' (Godley 1985, Poethig 1990, James and Bell 2001). A considerable number of New Zealand plants (greater than 200 species) exhibit heteroblastic development, showing distinct and dramatic changes in leaf morphology between the juvenile and adult stages (Godley 1985, Day *et al.* 1997). This is in contrast to homoblastic species such as those in the genera *Avicennia*, *Macropiper* and *Metrosideros* where a gradual transition between these life stages occur. For example, *Metrosideros excelsa* has characteristic phase–dependent leaf morphologies; leaves in adult compared with juvenile plants are typically more rounded and possess a characteristic downy tomentum on abaxial surfaces (Cockayne 1928, Dawson 1968).

In higher plants, the competence to undergo reproductive development is usually dependent upon attainment of an adult vegetative state (Hackett 1985). However, attainment of this adult vegetative state can be strongly influenced by propagation methodology. For example, seedlings of *Metrosideros* typically reach an adult reproductive phase within five to ten years, whereas cuttings from adult foliage are capable of flowering within one year (Oliphant *et al.* 1992). In light of the difficulties in rooting cuttings, such as in *Metrosideros*, an alternate and preferred method of propagation has been through micropropagation, where multiplication of clonal material can be obtained rapidly.

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During micropropagation, rejuvenation of parent material is a common occurrence in many woody plant species (Hackett and Murray 1992). Rejuvenation entails the ontogenetic reversion of adult material to the juvenile phase and results in plantlets bearing leaves that are morphologically and physiologically distinct from adult leaves (Oliphant 1988). This process is different to the reversal of physiological ageing, which can arise from cutting propagation, grafting or hedging and is often associated with renewed shoot vigour (Fortanier and Jonkers 1976, Wareing and Frydman 1976, Zimmerman *et al.* 1985). Rejuvenation following micropropagation is reported to occur in *Metrosideros* and the time until which plants can bear flowers can exceed three years (Oliphant *et al.* 1992). Moreover, floral development in *Metrosideros* occurs only on branches bearing adult morphology. This is in contrast to certain *Eucalyptus* species (also in the family Myrtaceae), where flowers have been reported to arise on branches bearing juvenile foliage (Wiltshire *et al.* 1991). Therefore, treatments applied to rejuvenated plants of *Metrosideros* that might accelerate the transition between the juvenile to adult phase might be expected to shorten the time to flower.

Application of various cultural practices can affect the timing of vegetative or reproductive phase change (Oliphant *et al.* 1992, Snowball *et al.* 1994). Acceleration of phase change as a result of manipulation of the growing environment (e.g. high light) or plant material (e.g. shoot restriction) has been attributed to conditions that promote vigorous growth (Wareing and Frydman 1976, Poethig 1990). Subsequently, treatments that tend to retard growth, such as water stress, low light and temperatures, and poor carbohydrate and mineral nutrition, have been associated with prolongation of the juvenile phase (Poethig 1990). In several studies, limiting growth to a single-stem by removal of axillary branches has been shown to hasten the onset of developmental phase change (Davis 1991). Additionally, root restriction has also been reported to accelerate phase change (Zimmerman 1972). However, in both these and other studies attainment of the adult phase has been assessed using flowering as an indicator of ontogenetic status and, therefore, no quantitative distinction was made of the progressive changes in vegetative characters and the onset of flowering.

In this study, vegetative phase change was characterised in plantlets of *Metrosideros excelsa* derived by micropropagation. The experiment was designed to assess the

relative effects of shoot and root restriction, applied in factorial combination, on accelerating vegetative phase change. Shoot restriction was imposed through removal of lateral shoots resulting in a single-stemmed plant, whereas a root restriction regime was afforded by using pots of different sizes. By applying a shoot restriction regime, the level of ontogenetic development in leaves from complementary height and node positions was assessed in branched (control) and single-stemmed plants through evaluation of leaf morphological characters.

Typically, phases of shoot development are permanently recorded as variation in the character of structures along the axis of the shoot (Poethig 1990). Thus, the capturing of these changes in morphological and optical properties of leaves was undertaken using an image analysis procedure. This technique was particularly suitable for examination of ontogenetic development of leaves in the homoblastic *M. excelsa*, where the transition between juvenile and adult leaf forms occurs gradually. Specifically, the use of optical parameters enabled quantification of the gradual accumulation of tomentum (increasing whiteness) on the abaxial surfaces of leaves undergoing ontogenetic development.

In this experiment, the following hypotheses regarding the effect of restriction treatments on phase change were tested. It is hypothesised that:

1) The restriction of shoots to a single-stem axis and/or application of a root restriction regime accelerates vegetative phase change in *M. excelsa*, as has been documented for several other woody species.

2.2 Material and Methods

2.2.1 Plant material

Micropropagated plants of *Metrosideros excelsa* `Scarlet Pimpernel´ growing in 50 mm square plastic were supplied by Lyndale Nurseries Auckland Ltd. Initial plant height, and total leaf, stem and root dry weights of a sample of five plants were 11.6 cm, 1.46 g, 0.63 g and 0.85 g, respectively. Plants were highly-branched, each bearing 8-9 terminal shoots. Experimental containers were made from 100 mm sections of PVC water pipe of

five different internal diameters. A synthetic, non-woven fabric (Syntex, Permathene Plastics Ltd., Auckland) was glued to the base of each section to give rooting volumes of 0.82, 1.7, 2.7, 4.1 and 7.2 l. Plants were transplanted on 29 August 1997 into the pots using a growing medium composed of split, expanded clay granules (1-4 mm diameter range, Hydroton, NZ Hydroponics International Ltd., Tauranga) and vermiculite (4 mm grade, Revertex Industries (NZ) Ltd.) (9:1 v/v). Each plant (regardless of pot size) received the same amounts of controlled release fertilisers (1.0 g 3-4 month release, and 3.0 g 8-9 month release Osmocote Plus, Grace Sierra, Heerlen, The Netherlands) placed adjacent to the root ball of the transplant. These application rates were comparable with those commonly used for woody plants grown for nine months in 2-3 L containers.

2.2.2 Experimental layout

In the first week after planting, all shoots with the exception of the tallest shoot on each plant were removed. On half of the plants grown in each of the five container sizes, the tallest shoot was allowed to grow unchecked and was trained in an upright position. Any branch development in these plants (referred to as single-stemmed plants) was prevented by removal of axillary buds as these swelled before bud break. On the remaining plants (referred to as branched plants), the single shoot was pinched once to encourage a branched growth habit. There were five blocks and two internal replicates (plants) of each of the 10 treatments, which were completely randomised within blocks. The plants were placed on capillary irrigation tables wetted four times daily for 15 minutes to ensure an uninterrupted supply of water to the plants. The experiment was conducted over nine months in a temperature controlled greenhouse set to vent at 23°C, and warmed when the temperature fell below 15°C.

2.2.3 Growth measurements

Plant height to the tip of the expanding leaves (shoot length) and number of nodes bearing leaves longer than 15 mm were recorded every 14 days on single-stemmed plants. Shoot length (the linear distance from the medium surface to the shoot tip of the longest shoot) and node number in the branched plants was recorded immediately before destructive harvest on 2 June 1998.

2.2.4 Image analysis

For image analysis, shoots from each single-stemmed plant were removed at the surface of the growing medium, and the longest shoot in each branched plant was removed at a point below which no green leaves were attached. Detached shoot bases were placed in vials of distilled water to maintain leaf turgor. Images were captured of both adaxial and abaxial surfaces of the four leaves at pairs of contiguous nodes at up to six locations within each shoot (node pairs 6-7, 16-17, 26-27, 36-37, 46-47, and 51-52). For shoots not bearing fully-expanded leaves to nodes 51-52, measurements were made at the same five locations up to nodes 46-47, and at the highest nodes bearing fully expanded leaves. In general, branched plants bore leaves on 35-40 nodes, and leaves on the lowest nodes, which had been heavily shaded by surrounding leaves, had abscised. Typically, image analysis was possible for leaves at nodes 16-17, 26-27 and 36-37 in these plants. In addition, images were captured for the lower surface of one leaf at every node for all plants grown in the intermediate container size (2.7 l).

2.2.5 Image analysis protocol

Leaf dimensional and optical properties were captured on a Sony DX3000P colour (3 chip) video camera mounted on a Polaroid MP4 copy stand containing four 75 W incandescent bulbs. The capturing and processing of leaf parameters was through PC installed hardware (Imaging Technology Colour Frame Grabber hardware) and software (Vision Image Processing System), respectively. Prior to any leaf measurements, the system was calibrated for aspect ratio and pixel size (Bailey 1995). The red, green and blue colour channels were normalised against a pre-calibrated white background.

Leaves were placed at the base of the copy stand below the camera lens and covered with a glass pane to prevent folding. Optical measurement were made of leaf length, width, length/width ratio, area, perimeter and roundness $(\text{leaf perimeter})^2/\text{leaf area}$), as described by Bailey and Hodgson (1988) and Bailey (1995). To make leaf colour measurements, the segmented leaf image was eroded by two pixels to avoid pixels at the leaf edge that may have included background. This was then used to mask the original colour image, which was transformed into HLS (Hue, Lightness and Saturation) colour

space (Foley and Van Dam 1982). The mean and standard deviation of leaf hue (scale 0-256), lightness (percentage scale) and saturation or strength of colour (percentage scale) were calculated.

2.2.6 Plant biomass and leaf mineral determination

Shoots were dissected into leaf and stem fractions. Leaf area per plant was measured, and the total number of terminal shoots >15 mm in length was recorded for branched plants. Root systems were washed clean of the growing medium, and fine roots recovered by sieving under running water. Leaves, stems and roots were dehydrated at 80°C for 24 h and weighed. Complete mineral analyses were obtained (Hill Laboratories, Hamilton, NZ) for four replicates of the most recently fully expanded leaves for single-stemmed and branched plants grown in 0.82 and 7.2 l containers. Fully expanded leaf samples were collected at nodes 17, 27, 37, 47 and 52 for single-stemmed and branched (on available nodes only) plants and from the canopy top of adult plants (~3 year-old plants) for determination of carbon isotope discrimination.

2.2.7 Carbon isotope analysis

Leaf samples were dried at 80°C for 48-72 h and were analysed for determination of $^{13}C/^{12}C$ isotope ratio at the Stable Isotope Unit, University of Waikato, New Zealand. The dry tissue samples were finely ground (<200 μm) and combusted, and the resulting CO_2 was analysed for δ_p using a Dumas Elemental Analyser (Europa Scientific Tracermass). A precalibrated C_4 sucrose reference was used that had been standardised relative to Pee Dee Belemnite by CSIRO, Canberra, Australia. Carbon isotope discrimination (Δ) was calculated from the sample (δ_p) and ambient carbon isotope ratios relative PDP (δ_a) (equated to –0.0078 ‰) using the formula (Farquhar *et al.* 1989):

$$\Delta = (\delta_p - \delta_a) / (1 + \delta_p)$$

2.2.8 Statistical analyses

Data were analysed by analysis of variance using the SAS statistical package (SAS Institute, Cary, N.C., USA). Time was included as a factor for repeated measures made of node number and plant height. Where appropriate, node position on single-stemmed plants was treated as a within-plant split plot for analyses of shape, size and colour parameters. Statistical comparison of leaves on single-stemmed and branched plants grown in 2.7 l containers were also made at a shoot length measured 280 mm from the surface of the growing medium. This was the maximum height at which fully expanded leaves were borne on harvested shoots of all plants, and corresponded with node positions 12 and 23 in single-stemmed and branched plants, respectively. Transformation of data to normalise variance was not required except for analysis of some mineral concentrations. A $\log_{10}(x+1)$ transformation before analysis of variance was used in these cases. Means were compared for significant differences at the 5% level.

2.3 Results

2.3.1 Growth parameters

Single-stemmed plants grew to ten times their initial height over the thirty-five week experimental period, each adding approximately 50 nodes (Figure 2.1). A key feature of the shoot restriction treatment was the greater number of nodes and greater shoot length that the single-stemmed plants attained compared to the branched plants. The longest shoot in branched plants was both shorter and bore fewer nodes (~40 cm and 32 nodes, respectively) than that in single-stemmed plants (~100 cm and >50 nodes) (Figure 2.1). Branched plants bore an average of 16-21 terminal shoots >15 mm long. Container size had no significant effect ($P>0.05$) on any of these growth parameters.

2.3.2 Image analysis: optical parameters

Measurements made at every node in both single-stemmed and branched plants grown in an intermediate container size (2.7 l) revealed contrasting trends in leaf development between these groups of plants as node number increased. Thus, while hue of the

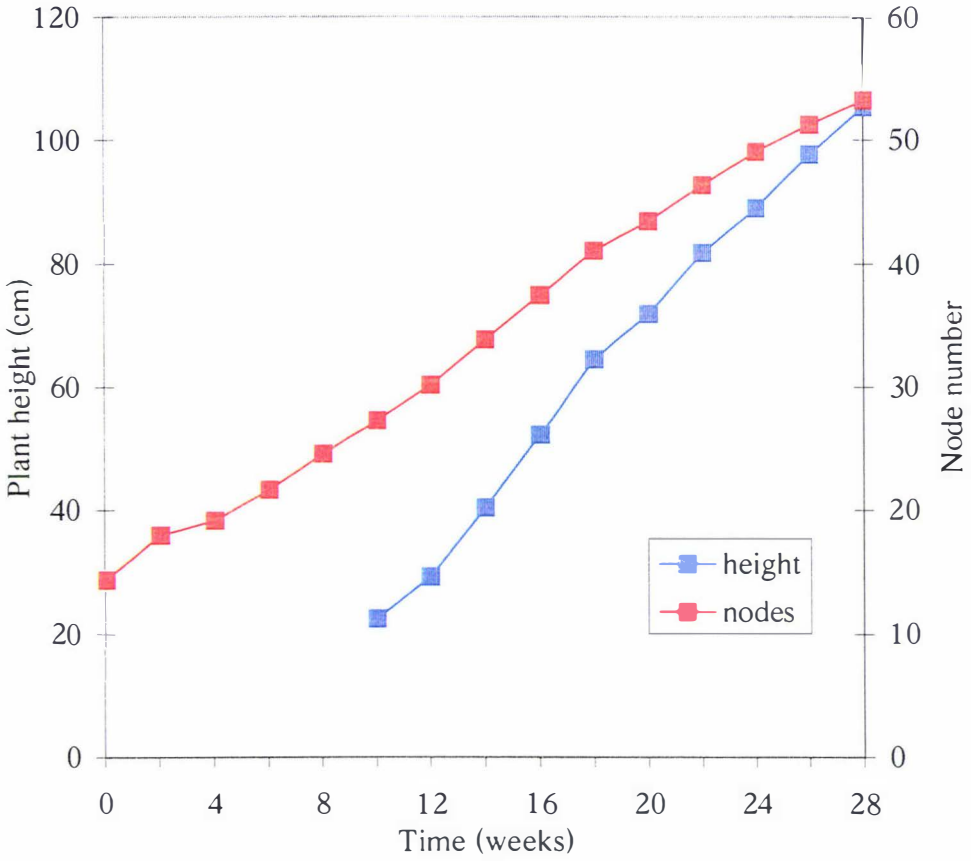

Figure 2.1 Increase in mean plant height and mean total number of nodes in single-stemmed plants of *Metrosideros excelsa* 'Scarlet Pimpernel' over the experimental period.

abaxial leaf surface decreased steadily in branched plants from approximately 60-65 units (yellow-green) at the basal nodes to 50-55 units (yellow) at nodes in the apical region of the shoot, that in the single-stemmed plants initially decreased slightly before increasing to above 70 units (approaching green) at node 50 (Figure 2.2 A). This trend of divergence between single-stemmed and branched plants above intermediate node positions was also observed for colour saturation of abaxial leaf surfaces. Leaves in the upper portion of single-stemmed plants became less saturated (had less colour) than those in branched plants (Figure 2.2 B). Leaf hue and saturation in single-stemmed plants attained or approached the corresponding values for leaves on adult plants. However, leaf hue and saturation in branched plants diverged from or did not approach these values (Figure 2.2 A-B).

Lightness (white {100%} as opposed to black {0%}) of abaxial leaf surfaces tended to increase in both single-stemmed and branched plants. Lightness in the basal portion of branched plants rose sharply so that leaves at nodes 30-35 in this group of plants were as light as those at nodes 50-55 in single-stemmed plants (Figure 2.2 C). Mean leaf lightness, which was comparable in the more apical leaves of both sets of plants at harvest, was approximately 95% that of leaves on adult plants (Figure 2.2 C). The 5% saturation attained for the abaxial surface of single-stemmed plants (approximately 25% that of branched plant leaves), coupled with a comparable lightness in the two sets of plants, gave the most recently expanded leaves of single-stemmed plants a greater "mealiness" or whiteness than those of branched plants.

Statistical analysis of leaf colour parameters for single-stemmed and branched plants were made across all root restriction treatments at the two node positions for which comparisons could be made (nodes 16-17 and nodes 26-27). There were highly significant differences ($P<0.0001$) for abaxial leaf surface hue between single-stemmed and branched plants at the two node positions (Table 2.1). Similarly for the saturation and lightness of abaxial leaf surfaces, there were highly significant differences ($P<0.0001$) between single-stemmed and branched plants at the two node positions tested (Table 2.1). Hue, saturation and lightness of adaxial leaf surfaces in single-stemmed plants were also significantly different ($P<0.0001$) from those in branched plants. Leaves on single-stemmed plants were greener, less saturated and lighter both at nodes 16-17 and 26-27 (Table 2.1). Container size did not have a significant effect on

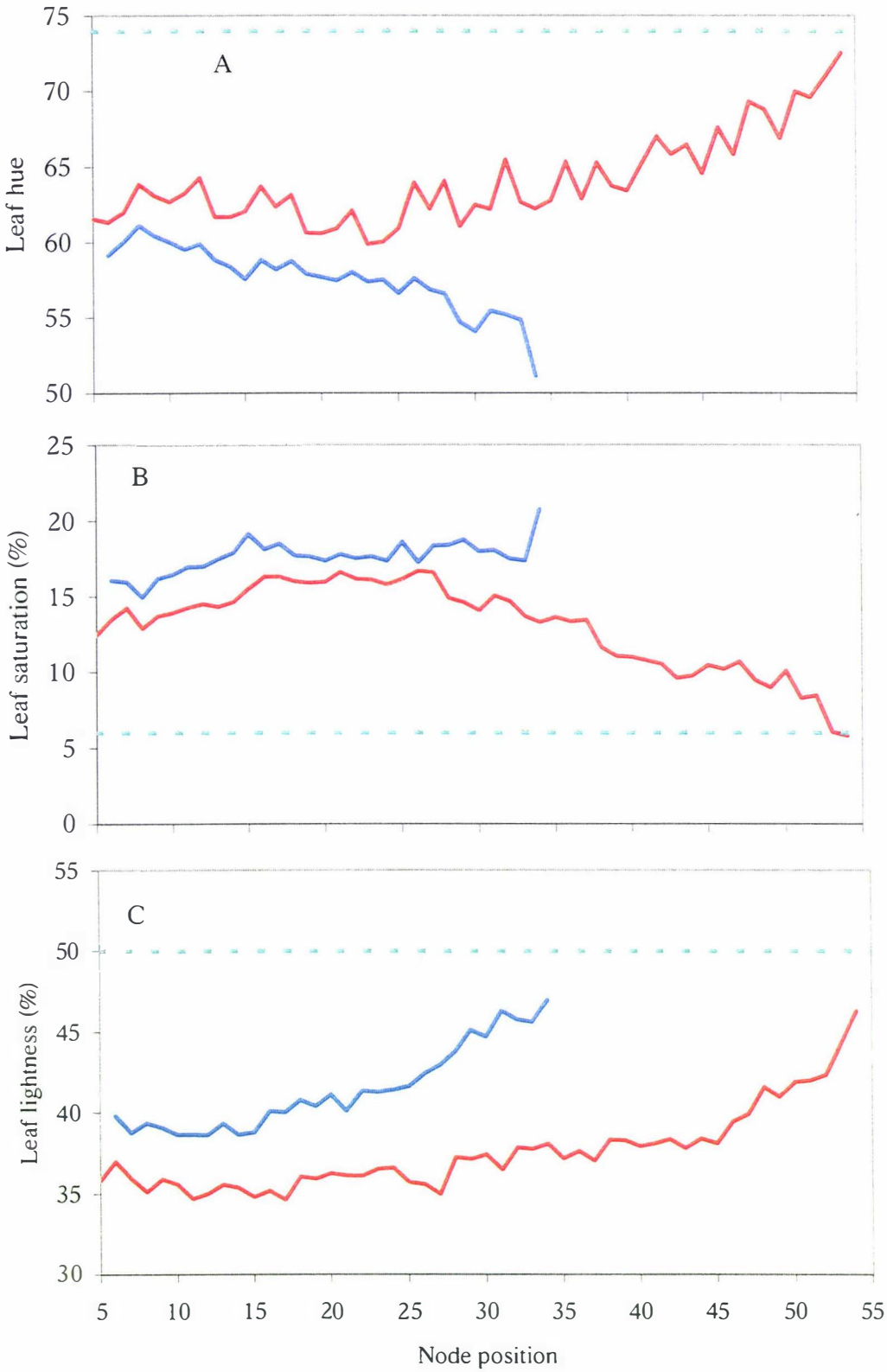

Figure 2.2 Changes in mean optical properties with increasing node position for the abaxial leaf surface in single-stemmed plants (red line) and branched plants (blue line) of *Metrosideros excelsa* 'Scarlet Pimpernel' grown in 2.7 l containers. A: hue. B: saturation. C: lightness. Green dotted line show the corresponding means for adult leaves.

Table 2.1 Comparison of single-stemmed and branched plants of *Metrosideros excelsa* 'Scarlet Pimpernel' with respect to leaf optical (abaxial and adaxial surfaces) and dimensional (adaxial surface) parameters at node positions 16-17 and 26-27. Means shown for single-stemmed and branched plants with each node position and leaf surface column were highly significantly different ($P<0.0001$). Data (mean ± s.e.) are for all container sizes.

Parameter	Leaves at nodes 16-17				Leaves at nodes 26-27			
	Abaxial surfaces		Adaxial surfaces		Abaxial surfaces		Adaxial surfaces	
	Single-stemmed	Branched	Single-stemmed	Branched	Single-stemmed	Branched	Single-stemmed	Branched
Hue (units)	63.17 ± 0.34	58.16 ± 0.31	102.18 ±1.46	74.56 ± 1.15	63.37 ± 0.44	57.34 ± 0.41	99.07 ± 1.92	65.70 ± 1.02
Saturation (%)	15.52 ± 0.22	17.53 ± 0.22	7.09 ± 0.11	12.57 ± 0.36	15.85 ± 0.26	16.76 ± 0.24	7.07 ± 0.10	17.10 ± 0.57
Lightness (%)	34.84 ± 0.19	40.12 ± 0.26	27.15 ± 0.19	32.29 ± 0.31	35.62 ± 0.18	42.86 ± 0.44	26.28 ± 0.21	35.32 ± 0.57
Width (mm)			27.27 ± 0.53	25.30 ± 0.54			33.64 ± 0.54	23.72 ± 0.61
Length/width ratio			n.s.	n.s.			1.58 ± 0.03	1.83 ± 0.02
Length (mm)			54.52 ± 1.18	48.99 ± 0.97			51.82 ± 0.65	42.85 ± 0.91
Area (mm^2)			1058 ± 37	874 ± 29			1221 ± 22	730 ± 28
Perimeter (mm)			132.4 ± 2.6	120.1 ± 2.2			139.2 ± 1.2	107.8 ± 2.3
Roundness			n.s.	n.s.			15.95 ± 0.08	16.35 ± 0.09

any of the three leaf colour parameters, nor was there a significant interaction between container size and shoot restriction.

For statistical comparisons of the six node positions within single-stemmed plants (from nodes 6-7 to nodes 46-47), there were highly significant effects ($P<0.0001$) of node position on abaxial leaf hue, saturation and lightness (Table 2.2). Node position also had a significant effect ($P<0.0001$) on adaxial leaf saturation and lightness (although not hue) in single-stemmed plants, the means generally converging on those for adult leaf adaxial surfaces (Table 2.2). There were no significant main effects of root restriction on the three leaf colour parameters, and generally no interactions between root restriction and node position.

2.3.3 Image analysis: dimensional parameters

With respect to shape and size parameters, leaves of single-stemmed plants tended to become more similar to those of adult plants with increasing node position than did those of branched plants grown in the intermediate container size (2.7 L). For example, leaf width and leaf length/width ratio in single-stemmed plants were comparable with those in adult plants above 30-40 nodes (Figure 2.3A and B). Leaf length, area and perimeter were greatest at nodes 10-40 in single-stemmed plants, and at 10-25 nodes in branched plants. Values for these parameters in single-stemmed plants became smaller at higher node numbers, and approached corresponding values for leaves of adult plants. In branched plants these parameters tended not to coincide with those for adult plants (Figure 2.3 C-E). Leaves became more rounded with increasing node number at a similar rate in single-stemmed and branched plants. However, leaves of branched plants had not attained the compactness of adult leaves by the end of the experiment (Figure 2.3 F).

Means for four of the six leaf size and shape parameters in single-stemmed and branched plants were highly significantly different in comparisons made at nodes 16-17 and 26-27 (Table 2.1). There was no significant difference between single-stemmed and branched plants at the lower node position for leaf length/width ratio and leaf compactness (Table 2.1), which was consistent with whole-shoot trends (Figure 2.3 B and F). There was no effect of container size or interaction of this factor with shoot

Table 2.2 Effect of node position within single-stemmed plants of *Metrosideros excelsa* 'Scarlet Pimpernel' on optical (abaxial and adaxial) and dimensional (adaxial) leaf parameters, and means for corresponding parameters in mature leaves. Means bearing the same superscript are not significantly different ($p<0.05$).

Parameter	p	Nodes 6-7	Nodes 16-17	nodes 26-27	Nodes 36-37	Nodes 46-47	Matures leaves
Abaxial hue (units)	0.0001	63.62^{a}	63.19^{a}	63.37^{a}	65.53^{b}	68.95^{c}	74.23
Adaxial hue (units)	n.s.	.	.	.	.	.	.
Abaxial saturation (%)	0.0001	13.02^{b}	15.49^{c}	15.83^{c}	12.44^{b}	9.11^{a}	6.21
Adaxial saturation (%)	0.0001	6.92^{a}	7.10^{a}	7.07^{a}	7.19^{a}	8.36^{b}	14.1
Abaxial lightness (%)	0.0001	35.91^{c}	34.90^{a}	35.69^{ab}	38.08^{c}	39.58^{d}	50.18
Adaxial lightness (%)	0.02	30.09^{c}	27.2^{b}	26.35^{a}	25.93^{a}	27.46^{b}	28.77
Width (mm)	0.0001	13.97^{a}	27.23^{b}	33.62^{c}	34.94^{c}	34.72^{c}	34.65
Length/width ratio	0.0001	2.21^{d}	2.01^{c}	1.57^{b}	1.31^{a}	1.25^{a}	1.15
Length (mm)	0.0001	30.63^{a}	54.36^{c}	51.65^{c}	45.03^{b}	43.07^{b}	39.62
Area (mm^2)	0.0001	323^{a}	1055^{b}	1219^{d}	1142^{bcd}	1108^{bc}	1045
Perimeter (mm)	0.0001	73.57^{a}	132.1^{bd}	138.8^{dc}	131.7^{bc}	127.6^{b}	124.6
Roundness	0.0001	17.98^{e}	16.93^{d}	15.93^{c}	15.26^{b}	14.72^{a}	15.06

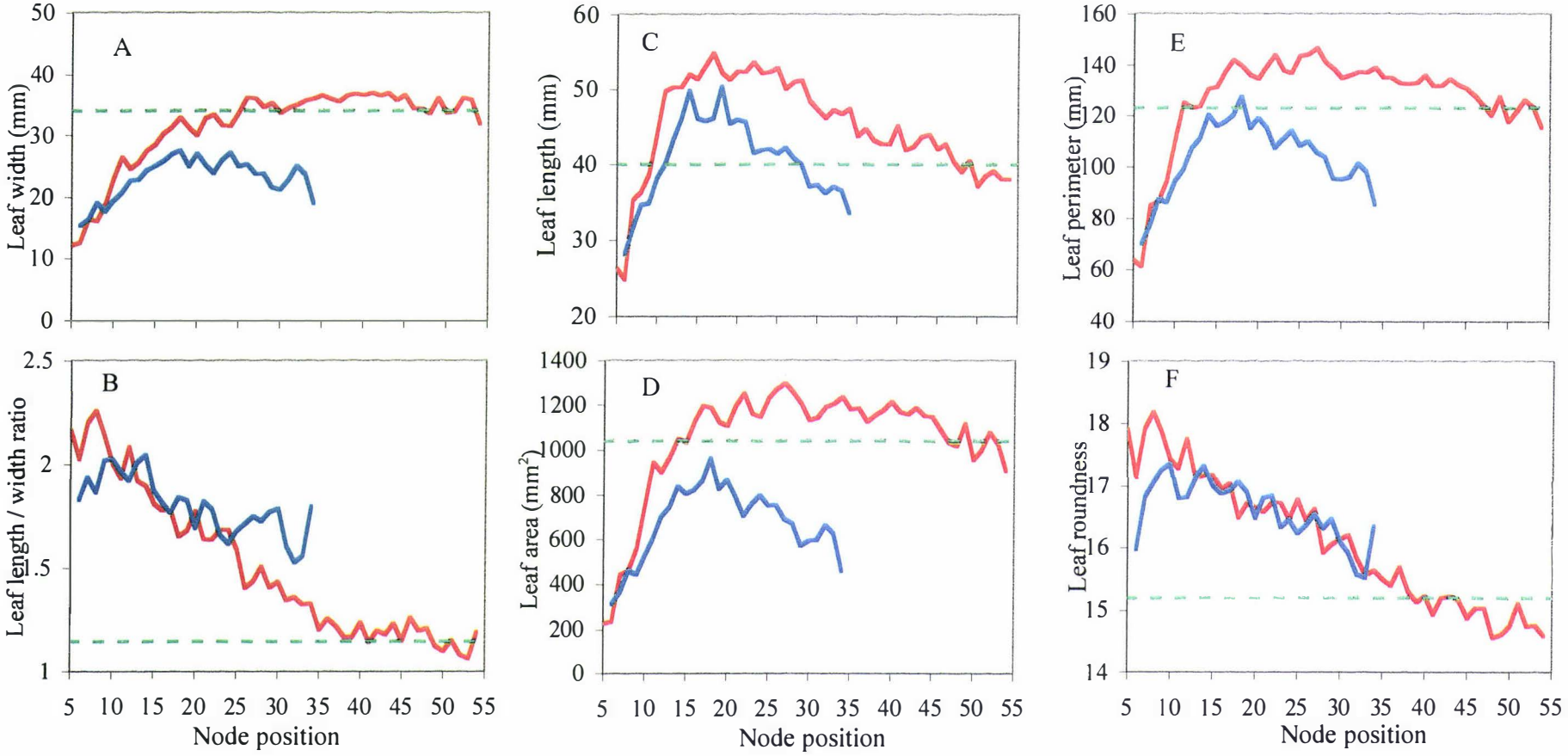

Figure 2.3 Mean dimensional changes with increasing node position for the abaxial leaf surface in single-stemmed plants (red line) and branched plants (blue lines) of *Metrosideros excelsa* 'Scarlet Pimpernel' grown in 2.7 l containers. A: with. B: length/width ratio. C: length. D: area. E: perimeter. F: roundness. Green dotted lines show the corresonding means for adult leaves.

restriction for shape and size parameters at the two node positions compared statistically.

There were highly significant effects ($P<0.0001$) of node position on shape and size parameters in single-stemmed plants when means at six locations ranging from nodes 6-7 to nodes 46-47 were compared (Table 2.2). As with the colour parameters, the means were consistent with the whole shoot trends shown by plants grown in the 2.7 L containers (Figure 2.3 A-F). There were no significant effects of root restriction or interaction between root restriction and node position for these parameters.

Comparing development at a linear shoot distance of 280 mm above the growing medium indicated no significant difference between leaves on single-stemmed and branched plants for any dimensional parameter. However, leaves of single-stemmed plants had higher hue, and lower saturation and lightness than branched plants (62.1 and 58.2, 14.7% and 17.1%, 35.5% and 41.5%, respectively).

2.3.4 Dry weight accumulation

Single-stemmed plants were significantly smaller than branched plants ($P<0.0001$) with respect to root dry weight (Figure 2.4 A), total leaf area (7.00 and 34.00 dm^2), leaf dry weight (16.3 and 61.0 g), stem dry weight (12.2 and 22.0 g) and total shoot dry weight (28.5 and 83.0 g). The most marked effect of container size was on the growth of roots in branched plants. Restricting roots caused a significant decline in root dry weight in these plants (Figure 2.4 A). However, an effect of container size on root dry weight was not seen in the single-stemmed plants (Figure 2.4 A). Container size did not have a significant effect on leaf area, leaf dry weight, stem dry weight or total shoot dry weight in branched or single-stemmed plants.

There was a significant interaction ($P<0.01$) between container size and shoot restriction treatments for shoot/root dry weight ratio. In both branched and single-stemmed plants shoot/root dry weight ratio increased as container size decreased, although the response to decreasing container size was more marked for the branched plants (Figure 2.4 B). Whereas the increase in shoot/root ratio in branched plants was due to the reduction in root dry weight with decreasing container size (Figure 2.4 A), that for the single-

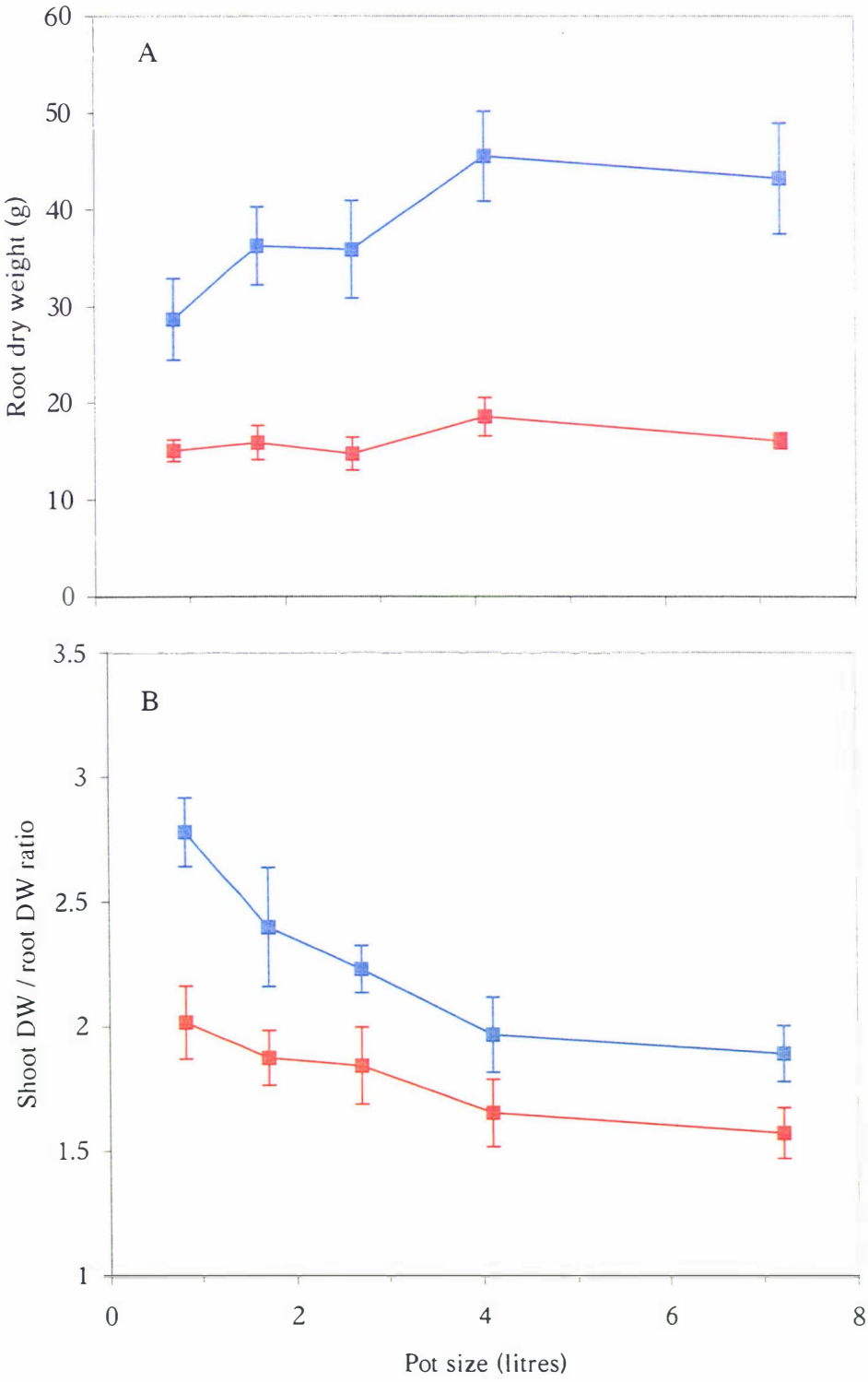

Figure 2.4 Effects of container size on growth of single-stemmed (red lines) and branched plants (blue lines) of *Metrosideros excelsa* 'Scarlet Pimpernel'. A: final root dry weight (mean ± SE). B: shoot dry weight/root dry weight ratio (mean ± SE).

stemmed plants was a result largely of a consistent (but non-significant) increasing trend in stem dry weight as container size decreased ($P>0.05$).

2.3.5 Leaf mineral concentrations

Leaves of branched plants contained significantly lower concentrations of most major nutrients (N, P, K, Ca and Mg) than single-stemmed plants (20-50% less); microelements Fe, Mn, Zn and B were also present at lower concentrations by 20-55% (Table 2.3). There was no interaction between shoot and root restriction treatments for these minerals, and no main effect of root restriction. The only main effect of root restriction was to significantly increase leaf Ca concentration ($P<0.0001$) in plants grown in the smallest pot size compared to those in the largest pot size (1.12% and 0.79%, respectively). There were also significant interactions between root and shoot restriction treatments for S and Na.

2.3.6 Carbon isotope discrimination

Carbon isotope discrimination (Δ) values differed between branched and single-stemmed plants, being significantly lower in single-stemmed than branched plants at nodes 27 and 37 ($P<0.01$) (Figure 2.5). Comparisons between plant types were not possible above node 37 since shoots in branched plants did not exceed this nodal position. There was also a significant main effect of node position ($P<0.0001$) but no interaction of plant type and node position on leaf Δ levels. In both single-stemmed and branched plants, mean leaf Δ values showed a diverging pattern away from those exhibited by adult plants, although values within branched plants did not differ significantly. In single-stemmed plants, a comparison of mean Δ levels at node position 7 with those at position 27 were significant ($P<0.05$), whereas comparisons with higher node positions showed an increase in significance (above node position 37, $P<0.0001$).

Table 2.3 Mineral nutrient concentrations in the leaves of single-stemmed and branched plants of *Metrosideros excelsa* 'Scarlet Pimpernel' grown in 0.82 and 7.2 l containers.

Shoot restriction Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe mg kg^{-1}	Mn mg kg^{-1}	Zn mg kg^{-1}	Cu mg kg^{-1}	B mg kg^{-1}
Branched	0.71± 0.05	0.07± 0.00	0.86± 0.04	0.79± 0.05	0.23± 0.01	25.5± 2.3	59.1± 2.1	7.0 ±0.5	3.75	18.8± 0.9
Single-stemmed	1.39± 0.06	0.11± 0.00	0.96± 0.02	1.12± 0.09	0.33± 0.01	35.0± 4.7	131.1± 6.4	10.5± 0.3	3.38	24.3± 0.5
P-value	0.0001	0.0001	0.046	0.0001	0.0001	0.04	0.0001	0.001	n.s.	0.001

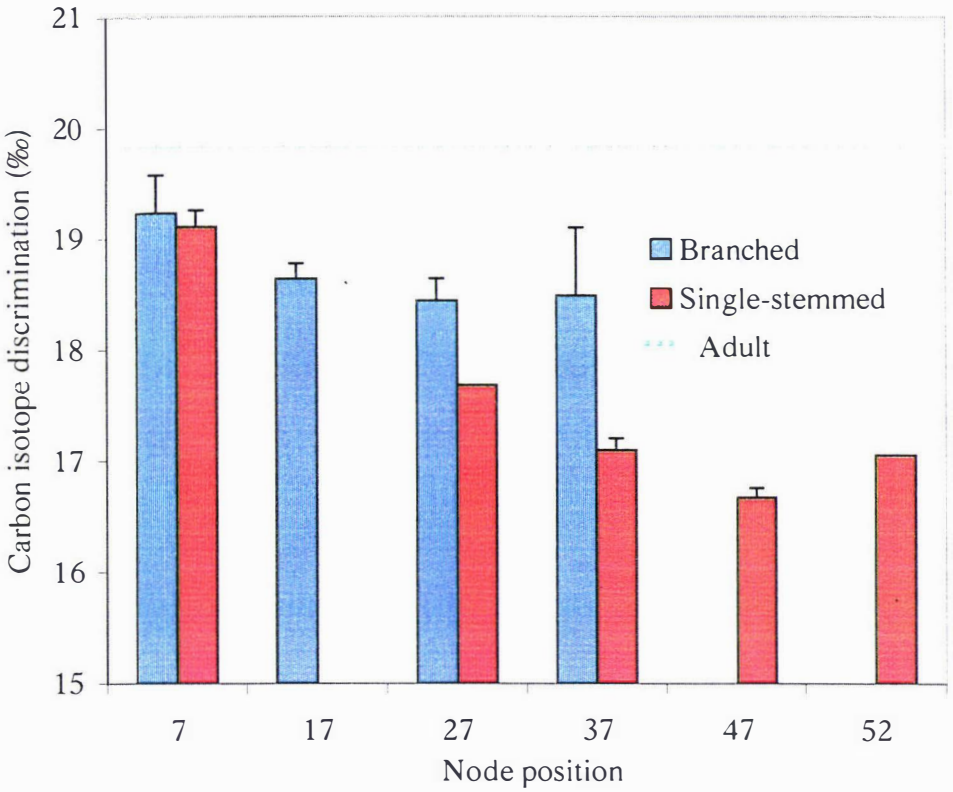

Figure 2.5 Mean carbon isotope discrimination values for leaves collected at different nodes for single-stemmed and branched plants and from the canopy top of adult plants (dashed line) of *Metrosideros excelsa* 'Scarlet Pimpernel' at the end of the experimental period.

2.4 Discussion

The hypothesis that restriction of shoots to a single-stem accelerated vegetative phase change was supported by this study, whilst root restriction appeared to have no significant effect. The progressive transition in single-stemmed plants of *Metrosideros excelsa* from juvenile to adult phenology for several optical and dimensional leaf parameters in typified homoblastic development (Goebel 1900). It was remarkable that leaves at a particular node position in branched plants did not display the same level of ontogenetic development as those in single-stemmed plants. Most notable of these features, was the progressive acquirement of a downy tomentum in single-stemmed plants evident by the increasing whiteness (less shade) or 'mealiness' in the abaxial leaf surfaces. In contrast, leaf optical parameters in branched plants above nodes 15-20 revealed a divergence in values for leaf saturation and hue away from those expressed in adult leaves.

The use of an image analysis procedure provided an effective and novel method for quantifying the level of ontogenetic development with ascending node position. Prior to this study, there had been no reports in which homoblastic behaviour had been fully characterised. Therefore, this protocol was particularly suitable for this homoblastic species where a gradual and subtle transition between juvenile and adult leaf forms occur. This is in contrast to a considerable number of native New Zealand species or certain closely related genera (e.g. *Eucalyptus*) where heteroblastic leaf development is exhibited and phenotypic expression of leaf morphological features from different life-stages are discernible (Day *et al.* 1997, James and Bell 2001).

The employment of a shoot restriction regime to accelerate phase change was consistent with findings in other woody species (Davis 1991, Snowball *et al.* 1994). Davis (1991), for example, reported a decrease in the length of the juvenile period in seed grown plants of *Actinidia deliciosa* (kiwifruit) following application of a shoot restriction regime. Similarly, phase change occurred with increasing node position in single-stemmed plants of *Citrus* (Snowball *et al.* 1994). In both these studies, however, phase change was assessed based on the ability of plants to flower and not on a quantification of the progressive change in vegetative characters exhibited along the shoot axis. Nonetheless, the increase in shoot length and accumulation of nodes with increasing

distance from the medium surface has also been reported to be a determinant of phase change in other woody species such as *Solanum aviculare* (James and Mantell 1997) and *Elaeocarpus hookerianus* (Day *et al.* 1997). This is in contrast to *Metrosideros* where changes in leaf parameters are less abrupt between node positions.

A common report with regards to juvenility in woody plants is that a minimum size (number of nodes) must be achieved before the juvenile phase is complete (Davis 1991, Snowball *et al.* 1994). In single-stemmed plants of *M. excelsa* the acquisition of adult leaf characteristics based on values for optical parameters were evident in leaves above node 50 where values were similar to those of adult leaves. Similar node heights were also required for stabilisation of most morphological parameters on par with values recorded in adult leaves. Thus, the requirement of vigorous growth for attainment of adult characteristics does not appear to be a requirement for ontogenetic development in *M. excelsa.* Interestingly, the size of the plant (linear distance from roots to shoot apex) also appeared to have little effect on vegetative phase change between single-stemmed and branched plants. Although leaf dimensional parameters did not differ between both sets of plants when measured at 280 mm, there were significant differences in colour parameters. Differences in morphological parameters, however, were further evident at higher node positions (or greater distance from roots) indicating a divergence in the two sets of plants rather than parallel development.

In contrast to single-stemmed plants, most leaf morphological parameters in branched plants displayed a divergence away from values expressed in adult leaves, despite having a greater leaf, stem and root mass than that of single-stemmed plants. While single-stemmed plants showed an increasing similarity in leaf properties with adult plants as node position increased, branched plant appeared to show the opposite pattern. This pattern of development was typified by a reversion in colour (decrease in hue: yellowing) and in most size and shape morphological parameters above nodes 15-20 towards values exhibited at lower nodes (nodes 5-10). Although shading in this experiment was kept to a minimum, it is possible that the reversion in these qualities was attributed to the environment in which the developing leaves were being formed. Increasing lateral growth and the successive formation of developing leaves along these lateral branches might cause a self-shading response on more plastic juvenile leaves (Poethig 1997, Day *et al.* 1998). Tsai *et al.* (1997) showed that the level of source-

strength in leaves of tobacco was a determinant of the level ontogenetic development achieved. Therefore, a relative increase in the source-strength in the less shaded leaves of single-stemmed plants may have been adequate for developmental phase change to occur.

Interestingly, container size did not appear to have an effect on leaf parameters in single-stemmed plants. Since root dry mass was significantly lower in single-stemmed plants compared to branched plants, an effect of container size would have been more pronounced in the latter plant type. Thus, if root restriction is associated with acceleration in vegetative phase change, then this phenomenon would have been expected to be marked in branched plants. This treatment, therefore, did not advance vegetative phase change in *M. excelsa*, although it may have an effect on the reproductive behaviour of adult plants (Zimmerman 1972).

Confinement of roots to small rooting volumes can affect plant performance (Webster *et al.* 1997). Moisture and nutrient stress are often associated with a reduction in rooting volume (Webster *et al.* 1997), although it is unlikely that either factor had an effect on plant growth in this study. This is in light of the fact that plants were irrigated with a more than sufficient level of water (four times daily for 15 minutes) and supplied at the start of the experiment with a standard nine-month slow-release fertiliser. Moreover, although leaves analysed from branched plants at the end of the experiment indicated a lower leaf nutrient concentration compared with single-stemmed plants, the absence of an effect of container size on shoot growth parameters and an associated reduction in shoot/root biomass and root dry weight confirmed that changes in root biomass were a result of root restriction, and not of moisture or nutrient stress associated with a small container size. Furthermore, although leaf mineral concentrations required for growth have not been reported in *Metrosideros*, comparable values regarded as adequate for growth have been reported in the closely related genera *Leptospermum* and *Psidium* (Reuter and Robinson 1986).

An examination of Δ values in leaves of single-stemmed plant showed a strong divergent pattern with increasing node position away from values expressed at lower node positions and away from those of adult plant leaves. This is in spite of the fact that leaves in single-stemmed plants were becoming increasingly more adult with increasing

node position. The use of stable isotopes from plant tissue can provide a long-term indication of plant performance (Farquhar *et al.* 1989), and has been strongly correlated with leaf gas exchange components (e.g. stomatal conductance) and features limiting carbon acquisition (Farquhar *et al.* 1989, Ehleringer *et al.* 1993, Sparks and Ehleringer 1997). Intra-specific differences in Δ values can vary between juvenile and adult plants, as previously acknowledged to occur in *M. polymorpha* (Cordell *et al.* 1998) and various other woody species (Donovan and Ehleringer 1991, 1994). However, the decline in leaf Δ values in single-stemmed plants away from those exhibited in adult plants appear anomalous, particularly since these leaves approach leaf characters exhibited by adult plants with increasing node position. It is possible that this phenomenon occurs only during phase change, and once a critical number of nodes have been attained, Δ values may return to similar values exhibited in leaves of adult plants. If this is the case, then Δ may be a reliable indicator of the level of phase change in *Metrosideros*. This supposition is evaluated further in Chapter 3, and discussed in relation to leaf gas-exchange processes during ontogenetic development in single-stemmed plants of *M. excelsa.*

In conclusion, this study supports the hypothesis that a shoot restriction regime effectively accelerates vegetative phase change in micropropagated plants of *Metrosideros excelsa.* As acknowledged by several authors (Zimmerman *et al.* 1985), the attainment of foliage in single-stemmed that phenotypically resembles that of adult leaves does not imply a competence to flower. However, exposure to inductive treatments which might include the addition of plant growth regulators (e.g. inhibitors of gibberellin biosynthesis) (Clemens *et al.* 1995), could be expected to be successful once a suite of vegetative characteristics that comprise the adult phase has been attained.

Chapter 3

Gas-exchange and carbon isotope discrimination characteristics during phase change in *Metrosideros excelsa*

3.1 Introduction

In woody perennials, developmental phase change from a juvenile to an adult vegetative state is frequently accompanied by changes in morphological and physiological properties of leaves (Hackett 1985, Poethig 1990, Greenwood 1995, Day *et al.* 1997). Morphologically, this can encompass variations in leaf dimensions between ontogenetic phases (James and Bell 1996). Anatomical features such as variation in leaf thickness, and trichome and stomatal properties can also vary between juvenile and adults leaves (Day *et al.* 1997, James *et al.* 1999, James and Bell 2000a). These factors, in turn, can influence gas exchange parameters within a leaf, such as limiting the diffusivity of external CO_2 into leaf tissue and affecting the loss of water vapour into the atmosphere (Choinski and Wise 1999, Niinemets *et al.* 1999, James and Bell 2000b). Thus, gas exchange relations are likely to be influenced by alterations to surface (tomentum), stomata and the intercellular properties associated with different phase-dependent states.

Numerous plant ecology and physiology studies have employed the use of stable isotopes for assessing plant performance (Farquhar *et al.* 1989, Donovan and Ehleringer 1991, 1994; Poss *et al.* 2000). Isotopic discrimination of ^{13}C relative to ^{12}C in plant material reflects the differential diffusivity and internal fractionation of CO_2 and, thus, has been correlated with a diversity of gas exchange components, including leaf conductance, transpiration, photosynthetic capacity and water-use efficiency (Farquhar *et al.* 1989, Ehleringer *et al.* 1993, Sparks and Ehleringer 1997). In many woody plant species, intra-specific differences in carbon isotope discrimination (Δ) values can vary between juvenile and adult plants (Donovan and Ehleringer 1991, 1994; Cordell *et al.* 1998). In these and other related studies, Δ is often reported to be higher in juvenile than in adult leaf tissue. Often associated with the higher leaf Δ levels in juvenile plants is an increase in several photosynthetic parameters such stomatal conductance, photosynthetic rate and stomatal transpiration, and an overall decrease in plant water-

use efficiency (WUE) (Hansen 1996, Cordell *et al.* 1998). However, comparisons of plants at different ontogenetic phases have not been conducted under unstressed conditions and controlled environments, but rather under field and/or common garden conditions, where interpretation of findings can be confounded by the prevailing environmental conditions (Donovan and Ehleringer 1991, Hansen 1996, Cordell *et al.* 1998).

The homoblastic species, *Metrosideros excelsa*, exhibits a gradual transition between juvenile and adult vegetative characteristics during phase change (Cockayne 1928, Dawson 1968). The most notable characteristic of the adult phase is the acquisition of rounder leaves with a downy (hairy) tomentum on leaf abaxial surfaces, which is otherwise lacking in juvenile plants (Dawson 1968). Previously, vegetative phase change in *M. excelsa* 'Scarlet Pimpernel' was characterised using leaf shape/dimension and optical parameters to define ontogenetic ageing (Chapter 2). Additionally, significant differences in Δ were found between ontogenetic states in *M. excelsa* as evidenced by leaf Δ values in juvenile plants undergoing vegetative phase change (along single-stemmed plants) diverging away from values exhibited by adult plants bearing leaves with adult morphology (Chapter 2). A similar response was observed for seedlings and plantlets of *M. excelsa* 'Scarlet Pimpernel', which exhibited adult leaf morphology at the end of four months when grown under a controlled temperature regime of 24/16°C (day/night) (M. Sismilich, P.E. Jameson and J. Clemens, unpublished data). A decline in leaf Δ values in juvenile plants undergoing phase change suggests an overall decrease in leaf stomatal conductance and an increase in WUE with increasing node position (Donovan and Ehleringer 1994, Hansen 1996, Cordell *et al.* 1998, Rundel *et al.* 1999). However, characterisation of physiological markers that may typify a phase-dependent state, e.g. photosynthesis, stomatal conductance and transpiration, have not been reported in *M. excelsa*. Moreover, whether or not the divergent pattern in Δ can serve as a reliable physiological marker of phase change merits further investigation.

To account for these observations it is hypothesised that:

1) Juvenile plants of *M. excelsa* undergoing vegetative phase change exhibit decreasing Δ because of changes in leaf morphology consistent with greater water use efficiency, notably more tomentose and rounder leaves. This may also be associated with a reduction in photosynthetic capacity with increasing node position.

In addition, with regards to leaf age, interpretation of leaf Δ data collected at the end of an experimental period would be limited, considering that leaves harvested along the axis of a single-stemmed plant at one point in time would be of different ages. Thus, an effect of age would be confounded with that of phase change. Therefore, an analysis of Δ for leaves of the same age collected from given node positions at different times during an experimental period would provide evidence complementary to analysis of leaves collected at one point in time. Therefore, it was further hypothesised that:

2) Changes in Δ are not a function of leaf age, but are the result of changes in leaf conductance relative to photosynthetic capacity at the time of leaf expansion.

To test these hypotheses, juvenile micropropagated plantlets of one cultivar of *M. excelsa* were grown as single-stemmed plants in order to accelerate vegetative phase change. Morphological and physiological changes in single-stemmed plants undergoing vegetative phase change were assessed using a complementary image analyses technique, and instantaneous (leaf gas exchange) and long-term integrated measurements (leaf Δ) of photosynthetic capacity conducted under controlled conditions on three sampling times. Comparative results were assessed against leaves from micropropagated plantlets that were allowed to branch freely (same genotypic population as single-stemmed plantlets), and adult leaves of >3 year-old plants of the same cultivar.

3.2 Material and Methods

3.2.1 Plant materials

Plants of *Metrosideros excelsa* 'Vibrance' were obtained at two developmental stages. Adult plants (approximately 1.5 m in height) supplied by Joy Plants, Hamilton, in 6 l bags in August 1998 were transplanted into 30 l containers. Sixty micropropagated plantlets were obtained from Lyndale Nurseries Ltd., Auckland, in 50 mm square plastic pots in April 2000 in a rejuvenated state, six months from exflasking. Plantlets were transplanted into 7 l containers in early June, 2000. The potting medium consisted of a mixture of peat and pumice (80:20 v/v) supplemented with a control release fertiliser (4.0 g of 8-9 month release Osmocote Plus Grace Sierra, Heerlen, The Netherlands). Plants were supplied twice daily with overhead irrigation and maintained under ambient outdoor conditions at the Plant Growth Unit (PGU), Massey University, Palmerston North.

3.2.2 Greenhouse environment

Adult and micropropagated plants were transferred in mid-June 2000, from outdoor (mid-winter) conditions into a heated greenhouse for the experimental duration. During this time, the greenhouse was vented at 26°C and warmed at night, when required, to ensure the minimum temperature was no lower than 14°C. Temperatures were monitored regularly using a Hobo shuttle logger (model H8, Scott Technical Instruments, Hamilton, New Zealand) to ensure that temperatures remained within the respective range. Photoperiod was extended to 14 h during the winter months (June – September) using overhead 100 W incandescent lights (~10 μmol m^{-2} s^{-1}). Plants were watered twice daily to container capacity using an automatic irrigation system with one microtube hose per plant container stemming from a central hose line. Four irrigation microtube hoses were fitted to containers holding adult plants.

3.2.3 Shoot treatments applied to greenhouse-grown plantlets

In the third week after transference to the greenhouse (18 June 2000), pruning treatments were applied to micropropagated plantlets. In half of the plantlets, all side branches were removed and the tallest shoot was trained in an upright position and allowed to grow unchecked. Branch development in these plants (referred to as single-stemmed plants) was prevented by pinching of axillary buds as these swelled before bud break. In the remaining plantlets (branched plants), the main stem was pinched once at the apical meristem region at approximately node 6-8, and commencement of axillary branching was not restricted in any way. Plants were randomly assigned to one of five blocks within the greenhouse based on a completely randomised block design. Within each block, there were six replicates each of branched and single-stemmed plants and two replicate adult plants. The experiment was conducted over a 10 month period. On three occasions, a random subset of plants per block was transferred to a controlled environment room for approximately 9 days for leaf gas-exchange measurements (described in Section 3.2.6) before transference back to the greenhouse. Diurnal leaf gas exchange measurements were also conducted at the end of the experimental period under ambient greenhouse conditions.

3.2.4 Measurements made on greenhouse plants

The height and the number of accumulated nodes was measured on micropropagated plants every 14 d. Shoot length was measured based on the linear distance from the medium surface to the shoot tip of the longest shoot. Measurements were also taken on the diameter of expanding buds in adult plants (10 replicate buds per plant) through to bud break (parting of the fourth pair of scales/bracts) and during elongation of the subsequent shoot (shoot length and node number).

Before bud break (approximately 6 weeks after the start of the experiment), an additional ten buds from four bud size classes (<4.0, 4-5.0, 5.1-6.0 and 6.1-7.0 mm) were randomly harvested from adult plants. Buds were excised with a razor blade and individually transferred to 1 ml Eppendorf tubes containing a 90% formalin, 5% acetic acid and 5% ethyl alcohol (FAA) fixative solution. The protocol for histological preparation of plant tissue follow Johansen (1940). Bud samples were placed under

vacuum for 48 h before being washed three times in 70% ethanol solution over 2 days. Samples were transferred to a wax infiltration procedure (described in Appendix I) before being finely sectioned (12 µm) on a microtome (Leica RM2145, Germany). After sections were transferred onto glass slides and allowed to dry (48 h), they were transferred through a safranin and fastgreen staining series (Appendix II) before viewing under a light microscope.

3.2.5 Controlled environment

Gas exchange measurements were conducted in a controlled environment room at the New Zealand Controlled Environment Laboratory, The Horticulture and Food Research Institute of New Zealand (HortResearch), Palmerston North. The environmental conditions within the controlled environment room were kept constant and were the same during each experimental period. Lighting in the growth room was provided by a water-screened array of four high intensity main 1 kW Metalarc lamps and four 1 kW tungsten halogen lamps (12 h) providing a photosynthetic photon flux (PPF) of 700 µmol m^{-2} s^{-1}. A photoperiod extension of 1 h was provided by four low intensity 150 W Tungsten auxillary lamps with a PPF of 7 µmol m^{-2} s^{-1}. Thermoperiod coincided with photoperiod. Day/night air temperature and relative humidity within the environment room were maintained at 24/19°C and 87/86%, respectively. This corresponded to a vapour pressure deficit of 0.4/0.3 kPa. CO_2 concentrations were maintained at approximately 350 ppm.

Within the room, plants were placed on one of two trolleys holding either three adult plants or 12 branched and single-stemmed plantlets (6 replicates per pruning type). Micropropagated plantlets were elevated to 1.5 m in height corresponding to the canopy height of adult plants to ensure an even exposure to environmental conditions within the room. Each trolley and individual plants on a trolley were rotated daily to ensure an even light distribution over time and to reduce any positional effects created by the room.

3.2.6 Gas exchange measurements in the controlled environment

Leaf gas exchange measurements were conducted within the controlled environment using an open photosynthetic system (LI6400, Li-Cor, Lincoln, Neb. USA). Gas exchange measurements were conducted on three separate occasions, occurring on 22–30 September, 1–9 December 2000, and 23 February–3 March 2001, and referred to as 'September', 'December' and 'March', respectively. During each sampling date, gas exchange measurements on all plants were conducted after an initial 48 h acclimatisation period. Leaf measurements were conducted on newly emerged (<2 months) but representative, fully expanded, whole leaves. In adult plants, this included leaves sampled from the top portion of the canopy. In plantlets, gas exchange measurements for respective sampling times, September, December and March, corresponded with new fully-expanded leaves for node position 10, 30 and 40 in single-stemmed plants, and node position 10, 20 and 30 in branched plants.

3.2.6.1 Light response curves

Leaf photosynthetic light response curves were analysed according to Greer and Halligan (2001). The selected leaf was placed in the Li-Cor Infra-Red Gas Analyser (IRGA) chamber. Chamber light intensity was provided by a Licor LED lamp. Chamber CO_2 was supplied via the LI 6400 CO_2 injection and scrubbing system and controlled at a concentration of 400 μmol CO_2 mol^{-1} at an air flow rate of 500 μmol s^{-1}. Photosynthesis was measured at 22°C starting at an initial PPF of 600 μmol m^{-2} s^{-1}. Once photosynthesis had stabilised, PPF was increased to 1200 μmol m^{-2} s^{-1}. The rate of photosynthesis (Ps) was then recorded in 10 steps of decreasing PPF until dark, when respiration was measured. Data were then analysed using a non-linear regression fitted to the following rectangular hyperbola equation:

$$Ps = (P_{max} * \text{Tanh} (PPF* \varphi_{app}/ P_{max})) - Rs$$

where, Tanh represents the hyperbolic tan function, φ_{app} the apparent photon yield, P_{max} the light saturated maximum rate of photosynthesis, and Rs the rate of respiration. To determine the maximum photosynthetic photon flux at 99% light saturation (PPF_{sat}), the

following equation was used, where Atanh represented the inverse hyperbolic tan function:

$PPF_{sat} = Atanh\ (0.99) * P_{max} / \varphi_{app}$

Gas exchange measurements from the light response curves included stomatal conductance, transpiration rate and intercellular CO_2 concentration. From these measurements, a measure of instantaneous water use efficiency (WUE) within the plant was calculated based on the difference in the maximum rate of CO_2 uptake per unit of transpired water.

3.2.6.2 CO_2 assimilation / intercellular CO_2 (A/Ci) curves

Leaf photosynthetic measurements were conducted on the same equipment described above for obtaining the light response curves. The selected leaf was placed in the leaf chamber where light intensity was provided by the LED system and the PPF was maintained at a constant 600 μmol m^{-2} s^{-1} and an air flow rate held at 500 μmol s^{-1}. Photosynthesis was measured at 22°C starting at an initial CO_2 concentration of 400 μmol CO_2 mol^{-1}. When photosynthesis had stabilised, the CO_2 concentration was decreased to 50 μmol CO_2 mol^{-1} and increased over nine steps to 900 μmol CO_2 mol^{-1} during which time photosynthesis was recorded. Based on the A/Ci response curves, biochemically based equations describing the potential limits to photosynthetic capacity such as the maximum Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) carboxylation rate (V_{cmax}) and the maximum rate of RuBP (ribulose-1,5-bisphosphate) regeneration mediated by electron transport (J_{max}) were calculated, according to the method of Walcroft *et al.* (1997).

3.2.7 Leaf image analysis

Image analyses were conducted on detached leaf samples harvested after each gas exchange sampling time, including additional samples taken from plants within the greenhouse. A total of ten leaf samples were collected from each plant type (one

representative replicate leaf per plant). Leaf samples corresponded to the node position examined during a given gas exchange sampling date.

Each leaf was cut from the plant at the base of the petiole and transported immediately in distilled water to the Image Analysis laboratory, Massey University. Petiole length was measured and excised before each leaf was imaged. Images were captured for determination of leaf shape, size and optical properties of abaxial and adaxial surfaces following the methodology described in Chapter 2.

Morphological measurements were taken of the petiole length, basal angle and total leaf mass. Basal angle was measured with a protractor between the leaf margin and the mid-vein at the base of the leaf blade. The specific leaf area was determined within 2 h of collection and dry mass was determined after drying at 80°C for 48-72 h. Leaf specific mass was determined by dividing the leaf dry weight by leaf area (obtained from the image analysis procedure). Leaf water content (%) was expressed as a function of the difference in fresh and dry mass, and as a function of total leaf area.

3.2.8 Carbon isotope discrimination

Collection of tissue for determination of carbon isotope discrimination (Δ) occurred at four separate times during the experimental period. In the first harvest, leaf tissue samples were collected from adult plants over an eight week period (July-August 2000). Tissue samples included those of (a) fully-expanded old leaves (growth from previous season) which subtended the tight bud, (b) unexpanded leaves enclosed within a tight bud (mean 5.1 mm in diameter), followed by (c) partly and fully expanded young leaves harvested from the second node of a newly derived shoot. Mean length/width measurements of partly (~15 day old) and fully expanded (~ 32 day old) leaves were 45/20 mm and 62/29 mm, respectively. In the three subsequent harvests, dry leaf tissue for determination of Δ was obtained from all plant types. The leaf samples corresponded to the nodes examined following each gas exchange sampling date. The leaf tissues collected during all four harvests were dried at 80°C for 72 h. Subsequent processing and determination of Δ of the dried samples followed the protocol described in Section 2.2.7.

3.2.9 Gas exchange measurements under greenhouse conditions

The daily photosynthetic response of leaves under greenhouse conditions was characterised over two consecutive sunny (<10% cloud cover) days occurring on 20-21 March 2001. Gas exchange measurements were conducted on terminal leaves from three adult plants and on branched (node 30) and single-stem (node 40) micropropagated plants. Measurements were started at 0600 h and ended at 1800 h with observations recorded at 2 h intervals. Gas exchange measurements were conducted with a LI6400 IRGA attached to a leaf chamber equipped with a transparent leaf chamber cover. Measurements were conducted under ambient CO_2 temperature and irradiance conditions within the greenhouse.

3.2.10 Leaf carbohydrate analyses

Leaf samples were collected between 0600-0630 h on 25 June 2001, from the three sets of plants growing in the greenhouse for determination of soluble sugars (sucrose, fructose and glucose) and starch concentrations. Extraction and determination of soluble sugars and starch are described below. Leaves sampled included those that were fully-expanded from nodes 10 and 40 in single-stemmed plants, node 30 in branched plants and from the canopy top of adult plants. Leaf samples consisted of eight to 12 replicates per node or plant type. Carbohydrate measurements were conducted on a Hitachi U-2000 spectrophotometer using 1.5 ml curvettes containing the sample solution.

3.2.10.1 Soluble sugars extraction and determination

Each leaf was cut from the plant and immediately snap-frozen in liquid nitrogen and stored on wet ice in tin foil sachets. Samples were transferred to the laboratory and freeze dried for 72 h before being finely ground to a powder (<200 μm). Samples (40 mg dry weight) were extracted in 2 ml 62.5% (v/v) methanol in a water bath at 55°C for 1 h while being vortexed every 15 min. Samples were centrifuged for 5 min at 3000 rpm and the pellet was retained for starch analyses. Chlorophyll, polyphenolics and other interfering substances were precipitated out with the addition of 10 μl of saturated

lead acetate per 400 µl of supernatant (Haslemore and Roughan 1976). The solution was centrifuged (15 min at 3000 rpm) and the supernatant containing the soluble sugars was decanted and used in the assay. Soluble sugar concentrations (mol/g dry weight) were determined using Sucrose/D-fructose/D-glucose enzyme kits (Boehringer Mannheim Biochemicals).

a) Determination of D-glucose and D-fructose

A sample of supernatant (30 µl) was pipetted into a 1.5 ml curvette well containing 400 µl of milli-Q water and 152 µl of solution 2 (triethanolamine buffer, pH 7.6, NADP 2.4 mg/ml, ATP 5.8 mg/ml), lightly vortexed (5 sec) and incubated for 3 min at room temperature before obtaining an absorbance reading (A_1) at 340 nm. After adding 20 µl of a six-fold dilution of solution 3 (hexokinase and glucose-6-phosphate dehydrogenase) to the curvette, samples were lightly vortexed (5 sec) and kept at room temperature for 15 min to complete reactions before a second absorbance reading (A_2) was obtained at 340 nm. A six-fold dilution of solution 4 (20 µl) (phosphoglucose isomerase) was added and a final absorbance reading (A_3) was taken at 340 nm after 15 min at room temperature. A standard curve was constructed from which sample amounts were calculated. Determination of D-glucose concentration was calculated by $A_2 - A_1$ and D-fructose concentration from $A_3 - A_2$.

b) Determination of sucrose

A sample of supernatant (30 µl) was pipetted into a 1.5 ml curvette with 30 µl of Solution 1 (β-fructosidase). Sample solution was lightly vortexed (5 sec) and incubated for 15 min at 25°C. Solution 2 (152 µl) and 400 µl milli-Q water were added and lightly vortexed (5 sec). Absorbance (B_1) was obtained at 340 nm after incubating for 3 min at room temperature. A six-fold dilution of Solution 3 (20 µl) was added, and after 15 min, a second absorbance reading (B_2) was taken at 340 nm. Sample contents were determined from a standard curve. Sucrose concentrations were then calculated by $B_2 - B_1$.

3.2.10.2 Starch extraction and determination

The sample pellet from the soluble sugar extraction procedure was placed in a 15 ml falcon tube to which 0.5 ml 8M hydrochloric acid (HCL) and 2 ml dimethyl sulphoxide (DMSO) was added. The solution was vortexed until the pellet was resuspended. The sample solution was heated at 60°C for 1 h in a water bath while being vortexed every 15 min. After being cooled on ice for 5 min., 0.5 ml NaOH was added to the sample solution and then vortexed for 20 sec. The solution was made up to 10ml with sodium citrate buffer (pH 4) and the supernatant was clarified by centrifuging at 2600 rpm for 5 min before being transferred to a fresh 1.5 ml curvette in preparation for the assay. Starch concentration (mol/g dry weight) was determined using a starch enzyme kit (Boehringer Mannheim Biochemicals).

a) Determination of starch

A sample (50 µl) was pipetted into a 1.5 ml curvette and mixed with 40 µl of Solution 1 (amyloglucosidase). The curvette was incubated for 20 min at 60°C for 15 min before 200 µl of Solution 2 (triethanolamine buffer, pH 7.6, NADP 75 mg, 190 mg ATP and 300 µl of milli-Q water was added. The solution was lightly vortexed (5 sec) and after 3 min absorbances of the solution (A_1) were read at 340 nm. Solution 3 (20 µl) (hexokinase, glucose-6-phosphate dehydrogenase) was added to the solution and after completion of the reaction (15 min), absorbances of the solution (A_2) were read at 340 nm. The sample contents were determined from a standard curve. Starch concentrations were then calculated by $A_2 - A_1$.

3.2.11 Statistical Analyses

Data analysis was performed using the SAS statistical package (SAS Institute, Cary, N.C., USA). A repeated measure Analysis of Variance (ANOVA) was used to compare differences in plant height, node position and internode length, with the repeated factor being time. Statistical comparisons of leaf shape, size, colour, carbon isotope discrimination, morphological and photosynthetic parameters were conducted using a two-way ANOVA for comparisons of the main effects of time (session date) and plant

type. Comparison of leaf gas exchange parameters from different plant types measured in the greenhouse over a 12 h diurnal period was conducted using a one-way ANOVA, with comparison of plant types analysed within each 2 h interval. Differences between carbohydrate samples were analysed using a one-way ANOVA. In all analyses, differences between treatments were assessed using a Least Square Means (LSM) multiple comparison test. Where appropriate, data were log-transformed to normalise variances and means were compared for significant differences at the 5% level.

3.3 Results

3.3.1 Characterisation of shoot growth

The average bud diameter in adult plants at the start of the experiment (16 June 2000) was 5.1 mm. Mean bud size increased significantly with time ($P<0.0001$) at a rate of 0.5 mm per 14 d at mean greenhouse temperatures of 22/18°C (day/night) over the first eight weeks ($P<0.0001$). By week 8, 96 % of buds had broken as emerging vegetative shoots elongated. Shoot length and node number continued to increase significantly over the duration of the experiment ($P<0.0001$) (Figure 3.1 A and B).

There was a significant effect of shoot restriction treatment on the length and number of nodes produced in micropropagated plants over the experimental period ($P<0.0001$). Shoot length and number of accumulated nodes in single-stemmed plants was significantly greater than in branched plants after week 16 (shoot length) and week 4 (node number) ($P<0.05$) (Figure 3.1 A-B). By the end of the experimental period, shoots from single-stemmed plants were longer and bore a greater number of nodes (~98 cm and 42 nodes) than branched plants (~48 cm and 30 nodes).

In adult plants, the mean internode length (averaged across the entire stem length for each record) showed a cyclic pattern in shoot growth (Figure 3.1 C). The highest rates of internode elongation occurred between weeks 12–18 and 22–28, with intermittent periods of 6–8 weeks during which time internode length did not increase significantly (weeks 18–22, 29–36) ($P>0.05$). Observations of terminal shoot apices during the first intermittent period where internode length did not increase (week 18–22) corresponded

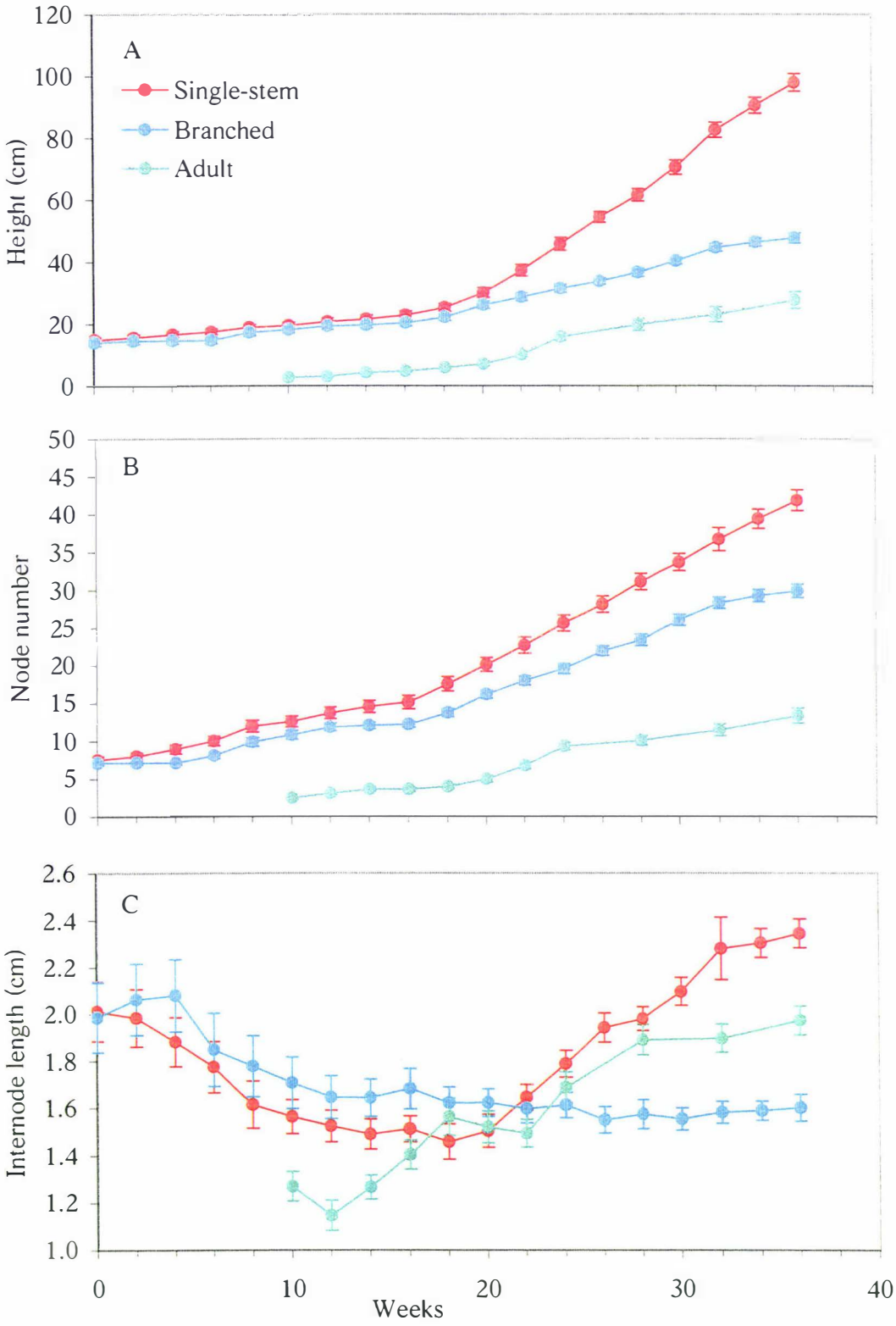

Figure 3.1 Changes in mean (±SE) plant height (A), node number (B) and internode length (C) of single-stemmed, branched and adult plants of *Metrosideros excelsa* 'Vibrance' over the experimental period.

to a time when a high percentage of terminal shoot apices had formed a 'resting bud' (77%), and a further 21% had aborted.

In micropropagated plants, there was a significant effect of time (week) on the mean internode length ($P<0.001$) (Figure 3.1 C). In branched plants, the overall mean internode length initially declined with increasing node position and remained relatively constant after week 8 (mean internode length ~1.6 cm between weeks 10-30). In single-stemmed plants, the overall mean internode length increased with each node attained after reaching node 20 (week 20), becoming increasingly longer than in branched plants after week 22 ($P< 0.05$). Additionally, internode growth in single-stemmed plants above node position 20 increased for the remainder of the experiment, with intermittent periods during which time internode length did not increase significantly (e.g. between weeks 22–26, 30–32) (Figure 3.1 C). A maximum mean internode length of 2.4 cm was recorded upon reaching 42 nodes.

3.3.2 Leaf development in adult plants

A histological examination of buds indicated that leaf meristems in quiescent buds from adult plants were produced continuously within the apical meristem region (Plate 3.1). There was a strong positive correlation of bud size (diameter) with the number of newly-formed pairs of leaves ($R^2 = 0.781$, $P < 0.0001$). Buds from the smallest diameter size class (<4.0 mm) averaged 0.8 pairs of leaves, whereas large buds (6.1–7.0 mm) averaged 8 pairs of leaves.

There was a significant difference in Δ of leaf tissue at different development stages of expansion ($P<0.001$) (Figure 3.2). Discrimination in leaf tissue was significantly higher in partly and fully expanded leaves (pooled mean 20.3‰) compared with bud tissue (18.6‰) ($P<0.05$). Isotope composition in older subtending leaves (previous growing season) did not differ from that of recently expanded leaves (both 50% and 100% expanded leaves) or newly formed bud tissue ($P>0.05$).

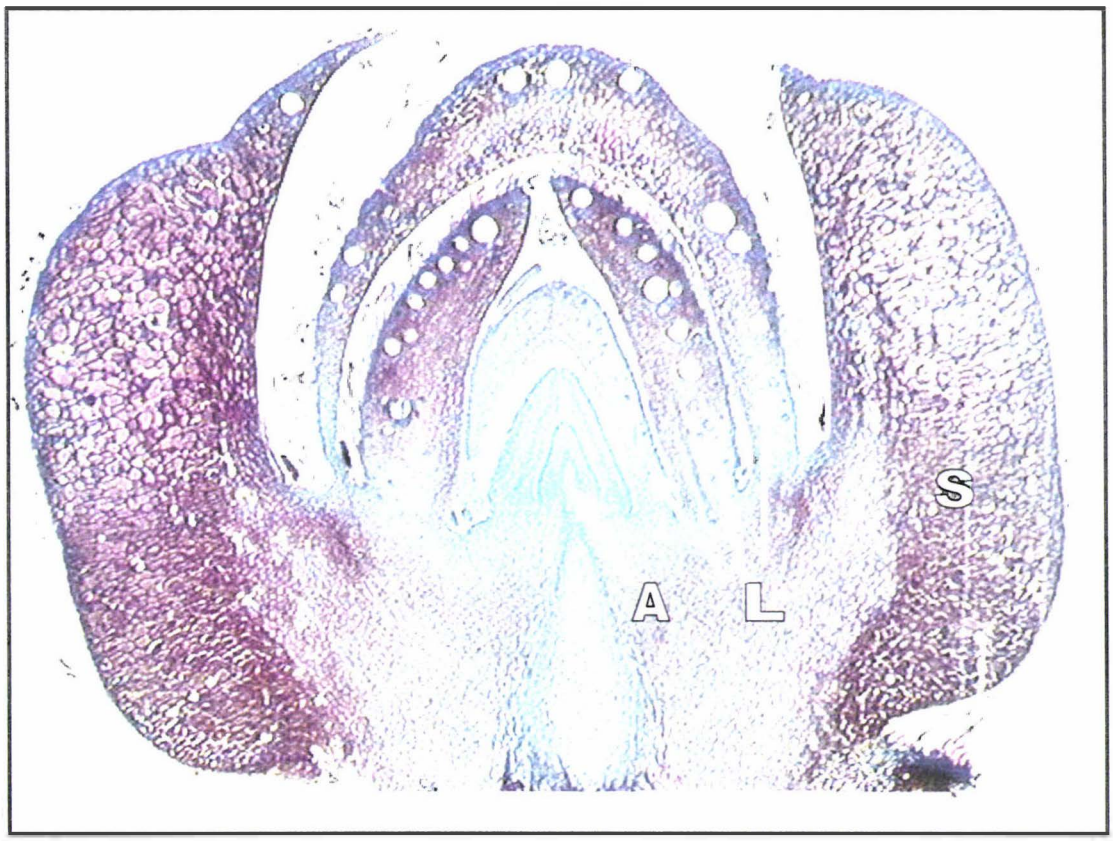

Plate 3.1 Cross-section of a vegetative bud (diameter before harvest: 4.2 mm) illustrating the continuous formation of new leaf primordia from the apical meristem. Bar = 500 μm. A = Apical meristem. L = Leaf / bract. S = Scale.

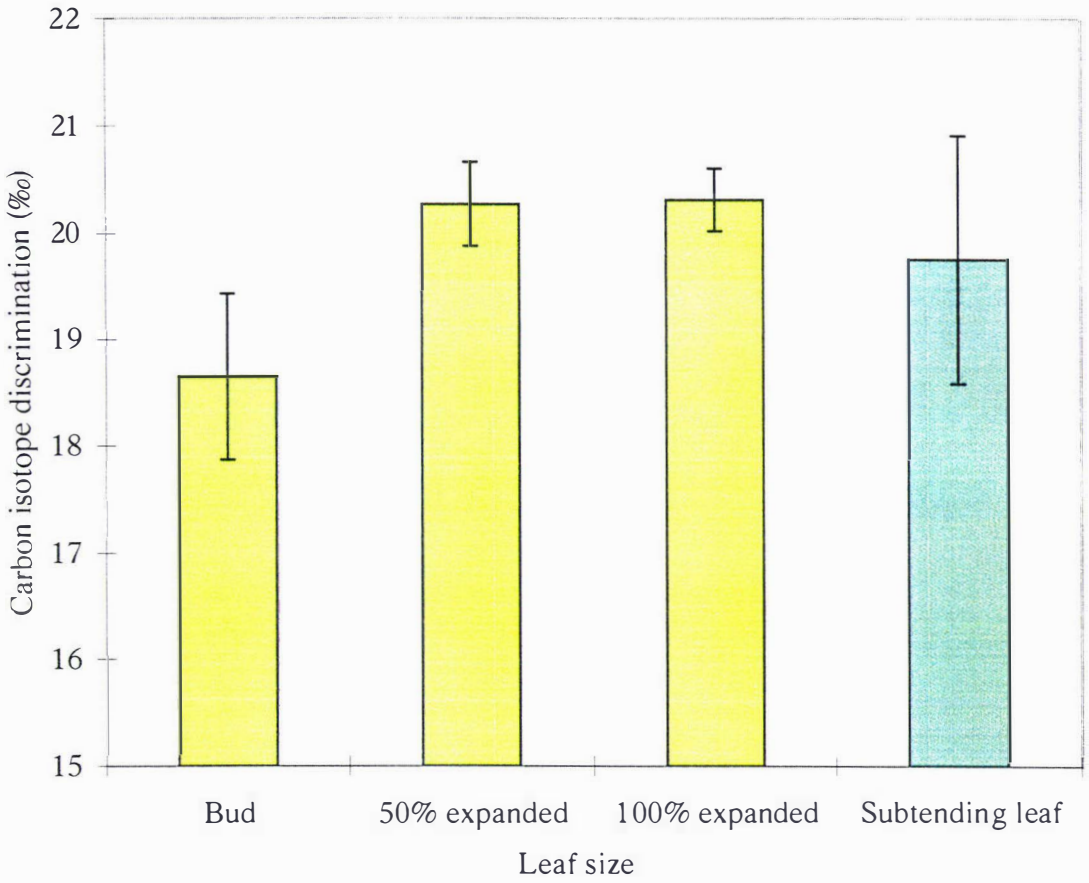

Figure 3.2 Difference in carbon isotope discrimination levels (mean ± SE) from bud and leaf tissue in adult plants of *Metrosideros excelsa* 'Vibrance' collected in July - August 2000. Tissue samples: tight bud, 50% and 100 % expanded leaves of current season's growth and subtending leaf from the previous season's growth.

3.3.3 Image analysis of leaf dimensional and optical properties

Imposition of a shoot restriction treatment on micropropagated plantlets of *Metrosideros excelsa* 'Vibrance' effectively accelerated vegetative phase change. Leaves in single-stemmed plants initially showed no significant differences from those in branched and adult plants in shape or size characteristics (node position 10 during September). At intermediate node positions (node position 30 during December), they tended to diverge away from leaf qualities expressed in juvenile as well as those of adult plants (Figure 3.3 A-F). This included a progression towards larger leaves, in terms of length, width, total leaf area and perimeter, with increasing node position ($P<0.0001$) (Figure 3.3 A-D). By node position 40 (March), however, leaves in single-stemmed plants began to approach qualities expressed in adult plants, resembling both the characteristic roundness and size parameters exhibited by adult leaves.

In contrast, branched plants in general did not differ significantly between sampling dates from adult plants with respect to leaf width and area ($P>0.05$) (Figure 3.3 B-C). However, with each subsequent sampling date (or increasing node number), leaf parameters such as length, length/width ratio, perimeter and leaf roundness in branched plants showed a divergent pattern away from that of adult leaves (significant interaction of time x plant type: $P<0.0001$). Leaves of branched plants therefore became less oval and more elongated with increasing node number (Figure 3.3 A, D-F).

Relative to adult plants, an assessment of leaf colour parameters on the abaxial surface of leaves in micropropagated plants provided an indication of the degree to which vegetative phase change (acquisition of white-coloured tomentum) occurred. There was a significant interaction of sampling date and plant type on hue and saturation values ($P<0.05$), and only a main effect of plant type on values for leaf lightness ($P<0.0001$) (Figure 3.4 A-C). Generally, leaf saturation and hue values in single-stemmed plants approached those of adult plants by March, whereas those of branched plants either remained constant or diverged away from values exhibited by leaves of adult plants (Figure 3.4 A-B). Leaf lightness in single-stemmed plants, however, did not differ from that in branched plants during the three sampling dates, although it did show a trend towards the values for adult plants by node 40 (March) (Figure 3.4 C).

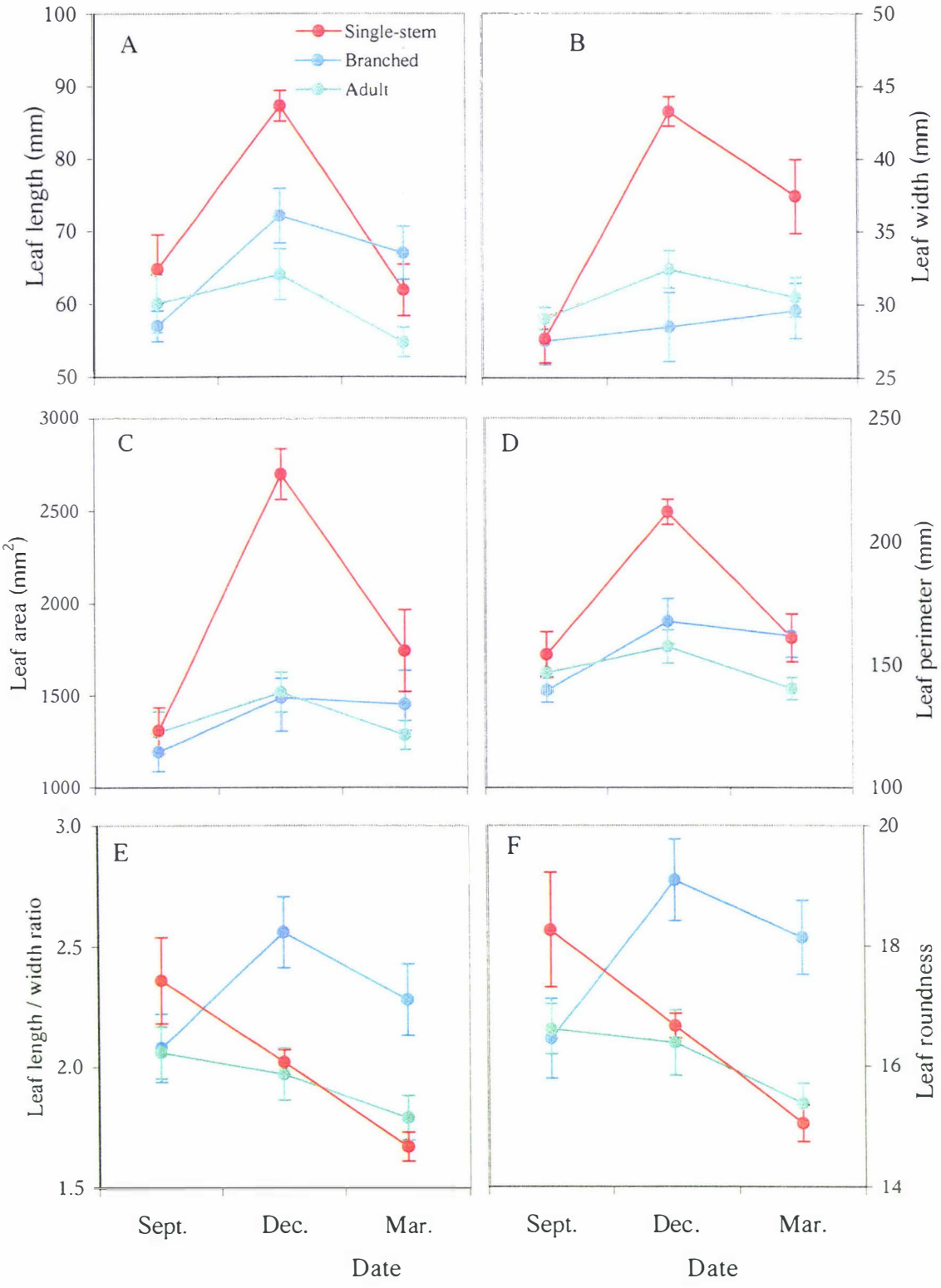

Figure 3.3 Changes in dimensional properties (mean ± SE) during successive sampling dates for leaf abaxial surfaces in single-stemmed, branched and adult plants of *Metrosideros excelsa* 'Vibrance'. A: length. B: width. C: area. D: perimeter. E: length / width ratio. F: roundness. Sampling dates for September (Sept), December (Dec) and and March (Mar) correspond to nodes 10, 20 and 30 in branched plants and nodes 10, 30 and 40 in single-stemmed plants, respectively. Leaves in adult plants harvested from canopy top. See Materials and Methods for specific sampling days.

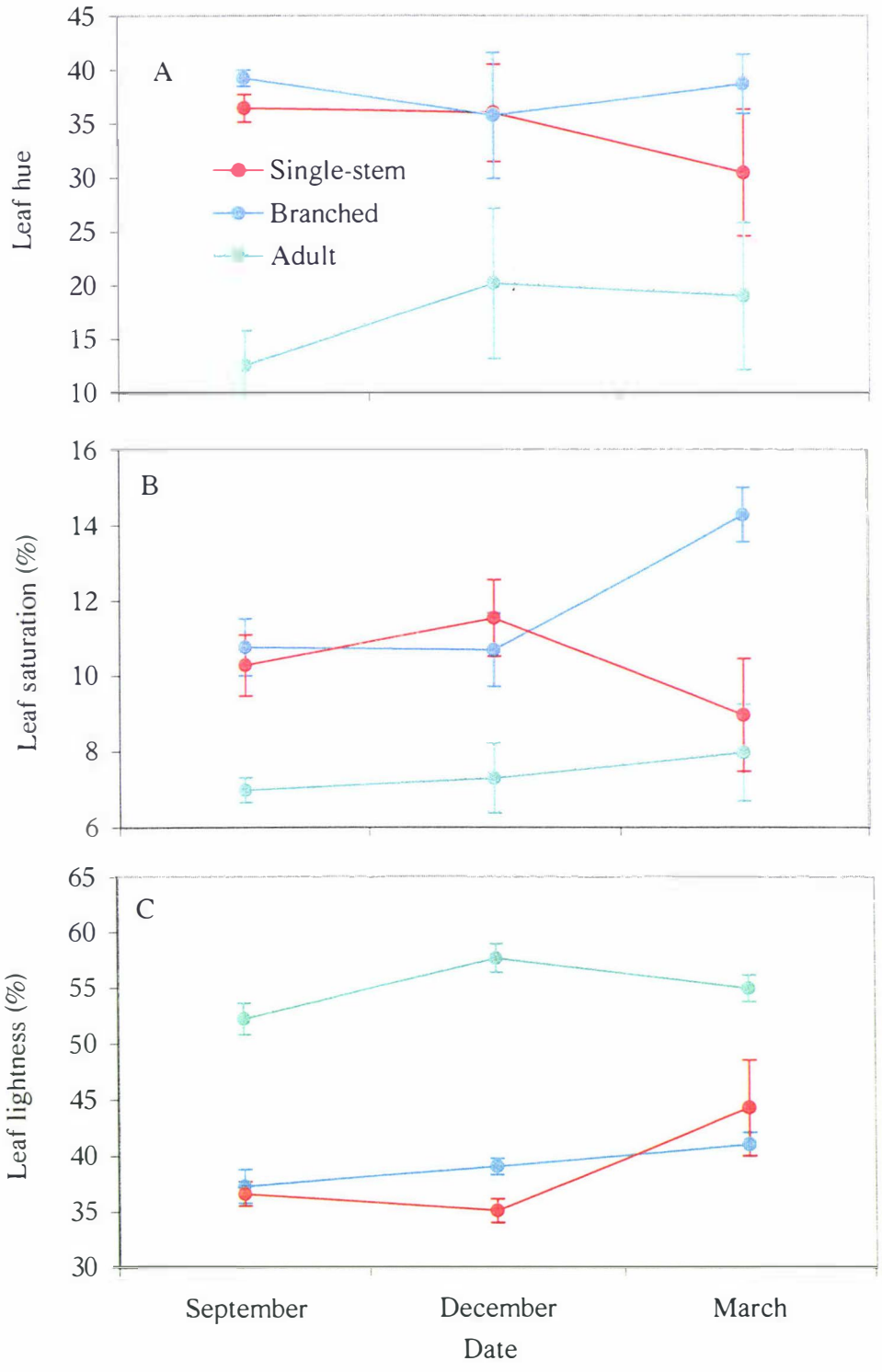

Figure 3.4 Changes in optical properties (mean ± SE) between successive sampling dates for leaf abaxial surfaces in single-stemmed, branched and adult plants of *Metrosideros excelsa* 'Vibrance'. A: hue. B: saturation C: lightness. Sampling dates for September, December and March correspond to nodes 10, 20 and 30 in branched plants and nodes 10, 30 and 40 in single-stemmed plants, respectively. Leaves in adult plants harvested from canopy top. See Material and Methods for specific sampling days.

3.3.4 Leaf morphological attributes

In micropropagated plants, leaf morphology varied depending on the sample date and plant type examined (Table 3.1). In single-stemmed plants, leaf petiole length increased with increasing node position, reaching a similar length by node position 40 as recorded in leaves of adult plants (pooled mean 7.3 mm in March). Leaf petiole length in branched plants also showed a similar trend to that of single-stemmed plants, although this was still significantly shorter than petioles in adult plants by March (node position 30 in branched plants). Values for leaf angle in single-stemmed plants were generally smaller at higher nodes compared with leaves of adult plants, whereas the opposite relationship occurred for leaves in branched plants. The total fresh and dry mass per leaf was generally higher in single-stemmed plants at higher nodes (node position 30-40) in comparison with leaves of branched and adult plants. Leaf specific mass in branched and adult plants increased between the first and last sampling date, whereas no trends were apparent in single-stemmed plants. Leaf water content (LWC) generally varied depending on plant type and sampling date. Percent LWC per given mass in micropropaged plants decreased with increasing node position, reaching similar values for adult leaves by March. LWC per given area in leaves of single-stemmed plants primarily was significantly higher than leaves of branched and adult plants during December and March.

3.3.5 Carbon isotope discrimination

Measurements of isotope composition taken during the three sampling dates revealed contrasting trends in the levels of leaf carbon isotope composition (Figure 3.5). There were significant main effects of sampling date ($P<0.0001$) and plant type ($P<0.0001$) on the level of isotope discrimination but no significant interaction between these factors ($P>0.05$). Mean carbon isotope discrimination levels in leaves of single-stemmed plants during the second and third sampling dates (December and March) did not differ, but were significantly lower than values recorded during the first date (September). The mean values for leaves in single-stemmed plants during the third sampling date were also lower than those recorded for branched and adult plants. In both adult and branched plants, mean carbon isotope discrimination values did not differ within and

Table 3.1 Comparison of leaf characteristics in single-stemmed, branched and adult plants of *Metrosideros excelsa* 'Vibrance'. Means shown for each plant type collected at the end of each sampling date (**Sept** = on 22 – 30 September, **Dec** = 1 – 9 December 2000, and **Mar** = 23 February – 3 March, 2001) correspond to leaves on nodes 10, 30 and 40 (shown in parentheses) in single-stemmed plants and 10, 20 and 30 in branched plants, respectively. Analysis for variables in each row conducted using a two-way ANOVA for assessing the effect of plant type (P), sampling time (T) and their interaction (PxT). Mean separation in rows by Least Square Means tests, 5% significance level.

Parameter	P-value	Single-stemmed			Branched			Adult		
		Date (node)			Date (node)			Date		
		Sept (10)	Dec (30)	Mar (40)	Sept (10)	Dec (20)	Mar (30)	Sept	Dec	Mar
Petiole length (mm)	PxT <0.0001	4.2^{f}	$5.6d^{e}$	6.7^{bc}	4.9^{ef}	4.9^{ef}	5.9^{cd}	7.6^{ab}	8.3^{a}	7.8^{ab}
Leaf basal angle (°)	PxT <0.05	36.9^{b}	23.4^{e}	22.8^{e}	42.9^{a}	36.2^{b}	34.2^{bc}	35.1^{b}	30.0^{cd}	28.6^{d}
Leaf fresh mass (mg)	PxT <0.0001	783.1^{c}	1616.9^{a}	1234.1^{b}	531.2^{d}	795.5^{c}	825.0^{c}	716.8^{c}	829.8^{c}	786.9^{c}
Leaf dry mass (mg)	PxT <0.001	236.1^{c}	461.9^{a}	447.8^{a}	152.2^{d}	245.3^{bc}	295.1^{bc}	264.9^{bc}	281.4^{bc}	305.4^{b}
Leaf specific mass (mg cm^{-2})	P, T<0.0001	0.19^{ab}	0.17^{a}	0.25^{a}	0.13^{e}	0.16^{de}	0.20^{bc}	0.20^{bc}	0.18^{cd}	0.24^{a}
Water content – mass (%)	PxT <0.0001	69.9^{a}	71.6^{a}	64.7^{bcd}	71.4^{a}	69.4^{a}	64.3^{c}	63.0^{cd}	66.1^{b}	61.2^{d}
– area (%)	P<0.0001	42.9^{ab}	43.1^{a}	44.9^{a}	32.5^{b}	36.9^{b}	36.4^{b}	34.9^{b}	36.1^{b}	37.4^{b}

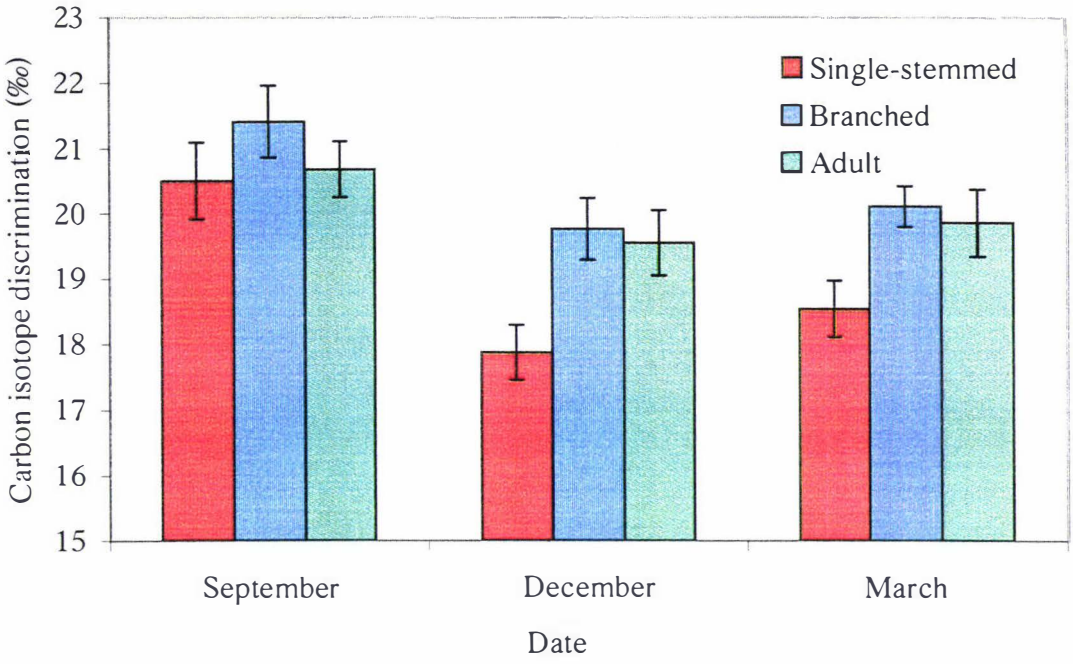

Figure 3.5 Comparison of leaf carbon isotope composition (mean ± SE) in single-stemmed, branched and adult plants of *Metrosideros excelsa* 'Vibrance'. Means shown for each plant type collected immediately after leaf gas exchange measurements conducted on three successive sampling dates. Specific dates described in Material and Methods.

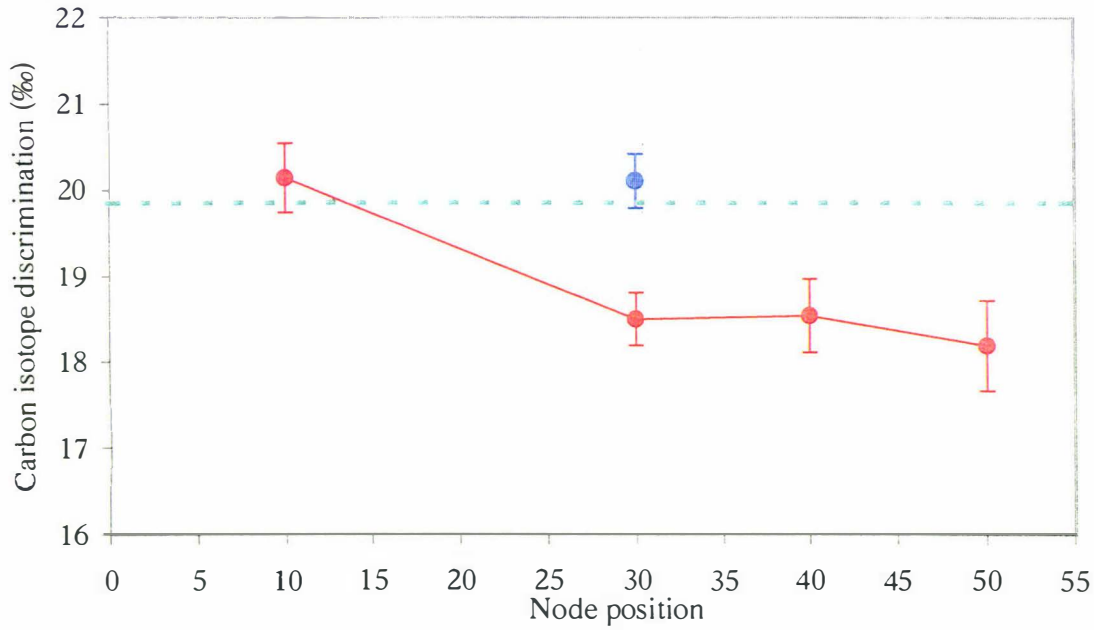

Figure 3.6 Comparison of carbon isotope composition (mean ± SE) from leaves harvested at different node positions for single-stemmed (red line) and branched (blue) plants with the mean value for adult plants (collected from canopy top) of *Metrosideros excelsa* 'Vibrance' collected at the end of the experimental period (March - May 2001).

between plant types during the second and third sampling date, although values from the second date were lower in comparison with the first.

During the third sampling date, carbon isotope composition was examined at different node positions in single-stemmed plants, and compared with values obtained from leaves of branched and adult plants. As node position increased in single-stemmed plants, carbon isotope values decreased, diverging away from values recorded in leaves for adult and branched plants ($P<0.001$) (Figure 3.6). Carbon isotope discrimination values at node 40 in single-stemmed plants decreased by approximately 12% compared with earlier values recorded at node 10. Additional leaf samples collected at node position 50 in single-stemmed plants also showed a continuation in this pattern of decreased carbon isotope discrimination values with increasing node position.
In single-stemmed plants, there was no significant effect of leaf age on the level of carbon isotope discrimination, based on an assessment of leaves collected from different nodes (ages) during the third sampling date with corresponding nodes collected during previous sampling dates ($P>0.05$) (Figure 3.5 and 3.6). Therefore, the older leaf samples collected in March from nodes 10 and 30 did not differ from corresponding node positions harvested in September and December, respectively, when leaves were relatively younger.

3.3.6 Relationship of carbon isotope discrimination and leaf characters

The number of leaf characters that were correlated with Δ values varied depending on the plant type (Table 3.2). In single-stemmed plants, all pre-defined features describing the size and shape characteristics of leaves were strongly correlated with Δ values. For instance, leaf Δ values decreased as leaves became rounder and smaller in size resembling a size and shape characteristic of adult leaves. Similarly, Δ values also decreased as the abaxial leaf surface became relatively lighter, indicative of an increase in the amount of tomentum accumulated. In branched plants, relatively few colour and size/shape parameters were correlated with Δ values. In adult plants, leaf morphological parameters were relatively constant with time and showed no relationships with Δ values, whereas only one colour parameter (leaf saturation) showed a negative correlation. The most notable differences in leaf mass and water content characteristics

Table 3.2 Correlation of leaf dimension, colour, mass and water content variables with leaf carbon isotope discrimination values in single-stemmed, branched and adult plants of *Metrosideros excelsa* 'Vibrance'. Pearson's correlation coefficient (r) and significance test (P) shown for each plant type were pooled for data collected at the end of three sampling dates (22 – 30 September, 1 – 9 December 2000, and 23 February – 3 March, 2001). Mean at 5% significance level. Symbol: n.s. not significant.

Leaf characteristic	Single-stem		Branched		Adult		All plants	
Size and shape	r	p	r	p	r	p	r	p
Basal angle (°)	0.546	0.002	0.444	0.014	.	n.s.	0.599	0.000
Petiole length (mm)	-0.575	0.001	.	n.s.	.	n.s.	-0.235	0.026
Length (mm)	-0.386	0.035	-0.625	0.000	.	n.s.	-0.381	0.000
Width (mm)	-0.619	0.000	.	n.s.	.	n.s.	-0.518	0.000
Length/Width Ratio	0.370	0.044	-0.580	0.001	.	n.s.	.	n.s.
Perimeter (mm)	-0.540	0.002	-0.471	0.009	.	n.s.	-0.487	0.000
Area (mm)	-0.646	0.000	.	n.s.	.	n.s.	-0.558	0.000
Roundness	0.464	0.010	-0.637	0.000	.	n.s.	.	n.s.
Colour								
Hue	0.438	0.050	.	.	.	n.s.	.	n.s.
Saturation (%)	0.464	0.039	-0.676	0.001	-0.444	0.050	.	n.s.
Lightness (%)	-0.500	0.025	-0.654	0.002	.	n.s.	.	n.s.
Mass and Water Content								
Total leaf dry mass (mg)	-0.419	0.021	-0.626	0.000	.	n.s.	-0.516	0.000
Specific mass (mg / cm^{-2})	n.s.	n.s.	-0.647	0.000	.	n.s.	-0.236	0.025
Water content - mass (%)	n.s.	n.s.	0.429	0.018	-0.552	0.002	.	n.s.
- area (%)	n.s.	n.s.	-0.579	0.001	.	n.s.	-0.380	0.000

were in branched plants, showing increases in leaf mass and water content per given area as values for Δ decreased.

In summary, leaves from single-stemmed plants displayed morphologically and anatomically similar characteristics to those of adult plants as node position increased, such as a transition to smaller and rounder leaves with tomentum on abaxial surfaces. This was also associated with a decrease in Δ values with increasing node position.

3.3.7 Photosynthetic response and carbon isotope discrimination relationship in plants under controlled conditions

Gas exchange measurements made on leaves on the three sampling dates revealed contrasting responses to changes in PPF. There was a significant interaction of plant type and sampling time on both P_{max} and φ_{app} ($P<0.05$) (Figure 3.7 A-B, Figure 3.8). Generally, in comparison with leaves of adult plants, leaf values for P_{max} and φ_{app} in micropropagated plants decreased at successive sampling dates, being significantly lower than values for adult leaves, especially at node 40. However, PPF_{sat} levels were similar in leaves from all plants (mean 1018±53 μmol m^{-2} s^{-1}), irrespective of plant type and sampling date ($P>0.05$) (Figure 3.7 C).

Based on analyses from the A/Ci curves (see Figure 3.9), there was a declining trend over time in values for micropropagated versus adult plants. There were significant main effects only of plant type and time ($P<0.05$) on values of Rubisco carboxylation activity (V_{cmax}) and maximum electron transport capacity (J_{max}), being primarily lower in single-stemmed and branched plants compared to adult plants on the March sampling date (Figure 3.7 D and E). However, in both types of micropropagated plants, mean values for V_{cmax} and J_{max} showed a declining (although not significant) trend with time. The general decline in these values was also reflected in the lower CO_2 assimilation curves for branched and single-stemmed plants compared with adult plants in March (Figure 3.9). Respiration rate increased with time in single-stemmed plants and remained relatively constant over time for branched and adult plants (significant time and plant type interaction, $P<0.05$) (Figure 3.7 F).

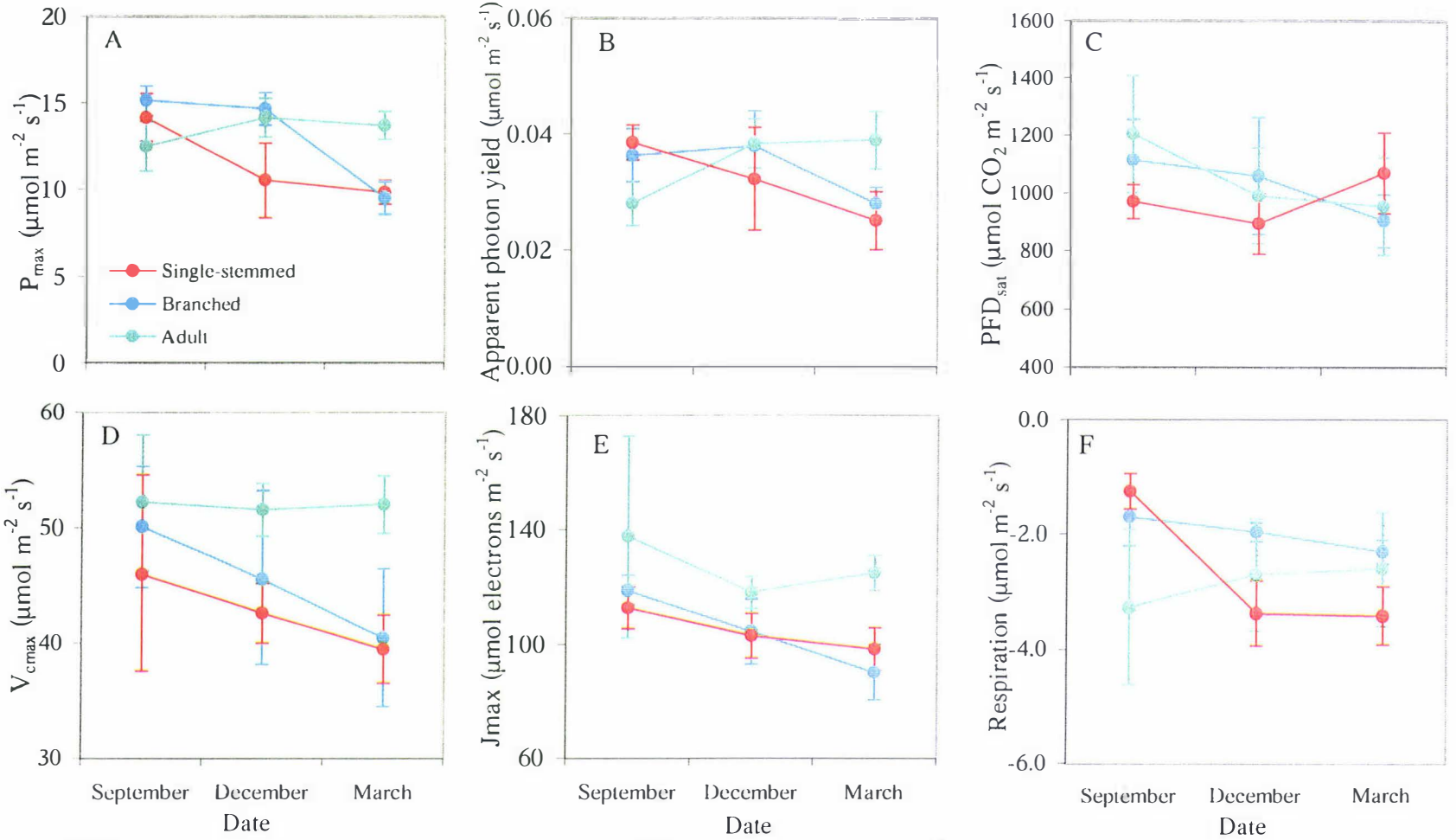

Figure 3.7 Comparison of variables from light response and CO_2 assimilation curves for leaves in single-stemmed, branched and adult plants of *Metrosideros excelsa* 'Vibrance'. Means (±SE) shown for each plant type collected during three sequential sampling dates in a controlled environment correspond to leaves on nodes 10, 30 and 40 in single-stemmed plants and 10, 20 and 30 in branched plants, respectively.

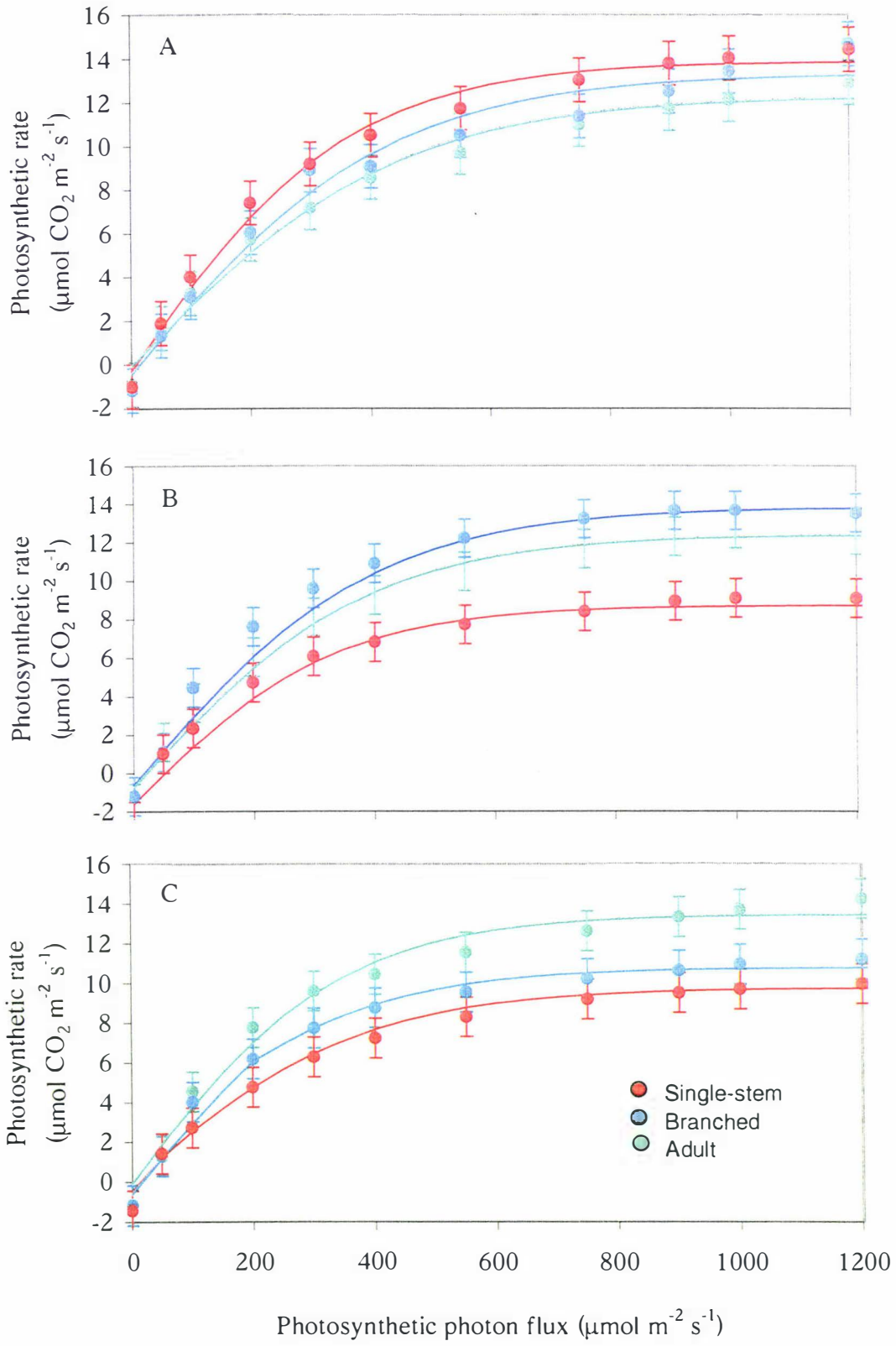

Figure 3.8 Mean (±SE) photosynthetic light response curves for branched, single-stemmed and adult plants of *Metrosideros excelsa* collected in a controlled environment on (A) 22 - 30 September, (B) 1 - 9 December 2000, (C) 23 February - 3 March 2001. Leaf node sampled for single-stemmed (A) 10, (B) 30 and (C) 40, and for branched plants nodes (A) 10, (B) 20 and (C) 30.

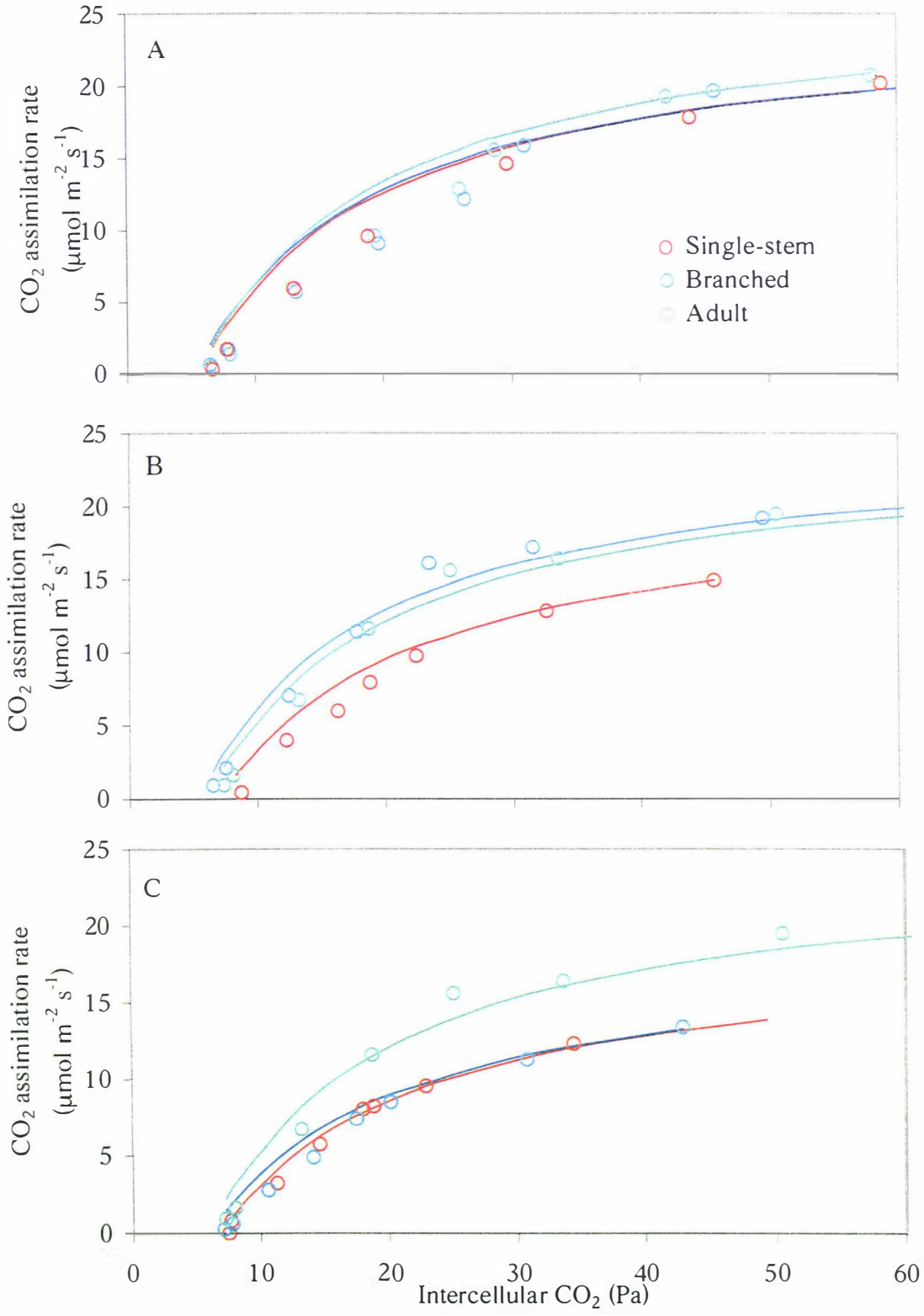

Figure 3.9 Mean representational CO_2 assimilation to intercellular CO_2 response curves for a leaf measured on single-stemmed, branched and adult plants of *Metrosideros excelsa* collected on (A) 22 - 30 September, (B) 1-9 December 2000, and (C) 23 February 3 - March 2001. Leaf nodes sampled for single-stemmed plants were (A) 10, (B) 30 and (C) 40, and for branched plants nodes (A) 10, (B) 20 and (C) 30. Leaves measured from the canopy top for adult plants.

An examination of photosynthetic performance at P_{max} showed some patterns related to plant type in gas exchange properties (Table 3.3). Leaf stomatal conductance and transpiration rates in single-stemmed plants decreased at higher node positions (nodes 30 and 40 in December and March, respectively), although these trends were not apparent in leaves of either adult or branched plants. Generally, intercellular CO_2 concentrations (Ci) were relatively constant in leaves of adult plants (mean 232±12 μmol CO_2 mol^{-1}) and to a lesser degree in single-stemmed plants (234±8 μmol CO_2 mol^{-1}). At all sampling times, the mean leaf chamber CO_2 was also relatively constant 400±1 μmol CO_2 mol^{-1}. In branched plants, however, Ci increased significantly with increasing node position, increasing by an initial 11% to 275±6 μmol CO_2 mol^{-1} by node 30 (March) ($P<0.01$).

Relationships in leaf WUE varied between plant types and time (Table 3.3). Generally, WUE in leaves of single-stemmed plants increased with node position to levels that were not significantly different from those in adult plants. Leaf WUE in single-stemmed plants showed a strong negative correlation with Δ ($R^2 = - 0.621$, $p<0.001$) (Figure 3.10). In contrast, leaves of branched plants became less water-use efficient at higher nodes (Table 3.3). There were significant correlations for values of Δ with stomatal conductance in single-stemmed plant ($R^2 = 0.557$, $p<0.001$) and branched plants ($R^2 = 0.352$, $p<0.05$) but not in adult plants ($R^2 = 0.005$, $p>0.05$). Based on data pooled across all plant and sampling time treatments, Δ was strongly and positively correlated with stomatal conductance ($R^2 = 0.416$, $p<0.0001$) and transpiration ($R^2 = 0.436$, $p<0.0001$), and negatively correlated with WUE ($R^2 = -0.394$, $P <0.0001$) (Figure 3.10).

3.3.8 Photosynthetic response of plants under greenhouse conditions

Rates of photosynthesis differed significantly between plant types depending on the time of day (Figure 3.11 A). Rates of photosynthesis were significantly higher in adult plants between 1000 and 1600 h, averaging a peak rate of 8.7 μmol CO_2 m^{-2} s^{-1} at 1000 h (mean PPF 1104±23 μmol m^{-2} s^{-1}). The highest level of PPF occurred at 1200 h with an average flux of 1678±14 μmol m^{-2} s^{-1}. Photosynthetic responses of leaves in branched and single-stemmed plants varied in accordance with the diurnal changes in

Table 3.3 Comparison of leaf gas-exchange parameters in single-stemmed, branched and adult plants of *Metrosideros excelsa* 'Vibrance'. Gas-exchange values represent means obtained at light saturated maximum rate of photosynthesis. Mean leaf chamber CO_2 across all treatments was 400±1 μmol CO_2 mol^{-1}. Means shown for each plant type collected during three sampling dates(**Sept** = on 22 – 30 September, **Dec** = 1 – 9 December 2000, and **Mar** = 23 February – 3 March, 2001) in a control room at the New Zealand Controlled Environment Laboratory, Horticulture and Food Research, Palmerston North, correspond to leaves on nodes 10, 30 and 40 (shown in parentheses) in single-stemmed plants and 10, 20 and 30 in branched plants, respectively. Analysis for variables in each row conducted using a two-way ANOVA for assessing the effect of plant type (P), sampling time (T) and their interaction (PxT). Mean separation in rows by Least Square Means tests, 5% significance level.

Parameter	P-value	Single-stemmed			Branched			Adult		
		Date (node)			Date (node)			Date		
		Sept (10)	**Dec (30)**	**Mar (40)**	**Sept (10)**	**Dec (20)**	**Mar (30)**	**Sept**	**Dec**	**Mar**
Stomatal conductance (mol H_20 m^{-2} s^{-1})	PxT <0.05	0.20^{a}	0.12^{b}	0.11^{b}	0.23^{a}	0.21^{a}	0.18^{a}	0.15^{ab}	0.18^{a}	0.19^{a}
Transpiration (mmol H_2O m^{-2} s^{-1})	PxT<0.001	2.7^{a}	1.8^{c}	1.6^{c}	3.0^{a}	2.8^{a}	2.3^{ab}	2.0^{bc}	2.4^{ab}	2.5^{ab}
Water use efficiency (μmol $mmol^{-1}$)	PxT<0.001	5.2^{bc}	6.3^{a}	6.1^{ab}	4.7^{c}	5.3^{b}	4.1^{d}	6.3^{ba}	5.5^{ab}	5.6^{abc}

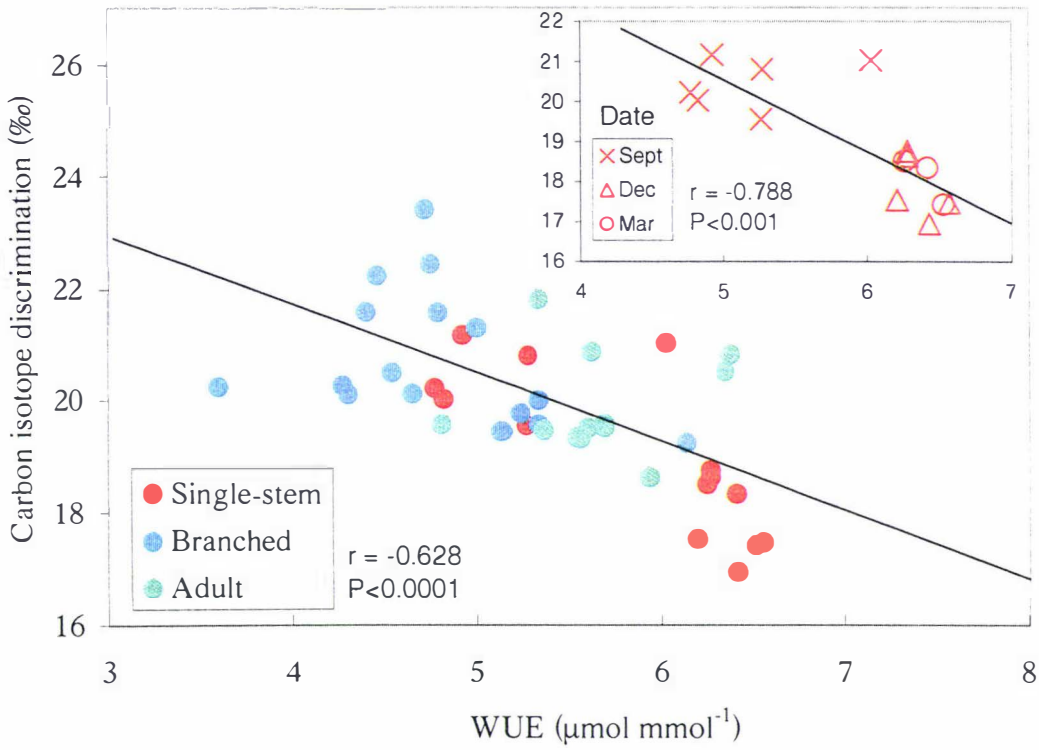

Figure 3.10 Correlation of water use efficiency (WUE) with carbon isotope discrimination for leaves from single-stemmed, branched and adult plants of *Metrosideros excelsa* pooled from the three sampling dates (22 - 30 September, 1 - 9 December 2000, 23 February - 3 March 2001). Small window: Correlation of water use efficiency with carbon isotope discrimination for single-stemmed plants with the three successive sampling dates displayed in descending order.

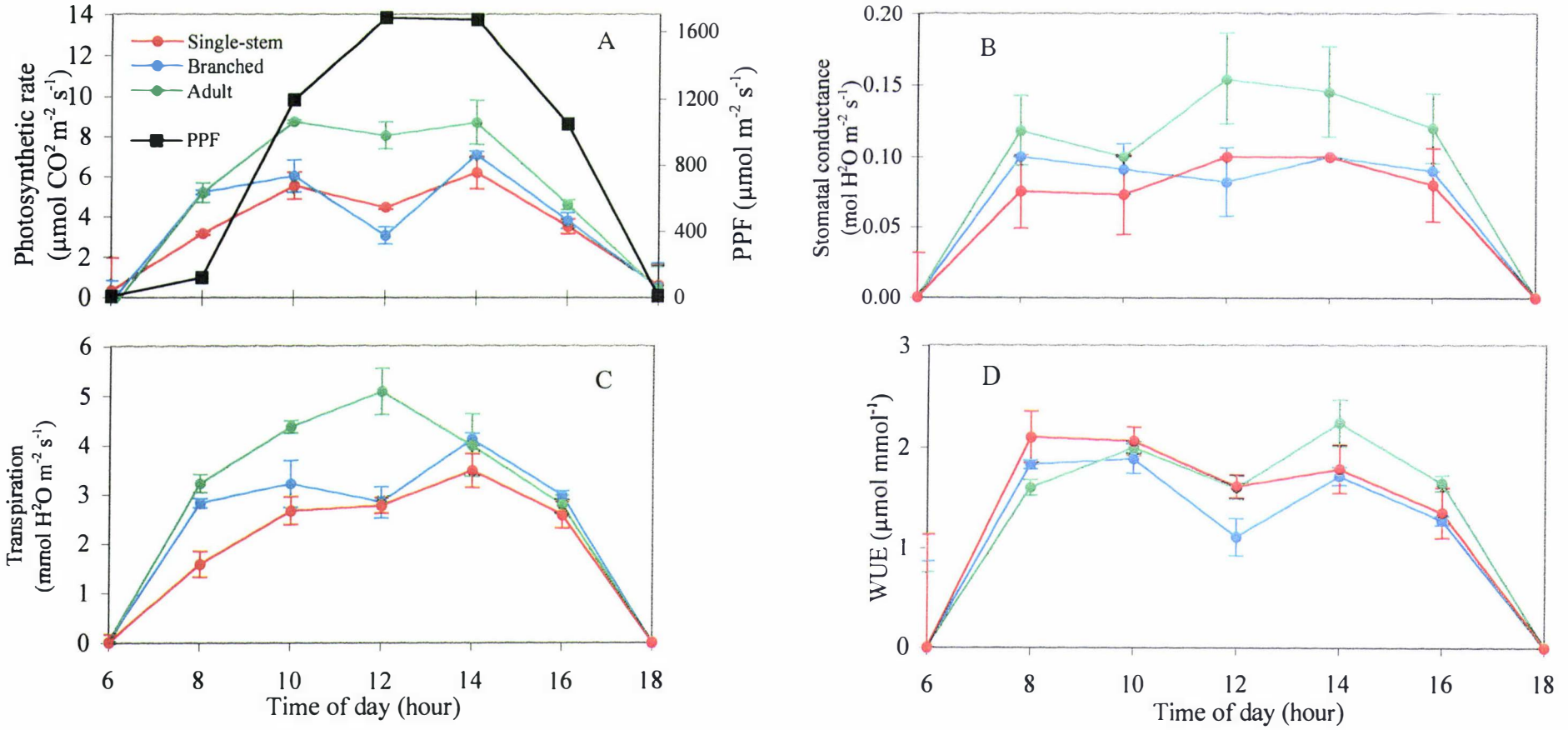

Figure 3.11 Diurnal comparison of leaf gas exchange parameters (mean ±SE) in single-stemmed, branched and adult plants of *Metrosideros excelsa* 'Vibrance'. Changes in (A) photosynthetic rate with photosynthetic photon flux (PPF), (B) conductance, (C) transpiration and (D) water-use efficiency (WUE) measured on 20 - 21 March 2001, within a greenhouse located at the Plant Growth Unit, Massey University, Palmerston North. Mean diurnal ambient CO_2: 358±0.07 μmol CO_2 m^{-2} s^{-1}. Diurnal air temperature range: 25-35°C.

PPF. Leaves in branched plants displayed a bi-modal photosynthetic pattern, with rates temporally decreasing significantly at mid-day (1200 h) before a subsequent resurgence to 7.1 μmol CO_2 m^{-2} s^{-1} (1400 h). A similar but less pronounced response also occurred in single-stemmed plants.

Several other photosynthetic parameters varied between plant types over the 12 h period. Generally, leaf stomatal conductance and transpiration rates were higher in adult plants during the early (0800–1200 h) and later (1200–1600) hours of the day, respectively, in comparison to values in leaves of micropropagated plants ($P<0.05$) (Figure 3.11 B and C). Water-use efficiency (WUE) in leaves of single-stemmed plants was significantly higher than in leaves of both branched and single-stemmed plants early in the day (0800 h), and only higher than branched plants at 1200 h ($P<0.001$) (Figure 3.11 D). In comparison with leaves from branched plants, those in adult plants became more WUE during the middle of the day (1200–1400), although these values did not differ from leaves of single-stemmed plants.

3.3.9 Carbohydrate analyses

There was a significant difference in the level of leaf carbohydrates between plant types (Table 3.4). Total leaf soluble sugar concentrations in single-stemmed (node 40) and branched plants (node 30) plants were almost twice as high compared with leaves harvested from adult leaves. This was attributed mainly to higher fructose and glucose but not sucrose concentrations in the micropropagated plants. Starch levels in leaves of single-stemmed plants and branched plants were similar between plant types, being approximately double the concentration recorded in leaves of adult plants.

Table 3.4 Comparison of carbohydrate concentrations in fully expanded leaves collected from nodes 10 and 40 in single-stemmed plants, node 30 in branched plants and from the canopy top in adult plants of *Metrosideros excelsa* 'Vibrance' on 25 June 2001, between 0600 – 0630 h. Replication consisted of 8-12 leaf samples per node or plant type collected within a greenhouse located at the Plant Growth Unit, Massey University, Palmerston North. Treatment comparisons were conducted with a one-way ANOVA with mean separation in rows by Least Square Means tests, 5% significance level. Symbol: n.s. not significant.

Carbohydrate (mol g^{-1} DW)	**p-value**	**Single-stemmed** node 40	**Branched** node 30	**Adult** canopy top
Sucrose	n.s.	0.028	0.029	0.035
Fructose	<0.01	0.026[a]	0.027[a]	0.015[b]
Glucose	<0.001	0.063[b]	0.046[a]	0.028[c]
Total soluble	<0.01	0.117[a]	0.102[a]	0.078[b]
Starch (mg g^{-1} DW)	<0.001	14.7[a]	17.5[a]	8.0[b]

3.4 Discussion

Imposition of a shoot restriction treatment on micropropagated plantlets of *Metrosideros excelsa* 'Vibrance' accelerated vegetative phase change. Not only did leaves in single-stemmed plants become smaller and rounder with increasing node position, but they also approached leaf optical values (representative of increasing tomentum on abaxial surfaces) similar to those of adult plants. This pattern of an acceleration in vegetative phase change is consistent with findings reported earlier in the single-stemmed plants of the cultivar 'Scarlet Pimpernel' (Chapter 2), and in similar experiments where phase change occurred with increasing node and height above the roots (Davis 1991, Snowball *et al.* 1994, James and Mantell 1997, Sismilich, Jameson and Clemens unpublished data). Unique to this study, however, was the characterisation of leaf gas exchange and Δ components during vegetative phase change in a woody species.

Variation in Δ values between adult and juvenile plants has been reported in a number of ecophysiological studies (Donovan and Ehleringer 1994, Hansen 1996, Cordell *et al.*

1998, Rundel *et al.* 1999). Typically, intraspecific differences in Δ values for juvenile plants are often reported to be higher than those of adult plants. A common theme in these and other studies has been the significance of Δ as a reliable indicator of long-term WUE, given the strong negative correlation between these two factors (Farquhar *et al.* 1989). For example, based on Δ values obtained from plants of *Chrysothamnus nauseosus* growing in common plots in semi-arid shrubland, Donovan and Ehleringer (1992, 1994) were able to assess the WUE between both sets of plants. Juvenile plants of *C. nauseosus* were found to be less WUE than adult plants, which coincided with higher rates of photosynthesis and increased stomatal conductance and transpiration in the former plant type. Similarly, comparable Δ and gas exchange responses have also been reported for different life stages in *Metrosideros polymorpha* (Cordell *et al.* 1998) and *Acacia* (Hansen 1996). The distinct ecophysiological characteristics expressed by each life stage may occur because juvenile plants in the wild are potentially under different selective pressures than adult plants (Donovan and Ehleringer 1991). Therefore, the relatively higher rates of photosynthesis expressed in many juvenile plant species, particularly those under severe conditions (e.g. arid conditions), would be consistent with the hypothesis of a 'go for broke' strategy (Bond 2000). This would provide the growth potential required for establishment, despite a greater risk of mortality (Bond 2000).

The results obtained from single-stemmed plants of *M. excelsa* that had been grown under well-watered conditions while undergoing vegetative phase change appeared to be consistent with the above studies. Not only did these plants exhibit short-term changes in gas-exchange characteristics, such as reduced rates of photosynthesis, transpiration and stomatal conductance with increasing node position, but also a long-term indicator of plant performance (i.e. Δ) suggested that these plants became more WUE as their leaves became more adult. Moreover, in single-stemmed plants, Δ values were also strongly correlated with most of the leaf morphological attributes, discriminating less as leaves became increasingly adult. Based on these observations, it would appear that these results support the hypothesis that juvenile plants exhibit decreasing Δ because of changes in leaf morphology consistent with greater water-use efficiency, notably the acquisition of more tomentose and round-shaped leaves similar to those born on adult plants.

The comparatively higher values of Δ in leaves of adult plants with those of single-stemmed plants, however, appeared anomalous. The contrasting responses in gas exchange parameters between single-stemmed and adult plants also highlighted this anomaly. This is in light of the fact that these plant-type specific differences in short-term (gas exchange) and long-term (Δ) responses were obtained consistently when obtained under both climate-controlled (light and CO_2 response curves) and ambient greenhouse conditions, respectively. Furthermore, Δ values did not differ between the three sets of plants at the beginning of the experiment, despite their differences in size, ontogenetic state and leaf dimensional qualities. These findings were also not confounded by age of the leaf samples given that harvested samples represented newly (< 2 months) expanded leaf tissue from all plants. Moreover, the hypothesis that the age of the leaf would not play a role in affecting the expression Δ was also upheld by this study, since leaves harvested from single-stemmed plants at similar nodes but at different times (leaf age) showed no significant differences. Therefore, this would suggest that Δ levels were established during a time of leaf expansion.

What factors may account for the discrepancy in photosynthetic rates between single-stemmed undergoing vegetative phase change and adult plants? It is possible that removal of axillary buds in single-stemmed plants induced a wound or stress response that limited stomatal conductance. However, this is unlikely because photosynthesis measurements were spatially and temporally separate from the times and positions at bud removal occurred along the stem axis. For instance, gas exchange measurements were conducted at least six days after buds had been excised. Furthermore, photosynthetic measurements were conducted on leaves that were below the position (~3–5 nodes) of the most recently excised axillary buds.

An alternative explanation relates to variation in source and sink relations as a result of differences in plant architecture. Following the removal of axillary meristems, the structural architecture of a single-stemmed plant would be expected to limit sink-demand within that plant to root and shoot apical regions, whereas in adult plants, this would be dispersed over numerous growing points, such as developing axillary buds/shoots within the canopy. Sink demand relative to source strength in the single-

stemmed plants would be expected to decrease further as these plants continued to grow and attain an increasingly greater number of source leaves (increased source strength). It has frequently been reported that treatments that reduce the translocation of photosynthates to sink regions (e.g. removal or reduction in sink demand) can lead to an excess accumulation and partitioning of these carbohydrates into starch within source leaves (Harlevy and Sharkey 1991, Stitt and Schulze 1994, Krapp and Stitt 1995, Myers *et al.* 1999, Nakano *et al.* 2000). Such experiments designed to induce alterations in sink demand have included excision of the sink material, including removal of shoot apices (Mondal *et al.* 1978), leaves (Myers *et al.* 1999), flower and fruiting bodies (Nautiyal *et al.* 1998, Nakano *et al.* 2000, Wünsche *et al.* 2000), and continuous removal of axillary buds (Gezelius *et al.* 1981, Sawada *et al.* 1999).

An increase in carbohydrate levels in source leaves has frequently been associated with a reduction in photosynthetic capacity (Krapp and Stitt 1995, Myers *et al.* 1999, Nakano *et al.* 2000). Nakano *et al.* (2000) and others have suggested that a decrease in photosynthetic activity is a result of a feedback inhibition on that activity caused by an accumulation of an end product (possibly starch) within the source leaves. The findings in *M. excelsa* are also consistent with this hypothesis. The higher concentrations of starch (and several soluble sugars) and the depressed rates of photosynthesis recorded in leaves of single-stemmed plants compared with those of adult plants, are suggestive of an end-product (possibly starch) induced feedback inhibition response on photosynthesis. Similar to *M. excelsa*, the accumulation of starch in source leaves in *Pinus taeda* has also been attributed to a reduction in carboxylation-related processes, such as in electron transport rates and Rubisco activity (Myers *et al.* 1999). This has been attributed to an extreme enlargement in starch grains within the leaf that physically damage chloroplasts, subsequently reducing photosynthesis and hindering CO_2 diffusion in chloroplast (Pritchard *et al.* 1997, Nakano *et al.* 2000). Similar reports of increased carbohydrate content and a down-regulation in photosynthesis in source leaves has also been observed under various sink-limited conditions following bud removal in seedlings of *Pinus silvestris* (Gezelius *et al.* 1981), terminal leader excision and girdling in *Pinus taeda* (Myers *et al.* 1999), seed pod removal in *Phaseolus vulgaris* (Nakano *et al.* 2000) and fruit removal in trees of *Malus sylvestris* (Wünsche *et al.* 2000).

Branched plants also exhibited a reduction in gas exchange parameters at higher node positions in conjunction with high starch level, although this was not reflected in the long-term WUE of the plant (e.g. Δ). This suggests that the actions or events that triggered a reduction in these parameters were of relatively recent origin and not of a long-term nature. The reduced photosynthetic rates in branched plants at high nodes may be attributed to the lack of axillary branches observed on long (ca. 30 nodes) individual shoots of branched plants from which samples were collected. It could be speculated that a similar sink-reduced effect in elongating shoots of branched plants might have occurred as node position increased. This would be consistent with the high levels of starch and reduced rates of photosynthesis at high node positions, as also observed in single-stemmed plants. Further evidence to support this hypothesis may have been attained if carbohydrate analyses of leaves in branched and single-stemmed plants were collected at different node positions and/or sampling times.

The decline in carbon isotope composition of single-stemmed plants is, therefore, consistent with alterations in gas exchange parameters. Not only was stomatal conductance and transpiration reduced in single-stemmed plants at higher nodes, but they also became increasing more water-use efficient. Both stomatal conductance and WUE, particularly in single-stemmed plants, were also strongly correlated with Δ. This response is suggestive of either greater stomatal closure or more heterogeneity in stomatal apertures over the leaf surface during photosynthesis, ultimately limiting atmospheric and intercellular CO_2 gas exchange (Lauteri *et al.* 1993, Patterson and Rundel 1993). Thus, the reduced supply of atmospheric CO_2 in a stomatal-limited photosynthetic system would, theoretically, account for the decline in Δ values observed in single-stemmed plants. Therefore, the decline in leaf Δ values in single-stemmed plants undergoing phase change does not support the hypothesis that it is associated with changes in leaf morphology, but rather appears to reflect the physiological responses associated with a disruption in source and sink relations.

Characterisation of shoot and leaf growth was also described in this study. It was interesting to note that the pattern of vegetative growth in adult and micropropagated plants differed significantly. Both single-stemmed and branched plants exhibited continuous elongation of either a main or an axillary shoot, respectively, whereas in

adult plants, shoot elongation was episodic. This episodic pattern of vegetative growth is typical of adult plants in *Metrosideros* (Gerrish 1989, Clemens *et al.* 1995), whereby growth is interrupted by intermittent periods during which time resting buds are formed (Sreekantan *et al.* 2001). Histological observations of adult buds of *M. excelsa* suggested that leaves were preformed inside the bud. This is in contrast to micropropagated plants where *de novo* leaf organogenesis appeared to arise continuously from the apical meristem.

Within the developing buds on adult plants, the number of preformed leaves was strongly correlated with the size of the bud itself. It is possible that the number of subsequent leaves and internodes laid down following bud break is related to the number of preformed leaves within the bud, as thought to occur in various temperate and tropical tree species (Steeves and Sussex 1989). In the event that only preformed leaves expand, the first primordia formed at the shoot apex may begin to differentiate as cataphylls that would surround the next terminal bud, subsequently containing the next set of pre-formed leaves (Steeves and Sussex 1989). Presumably, the size of the shoot, being the number of nodes formed, would be a function of the size of the bud at the time of bud-break. Therefore, bud-break of relatively small or large buds may result in shoots containing few or many nodes, respectively.

Relatively little attention has been given to the evaluation of carbon isotope signatures conducted during bud and subsequent shoot and leaf ontogeny. The fact that carbon isotope composition in partly to fully-expanded leaves of *M. excelsa* were similar to those expressed in older leaves (~6 month in age), suggests that discrimination levels were not affected by the age of the leaf. However, Δ values in buds containing pre-formed leaves, although not differing from that of old subtending leaves, were significantly lower than that of the newly expanded leaves. This difference is probably related to the origin of organic matter obtained during bud development. Damesin *et al.* (1998) suggested that the origin of fractionated leaf-external organic matter differed between deciduous and evergreen species. In deciduous oak species, for example, this organic matter comprises reserves (mostly starch) stored from previous growth seasons, whereas leaf organic matter in evergreen species is mostly comprised of recently assimilated sugars (Damesin *et al.* 1998). Considering that *M. excelsa* is an evergreen species, it was surprising that Δ values were comparatively lower in bud tissue,

particularly since recently synthesised photoassimilates deriving from subtending leaves would be expected to have a similar isotopic signature. It is possible that some of the carbohydrates acquired during bud growth were obtained from storage carbohydrates, given that complex carbohydrates, such as starch, can have a higher affinity for ^{13}C than soluble sugars (Brugnoli *et al.* 1988). This would explain the relatively low Δ values recorded in the developing bud. Further studies, however, using ^{13}C labelling would confirm this hypothesis.

In conclusion, the role of carbohydrates during the phase change process has, as of yet, received very little scientific attention. It has been suggested that treatments that promote carbohydrate accumulation in various parts of the plant can also affect the transportation and accumulation of other substances (e.g. hormones), which would subsequently complicate interpretation of carbohydrate effects on plant developmental and physiological responses. Nevertheless, the evidence thus far suggests that carbohydrates do play a role during phase change (Tsai *et al.* 1997), although further studies incorporating molecular technology would elaborate on their role during vegetative phase change. The results of this study suggest that shoot restriction accelerates vegetative phase change in *M. excelsa.* In future work, examination of these trends over a longer growing period would provide a useful comparison between branched and single-stemmed plants. The hypotheses that a reduction in photosynthetic and Δ parameters in single-stemmed plants undergoing phase change were attributed to either the age of the leaf or to differences expressed between juvenile and adult plants were not supported by this study. Instead, the decline in these parameters, although coinciding with vegetative phase change, were consistent with studies linking these phenomena to alterations in source and sink relations. Therefore, based on the evidence presented in this study, the decline in leaf Δ values with increasing maturity in single-stemmed plants would not serve as a reliable marker of phase change in *M. excelsa*. Future research in this area may profit from a better understanding of the role of carbohydrates during vegetative phase change in woody species.

Chapter 4

Effects of photoperiod, temperature and bud size on flowering in *Metrosideros excelsa*

4.1 Introduction

Manipulation of flowering time has been used extensively within the horticultural industry (Atherton 1987), particularly in the scheduled production of ornamental pot-plants. Most research in this area has focused on a limited number of herbaceous species or woody plants cultivated for many decades. However, relatively few studies have considered development of practical methods for controlling flowering in woody angiosperm species new to cultivation. Of those that have, manipulation of temperature and/or photoperiod conditions have been successful in promoting flowering in *Leptospermum* (Zieslin 1985), *Chamelaucium* (Shillo *et al.* 1985), *Eucalyptus* (Moncur 1992), *Hypocalymma* (Day *et al.* 1994a), *Pimelea* (King *et al.* 1996) and *Hardenbergia* (King 1998).

Specific temperature and photoperiodic treatments required to initiate flowers vary between species. In *Leptospermum* and *Chamelaucium* (family Myrtaceae), sustained cool temperatures (mean 15-20°C) and short days (≤12 h) were conducive for floral initiation (Shillo *et al.* 1985, Zieslin and Gottesman 1986, Dawson and King 1993). However, flowering in *Eucalyptus* and *Hypocalymma* was achieved using short (5-10 week) exposure to cold (12-13°C) temperatures, irrespective of photoperiod (Moncur 1992, Moncur and Hasan 1994, Day *et al.* 1994a). Floral induction in response to relatively cool temperatures has also been reported for *Pimelea* (King *et al.* 1996).

The bud contains the active site (apical meristem) where endogenous signals for flowering are received from the subtending leaf (Bernier *et al.* 1993). However,

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relatively little attention has been paid to the effect on floral initiation that bud size at the time inductive conditions are applied might have (Patten and Wang 1994). In cranberry (*Vaccinium macrocarpon*) buds >1 mm in diameter in autumn had a higher probability of becoming floral the following summer than did buds <1 mm in diameter (Patten and Wang 1994). Therefore, it is important to understand the influence bud size can have on developmental outcome of buds.

The unpredictable timing and non-uniform patterns of flowering (Clemens *et al.* 1995) have hindered the development of *Metrosideros* for the floricultural market. The aim of this study was, therefore, to determine the effect of photoperiod and temperature on floral initiation and development, and to examine the effect of bud size on flowering in two important cultivars of *M. excelsa.* The approach taken in this experiment was to study potentially inductive combinations of lowered temperature and short photoperiod applied over a number of weeks in controlled environments. In a complementary experiment, other plants were grown through winter either under naturally inductive conditions, or in greenhouses to determine the deleterious effects on flowering of elevated temperature and/or daylength extension.

Based on the current literature on factors that promote floral induction in species within the Myrtaceae, it is hypothesised that:

1) Both low temperature and/or short daylength are important in promoting floral induction in *M. excelsa.*

2) The size of the developing bud at the time of application of an inductive treatment will determine whether or not floral initiation will take place, with larger buds becoming floral more readily than smaller buds.

4.2 Materials and Methods

4.2.1 Plant materials

Commercial growers supplied two year-old, cutting-grown plants of *Metrosideros excelsa* 'Scarlet Pimpernel' and 'Vibrance' that had been grown under ambient conditions. In August 1997, when the plants were approximately 1.5 m high,

they were potted into 7 l containers using a peat and pumice growing medium (80:20 v/v) and appropriate control-release fertilisers. The plants were maintained in a greenhouse from November 1997 (early summer) until the start of experimentation in February 1998. During this time the greenhouse was vented at 24°C and warmed at night when required ensuring the minimum temperature was no lower than 17°C. Daylength was extended to 16 h using incandescent lighting (10 μmol m^{-2} s^{-1}). Two experiments were conducted using a single population of each cultivar for both experiments.

4.2.2 Controlled environment experiment

In the first experiment, temperature and photoperiod treatments were applied in four controlled environment rooms located at the New Zealand Controlled Environment Laboratory, The Horticulture and Food Research Institute of New Zealand (HortResearch), Palmerston North. Plants were grown using a factorial combination of two temperatures and two photoperiods. Day/night temperatures were maintained at either 12/9°C or 17/14°C (mean temperatures 10°C and 15°C, referred to below as "cold" and "cool") in combination with 10 h (short day) or 16 h (long day) photoperiods. Lighting in the rooms was provided by four high intensity main 1 kW Metalarc lamps with four 1 kW tungsten halogen lamps (8 h) with a photosynthetic photon flux (PPF) of 680-700 μmol m^{-2} s^{-1}. A photoperiod extension of either 2 h (short days) or 8 h (long days) was provided by low intensity 150 W Tungsten auxillary lamps (PPF 7 μmol m^{-2} s^{-1}). In growth rooms maintained under cool conditions, the relative humidity (RH) was maintained at (day/night) 79/81% with a dew point (DP) at 13/11°C. In cold rooms, RH was maintained at 71/74% with a DP at 7/5°C. In all rooms, CO_2 concentration was 350 ppm and vapour pressure deficit was 0.4/0.3 kPa (day/night) (Fulton 1998).

Plants were assigned to one of four blocks and subsequently to one of the four treatments. Each block contained seven plants, three of which were 'Scarlet Pimpernel' and four of 'Vibrance'. Due to the low number of 'Scarlet Pimpernel' plants, they were placed in blocks per treatment using a balanced incomplete block design. Plants were blocked on four trolleys and were rotated within the rooms every seven days to ensure an even light distribution over time and to reduce any positional effects created by the rooms. Subsets of plants were subjected to the four environments for 0, 5, 10 or 15 weeks before being transferred to a greenhouse maintained at 24/17°C (day/night) under

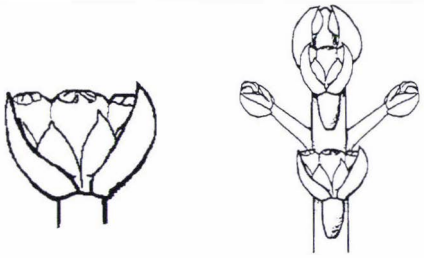
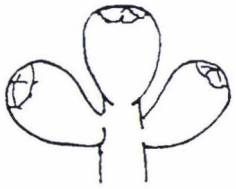
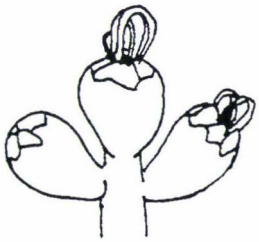
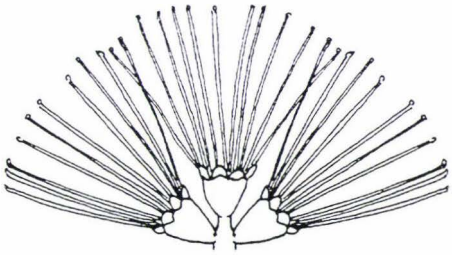
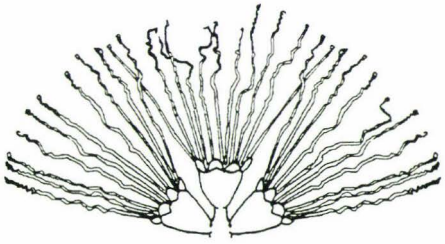
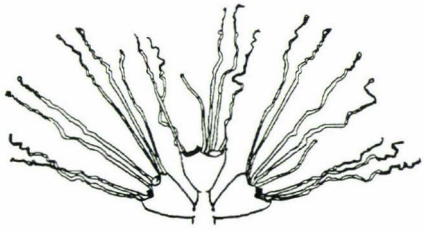
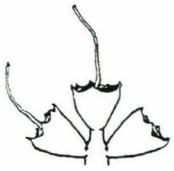
natural illumination with photoperiod extended to 16 h using 100 W incandescent lights (10 μmol m^{-2} s^{-1}).

At the outset of the experiment, 10 pairs of terminal axillary buds from each plant were selected. Selection of buds was designed to capture a proportional range of bud sizes from each plant so that their fate could be established at the completion of the experiment. Initial measurements were made of bud diameter perpendicular to the petiole using digital calipers. Upon completion of the experiment in January 1999, buds were scored as floral, aborted, vegetative, or not broken (fewer than two pairs of scales parted). Records were kept of the number of plants that flowered, the number of inflorescences per plant, the number of cymules per inflorescence and the timing of anthesis. Changes in floral development were tracked every seven days using seven recognisable stages (Stages 1-7, Table 4.1).

4.2.3 Greenhouse experiment

Plants were maintained in four greenhouses for the duration of the experiment (February 1998–January 1999) using factorial combinations of temperature and daylength. The two temperature regimes were those occurring under ambient conditions, and maintenance of a warm temperature regime (24/17°C) (day/night). Mean ambient day/night temperatures during mid-winter were 13/8°C, rising to 25/17°C in mid-summer. Photoperiod treatments were ambient conditions (declining through mid-winter to 9 h and rising to 13 h in mid-summer), and maintenance of a 16 h photoperiod. Photoperiod was extended using 100 W incandescent lights (10 μmol m^{-2} s^{-1}). Plants held under ambient temperature conditions were housed in semi-transparent plastic tunnels that provided frost protection and unrestricted air-flow from both ends. Temperatures were monitored using Hobo shuttle loggers (model H8, Scott Technical Instruments, Hamilton, New Zealand). Treatments were replicated using four blocks, each containing six individuals of 'Vibrance' and one of 'Scarlet Pimpernel'. Observations of plant development were made as for the controlled environment experiment.

Table 4.1 Classification of stages of floral development in *Metrosideros excelsa*.

Developmental stage	Description	Diagram
1: Bract shedding	• Subtending bracts shed from cymule • Individual flower buds visible	
2: Tight bud	• Remaining bracteoles shed • Red petals visible between calyx lobes	
3: Flower opening	• Petals reflexing • Style and stamens expanding	
4: Anthesis	• Stamens and style fully elongated • Pollen shedding	
5: Stamen wilting	• Stamens starting to wilt	
6: Stamen abscission	• Stamen abscission occurring • Petals abscising	
7: Senescence	• All stamens and petals abscised • Style may have abscised	

4.2.4 Statistical Analyses

Statistical analyses were conducted using SAS System statistical package (SAS Institute, Cary, N.C., USA). For each experiment, the main and possible interaction effects of temperature and daylength on the proportion of flowering plants and the number of inflorescences per plant were analysed using analyses of variances with the GENMOD procedure. The GENMOD procedure fits a generalised linear model to the data by maximum likelihood estimations, where the mean of a population is dependent on a linear predictor through a nonlinear link function. The response probability distribution was based on a Poisson distribution for count data, and a logistic regression for proportional data. An examination of differences in the rates of flower development was conducted using a repeated measures analysis of variance with treatment and time as the main factors. Differences between treatments were assessed using Tukey multiple comparison tests. Data were log transformed prior to analyses.

4.3 Results

4.3.1 Controlled environment experiment

For the cultivar Vibrance, flowering after transfer to the forcing greenhouse occurred only in plants held for 15 weeks under cool (mean 15°C), short days (10 h). No plants of this cultivar flowered after being held under cool, long days (16 h), or under cold (mean 10°C) conditions with either long or short days (Figure 4.1 A). Sixty-seven percent of 'Scarlet Pimpernel' plants flowered after being held for only 10 weeks under cool, short days before transfer to the forcing greenhouse. After 15 weeks, 33-67% of 'Scarlet Pimpernel' plants flowered in all treatment combinations (Figure 4.1 A). Both temperature and photoperiod main effects were significant ($P<0.05$), with cold and short days treatments having the higher proportion of flowering plants. Plants of either cultivar remaining in the forcing greenhouse or treated for only five weeks before transfer to the greenhouse did not flower.

'Vibrance' plants in the cool, short day treatment bore 8-9 inflorescences per plant (Figure 4.1 B). In 'Scarlet Pimpernel', temperature and photoperiod significantly affected ($P<0.05$) the number of inflorescences per plant, with the cool, short day treatment giving rise to the greatest number of inflorescences ($P<0.05$) (Figure 4.1 B).

Figure 4.1 Effects of temperature and photoperiod on flowering in plants of *Metrosideros excelsa* cvs. Scarlet Pimpernel (dark bars) and Vibrance (white bars). (A) Percentage of plants flowering, and (B) number of inflorescences per plant, in plants treated for 15 weeks under cool (mean 15°C) or cold (mean 10°C) temperatures in factorial combination with 10 h (SD) or 16 h (LD) photoperiods. (C) Percentage of plants flowering, and (D) mean number of inflorescences per plant, in plants grown continuously in greenhouses maintained at either ambient temperatures (Amb T) or under warm conditions (24/17°C) in factorial combination with ambient daylengths (Amb DL) or a 16 h (LD) photoperiod.

A
Plants flowering (%)
100
75
50
25
0
Cool
Cold
LD
SD
B
Inflorescences/plant
10
8
6
4
2
0
Cool
Cold
LD
SD
C
Plants flowering (%)
100
75
50
25
0
Amb T
Warm
LD
Amb DL
D
Inflorescences/plant
30
25
20
15
10
5
0
Amb T
Warm
LD
Amb DL

There was no interaction between the main effects for number of inflorescences per plant. 'Scarlet Pimpernel' inflorescences were significantly larger ($P<0.01$) in the cool, short day than in the cold, short day treatment (6.9 and 3.4 cymules per inflorescence, respectively).

Plants from the controlled environment experiment reached anthesis (Stage 4) in late spring (Julian day 290-340). Inflorescences of 'Scarlet Pimpernel' took 3-4 weeks to pass from Stage 2 (tight bud) to Stage 6 (stamen abscission), Stage 4 (anthesis) lasting 5-6 days, regardless of treatment. However, there was a significant effect of treatment ($P<0.05$) on the time at which these stages of floral development took place (Figure 4.2). Inflorescences of plants held for 10 weeks under cool, short days before transfer to the forcing greenhouse developed significantly earlier (by two weeks) than those of plants treated for 15 weeks under the same conditions. Plants treated for 15 weeks with cool, long days developed significantly later (by approximately five weeks) than those given the cool, short day treatment (Figure 4.2). However, there was no difference between the timing of floral development in plants treated with long or short days under cold conditions. The timing of floral development in 'Vibrance' plants treated with cool, short days was the same as that of 'Scarlet Pimpernel' plants given the same treatment for 15 weeks.

The diameter of 'Scarlet Pimpernel' buds that became floral after 15 weeks treatment with cool, short days was in the range 2.1-4.0 mm (mean 2.6-3.0 mm size class) (Figure 4.3). By contrast, mean bud diameter for those that remained vegetative was centered on the 1.6-2.0 mm size class. The highest proportion of unbroken buds was in the smallest size class (1.1-1.5 mm diameter) (Figure 4.3). Breaking buds of 'Scarlet Pimpernel' in the smaller size classes also tended to be vegetative rather than floral in the other controlled environment treatments, although the numbers of floral buds in the sample was low because the treatments were relatively ineffective for floral induction.

4.3.2 Greenhouse experiment

Temperature and daylength treatments significantly affected the proportion of plants that flowered ($P<0.01$), and there was also a significant interaction between the main effects ($P<0.05$) in both cultivars. The highest proportion of plants that flowered occurred under conditions of ambient temperature and ambient daylength (100% and

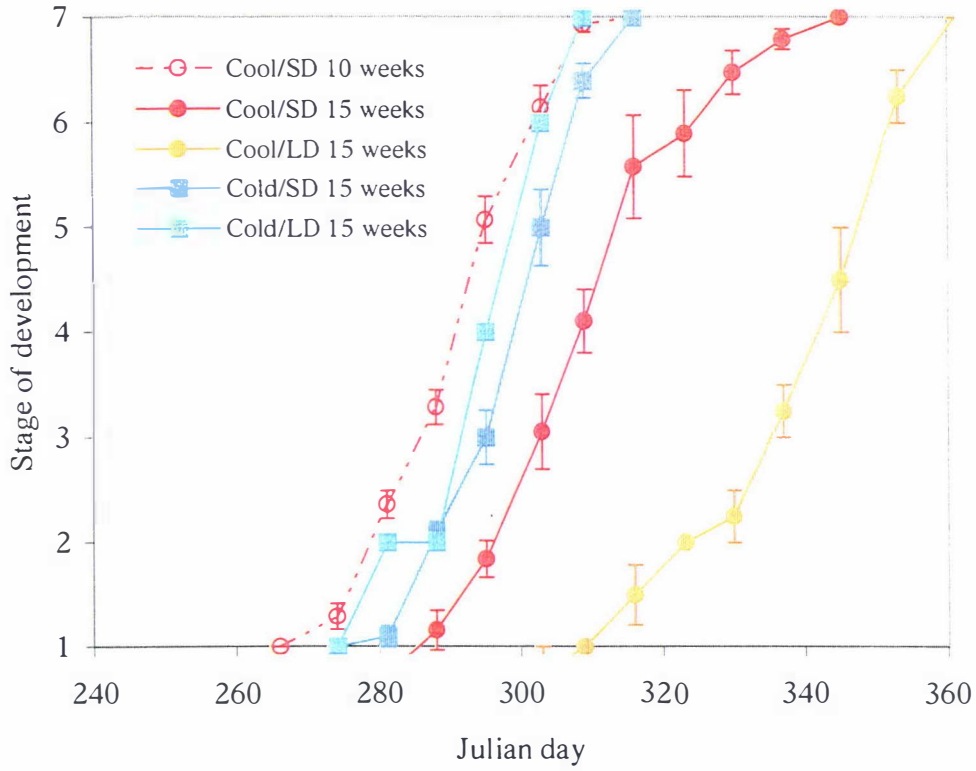


Figure 4.2 Mean (±SE) rate of floral development in plants of *Metrosideros excelsa* 'Scarlet Pimpernel' treated for 10 or 15 weeks in controlled environments before being transferred to a forcing greenhouse. Plants were grown under cool (mean 15°C) or cold (mean 10°C) temperatures in factorial combination with 10 h (SD) or 16 h (LD) photoperiods.

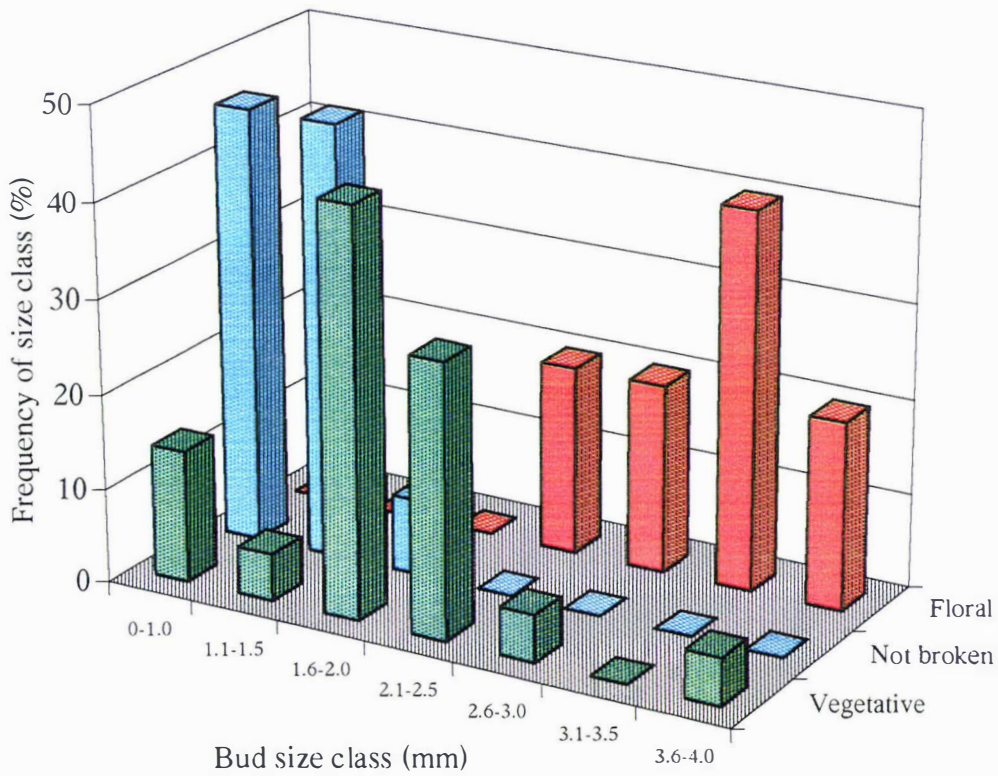


Figure 4.3 Percentage of *Metrosideros excelsa* 'Scarlet Pimpernel' buds in each of seven bud size classes that were floral, remained vegetative, or did not break in plants transferred to a forcing greenhouse after 15 weeks treatment with cool (15°C), short days (10h).

83% for 'Scarlet Pimpernel' and 'Vibrance', respectively) (Figure 4.1 C). The proportion of 'Scarlet Pimpernel' plants that flowered was also high (100%) in the ambient temperature and long day treatment, although this was not the case for 'Vibrance' (12.5%). No flower buds developed under warm (24/17°C), long days for either cultivar.

Temperature and daylength significantly affected the number of inflorescences per plant ($P<0.001$), with ambient temperature rather than warm conditions, and ambient daylength rather than long days, resulting in more inflorescences per plant in both cultivars. There were also significant interactions between the main effects ($P<0.001$) in both cultivars. Significantly more inflorescences per plant occurred in the ambient temperature and ambient daylength treatment for both cultivars (Figure 4.1 D). Number of cymules per inflorescence across all treatments was in the range 5.8-7.0.

Both cultivars in the greenhouse experiment reached anthesis at approximately the same time in early summer (Julian day 355-365). In general, the time taken for inflorescences to pass through Stages 1-7 was similar to that shown for inflorescence of plants from the controlled environment experiment (Figure 4.2). At no time did treatment have an effect on the rate of inflorescence development in 'Scarlet Pimpernel' (Figure 4.4 A). However, inflorescences of 'Vibrance' plants in the warm temperature/ambient daylength treatment were more advanced by ~10 days than those in the ambient temperature treatment with either ambient or short daylength (Figure 4.4B). These significant effects ($P<0.05$) were evident between 337 and 361 Julian days.

As in the controlled environment experiment, 'Scarlet Pimpernel' and 'Vibrance' buds that initially had a mean diameter of 2.1–2.5 mm were more likely to become floral than those of any other size class. The proportion of buds that remained vegetative increased with decreasing bud size class (highest proportion below 1.0 mm). The largest proportion of unbroken buds in both cultivars was usually below 1.0 mm in diameter.

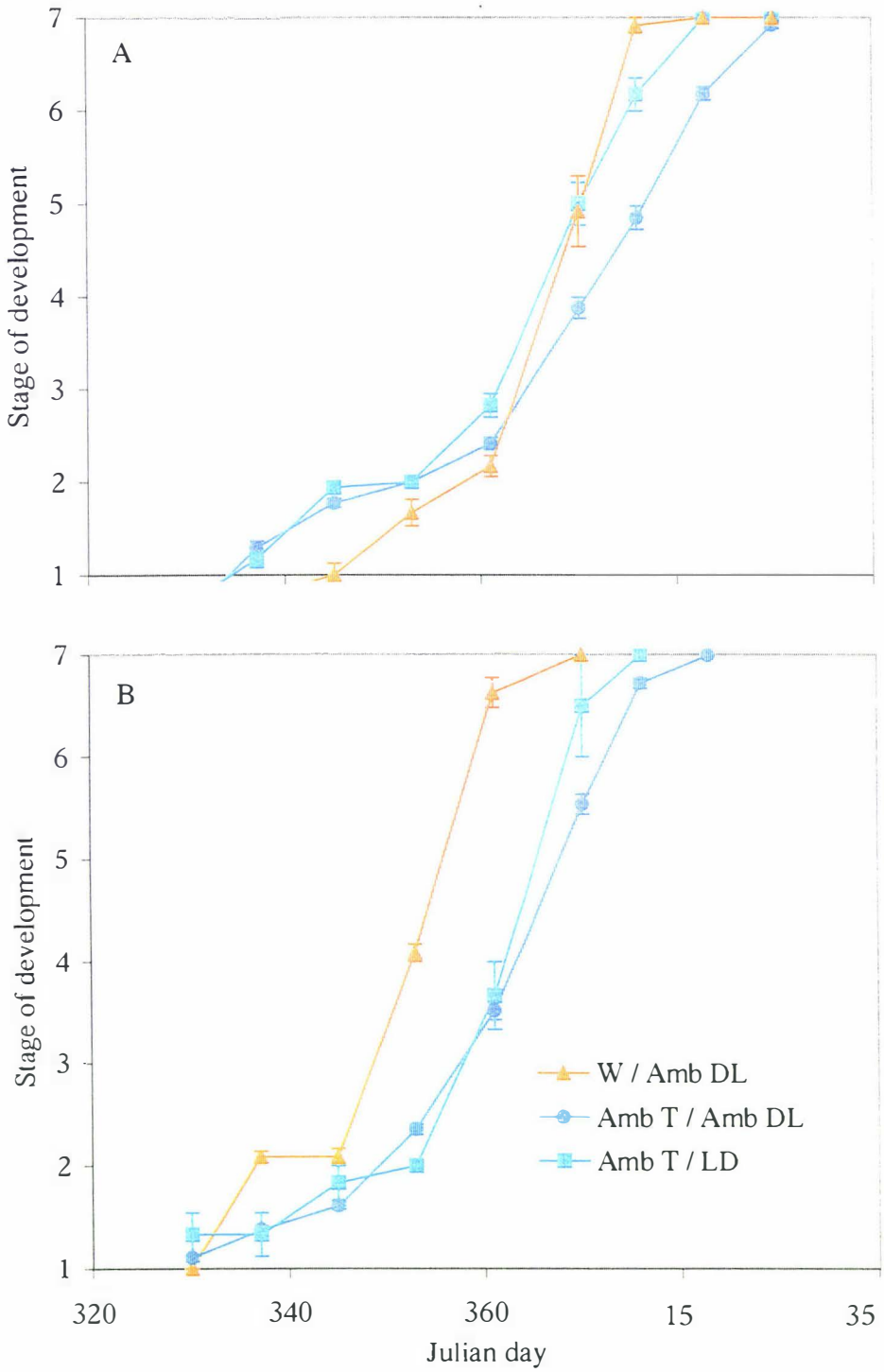


Figure 4.4 Mean (±SE) rate of floral development in plants of *Metrosideros excelsa* (A) 'Scarlet Pimpernel' and (B) 'Vibrance' grown continuously in greenhouses maintained at either ambient temperature (Amb T) with either ambient daylengths (Amb DL) or a photoperiod of 16 h (LD), and at a day / night temperature of 24/17°C (Warm) with ambient daylength (Amb DL).

4.4 Discussion

Metrosideros excelsa 'Vibrance' was highly responsive to photoperiod. While a significant number of 'Scarlet Pimpernel' plants flowered under both short days and long days, few or no 'Vibrance' plants flowered under long days. However, in both cultivars the intensity of flowering was significantly reduced by exposure to long days. Consequently, *Metrosideros excelsa* can be considered to be a facultative short-day plant. Temperature also had a significant effect on flowering, with the controlled environment cold treatment completely inhibiting flowering of 'Vibrance' and reducing the intensity of flowering of 'Scarlet Pimpernel'. A combination of lowered temperatures and short photoperiods are also important for floral induction in a number of temperate and tropical species within Myrtaceae. Shillo *et al.* (1985), for example, showed that *Chamelaucium uncinatum* required a four week treatment of short days and cool (20/14°C) temperatures for flower initiation and development to occur. Similarly, combinations of short days and lowered temperatures caused flowering in *Leptospermum scoparium* (Zieslin 1985) and *Hardenbergia* (King 1998). In contrast to *M. excelsa*, some species in Myrtaceae appear to be responsive to either photoperiod or temperature. Bolotin (1975), for instance, reported that seedlings of *Eucalyptus occidentalis* (less than one year old) were capable of precocious flowering when grown under a long-day regime of 16 h or longer, whereas temporary exposure (4-6 weeks) to a relatively low temperature regime in *E. lansdowneana* was sufficient for promoting floral initiation, regardless of the daylength (Moncur 1992). Moncur and Hasan (1994) also suggested that low temperature rather than a change in photoperiod is a strong stimulus for flowering in *E. nitens*.

The variability in flowering responses exhibited by the different cultivars of *M. excelsa* was not unexpected. Variation in responses may reflect the different parental origins of cultivars. Noack *et al.* (1996), for example, investigated the influence of different low temperature treatments on the timing and degree of flowering in three New Zealand *Hebe* cultivars. For *Hebe* 'Variegata', the duration of the low temperature requirement for obtaining an equivalent number of flowers was longer than that in 'Inspiration' and 'Waikiki', and was attributed to the long chilling period experienced in the natural environment by the parental stock of the former cultivar. King *et al.* (1995) also reported on the flowering responses of different ecoytpes of *Pimelea ferruginea* grown under controlled conditions and found that selections from cooler southerly sites

flowered with a lower temperature optimum than those from northerly sites. Thus, between-cultivar differences in *M. excelsa* were more likely due to differences in cultivar responsiveness rather than to differences in the conditions under which the plants were grown before experimentation began. Plants of both cultivars were grown under identical conditions for several months during the previous year, including the time of the spring growth flush when potential floral meristems would have been laid down.

The optimum conditions for floral initiation within the controlled environment experiment were Cool (17/14°C) temperatures and Short Daylength. In regards to the number of inflorescences per plant, 'Vibrance' induced under Cool/Short Day conditions showed a similar response to those grown in the ambient temperature/ambient daylength greenhouse environment, suggesting that a minimum requirement of 15 weeks was suitable for maintaining a comparable flower intensity. 'Scarlet Pimpernel' appeared to be more responsive to a range of treatments and durations (10 and 15 weeks) to induce flowering. However, in both cultivars the optimum number of inflorescences per plant was achieved with the 15 week Cool/Short Daylength treatment. The effect of this treatment imposed during the controlled environment experiment may have been greater had the treatments been imposed for longer than 15 weeks, especially in the 'Vibrance'. In addition, plants may have borne more flowers had the environment in the forcing house to which the plants were transferred been optimised for floral development subsequent to floral initiation. However, because treatment effects were maintained during forcing, it can be considered that the forcing house environment was satisfactory for its purpose. For this reason, the lack of floral response in plants held continuously in the forcing house can be ascribed to the unsuitability of its warm, long days for floral induction, rather than for subsequent floral development.

In field-grown plants of *M. excelsa*, floral initiation is observed over a 6-12 week period during the time of most rapid decline in daylength in autumn (Sreekantan *et al.* 2001). It is therefore likely that floral initiation in our controlled environment experiment occurred before plants were transferred to the forcing house. The delay of floral development in 'Scarlet Pimpernel' plants in the cool, long day treatment relative to the cool, short day treatment may have arisen directly from a delay in floral initiation in the former treatment.

Exposure of plants to inductive conditions for up to 15 weeks in the controlled environment experiment advanced flower development by eight weeks compared to that of plants grown continuously under ambient conditions. Floral initiation was probably well advanced in both sets of plants before the plants from the controlled environment were transferred to the forcing greenhouse. Therefore, the difference in flowering time was due to a shorter floral developmental time between initiation and anthesis in plants transferred to the forcing house. It was interesting to note that 'Scarlet Pimpernel' plants transferred from the 10 and 15 week cool, short day treatment reached anthesis only two weeks apart. Similarly, Noack *et al.* (1996) found that *Hebe* plants, subjected to longer low temperature treatments followed by a transfer to forcing conditions flowered more rapidly compared to those exposed to shorter low temperature treatments.

In the greenhouse experiment, flowering intensity was greater in the ambient temperature and daylength environment, one in which vegetative growth was minimal during winter. The relationship between reduced vegetative growth and enhanced flowering is a common trend in woody species (Bernier *et al.* 1981b). Developing floral meristems are intensive centres of growth, requiring assimilates and other metabolites. A reduction in shoot growth enables the plant to channel these nutrients to the growing bud (Halevy 1987).

'Scarlet Pimpernel' plants from the Ambient Daylength and Ambient Temperature/ Long Day greenhouse treatments reached anthesis simultaneously despite their daylength or temperature treatments. This lack of treatment response may be due to an overriding effect of irradiance, photosynthetic photon flux levels would have increased similarly across all greenhouse treatments. Although 'Vibrance' plants receiving the Ambient Temperature treatment reached anthesis at approximately the same time as those of 'Scarlet Pimpernel', floral development in this cultivar was more accelerated by the Warm temperature treatment. For instance, 'Vibrance' inflorescences in the forcing Warm/Ambient Daylength environment reached anthesis and senesced significantly faster compared to the other environments, suggesting a greater cultivar-specific sensitivity to temperature for flower opening than that of 'Scarlet Pimpernel'. King *et al.* (1992) demonstrated a similar response in *Pimelea ferruginea*, whereby plants transferred to a warm environment (24/19°C) following seven weeks of cool

inductive conditions (15/10°C) accelerated floral development relative to those held continuously in the cool environment. Similarly, opening patterns in the *Portulaca* species from Japan are influenced strongly by temperature and, less so, by light (Ichimura and Suto 1998). Rapid flower opening occurred when temperatures were increased to 20°C. This response was also intensified when light (as opposed to dark) conditions were applied.

This study supported the hypothesis that the size of the bud during induction can significantly affect its competency to become an inflorescence. Interestingly, resting buds of an intermediate diameter at the time of their treatment were more responsive to inductive signals. Such a positive correlation between bud size and probability of buds becoming floral has been observed for field-grown *Metrosideros excelsa* trees (Clemens *et al.* 1998, Sreekantan *et al.* 2001). Sreekantan *et al.* (2001) observed that larger buds were more likely to be floral and bear more cymules in each inflorescence than smaller buds. However, they also noted that some of the largest buds borne on vigorous shoots tended to be vegetative. They attributed this to the early development of leaves within the resting bud before the onset of inductive conditions. A few studies on other species have shown a similar relationship between bud size and floral competency, such as in *Iris* (Doss and Christian 1979), *Vaccinium macrocarpon* (Patten and Wang 1994) and *Eryngium planum* (Ohana and Weiss 1998). Physiological processes underlying this phenomenon have not been reported, although in the case of *Metrosideros* this effect may be due to the timing of shoot developmental events during the previous vegetative growth flush (Clemens *et al.* 1998, Sreekantan *et al.* 2001). Larger buds presumably contain more developmentally advanced meristems that are better able to respond to environmental signals than those in smaller buds.

In conclusion, this study supported the hypothesis that flowering in *Metrosideros excelsa* can be manipulated by applying or withholding cool temperatures and short days, and by advancing flowering time using warm, long day conditions after inductive conditions have been applied. Cultural treatments that synchronise development of quiescent bud populations within plants could be used to increase the proportion of buds that become floral, provided inductive treatments are applied when buds are of an appropriate size.

Chapter 5

Effect of irradiance on floral induction and development in *Metrosideros excelsa*

5.1 Introduction

The influence of temperature and photoperiod on flowering has been a recurrent theme in the literature over the past century. In many species, plant responses to changes in temperature and/or photoperiod can precede the onset of floral activity in meristematic regions. However, less emphasis has been placed on other environmental factors such as irradiance which can have a significant effect on flower number and quality in many species. In several myrtaceaous woody plants, the manipulation of temperature and/or photoperiod has been successful in promoting flowering in genera such as *Leptospermum* (Zieslin 1985), *Chamelaucium* (Shillo *et al.* 1985) and *Eucalyptus* (Moncur 1992), whereas only in *C. uncinatum* (Dawson and King 1993) has the effect of irradiance been examined. In this species, the level of irradiance applied during induction (up to 750 μmol m^{-2} s^{-1}) was linearly related to the number of flowers produced per plant.

A common theme demonstrated in various woody plants has been this positive relationship between flower number and irradiance. Ornamentals such as *Begonia* x *hiemalis* (Myster 1999), *Hibiscus rosa-sinensis* (Neumaier *et al.* 1987), *Kalanchoë blossfeldiana* (Mortensen 1994) and *Rosa* species (Anderson 1993) produce a greater number of flowers under high compared to low irradiance treatments. Associated with plants growing under these different irradiance treatments are changes in the status of leaf chlorophyll and carbohydrate concentrations, often being features associated with flowering ability and number (Jiao and Grodzinski 1998, King and Ben-Tal 2001). In some ornamentals such as *Leucospermum* or *Hamelia patens*, exposure to low light can also affect flower morphogenesis or delay the time to anthesis, respectively (Jacobs and Minnaar 1980, Armitage 1995). For instance, flower stem length in various woody species such as *Boronia heterophylla* and *B. megastigma* (Plummer *et al.* 1998),

Begonia x *hiemalis* (Myster 1999) and various cultivars of *Rosa* (Bredmose 1993) were significantly increased when grown under low irradiance.

The effects of varying levels of irradiance on flowering and vegetative growth has been documented in number of other woody perennials, such as *Boronia megastigma* (Roberts and Menary 1989), *Garcinia mangostana* (Wiebel *et al.* 1994) and *Averrhoa carambola* (Marler *et al.* 1994). The emphasis of these and other studies has been on manipulation of irradiance either outdoors or in greenhouse environments using artificial lighting or through the use of neutral density shade-cloth. However, the use of controlled and manipulated environments can provide the most satisfactory results for interpreting irradiance effects on flowering and vegetative performance by removing any confounding environmental factors, such as temperature.

This study investigated the role of irradiance on floral and vegetative features under controlled inductive conditions using a range of artificially supplied photosynthetic photon fluxes (PPF) on adult pot plants of *M. excelsa.* The aim was to quantify the effects of irradiance on floral induction/initiation and subsequent development, and relate both flowering and vegetative growth patterns to physiological changes within the plant during induction. It is hypothesised that:

> Irradiance is positively related to the number of flowers produced in *M. excelsa* when applied in conjunction with treatments inductive for flowering.

5.2 Materials and Methods

5.2.1 Plant material

Experiments were conducted using two commercially available cultivars ('Lighthouse' and 'Scarlet Pimpernel') of *Metrosideros excelsa*. Thirty-two mature 1.5 m (in height) 'Lighthouse' plants were obtained in August 1998 from Lyndale Nurseries, Auckland, and were transplanted into 30 l containers using a peat and pumice growing medium (80:20 v/v) supplemented with control-release fertilisers. Plants were maintained under ambient outside conditions and were irrigated daily.

In addition, 32 micropropagated 'Scarlet Pimpernel' plants, obtained in August 1997 from Lyndale Nurseries Auckland Ltd., grown in 50 mm square plastic pots were transplanted into 0.82-7.2 l containers containing a 90:10 (v/v) growing medium composed of expanded clay granules (1-4 mm diameter, Hydrotron, NZ Hydroponics International Ltd., Tauranga) and vermiculite (4 mm grade, Revertex Industries Ltd). Each plant was supplied with 4.0 g controlled-release fertiliser. Plants were grown in a temperature controlled greenhouse (range 15-23°C) for 9 months. Plants were trained up as a single-stem shoot by pinching all developing axillary buds, a technique shown to promote early vegetative phase change (Chapter 1). Upon reaching an adult vegetative state, as indicated by the attainment of leaf shape, size and texture characteristics indicative of adult plants, they were transplanted into 7 l containers using a 80:20 (v/v) mixture of peat and pumice, and supplemented with Osmocote. The single-stem plants were transferred in June 1998 to an outdoor environment experiencing ambient late-winter conditions where plants were allowed to form side branches. Both cultivars were maintained under these ambient conditions until being transferred into their experimental environments on 12 March 1999.

5.2.2 Experimental environments

Plants of both cultivars were transferred to controlled environment rooms to receive one of four irradiance treatments provided during inductive 8 h days, for 20 weeks. The four controlled environmental rooms were located at the New Zealand Controlled Environment Laboratory, The Horticulture and Food Research Institute of New Zealand (HortResearch), Palmerston North. Plants assigned to each room were arranged in a completely randomised block design using four blocks each containing two replications of both cultivars. Plants were assigned to each block based on a stratified randomised sample of the total number of terminal axillary buds.

Lighting in each of the rooms was provided by either two, four or six 1kW Metalarc lamps (8 h) combined with either one, two or six 1kW Tungsten halogen lamps (8 h) producing a PPF output of either 174, 567, 961 or 1355 μmol $m^{-2}s^{-1}$ per room (PPF increments of 394 units). In each room, the temperature was maintained at (day/night) 17/14°C with a vapour pressure deficit at 0.4/0.3 kPa, a relative humidity at 79/81% and

a dew point at 13/11°C. The concentration of CO_2 was maintained at 350 μl l^{-1}. Downward airflow through the plants was at a velocity of 0.3-0.5 m s^{-1}. Watering to container capacity was conducted for 10 min twice daily using a microtubule irrigation system. Plants were rotated every 7 d to ensure an even light exposure and to avoid any positional effects within the room.

Concurrently, eight plants of each cultivar serving as the control treatment were placed outside under ambient conditions (early-autumn through to mid-winter) for 20 weeks at the Plant Growth Unit, Massey University, Palmerston North. The arrangement of plants in the ambient treatment followed the same criteria as for those in the controlled environments. Temperatures were monitored outside using a model H8 Hobo shuttle logger (Scott Instruments, Hamilton, NZ). The mean monthly day/night temperatures over the 20 week period for 12 March through to 30 July 1999 were 21.6/16°C, 16.6/11.9°C, 13.3/9.3°C, 10/9.6°C and 10.2/8.4°C, respectively.

Following 20 weeks exposure to the four irradiance treatments and the ambient treatment, all plants were transferred on 30 July 1999 to a forcing greenhouse maintained under warm, LD conditions. The greenhouse was vented when temperatures exceeded 21°C and heated below 15°C. Daylength was extended in the greenhouse using 100 W incandescent lights (10 μmol m^{-2} s^{-1}). Plants were maintained in this environment until plants had reached anthesis, during which time anatomical measurements were made on vegetative and floral shoots.

5.2.3 Bud measurements

At the start of the experiment, the diameter of all distal axillary buds was measured so as to determine the fate of those buds during (e.g. histological examination) and at the completion of the experiment (e.g. presence/absence of an inflorescence). Leaves subtending every pair of axillary buds from each plant were labelled. The initial diameter measurements were also used to classify the buds into one of three size classes from which later samples were taken for histological examination. The three size classes were defined as small (<1.5 mm), medium (1.6-2.0 mm) and large (>2.0 mm) buds.

5.2.4 Bud histology

Bud samples from the three bud size classes were collected after 13, 20 and 23 weeks from the start of the experiment, which occurred during and at the end of the application of the irradiance treatments, and after three weeks in the forcing greenhouse, respectively. During each of these sampling times, one distal axillary bud per size class was randomly selected and carefully removed from each 'Lighthouse' plant using a razor blade. Bud samples were individually transferred to 1 ml Eppendorf tubes containing a FAA fixative solution and subsequently processed following the protocol described in Section 3.2.4 for viewing under light microscope.

5.2.5 Inflorescence measurements

At 11 weeks after plants were transferred to the forcing greenhouse and most inflorescences (89%) were at Stage 1 (October 13-15, 1999), a number of records were obtained for each inflorescence. This included records of the number of inflorescences per plant from each cultivar. For each inflorescence, the bud size class (as determined at the start of the experiment) from which the inflorescence had been derived was determined. In addition, quantitative measurements were made along the axis of each inflorescence. This included count data for (a) the number of individual nodes bearing cymules (≥5 mm in length), (b) the number of cymule pairs, and (c), if present, the number of fully expanded leaves along the actively growing vegetative axis originating from the terminal bud of the indeterminate inflorescence.

Linear measurements using a hand-held digital caliper (Sylvac, Industrial Tooling Ltd, NZ) were made of the regions from which count measurements were conducted, included the length (a) from the base of the inflorescence peduncle to the point of attachment of the first cymule (referred to as the *peduncle*), (b) from the point of attachment of the first cymule through to that of most apical cymule (referred to as the *inflorescence axis*), and (c) of the actively growing vegetative axis originating from the terminal vegetative bud (referred to as the *terminal vegetative axis*). Qualitative data describing the level of activity of the terminal vegetative bud was used to classify the bud as either actively growing, aborted or not broken/broken (>4 bud scales shed).

The stage of floral development was scored weekly starting from the time all cymules on inflorescences were macroscopically visible (Stage 1) through to anthesis (Stage 4). Specific classification of stages of floral development were based on those in Table 4.1 of this thesis. This included stages corresponding to the shedding of bracts sheathing the floral receptacles (Stage 1), expansion of floral receptacle girth (Stage 2), elongation of stamens/style (Stage 3), attainment of anthesis (Stage 4), onset of stamen wilting (Stage 5), onset of stamen abscission (Stage 6) and abscission of all stamens (Stage 7).

5.2.6 Vegetative measurements

On the eleventh week after plants had been transferred into the forcing greenhouse, the six vegetative shoots were selected randomly from each plant of both cultivars. The length and number of nodes were recorded on each shoot.

5.2.7 Chlorophyll determination

One leaf per plant was harvested between 0900-1000 h from each 'Lighthouse' plant on week 20, immediately before plants were transferred to the forcing greenhouse. A representative leaf located on each plant's upper canopy was randomly selected on the second or third node proximal to an axillary branch. The mid-rib which lacks chlorophyll was discarded and a 0.2 g leaf blade sample was weighed and transferred into a glass cuvette containing 4 ml of dimethylformamide (DMF). Samples were chilled temporarily on wet ice before being transferred to a dark 4°C refrigerator for 48 h. Each sample was diluted with 1/10 DMF solution and the extinction coefficient was measured on a spectrophotometer at wavelengths 664 nm (A_{664}) and 647 nm (A_{647}), corresponding to chlorophyll a and b, respectively (Inskeep and Bloom, 1985). Chlorophyll content was measured in mg/g FW using the following formula:

Chlorophyll a (Chl *a*) = $12.7*0.007+A_{664} - 2.79*A_{647}$

Chlorophyll b = (Chl *b*) $20.7*A_{647} - 4.62 * 0.007 + A_{664}$

Total Chlorophyll = Chl *a* + Chl *b*

5.2.8 Carbohydrate extraction and determination

A second leaf sample was collected concurrently on 'Lighthouse' plants following the same selection and harvest criteria as described for the chlorophyll analyses. Leaf blade tissue were snap frozen in liquid nitrogen and stored on wet ice in tin foil sachets. Subsequent processing, extraction and determination of soluble sugars (sucrose, fructose and glucose) and starch concentration follow the protocol described in Section 3.2.10. The methodology was modified by Dr. Jocelyn Eason (The Crop and Food Research Institute) to accommodate the use of micro-amounts that could be read on a plate reader as opposed to a spectrophotometer.

5.2.9 Statistical analyses

All biometrical analyses were conducted using the SAS program (SAS Institute, Cary, N.C., USA). One-way Analyses of Variances (ANOVA) were used to determine the effect of irradiance treatment on the size of microscopic floral meristems and leaf concentrations of chlorophyll, starch and soluble sugars. Multivariate ANOVA were used to test the effect of irradiance and cultivar treatments on a number of floral and vegetative characteristics. The main and possible interaction effects of irradiance and bud size on the proportion of flowering plants and the number of inflorescences per plant were analysed using ANOVAs with the GENMOD procedure (procedure described further in Section 4.2.4). To test the effect irradiance and cultivar treatment on the rate of floral development, a repeated measure ANOVA was used using time as the repeated measure. Data was log transformed when necessary in order to normalise variances.

5.3 Results

5.3.1 Histological examination of buds

Buds from 'Lighthouse' plants were harvested at 13, 20 and 23 weeks from the start of the experiment in order to assess the effects of irradiance and bud size on the presence (or absence) of cymule primordia and, if present, on the level of floral meristem initiation and differentiation. At 13 weeks (seven weeks before removal from the

inductive irradiance treatments), a histological study of buds showed no evidence to suggest that floral initiation had occurred. There was a lack of cellular activity in the axils of bud scales (Plate 5.1 A).

By week 20 (the end of the inductive irradiance treatments), there was a significant effect of irradiance environment on the proportion of buds with cymule primordia that were microscopically visible ($P<0.05$), although there was no effect of bud size ($P>0.05$) or interaction of bud size with irradiance environment ($P>0.05$) (Figure 5.1 A and Plate 5.1 B). Buds with cymule primordia were observed only in the 567, 961 and 1355 $\mu mol\ m^{-2}\ s^{-1}$ controlled environments, although their proportions did not differ significantly between these three environments ($P>0.05$).

At 23 weeks (third week in the forcing greenhouse), there was a significant interaction effect of bud size and irradiance environment ($P<0.05$) on the proportion of harvested buds with cymule primordia, although no main effects of either treatment ($P>0.05$) (Figure 5.1 B). Buds in the 567 $\mu mol\ m^{-2}\ s^{-1}$ PPF environment that were from the medium size class (1.6-2.0 mm at the start of the experiment) had the highest proportion of buds with cymule primordia (50% of buds per plant), whereas low levels (6%) occurred in the 174 $\mu mol\ m^{-2}\ s^{-1}$ PPF environment, and only from the large bud size class. No cymule primordia were observed at 23 weeks in the small and medium bud size classes from either the 174 $\mu mol\ m^{-2}\ s^{-1}$ or ambient environment, or from the small bud size class from the 961 $\mu mol\ m^{-2}\ s^{-1}$ environment.

An examination of floral meristem dimensions between the 20 and 23 week period showed that growth during this period was primarily directed towards elongation of the cymule primordia as shown by a significant increase in meristem length ($P<0.05$) rather than width ($P>0.05$) over that three week period. During this time span, the mean length and width dimensions of cymule primordia had increased from 130±51 µm to 369±144 µm and from 163±45 µm to 269±70 µm, respectively. Meristem size (both length and width parameters) was significantly and positively correlated with the diameter of the bud at the time of harvest (both $R^2=0.487$, $P<0.05$) (Figure 5.2 A-B).

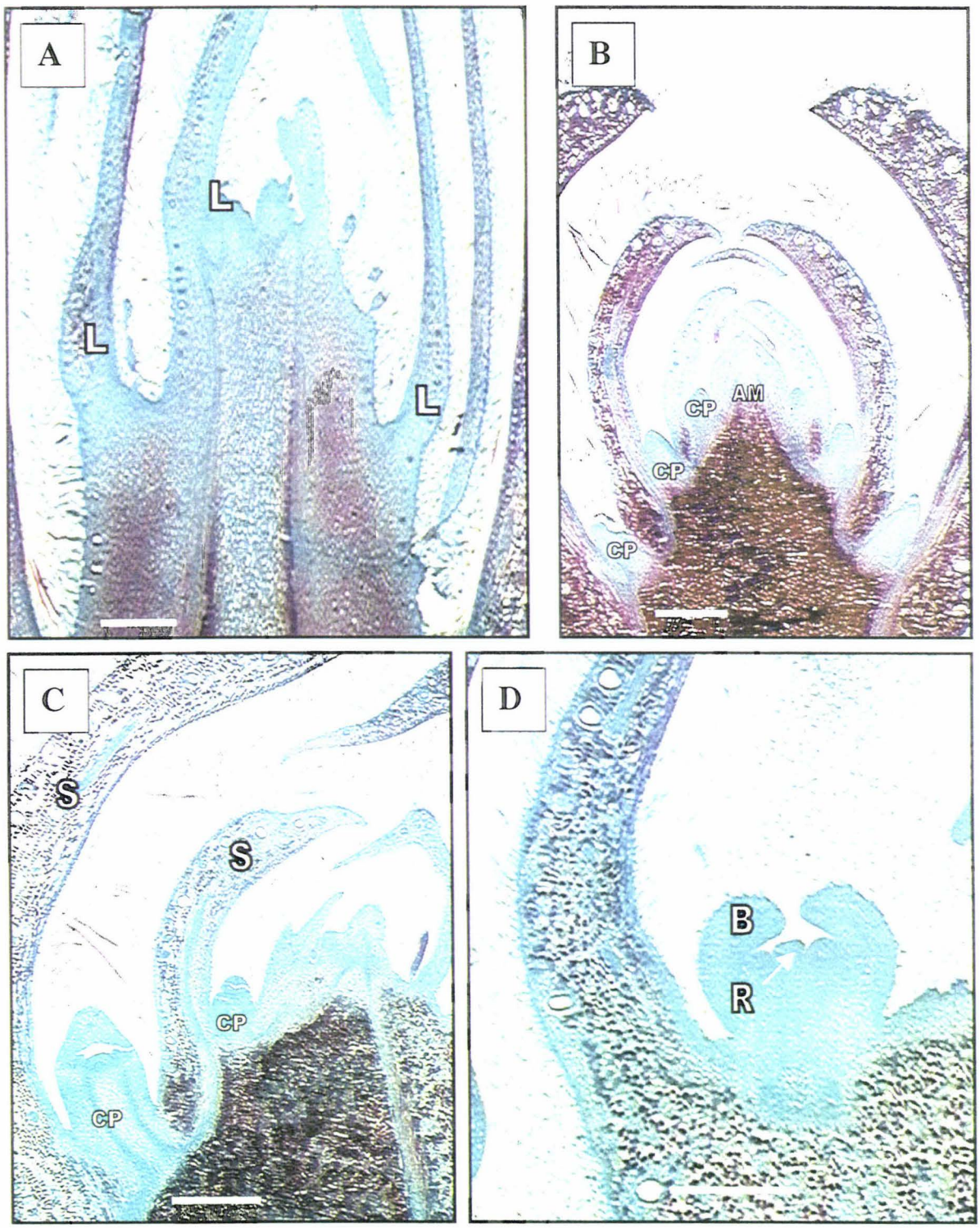


Plate 5.1 Microscopic study of vegetative and floral initiation and development in buds collected at 20 and 23 weeks after the start of the experiment with 20 weeks treatment to different inductive irradiances. A: Vegetative meristem showing a lack of floral meristematic activity in leaf axils at 23 weeks (174 μmol m^{-2} s^{-1}). B: Initiation of floral primordia in scale axils at 20 weeks (567 μmol m^{-2} s^{-1}). C: Development of cymule primordia at 23 weeks (567 μmol m^{-2} s^{-1}). D: Floral receptacle and subtending bract differentiation evident at 23 weeks (567 μmol m^{-2} s^{-1}). Bar = 500 μm. AM = Apical meristem. B = Bract. CP = Cymule primordium. L = Leaf. R = Floral receptacle. S = Scale.

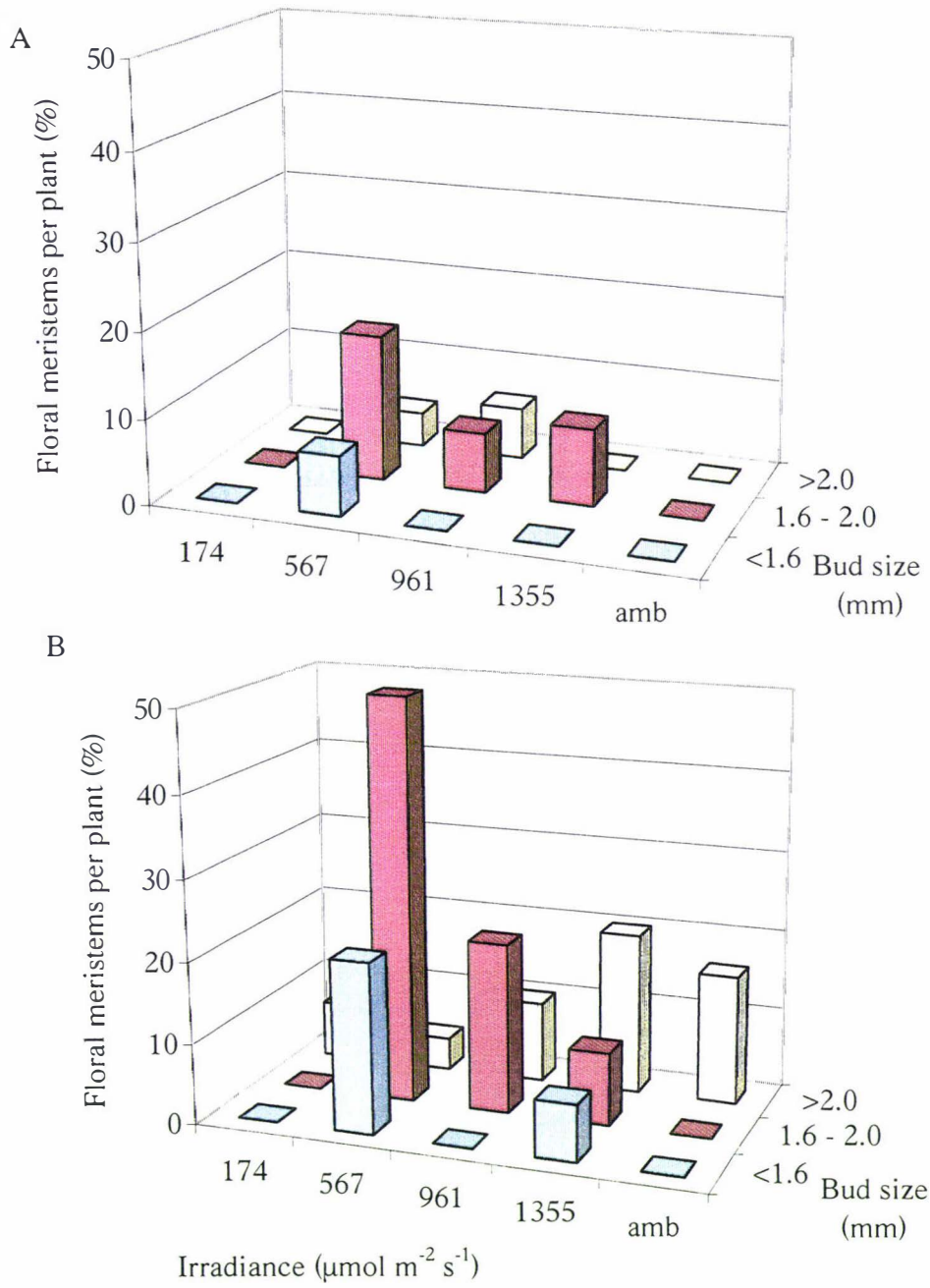


Figure 5.1 Effect of irradiance and ambient conditions (amb) on the cumulative proportion of floral meristems per plant at (A) 20 and (B) 23 weeks from the start of the experiment in three bud size classes of *M. excelsa* 'Lighthouse'. Week 20 corresponded to the last week plants were held under inductive irradiance conditions and week 23 to the third week plants were held under forcing conditions.

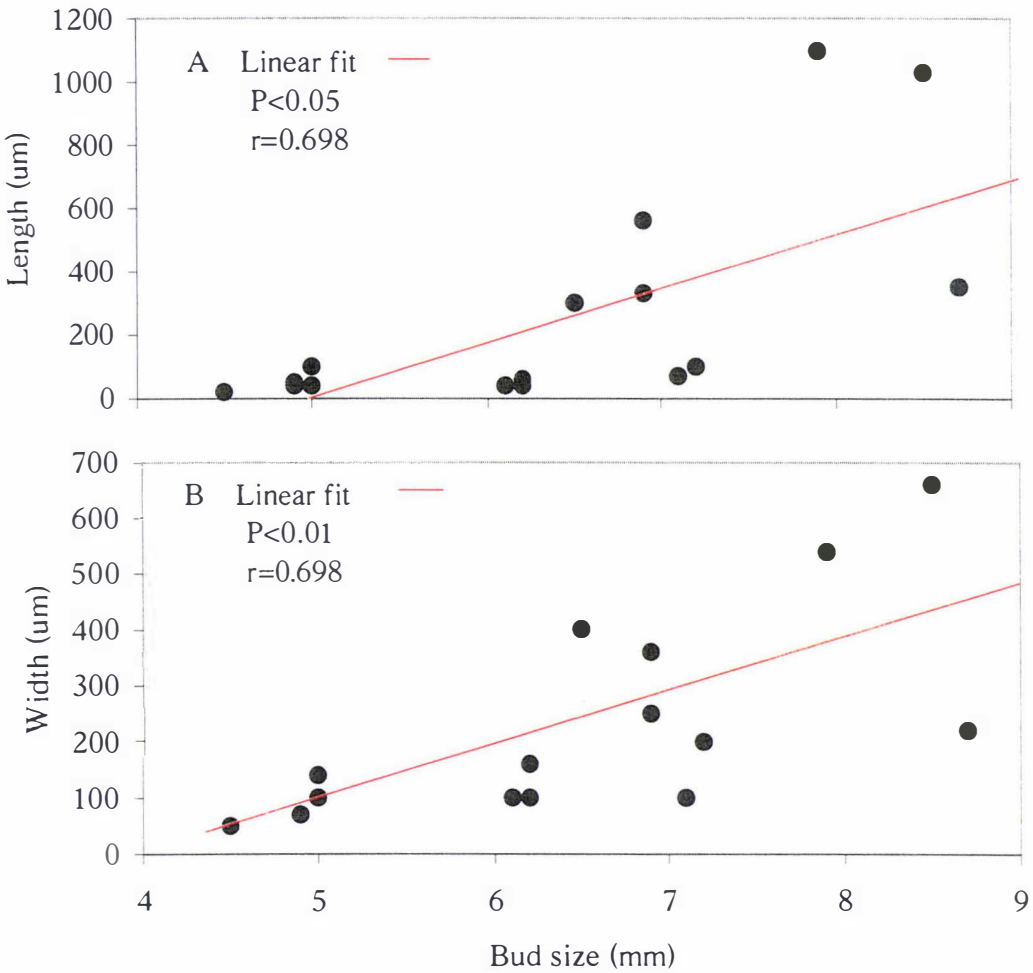


Figure 5.2 Correlations between bud size at time of harvest with maximum (A) length and (B) width of floral meristems in *Metrosideros excelsa* Lighthouse'. Data pooled for buds collected at 20 and 23 weeks from the start of the experiment.

At 23 weeks, on the terminal regions of the cymule primordia, three receptacles and opposing bracts on either side of the three receptacles were beginning to differentiate (Plate 5.1 C-D). Also by this time, the size of the floral meristems were significantly affected by irradiance, being larger, at least in terms of length, in the 567 μmol m^{-2} s^{-1} treatment than in the other treatments (Figure 5.3).

5.3.2 Proportion of plants flowering

Flowering occurred in both cultivars and in all irradiance treatments (including control plants induced under ambient conditions) with the exception of 'Lighthouse' plants in the 174 μmol m^{-2} s^{-1} PPF treatment. Both cultivars grown under the differing PPF treatments displayed a similar unimodal pattern of flowering, with the modal peak occurring at an irradiance treatment of 567 μmol m^{-2} s^{-1} (Figure 5.4). The main effects of both irradiance and cultivar type were highly significant ($P<0.001$) although there was no significant interaction effect ($P>0.05$). Across all irradiance treatments (but not in the ambient controls), 'Scarlet Pimpernel' consistently produced a greater number of inflorescences than 'Lighthouse' with both cultivars averaging the highest yields of 5.6 and 2.5 inflorescences per plant, respectively, at the 567 μmol m^{-2} s^{-1}. There was sufficient data from the 'Scarlet Pimpernel' cultivar to test whether the size of buds at the start of the experiment had a significant effect on the proportion of inflorescences that developed within each environment. The main effects of irradiance ($P<0.0001$) and initial bud size ($P<0.05$) but not their interaction ($P>0.05$) significantly affected the proportion of buds that produced inflorescences (recorded at Stage 1 of floral development) (Figure 5.5). The irradiance environment and bud size class with the highest proportion of inflorescences were in the 567 μmol m^{-2} s^{-1} and large size class treatments, respectively. Intermediate levels in the proportion of inflorescences from each size class occurred in the 961 μmol m^{-2} s^{-1} environment (<6%) and, to a lesser extent, in the 1355 μmol m^{-2} s^{-1} environment (<4%) whereas, relatively few inflorescences per bud size class (<3%) occurred in the 174 μmol m^{-2} s^{-1} and ambient control environments.

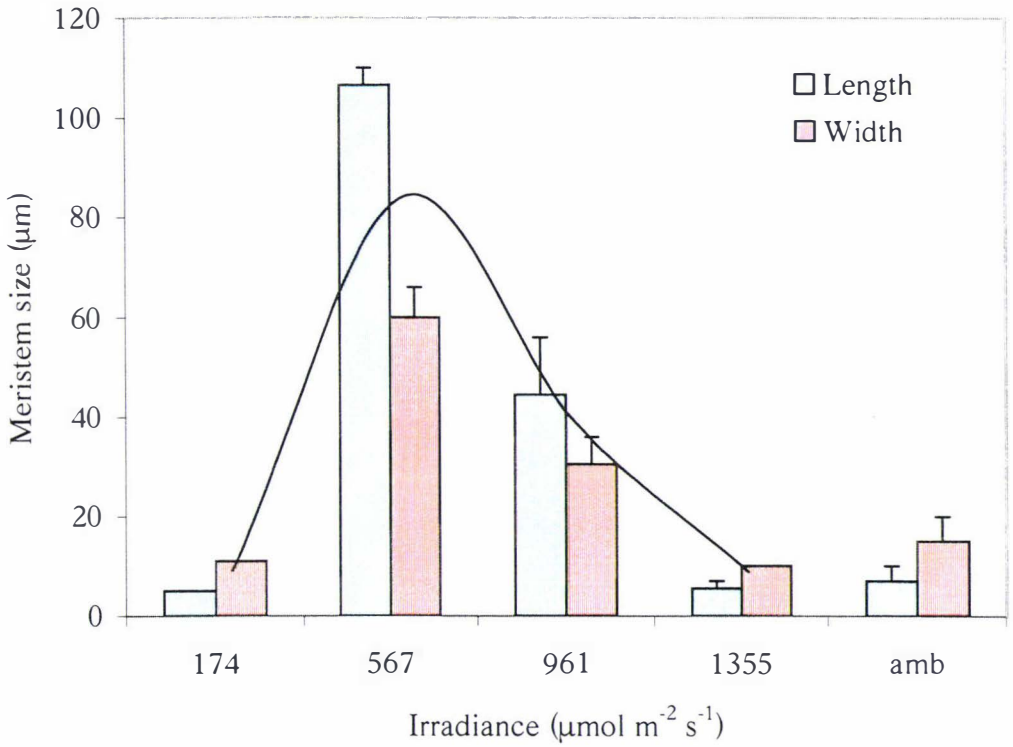


Figure 5.3 Effect of irradiance treatments and ambient conditions during floral induction on the mean (± SE) size of floral meristems at 23 weeks after the start of the experiment. Plants held under ambient inductive conditions denoted "amb".

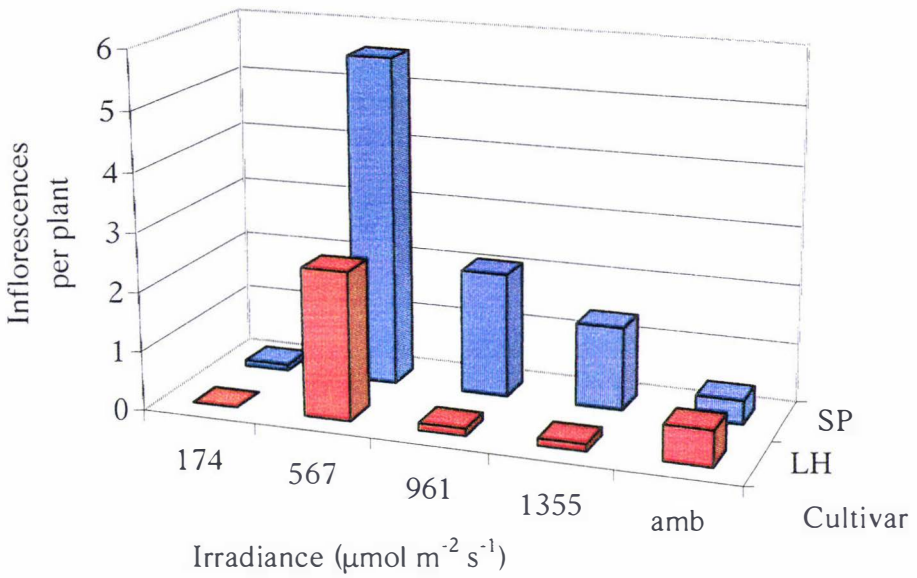


Figure 5.4 Effect of inductive irradiance and ambient conditions on the mean number of inflorescences per plant in *Metrosideros excelsa* 'Lighthouse' (LH) and 'Scarlet Pimpernel' (SP).

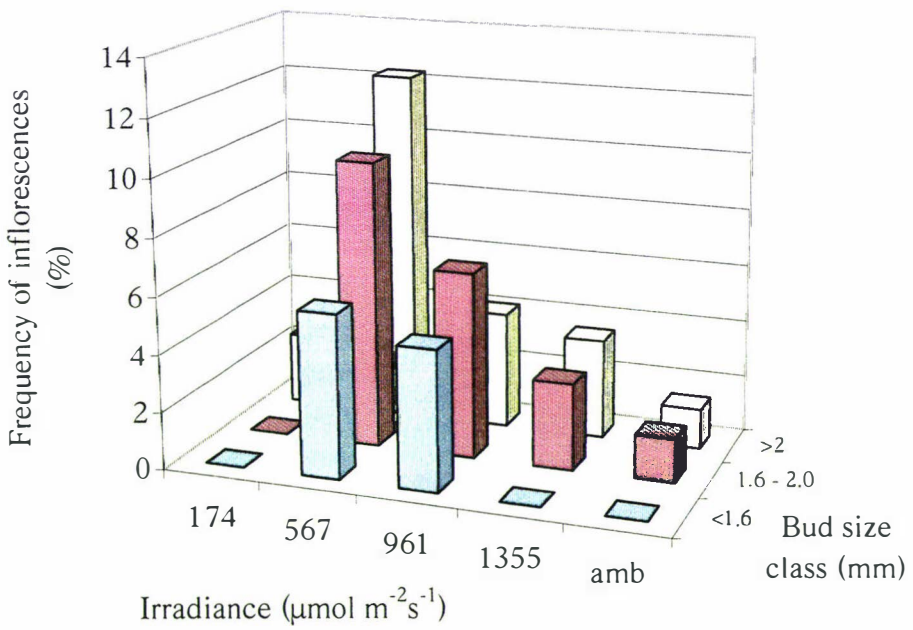


Figure 5.5 Effect of irradiance and ambient conditions (amb) on the cumulative proportion of inflorescences per three bud size classes per plant of *Metrosideros excelsa* 'Scarlet Pimpernel' at 11 weeks after transference to the forcing greenhouse environment.

5.3.3 Flowering time

Both cultivars from all treatments reached anthesis at approximately 15 weeks after being transferred to the forcing greenhouse (mid-November). A repeated measures analysis of variance showed no significant effect of either inductive irradiance environment ($P>0.05$) or cultivar type ($P>0.05$) on the rates of floral development. Inflorescences took approximately seven weeks to progress from a stage where flower buds were visible and bracts had shed (Stage 1) through to anthesis (Stage 4). A further five weeks passed before all inflorescences had senesced (Stage 7).

5.3.4 Inflorescence morphology

A comparative assessment of inflorescence morphological characteristics was conducted between cultivars and across irradiance treatments at 31 weeks (11 weeks after transference to the forcing greenhouse) when most (89%) of inflorescences from both cultivars had reached Stage 1. With the exception to the length of the vegetative shoot arising from the terminal bud of the inflorescence, the inductive irradiance treatments had little to no effect on a number of inflorescence morphological characteristics and those that were significant were primarily cultivar specific (Table 5.1). For example, measurement of several regions in inflorescences of 'Scarlet Pimpernel' plants were significantly longer than those of 'Lighthouse', including the mean length of the peduncle, inflorescence and overall terminating vegetative shoot regions of the inflorescence. Similarly, the mean number of individual cymule nodes (and cymule node pairs) per inflorescence and leaf pairs on the terminating vegetative shoot region were significantly greater in inflorescences of 'Scarlet Pimpernel'.

The frequency of vegetative shoots elongating from the terminal bud of inflorescences within each environment varied between cultivars (Figure 5.6 A-B). No fewer than two thirds of 'Scarlet Pimpernel' plants produced terminal vegetative shoots in all of the inductive environments. In contrast, 100% of 'Lighthouse' plants produced terminal vegetative shoots only from the ambient inductive environment. The highest proportion of aborted terminal apices in inflorescences occurred in approximately 75-100% of inflorescences in 'Lighthouse' and 6% in 'Scarlet Pimpernel from both the 567 and 961 $\mu mol\ m^{-2}\ s^{-1}$ PPF environments.

Table 5.1 Effect of inductive environment and cultivar on inflorescence morphological characteristics. Data are mean ± standard error of count and length measurements. Significance tests for cultivar (CV) and environment (ENV) at 5% level.

Inflorescence characteristic	Cultivar		Significance	
	Lighthouse	Scarlet Pimpernel	CV	ENV
Counts				
Cymule number	3.6 ± 0.4	5.8 ± 0.5	<0.01	n.s.
Cymule nodes	2.4 ± 0.2	3.6 ±0.2	<0.01	n.s.
Terminal shoot nodes	1.9 ± 0.5	4.2 ± 0.4	<0.001	n.s.
Length (mm)				
Peduncle axis	9.8 ± 0.9	12.0 ± 0.5	n.s.	n.s.
Inflorescence axis	14.8 ± 1.6	21.9 ± 1.5	<0.01	n.s.
Terminal shoot	7.7 ± 2.2	15.9 ± 1.4	<0.01	<0.0001

5.3.5 Leaf chlorophyll concentrations

Chlorophyll *a* and *b*, and total chlorophyll concentration were significantly affected by irradiance treatment ($P<0.001$) (Table 5.2), showing a negative correlation with the level of PPF ($r= -0.386$, $P<0.05$). Chlorophyll concentration in plants induced under ambient conditions did not differ statistically from any of the irradiance treatments, except being significantly lower than plants grown at 174 μmol m^{-2} s^{-1}.

5.3.6 Leaf carbohydrate concentrations

Leaf total soluble sugars differed significantly in plants from the different irradiance environments, there being higher and lower concentrations, respectively, in the ambient control and the 174 μmol m^{-2} s^{-1} treatments than the other irradiance treatments (Table 5.2). This result was primarily attributed to differences in sucrose and, to a lesser extent, to fructose, concentrations between inductive treatments. The highest and lowest sucrose concentrations were in plants from the ambient control and 174 μmol m^{-2} s^{-1} PPF environments, respectively, while intermediate concentrations were from the other treatments. Fructose concentration differed only between plants from the ambient control and the 174 μmol m^{-2} s^{-1} PPF environments, being significantly higher in the former. There were no differences in fructose levels among the other irradiance

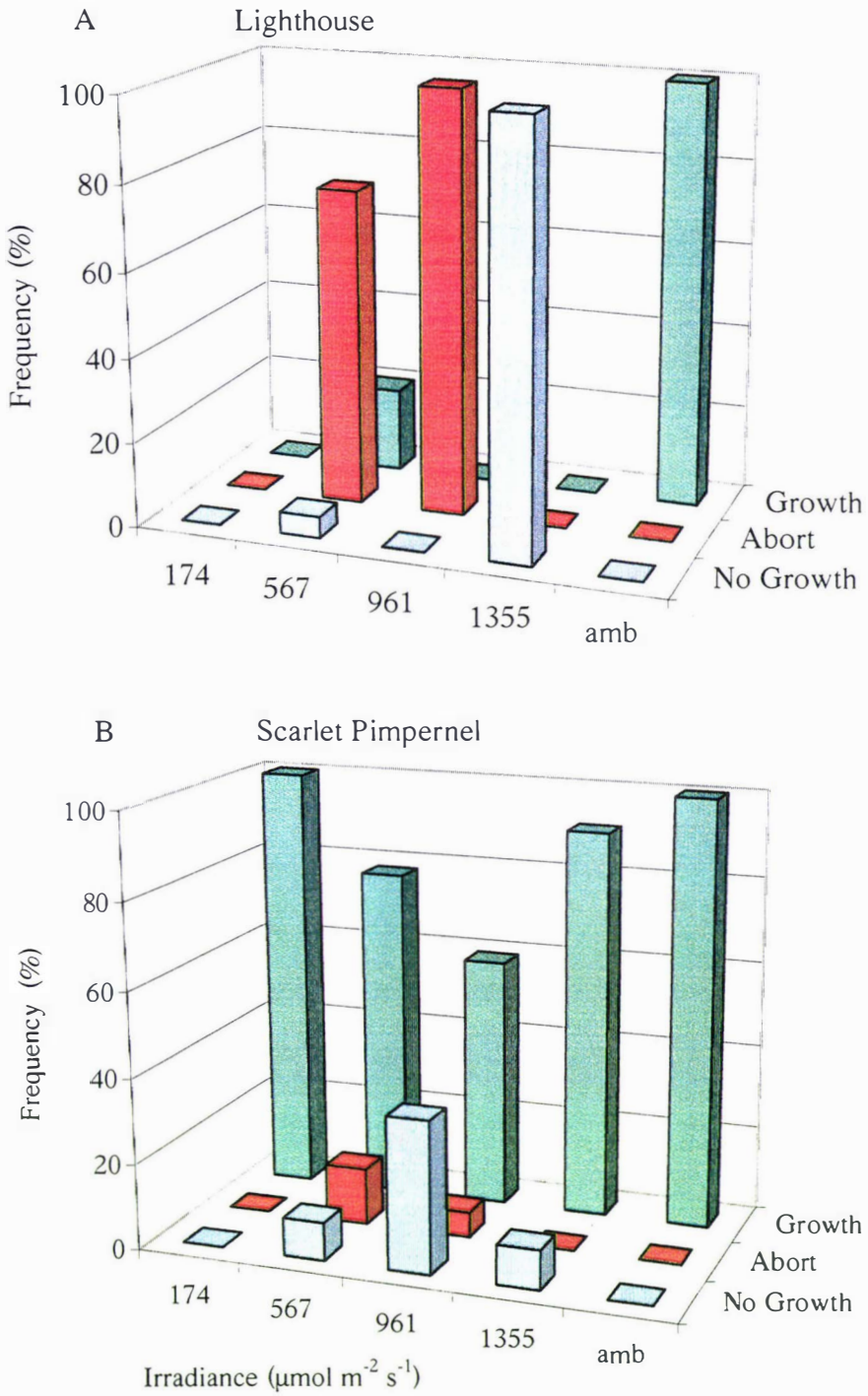


Figure 5.6 Effect of inductive environment on the percentage of terminal buds of inflorescences that had broken and from which vegetative shoots were elongating (growth), that had aborted (abort), or that had not broken (No growth) in *Metrosideros excelsa* (A) 'Lighthouse' and (B) 'Scarlet Pimpernel assessed at Stage 1 of inflorescence development. Plants held under ambient inductive conditions denoted "amb".

treatments. Of all sugars tested, only sucrose showed a relationship with chlorophyll concentration, being significantly and inversely correlated (R^2= -0.510, P<0.001).

The concentrations of starch differed between environments (P<0.001), being significantly higher in leaves collected from plants treated at and above a PPF of 567 μmol m^{-2} s^{-1} in comparison to the other environmental treatments (Table 5.2). Starch concentration in leaves of the control plants was significantly lower than those in all other treatments except in the lowest PPF (174 μmol m^{-2} s^{-1}) environment. Overall, starch concentration was positively correlated with PPF (R^2=0.398, P<0.001) and negatively correlated with leaf total chlorophyll concentration (R^2= -0.321, P<0.01).

Table 5.2 Effect of irradiance and ambient conditions during floral induction on leaf chlorophyll and carbohydrate concentrations in *Metrosideros excelsa* 'Lighthouse' after 20 weeks in induction treatments. Data are mean concentrations for leaves harvested from each environment. Values followed by different letters (along rows) are significantly different according to a Tukey's multiple range test at a 5% significance level.

	Inductive environment					
		Irradiance (μmol m^{-2} s^{-1})				Significance
Variable	Ambient control	174	567	961	1355	P-value
Chlorophyll (mg dm^{-2})						
Chlorophyll *a*	134.6a	279.0^{b}	204.1^{ab}	120.3^{a}	102.9^{a}	<0.0001
Chlorophyll *b*	54.3^{a}	102.1^{b}	73.9^{ab}	46.9^{a}	42.4^{a}	<0.0001
Total Chlorophyll	188.9^{a}	381.2^{b}	278.0^{ab}	167.1^{a}	145.3^{a}	<0.0001
Carbohydrates (mol g^{-1} DW)						
Sucrose	0.028^{a}	0.012^{c}	0.019^{b}	0.021^{b}	0.022^{b}	<0.0001
Glucose	0.018^{a}	0.012^{a}	0.027^{a}	0.020^{a}	0.014^{a}	n.s.
Fructose	0.056^{a}	0.036^{b}	0.042^{ab}	0.045^{ab}	0.045^{ab}	<0.01
Total Soluble	0.103^{a}	0.071^{c}	0.097^{b}	0.088^{b}	0.081^{b}	<0.0001
Starch (mg g^{-1} DW)	12.3^{a}	4.3^{b}	26.7^{c}	42.4^{c}	33.7^{c}	<0.0001

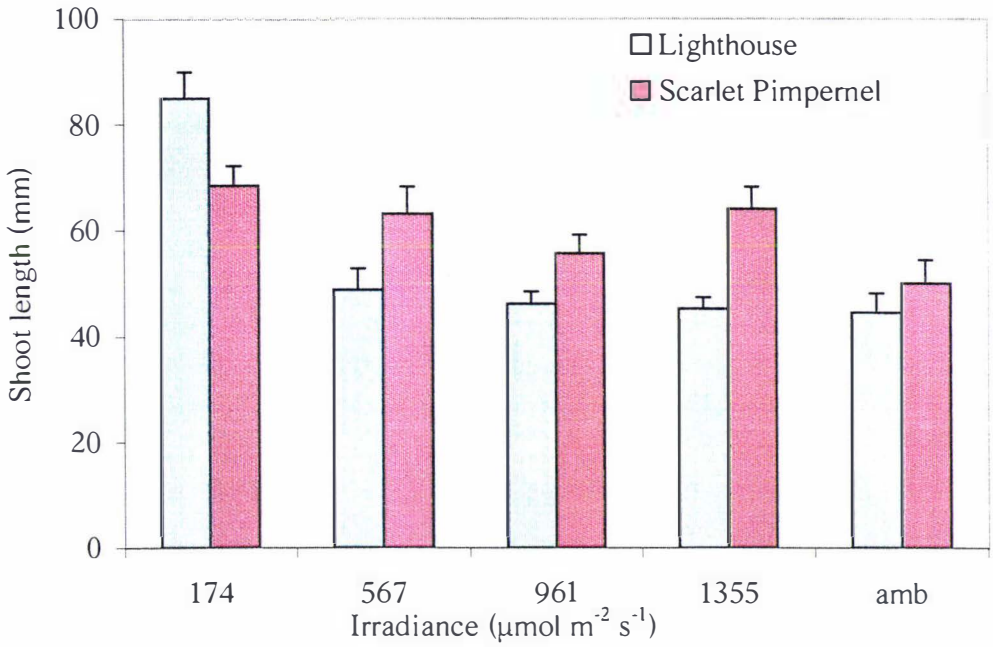


Figure 5.7 Effect of irradiance and ambient conditions during floral induction on vegetative shoot length (mean ± SE) in two cultivars of *Metrosideros excelsa* determined 11 weeks after transference to forcing conditions. Plants held under ambient inductive conditions denoted as "amb".

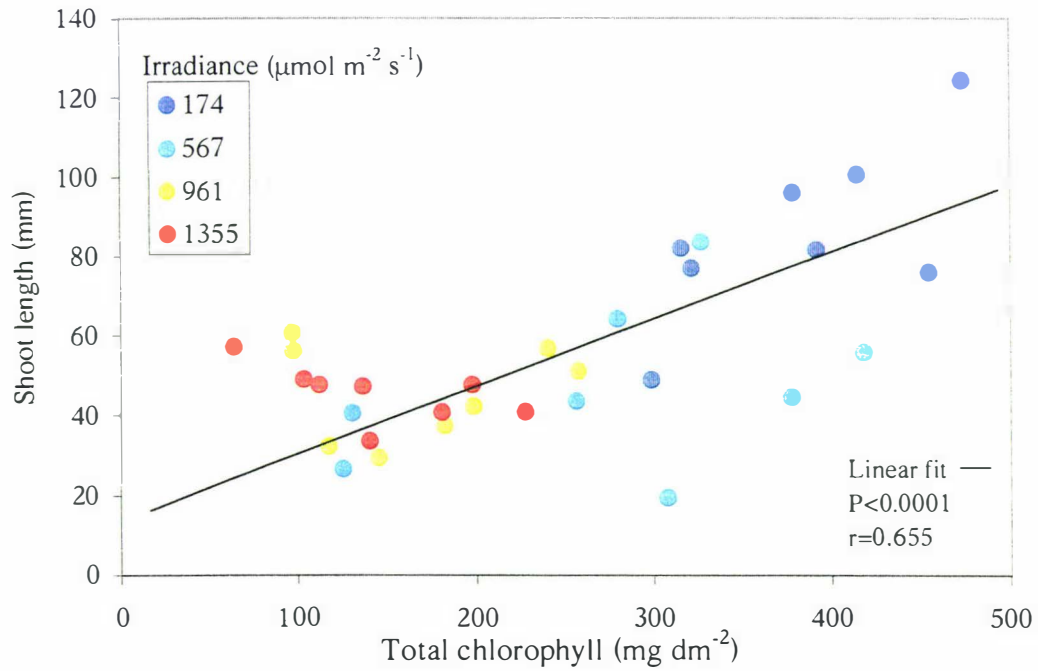


Figure 5.8 Relationship between vegetative shoot length and leaf total chlorophyll concentration in *Metrosideros excelsa* 'Lighthouse' grown under different inductive irradiance treatments.

5.3.7 Vegetative Growth

There was a significant interaction effect of cultivar with inductive environment on the length of vegetative shoots assessed eleven weeks after transferring to the forcing greenhouse ($P<0.001$). In comparison to all other treatments, 'Lighthouse' plants within the lowest PPF treatment bore longer vegetative shoots ($P<0.001$), which was a result of both a greater number of nodes ($P<0.001$) and an increased internode length ($P<0.001$) (Figure 5.7). Shoot growth in 'Scarlet Pimpernel' was less responsive to irradiance than in 'Lighthouse', and there were no significant differences ($P<0.05$) in the length, node number or internode length for this cultivar. In comparison with the plants in the ambient control environment, only 'Scarlet Pimpernel' plants within the lowest PPF treatment bore shoots that were significantly longer ($P<0.001$). This was attributed to shoots having a greater number of nodes along the shoot axis ($P<0.05$), but not to the length of internodes ($P>0.05$).

Patterns in vegetative growth were associated with chlorophyll concentration as assessed from leaf tissue collected from 'Lighthouse' plants at 20 weeks. The mean length of vegetative shoots showed a strong positive correlation with total chlorophyll ($R^2=0.429$, $P<0.001$) (Figure 5.8).

5.4 Discussion

In the present study, the flowering response in two cultivars of *Metrosideros excelsa* was clearly not linearly related to irradiance. Both cultivars of *M. excelsa* responded similarly to the different PPF treatments, producing the highest proportion of inflorescences under an intermediate PPF level of 567 $\mu mol\ m^{-2}\ s^{-1}$, whereas excessively high or low irradiance imposed during inductive treatments detrimentally affected subsequent floral development in *M. excelsa*. Thus, a unimodal PPF response in flower number suggests that inductive treatments with intermediate levels of PPF, in combination with short daylengths and cool temperatures, were essential for maximum flower yield in this species. Thus, the hypothesis that irradiance is positively related to the number of flowers produced was not upheld by this study.

A similar uni-modal flowering pattern in response to different irradiances has also been reported for *Boronia heterophylla* grown under ambient conditions. Plants of *B. heterophylla* maintained continuously outside (during cool SD conditions) under a shade cloth (75% sunlight or 1022 μmol m^{-2} s^{-1}) produced the highest yield of flowers (mean 347 flowers per stem), whereas no shading or shading treatments below 75% sunlight produced significantly fewer flowers (≤ 218 flowers per stem) (Plummer *et al.* 1998). This in contrast to a number other studies examining the effect of irradiance on flowering whereby a strong linear correlation between flower number and irradiance are typically reported (e.g. Bredmose 1993, Dawson and King 1993, Mortensen 1994). The results are often attributed to a lack of a broad range of PPF levels, or to a failure to subject plants to supra-optimal levels of PPF, which might otherwise provide an accurate response curve for determination of conditions optimal for flowering.

In general, flowering in many species, however, appears to be limited more under low than high irradiance (Dawson and King 1993). The current study is in accordance with these observations to the extent that both cultivars produced a greater number of inflorescences in PPF treatments at and above 567 rather than at 174 μmol m^{-2} s^{-1}. For example, the 'Lighthouse' produced no flowers that reached Stage 1 in floral development under the 174 μmol m^{-2} s^{-1} treatment, whereas flowering was recorded in all other PPF treatments. In *Chamelaucium uncinatum* (also Myrtaceae), the number of flowers produced was linearly related to the PPF applied during 8 weeks of inductive conditions, yielding approximately 400 flowers per plant at ~750 μmol m^{-2} s^{-1}, whereas few to no flowers were observed below 160 μmol m^{-2} s^{-1} (Dawson and King 1993). Low light levels have also been reported to decrease the flowering response in other SD species, such as *Chrysanthemum* (De Jong 1986), *Pimelea ciliata* (Slater *et al.* 1994) and in various crop plants such as grape and kiwifruit (Morgan *et al.* 1985).

In various species, floral induction and subsequent development at different irradiances have been strongly associated with carbohydrate status (Bernier *et al.* 1981a, Zieslin and Mor 1990, King and Evans 1991, King and Ben-Tal 2001). In rose, for example, the lack of assimilates due to low light conditions has generally been assumed to cause flower abortions (Zieslin and Moe 1985), and only a shift in assimilate partitioning within the plant (subtending leaves) without an increase in photosynthesis can promote

shoot or flower development (Maas and Bakx 1997). The data for *Metrosideros* support the suggestion that there was a lack of an adequate supply of photoassimilates at 174 μmol m^{-2} s^{-1} for promotion of long-term development in floral meristems. This was evident by the sucrose and storage carbohydrate (starch) concentrations, which were significantly reduced in plants grown in that environment. The reduction in the number of inflorescences in plants exposed to high irradiance treatments did not appear to be directly related to differences in the concentration of carbohydrates, since similar values were reported in these treatments (PPF of 961 and 1355 μmol m^{-2} s^{-1}) with that in which flower number was highest (PPF of 567 μmol m^{-2} s^{-1}). Therefore, this supports the hypothesis that while carbohydrates, such as sucrose, may have a 'florigenic' role during the flowering process (e.g. increased number of flowers under favourable irradiances), they are unlikely to be the only factor mediating this process (Bernier *et al.* 1993, King and Ben-Tal 2001).

Evidence to suggest that floral buds in the other PPF environments may have undergone abortion comes from comparisons of the proportion of buds that were microscopically visible at an early stage of development (week three in the forcing greenhouse) with those at Stage 1 of development (week 11 in the forcing greenhouse). Within 'Lighthouse', approximately 6% of buds from the large size class in plants grown at 174 μmol m^{-2} s^{-1} contained floral meristems on the third week while in the forcing greenhouse. However, by the eleventh week, no inflorescences had reached Stage 1 of development. A between-cultivar comparison also suggests a similar fate. The proportion of buds that contained microscopically visible floral meristems (e.g. in'Lighthouse') with the proportion of buds that reached Stage 1 of floral development (e.g. in 'Scarlet Pimpernel') suggests that a considerable reduction occurred in the number of flowers that reached this later stage of development. For instance, this was clearly evident for medium-sized buds in the 567 μmol m^{-2} s^{-1} environment for which approximately half of all buds in that size class contained floral meristems but only around 10% reached Stage 1 of floral development. If this response was not an artefact due to differences between cultivars, it would not be unreasonable to conclude that a large proportion of buds from different environments and bud size classes had undergone abortion while under the warmer forcing greenhouse conditions. It is possible that the considerably warmer conditions within the forcing greenhouse may

have inhibited floral development during a critical stage(s) of development. A similar suggestion has been made for developing floral primordia in *Acacia pycnantha*, whereby both low light and high temperatures can block different steps in the process of floral differentiation (Sedgley 1985). Thus, transference of *M. excelsa* plants from the inductive conditions to a forcing greenhouse with lower mean temperatures may have increased the number of inflorescences that would have developed through to anthesis.

Despite the possibility of floral abortions, irradiance clearly had a differing effect on buds of different size that eventuated into inflorescences. In 'Scarlet Pimpernel', the highest proportion of buds that produced inflorescences occurred in plants containing intermediate and large sized buds treated at 567 μmol m^{-2} s^{-1}. Similarly, in field grown plants of *M. excelsa*, Sreekantan *et al.* (2001) observed that flowering capacity was also dependent on buds being within a certain size class range during the inductive winter season, and that parts of the canopy containing large floral buds were those that were brightly lit. The effect of bud size in determining competency for flowering has also been acknowledged in various other species, including *Iris* (Doss and Christian 1979), *Vaccinium macrocarpon* (Patten and Wang 1994) and *Eryngium planum* (Ohana and Weiss 1998). However, the physiological processes underlying this phenomenon have not been reported, let alone the influence of irradiance during induction.

It was surprising that the proportion of flowers in plants treated to the ambient control environment was significantly reduced in comparison with those under optimal controlled irradiance conditions. The lack of a strong flowering response in this treatment was probably due to the comparatively lower temperatures experienced by these plants under ambient inductive conditions (mean day/night of 10.2/8.4°C in July) in comparison with those in controlled inductive conditions (mean day/night of 17/14°C). A reduction in temperature would, therefore, have reduced growth as evident in the decreased size and number of floral meristems at three weeks after transference into the common forcing greenhouse environment in comparison with those previously treated under controlled inductive conditions. Similar to the response in *A. pycnantha*, shifting the *M. excelsa* plants from the colder ambient conditions to the heated forcing greenhouse at a critical stage in floral primordia development may have led to the early abortion of flower buds (Sedgley 1985).

Irradiance can significantly affect the morphology of inflorescences, such as the effect of low-light in promoting flower stem length (Bredmose 1993, Myster 1999). In *M. excelsa*, the proportion of inflorescences with elongating terminal vegetative shoots was considerably higher in 'Lighthouse' (but not 'Scarlet Pimpernel') plants grown under ambient inductive conditions (100%), in comparison with those from the other controlled inductive environments (<20%). However, this response is unlikely to be related to irradiance, otherwise, it probably would have been observed in plants grown in one or more of the controlled environments with different irradiances. It is likely that this response was due to the cooler temperature regime experienced by plants under ambient winter conditions, which may have provided chilling favourable for subsequent vegetative growth of the terminal apex of the inflorescence.

Vegetative shoot length was significantly affected by irradiance, at least in the 'Lighthouse'. By the eleventh week within the forcing conditions, 'Lighthouse' plants which had been grown under low light conditions (174 μmol m^{-2} s^{-1}) had produced significantly longer shoots containing a higher number of nodes. The increased size of the shoots in 'Lighthouse'was probably due to the increased capacity of plants to produce photoassimilates as a result of having obtained higher chlorophyll concentrations, and presumably a higher chloroplast density, while being grown in the low light inductive environment. Acclimation of plants to shady conditions has been reported widely in the literature, and include modifications to a number of leaf anatomical and morphological characteristics, such as the presence of larger leaves with reduced thickness, stomatal density and conductive tissue (Morgan *et al.* 1985). Moreover, physiological modifications can include increases in total chlorophyll content (Marler *et al.* 1994, Marks and Simpson 1999). These modifications allow for a maximisation of photosynthetically active area over the total plant mass, a strategy often employed in low light environments (Marler *et al.* 1994). Therefore, these conditions probably provided an added advantage for vegetative growth once plants of 'Lighthouse' were transferred to the relatively high light conditions in the forcing greenhouse. Moreover, the strong negative correlation between chlorophyll concentration and irradiance also suggests that plants transferred from the high light environments may have suffered from photoinhibition, a feature acknowledged to cause a lowering of chlorophyll concentrations and damage to the photosynthetic apparatus

(Krause 1988, Marks and Simpson 1999). The lack of a similar low-light response in 'Scarlet Pimpernel', in terms of an increase in vegetative shoot growth, may have been due the diversion of photoassimilates towards floral development, considering that some flowering was recorded in this cultivar in the low PPF treatment. This would be consistent with the diversion of assimilates towards stronger sinks such as those that favour floral growth, as postulated by several authors (Sachs and Hackett 1983, Kinet *et al.* 1985).

In conclusion, in inductive cool, short-day conditions, irradiance had a significant effect on not only the rate of floral primordium growth but also on the proportion of buds within a size class that produced floral meristems. Intermediate levels of irradiance (~567 μmol m^{-2} s^{-1}) clearly enhanced the number of inflorescences that were produced, while excessively low or high irradiance imposed during inductive treatments detrimentally affected subsequent floral development in *M. excelsa.*

Chapter 6

The postharvest characteristics of *Metrosideros excelsa* as a cut flower

6.1 Introduction

Within the family Myrtaceae, the main postharvest and display problems are associated with ethylene-related abscission of whole flower and/or floral organs (Zieslin and Gottesman 1983, Joyce and Poole 1993), and adverse water relations within cut flowers (Joyce and Jones 1992, Jones *et al.* 1993a, Burge *et al.* 1996). For example, in cut flowers of *Metrosideros collina* stamen wilting and abscission readily occur in response to unfavourable holding solutions and to exogenous and/or endogenous ethylene (Sun *et al.* 2000). Generally, inclusion of sucrose to vase solutions at moderate to low concentrations (≤10%) can improve the flower quality and extend vase life (Jones *et al.* 1993a, Sun *et al.* 2000). However, the response of cut flowers to different sucrose solutions can be variable even in closely related species. For instance, continuous application of 0.5-5% sucrose in a holding solution reduced vase life of *Eucalyptus tetragona*, whereas concentrations up to 2% had no effect in *E. youngiana* (Delaporte *et al.* 2000). In *E. ficifolia*, increasing concentrations up to 2% sucrose were associated with decreased stamen wilting, a factor limiting vase life (Sun *et al.* 2001).

The addition of biocides such as hydroxyquinoline (HQ) based compounds to holding solutions usually improves hydraulic conductivity within cut stems (Ichimura *et al.* 1999). Their suggested mode of action has been through the inhibition of vascular occlusions of microbial origin or deposition of organic substances (Put and Clerkx 1988). In *M. collina*, the vase solution that successfully reduced the level of stamen wilting was a 2% sucrose with 200 mg l^{-1} HQ citrate (Sun *et al.* 2000). Whether similar combinations of additives and/or concentrations are effective in other *Metrosideros* species are not known.

The sensitivity of cut flowers in Myrtaceae to ethylene can vary depending on genera and species. Cut flowers in *M. collina*, for example, were found to be highly sensitive to the effects of this hormone. Application of exogenously applied ethylene (0.1 μl l^{-1})

resulted in 80% stamen abscission by day 5 after harvest, and higher concentrations caused whole flower abscission (Sun *et al.* 2000). In contrast, higher concentrations of ethylene (8.6 $\mu l\ l^{-1}$) caused no floral abscission in *Verticordia chrysantha*, *V. plumosa* or *V. densiflora* but substantially induced flower, pedicel and leaf abscission in *V. nitens* (Joyce and Poole 1993). Furthermore, environmental conditions such as high relative humidity (RH) can induce floral abscission possibly through the enhancement of endogenous ethylene effects (Zieslin and Gottesman 1986). Thus far, there have been only a few studies examining the effect of RH on ethylene production and/or vase life characteristics.

The use of chemical agents that inhibit ethylene action have successfully extended the vase life and preserved floral qualities in a wide variety of cut flowers. Application of silver thiosulfate (STS) has been used consistently and applied successfully to intact and excised flowers over recent decades (Cameron and Reid 1983, Joyce and Beal 1999). Relatively recent concerns of the toxic effects of silver and its release into the environment, however, have prompted a search for safe but equally effective alternatives (Halevy 1994). Thus far, 1-methylcycloporopene (1-MCP), a gaseous compound that also inhibits ethylene action appears to be a promising alternative to STS, and affords protection from ethylene in a number of flowering species (Serek *et al.* 1995b, Macnish *et al.* 2000). In various genera within Mrytaceae, 1-MCP prevented floral abscission and generally delayed ethylene-induced senescence, such as in *Chamelaucium* (Serek *et al.* 1995a) and *Leptospermum* (Macnish *et al.* 2000). In contrast, 1-MCP applied to cut flowers of *Metrosideros* was relatively ineffective in preventing ethylene-induced abscission of flowers and stamens, and instead, stimulated endogenous ethylene production (Sun *et al.* 2000). Whether this response was specific for *M. collina* or occurs within other congeneric species merits an investigation.

The aim of the present study was to investigate the postharvest physiology and vase life characteristics of cut flowers of *Metrosideros excelsa.* In light of the current literature, it is hypothesised that:

1) The vase life of *M. excelsa* is limited by adverse water relations and by the ethylene-related abscission of whole flower and floral organs. Appropriate holding solutions

and inhibitors of ethylene action may, therefore, provide effective treatments for extending the vase life and minimising ethylene-related damage.

6.2 Materials and Methods

6.2.1 Plant material

The postharvest experiments were conducted using inflorescences harvested from two cultivars of mature (~1.5 m in height) *Metrosideros excelsa* plants, 'Lighthouse' and 'Vibrance'. Liner plants obtained from Lyndale Nurseries, Auckland, in August 1998 were potted up into 30 l containers with an 80:20 v/v peat and pumice media, and supplemented with a slow-release (6 month) fertiliser. Plants were grown outside under ambient conditions at the Plant Growth Unit, Massey University, Palmerston North, New Zealand, and supplied daily with overhead sprinkler irrigation. Plants were arranged into four blocks containing approximately 10-15 plants of each cultivar from which inflorescences were harvested over two consecutive summers, being late December 1999/early January 2000 and December 2000 / January 2001.

6.2.2 Harvest and experimental preparations

Inflorescences containing three to four pairs of cymules were harvested between 0700 and 0800 h from the experimental plants. Cut inflorescence stems were transported in buckets with distilled water to the laboratory in preparation for each experiment. Cymules selected included those containing a long pedicel (>5 mm) with three flower receptacles at late Stage 2 (tight bud with petals beginning to reflex) (See Table 4.1). Cymules were detached by hand from the floral stem. Each cymule was randomly selected and the base of the petiole cut under distilled water using a sterile razor blade, and assigned at random to a treatment.

Each explant (individual cymule) was suspended individually in a 40 ml beaker supported by an aluminium foil cover so that the end of the pedicel was immersed in a holding solution. Standard holding solutions, unless otherwise stated, contained 2% sucrose and 200 mg l^{-1} of hydroxyquinoline citrate (HQC), as recommended by Sun *et al.* (2000).

6.2.3 Vase life room

Throughout the experiments, cymules were maintained under controlled conditions in a vase-life evaluation room at the Plant Growth Unit, Massey University, following the conditions prescribed by Halevy (1976). The vase life evaluation room was maintained at a constant temperature of 20°C with a relative humidity (RH) at approximately 70%. Overhead lighting was provided by four cool white fluorescent tubes (36 W Philips TLD) (15 μmol m^{-2} s^{-1}) on a 12 h photoperiod.

6.2.4 Scoring flower condition

Flower quality was assessed daily using several methods. The developmental stage of flowers on each cymule was scored based on a modified version of the classification scheme outlined in Chapter 4. This included stages that corresponded to the expansion of flower bud girth and petal reflexing (Stage 2), elongation of the stamens/style (Stage 3), attainment of anthesis and stamens fully elongated (Stage 4), dehiscing of anthers and visibility of pollen (Stage 5), onset of stamen abscission (Stage 6), and abscission of all stamens and petals (Stage 7). Flower quality was assessed based on the level of wilting or abscission in stamens and petals (percentages), and on the abscission on floral receptacles (numeric counts). The vase life of cut cymules was defined as time taken for flowers to develop from the onset of Stage 2 through to the attainment of Stage 7.

Flower mass, water uptake and the rate of transpiration of each cymule was measured daily. This was undertaken by recording the mass of the holding solution with and without the cymule, both before and after the addition of any supplementary solution, required as either part of the treatment or to replenish a depleted holding solution. Based on these data, cymule mass (fresh weight) was determined. Comparisons with data collected from the previous 24 h session enabled calculation of the difference in daily water uptake and transpiration.

6.2.5 Experiments 1 A-C: Holding solutions: effects on flower quality

Cymules of 'Lighthouse' and 'Vibrance' were harvested at late Stage 2 and assigned to one of five treatments within five blocks based on a Randomised Block Design (RBD).

The holding solution treatments included (1) distilled water (control), (2) 200 mg l^{-1} HQC solution, (3) 2% sucrose and 200 mg l^{-1} HQC solution (referred to as 2% sucrose), (4) a 24 h pulse solution of 10% sucrose and 200 mg l^{-1} HQC before transference to a 200 mg l^{-1} HQC solution (referred to as 10% sucrose pulse), or (5) a 24 h pulse solution of 10% sucrose and 200 mg l^{-1} HQC before transference to a 2% sucrose and 200 mg l^{-1} HQC solution (referred to as 10% sucrose pulse, 2% holding). Cymules were held in the vase life evaluation room continuously and measurements on flower quality were conducted daily using the characteristics defined above. The effects of holding solution on the vase life of 'Lighthouse' and 'Vibrance' were examined in separate experiments due to the variation in flowering times between the cultivars.

A third experiment was undertaken using cut cymules of 'Lighthouse' to examine whether the effects on flower quality were attributed to the HQC itself, or to the pH effect of HQC. The experiment was conducted using a completely randomised block design with two treatments and four blocks. The first series of treatments consisted of holding solutions of only distilled water (control) (pH 5.0) and distilled water with HQC added (100, 200 and 300 mg l^{-1}) corresponding to pH levels of 3.8, 3.7 and 3.6. The second series consisted of distilled water containing HQC (100, 200 and 300 mg l^{-1}) but with pH adjusted to 5.0 using additions of sodium hydroxide (0.1 M). Flower quality was scored based on the stages of floral development, level of wilting and stamen abscission over the duration of the experiment.

6.2.6 Experiment 2: Effect of humidity on flower quality and endogenous ethylene production.

The experiment was conducted using explants harvested from *M. excelsa*, 'Lighthouse'. The experiment was designed based a repeated measures randomised block design with two humidity environments and seven assessment (time) treatments within four blocks. The two environments consisted of either a bench top within the vase-life evaluation room (RH ~ 70%) or one of four glass aquarium enclosures (RH >90%). Each glass enclosure consisted of 50 L, semi-sealed aquarium tank outfitted with a narrow (1 x 10 cm) opening on the top to provide unrestricted airflow and to prevent ethylene accumulation. Beakers containing two cymule explants were placed inside each enclosure along with 20 g soda lime (Carbasorb, BDH). An aquarium air pump was

used to discharge humidified air (>90%) into each enclosure. Hygrometers were used to ensure that the desired RH levels within each enclosure and in the vase-life room were maintained continuously. Air samples from both the environments were taken every 24 h to ensure that ethylene concentrations did not differ significantly from ambient.

The experiment was conducted using eight assessment (time) treatments in order to quantify the level of exogenously produced ethylene at various stages of floral development. Daily, over the course of the eight days, beakers with two explants were transferred from either the bench or the aquarium enclosures to 0.5 l jars. To each of the jars, 5 g of soda lime was added for absorption of respiratory CO_2, and lids were tightly sealed. Lids on each of the jars were outfitted with a gas-impermeable rubber plug (septum) that allowed the extraction of 1 ml of internal air via a needle syringe at 0, 24 and 48 h after being sealed. The concentration of ethylene in the air was measured using a gas chromatograph (Hewlett Packard 5890A, USA). Flower quality was assessed daily from cymules in both environments and within the glass jars.

6.2.7 Experiment 3: Effect of applied exogenous ethylene on flower quality and endogenous ethylene production.

This experiment was arranged following a factorial design with four exogenous ethylene treatments and four subsequent incubation periods. Each explant of 'Lighthouse' was held in a beaker placed in 1 l jar along with 10 g soda lime [and 10 g of Purafil (Ipsco Ltd., Auckland) held in a paper sachet (control jars only)]. Jars were tightly sealed using lids outfitted with septa. Jars were treated with one of four concentrations of exogenous ethylene (0, 0.1, 1 and 10 $\mu l\ l^{-1}$) injected via the rubber septa and held in darkness for 24 hours. After ethylene treatment, beakers containing explants were transferred to a bench in the vase-life evaluation room. Explants were then transferred at time 0, 24, 48 and 96 h to clean 1 l jars containing only soda lime (5 g) for determination of ethylene production over 24 h. The vase-life characteristics of explants were assessed daily.

6.2.8 Experiment 4: Efficacy of 1-methylcyclopropene (1-MCP) and silver thiosulfate (STS) on ethylene-induced responses

The effectiveness of STS and 1-MCP was evaluated in explants of 'Lighthouse' subsequently treated with exogenous ethylene. The experimental layout followed a factorial design with two main factors, comprising four levels of an ethylene inhibitor treatment and two levels of an exogenous ethylene treatment. Replication consisted of two explants per treatment within each of the five blocks.

6.2.8.1 Protection treatment

Explants in holding beakers were sealed in 1 l jars outfitted with septa through which 1-MCP (ElthylBloc, Yates, New Zealand Ltd.) was injected to provide one of three concentrations (0, 15, and 150 nl l^{-1}). Additional explants were pre-treated with 2 mM STS sprayed to incipient run-off, and allowed to dry for 1 h before being placed in jars. Jars containing both 1-MCP and STS treated explants were sealed and stored for 6 h. After 6 h, the jars were opened and the gaseous 1-MCP was dissipated for 1 h.

6.2.8.2 Ethylene treatment

The jars were re-sealed after adding 5 g soda lime and injected with ethylene to provide one of two concentrations (0 and 5 µl l^{-1}) and stored in darkness for 24 h. After incubation for 24 h, beakers with explants were removed from the jars and placed on a bench in the vase-life evaluation room, and vase life characteristics were monitored daily. In addition to the vase life characteristics described earlier, a visual measure of the level of petal in-rolling was recorded daily based on a 0-100% scale, using 10% units. This range included petals that were fully- to semi-turgid (0-30%), partially turgid/desiccated (40-60%), and completely desiccated (70-100%).

6.2.9 Statistical analyses

Biometrical analyses were conducted using the SAS (SAS Institute, Cary, N.C., USA) statistical program. A repeated measures analysis of variance was used to analyse daily

changes in stages of floral development, cymule mass, transpiration, water uptake and the proportion of organ abscissions. Percent data (stamen wilting level) was transformed using a log transformation. Treatments incorporating two or more main treatment set up in factorial combination were analysed using a factorial repeated measures. In all analyses, a Least Square Means (LSM) test was used to compare differences between treatments at specific time points.

6.3 Results

6.3.1 Experiment 1 A: Holding solutions: effects on flower quality in 'Lighthouse'.

6.3.1.1 Floral development

Holding solution had a small but significant effect on the stage of floral development over the 12 day experimental period ($P<0.05$) (Figure 6.1). All treatments reached Stage 4, 5 and 6 on Days 3, 5 and 7-9, respectively. Differences in the rate of flower development differed significantly only during Days 7-9. On these days, cymules treated with both 10% sucrose pulse treatment showed a decrease in the rate of senescence compared with cymules held in a 2% sucrose solution.

6.3.1.2 Water relations

There was a main effect of holding solution treatment ($P<0.05$) and time ($P<0.0001$) on water uptake but no significant interaction ($P>0.05$) (Table 6.1). Generally, water uptake was lower in all treatments in comparison with controls, except in the 2% sucrose treatment. Data pooled for all treatments showed that water uptake declined steadily over the experimental period starting at 0.26 g day^{-1} (Day 1) and decreased to 0.15 g day^{-1} (Day 12) with a peak uptake on Day 4 of 0.39 g day^{-1} compared with Day 1 ($P<0.05$) (Figure 6.2 A).

The main effects of holding solution treatment ($P<0.01$) and time ($P<0.0001$) affected transpiration, although there was no significant interaction ($P>0.05$) (Table 6.1). Transpiration in the 2% sucrose treatment did not differ from that of control, and was lower in the other treatments. Generally, transpiration rates mirrored the response in

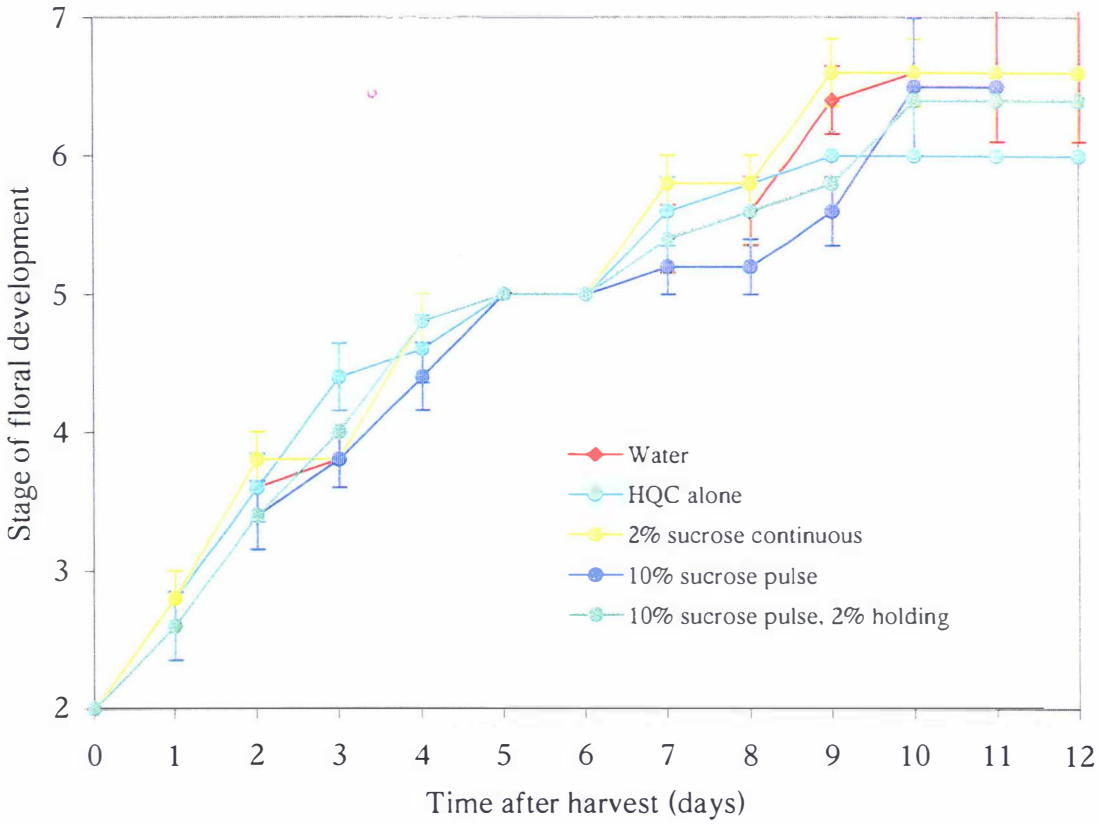


Figure 6.1 Effect of holding solution on the mean (±SE) stage of floral development in cut cymules of *Metrosideros excelsa* 'Lighthouse'.

Table 6.1 Effect of holding solution on mean water uptake and transpiration in cut cymules of *M. excelsa* 'Lighthouse' over the experimental period. Mean separation in columns by Least Square Means test, 5% level.

Holding solution	Water uptake ($g\ d^{-1}$)	Transpiration ($g\ d^{-1}$)
Water (control)	0.31^{a}	0.33^{a}
HQC alone	0.19^{c}	0.20^{c}
2% sucrose continuous	0.26^{ab}	0.28^{ab}
10% sucrose pulse	0.22^{bc}	0.22^{c}
10% sucrose pulse, 2% holding	0.21^{bc}	0.22^{bc}

water uptake (Figure 6.2 A), although the mean rate of transpiration was higher than that of water uptake from Day 6 onwards.

Cymule mass was significantly affected by the different holding solutions over time ($P<0.0001$). All treatments displayed a uni-modal pattern with cymule mass peaking between Days 5-6 (Figure 6.2 B). Generally, differences in cymule mass between treatments occurred after Day 7, when the mass of HQC-treated cymules was lower in comparison with both pulse treatments ($P<0.05$), although these did not differ from the mass of control cymules. The start of the decline in cymule mass (for all treatments) occurred after Day 5, corresponding to the same day that the mean rate of transpiration exceeded water uptake (Figure 6.2 A).

6.3.1.3 Stamen wilting

Stamen wilting increased significantly between treatments over time ($P<0.05$), with the onset of wilting occurring after Day 7 (Figure 6.3 A). Between Days 11-12, the level of stamen wilting in all treatments (except in 10% sucrose pulse) was inversely related to the proportion of stamen abscissions (Figure 6.3 B). Therefore, most treatments with low stamen abscission rates had the highest incidence of wilting (except the 10%

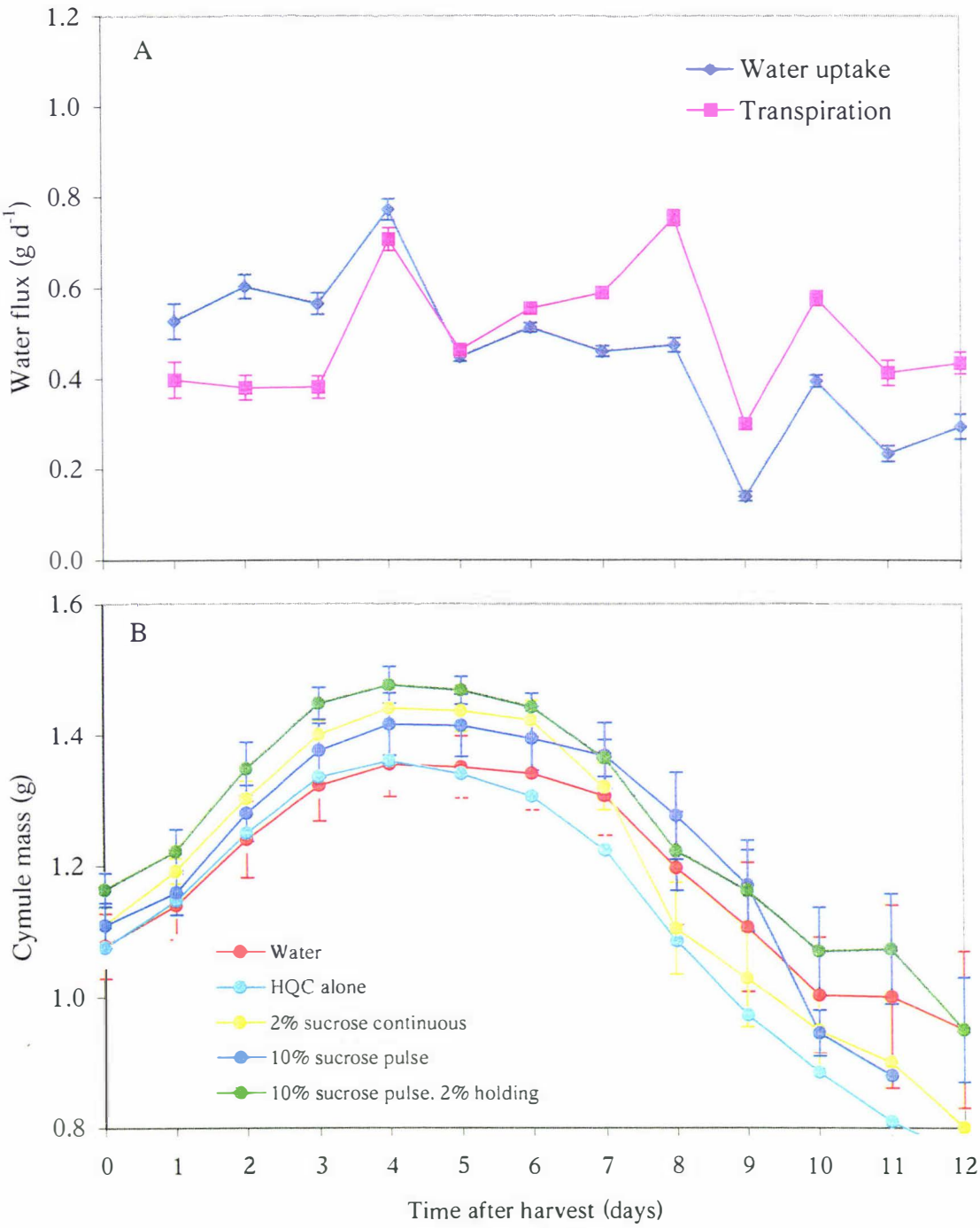


Figure 6.2 Changes in (A) mean (±SE) water flux (uptake and transpiration) pooled for all holding solution treatments and (B) mean (±SE) cymule mass for individual treatments in cut cymules of *Metrosideros excelsa* 'Lighthouse'.

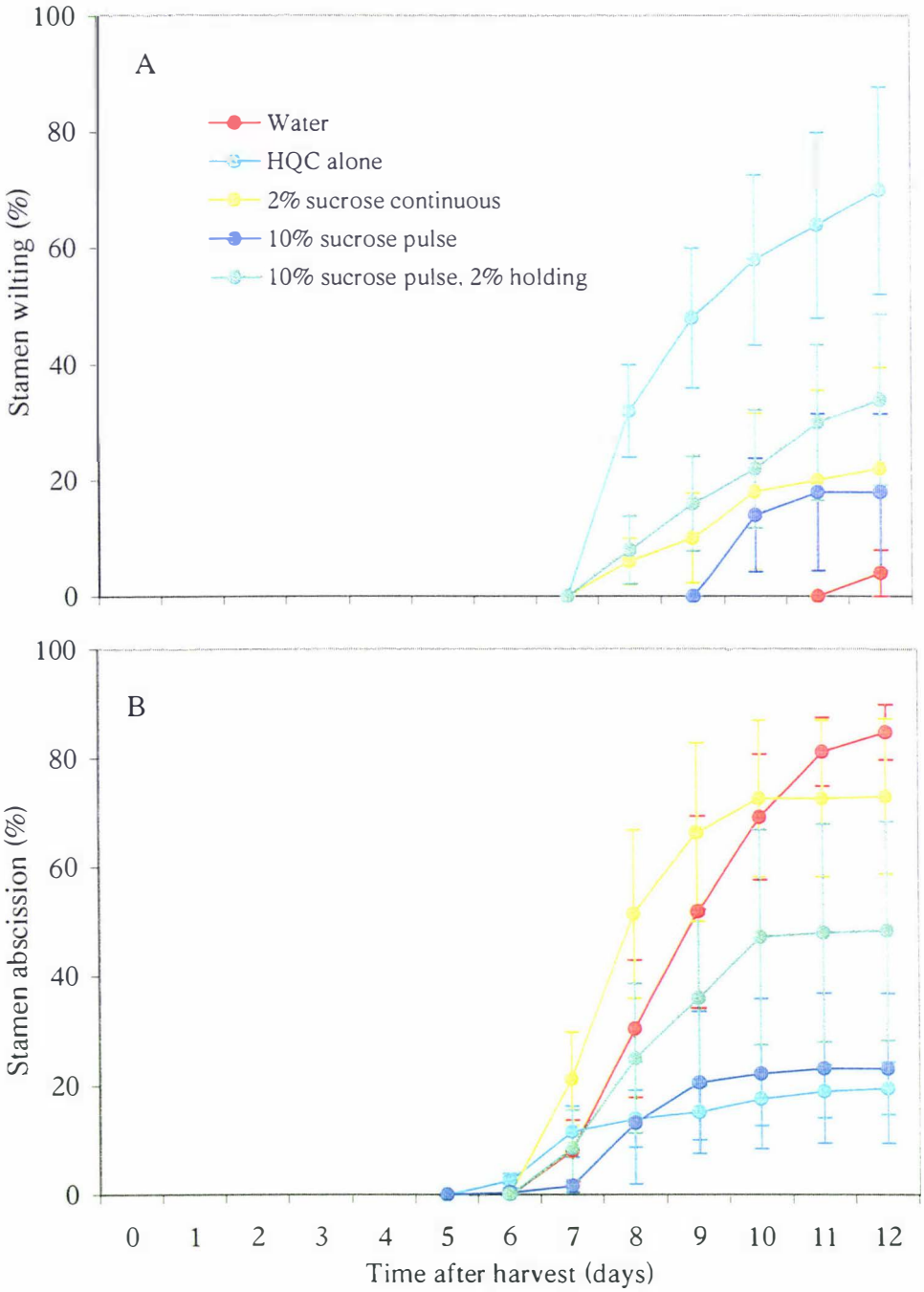


Figure 6.3 Effect of holding solution on mean (±SE) (A) stamen wilting and (B) abscission in cut cymules of *Metrosideros excelsa* 'Lighthouse'.

sucrose pulse treatment). In particular, stamen wilting in the HQC solution was significantly higher in comparison with all other treatments ($P<0.05$), attaining a 70% wilting level by Day 12. In comparison, only a 4% wilting level was recorded in the distilled water treatment (control). Generally, both the pulse (10% sucrose pulse and 10% sucrose pulse, 2% holding) and the 2% sucrose treatments were higher than the control treatment (except Day 12), attaining a wilting level of 18-34% by Day 11.

The level of stamen wilting in all treatments pooled between Days 8-12 was negatively correlated with cymule mass ($P<0.001$, $r = -0.537$). In particular, stamen wilting with solutions containing only HQC (HQC alone and 10% sucrose pulse) showed the strongest relationship with cymule mass compared to either the control (water) or the addition of sucrose (2% sucrose and the 10% sucrose pulse, 2% holding) (Table 6.2). However, the lack of a possible significant correlation in the control group was due, at least in part, to the lack of wilting data in that treatment.

Table 6.2 Correlation of mean stamen wilting and cymule fresh weight between Days 8-12 for each holding solution treatment for cut cymules of *Metrosideros excelsa* 'Lighthouse'. Symbol: n.s. not significant at 5% level.

Holding solution	Pearson correlation F - value	P - value
Water (control)	-0.012	n.s.
HQC alone	-0.915	<0.0001
2% sucrose continuous	-0.365	n.s.
10% sucrose pulse	-0.627	0.005
10% sucrose pulse, 2% holding	-0.332	n.s.
Total	-0.537	0.0001

6.3.1.4 Floral abscission

There was a significant interaction of the main effects of treatment and time on the proportion of stamen abscissions ($P<0.000$) that ensued from Day 7 onwards (Figure 6.3 B). After Day 9, abscission rates from the HQC alone and 10% sucrose pulse treatments were lower than the 2% sucrose or the control water treatment ($P<0.05$). The 10% sucrose pulse 2% holding treatment displayed an intermediate level of stamen abscission and did not differ from both of the HQC treatments (HQC alone and 10% sucrose pulse) or the 2% sucrose and control treatment ($P>0.05$).

Petal abscission ensued in all treatments from Day 7 onwards, increasing up to a mean abscission rate of 44% of petals per cymule by Day 12. There was no significant interaction of the main effects of treatment and time on petal abscission ($P>0.05$).

6.3.2 Experiment 1 B: Holding solutions: effects on flower quality in 'Vibrance'.

6.3.2.1 Floral development

There was no significant interactive effect of holding solution treatment and time on changes in floral development over the 16 day experimental period ($P>0.05$). Cymules from all treatments reached Stage 4 and 5 on Days 4-5, and 6-8, respectively. Most cymules reached Stage 6 (onset of stamen abscission) by Day 14, with the exception of those in the HQC solution, since stamen abscission was absent in this treatment throughout the experimental period.

6.3.2.2 Water relations

Mean water uptake did not differ significantly between holding solutions over the 15 day experimental period ($P>0.05$). Over this time period, water uptake per cymule averaged for all treatments peaked on Day 5 (0.50 g day^{-1}) before decreasing steadily to 0.08 g day^{-1} by Day 15 (Figure 6.4 A).

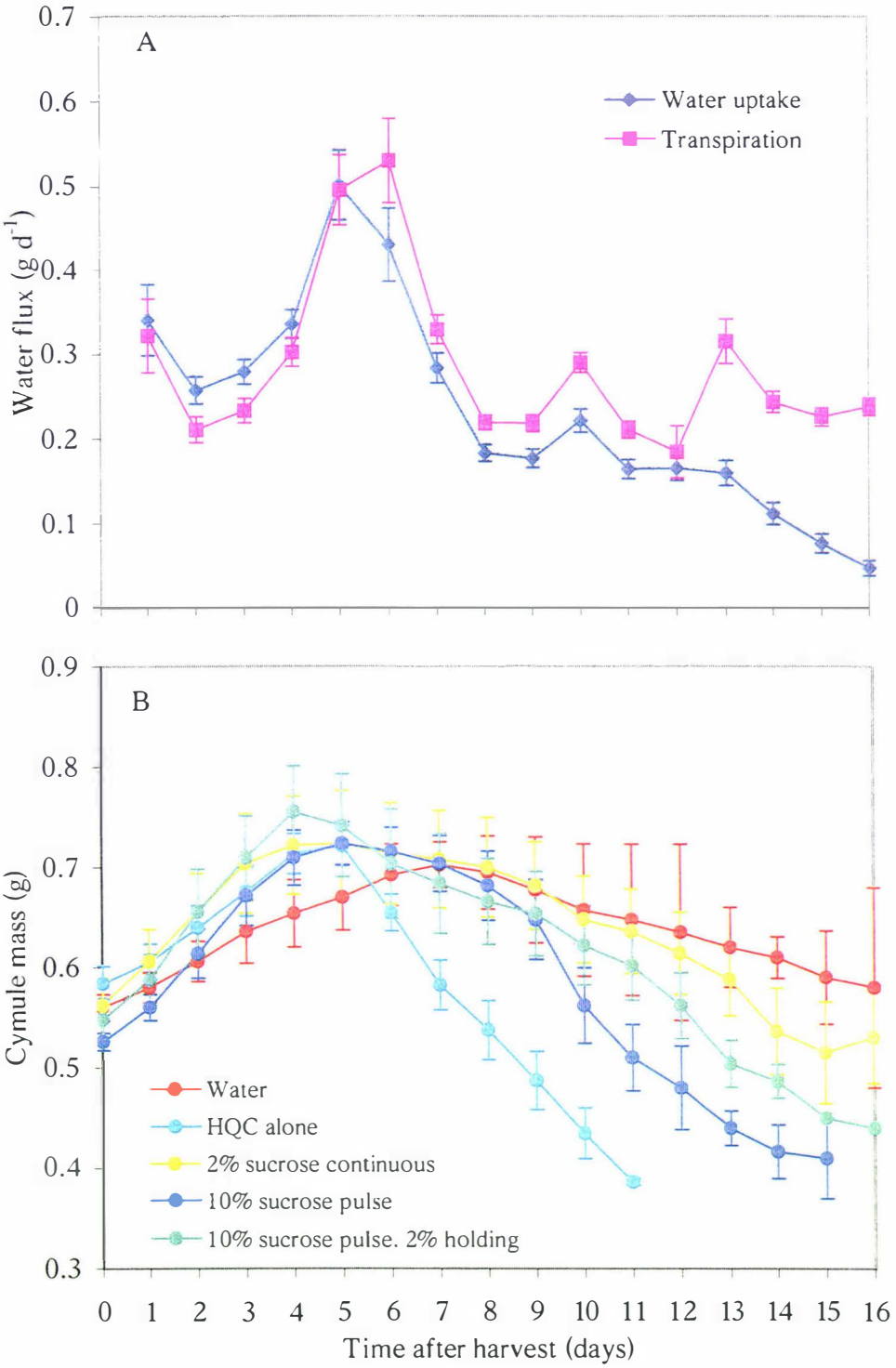


Figure 6.4 Changes in (A) mean (±SE) water flux (uptake and transpiration) pooled for all holding solution treatments and (B) mean (±SE) cymule mass for individual treatments in cut cyumles of *Metrosideros excelsa* 'Vibrance'.

There was no significant interaction effect of treatment and time on the rate of transpiration ($P>0.05$). Mean transpiration per cymule peaked on Days 5-6 at a rate of approximately 0.51 g day^{-1} and subsequently declined and remained relatively constant at ca. 0.24 g day^{-1} for the duration of the experiment (Figure 6.4 A). Transpiration rates exceeded water uptake after Day 5.

Cymule mass differed significantly between holding solutions over time ($P<0.0001$). All treatments showed a uni-modal pattern in weight change with a mean peak of 0.72 g per cymule (pooled data) on Day 5 before declining to 0.52 g day^{-1} by Day 15 (Figure 6.4 B). From Day 7 onwards, cymule mass in the HQC holding solution declined significantly (loss of 0.05 g day^{-1}) in comparison with all other treatments (loss of 0.03 g day^{-1}) ($P<0.05$). After Day 10, cymule mass from the 10% sucrose pulse treatment also decreased significantly in comparison to the control, 2% sucrose and the 10% sucrose 2% sucrose pulse treatments ($P<0.05$).

6.3.2.3 Stamen wilting

Treatments associated with lowest incidence of stamen abscission produced the highest levels of wilting over time ($P<0.0001$) (Figure 6.5 A). This included higher levels of wilting in both the pulse and constant HQC solutions throughout most of the experimental period. Initially, the highest rates of stamen wilting were recorded in the HQC solution, which reached a maximum wilting level of 76% by Day 10. Up until Day 13, both the HQC and 10% sucrose pulse solutions were significantly higher (mean 88% wilting level) compared to control and sucrose treatments (mean 31% wilting level) ($P<0.05$). From Day 13 onwards, the level of wilting in both sucrose treatments (2% sucrose and the 10% sucrose pulse 2% holding) did not differ from the HQC treatment ($P>0.05$).

Stamen wilting for all treatments pooled was strongly correlated with cymule mass during the days that wilting occurred (Day 4-16) ($r = -0.781$, $P<0.0001$). For individual treatments, the level of wilting showed a strong negative correlation with cymule mass (Table 6.3). In particular, both the HQC and 10% sucrose pulse treatments showed the strongest linear fit ($r <-0.900$). This corresponded to the lower mean levels of cymule

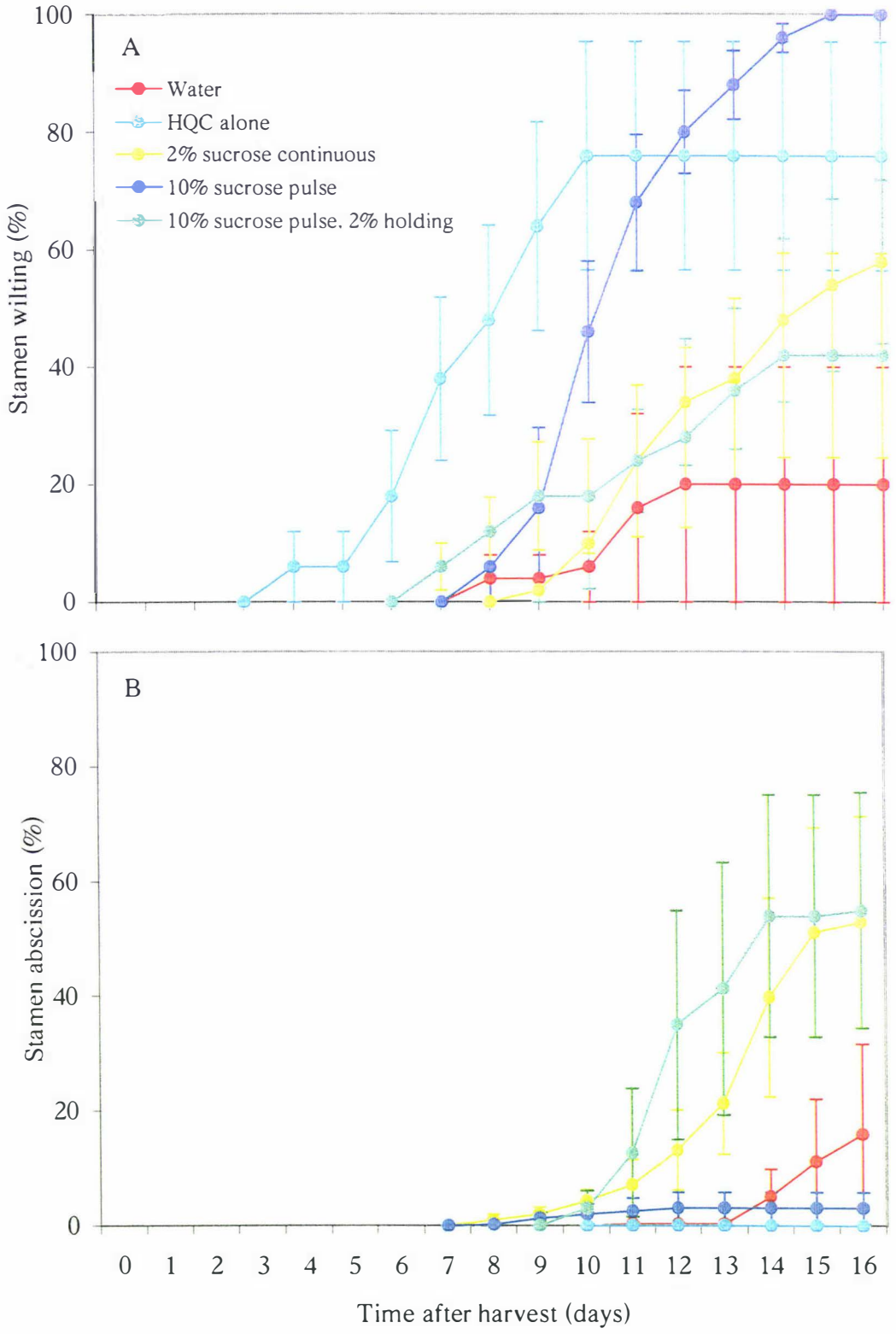


Figure 6.5 Effect of holding solution on mean (±SE) (A) stamen wilting and (B) abscission in cut cymules of *Metrosideros excelsa* 'Vibrance'.

mass per day recorded for HQC and 10% sucrose pulse after Day 5 and 10, respectively (Figure 6.4 A).

Table 6.3 Correlation of mean stamen wilting and cymule fresh weight between Days 4-16 for each holding solution treatment for cut cymules of *Metrosideros excelsa* 'Vibrance'.

Holding solution	Pearson correlation value	F - P - value
Water (Control)	-0.724	<0.0001
HQC alone	-0.929	<0.0001
2% sucrose continuous	-0.676	<0.0001
10% sucrose pulse	-0.906	<0.0001
10% sucrose pulse, 2% holding	-0.699	<0.0001
Total	-0.781	<0.0001

6.3.2.4 Floral Abscission

The onset of stamen abscission occurred from Day 7 onwards in all treatments, except within the HQC holding solution where stamen abscission remained absent (<1%) during the entire 16 day experimental period (Figure 6.5 B). There was a significant interaction between the main effects of treatment and time on the proportion of stamen abscissions ($P<0.001$). By the end of the experimental period (Days 13-16), the highest levels of stamen abscission occurred in both the pulse and constant 2% sucrose solutions in comparison with both HQC solutions where stamen abscission was significantly lower ($P<0.001$). Stamen abscission in the water control treatment did not differ from both HQC treatments.

Petal abscission did not differ significantly between treatments over time ($P>0.05$). The level of abscission was low for all treatments by Day 16, averaging a loss of 4% of petals per treatment.

6.3.3 Experiment 1 C: Holding solutions: effect of HQC and pH on flower quality in 'Lighthouse'.

The highest levels of stamen abscission occurred after Day 6 in the control solution containing only water (pH 5.0) in comparison with all solutions containing HQC ($P<0.01$) (Figure 6.6 A). By Day 10 onwards, approximately 50% of all stamens in the control treatment had abscised in comparison to HQC-treated cymules (mean ≤ 5%), regardless of pH adjustment. There was also a strong significant interaction effect of holding solution treatment and time on stamen wilting ($P<0.0001$). Holding solutions that showed the least amount of stamen abscission had the highest level of wilting and vice-versa (Figure 6.6 B).

6.3.4 Experiment 2: Effect of humidity on flower quality and endogenous ethylene production.

6.3.4.1 Floral development and abscissions

Flower quality was assessed in explants maintained continuously in environments with either high (>90%) or intermediate (~70%) levels of relative humidity (RH) over the 8 day experimental period. Environment had a significant effect on the stage of floral development over time ($P<0.01$) (Figure 6.7). A higher humidity level (>90%) slowed flower bud opening by 2 days in comparison to cymules at 70% RH. For instance, cymules maintained at RH 70% reached Stage 4 (anthesis) on Day 4, whereas those at 90% RH only reached this stage on Day 6.

Flower (as opposed to stamen) abscission occurred only in the high RH environment. The onset of flower abscission in this 90% RH environment occurred on Day 5 (mean 63% abscission). By Day 7, 100% of flowers in this environment had abscised. No flower abscission was recorded for cymules maintained in the 70% RH environment over the 8 day experimental period.

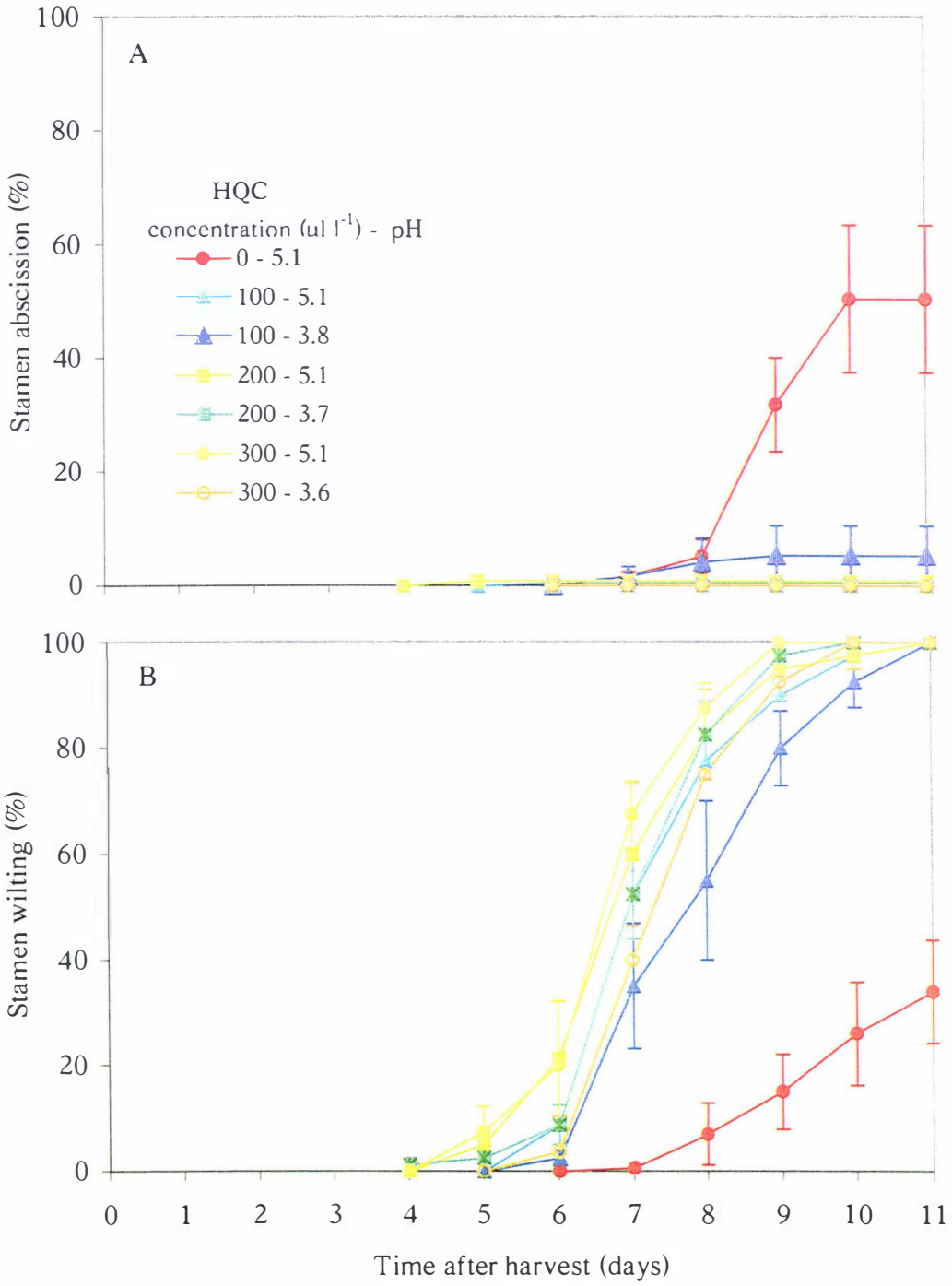


Figure 6.6 Effect of HQC and pH holding solutions on mean (±SE) (A) stamen abscission and (B) wilting in cut cymules of *Metrosideros excelsa* 'Lighthouse'.

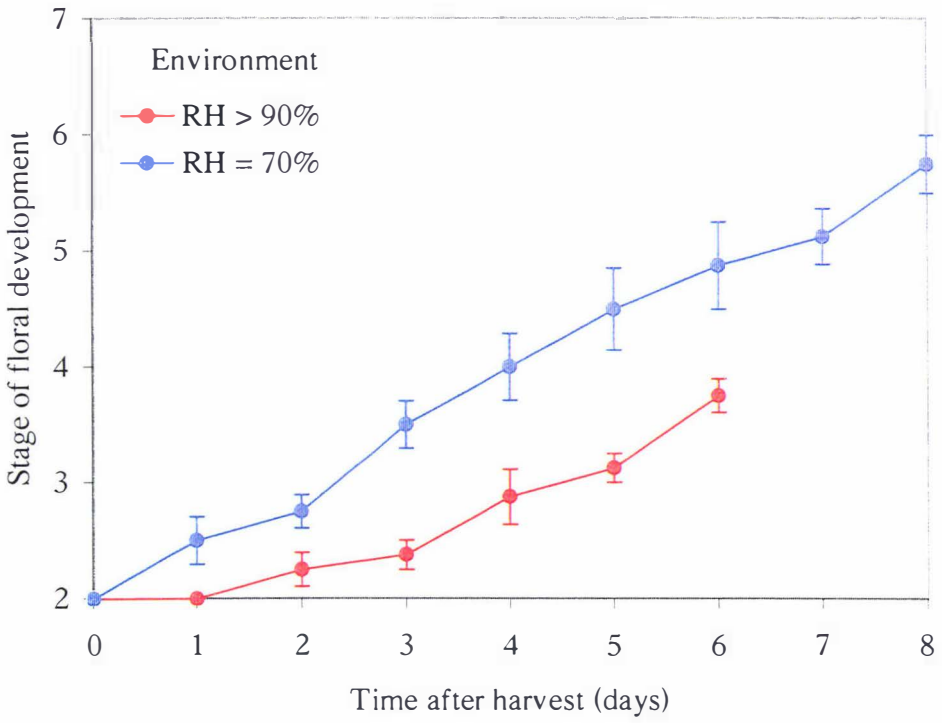


Figure 6.7 Effect of relative humidity (environment) on mean (±SE) stag development in cut cymules of *Metrosideros excelsa* 'Lighthouse'.

Stamen abscission occurred in cymules maintained only in the 70% RH environment starting from Day 5 and proceeding through to the end of the experiment (Table 6.4). Similarly, petal abscission was also recorded for cymules only in the 70% RH environment (Table 6.4).

Table 6.4 Effect on humidity on percentage of stamen and petal abscission in cut cymules of *Metrosideros excelsa* 'Lighthouse'. Days in which all flowers in a treatment had abscised are indicated by a ' - '.

Abscission type	Environment: Relative humidity (%)	Days				
		4	5	6	7	8
Stamen	70	0	0.25	7.4	26	33.8
	> 90	0	0	0	-	-
Petal	70	0	0	13.3	13.3	13.3
	> 90	0	0	0	-	-

6.3.4.2 Water relations

Relative humidity significantly affected the rate of water flux (water uptake and transpiration) in cymules, as indicated by a strong interaction effect of environment and time ($P<0.0001$) (Figure 6.8 A). Comparatively, water flux was lower for cymules maintained in the high humidity environment.

The extent of water flux within each of the environments affected cymule mass. A high (>90%) RH environment significantly reduced the water flux in the cut cymules ($P<0.01$) (Figure 6.8A). Cymule mass was also affected and was significantly lower in the high RH environment ($P<0.01$) (Figure 6.8 B). Within the 70% RH environment, mean transpiration exceeded mean water uptake after Day 5, which coincided with a decline in mean cymule mass. In contrast, transpiration in the >90% RH environment remained lower than water uptake and cymule mass subsequently increased with time.

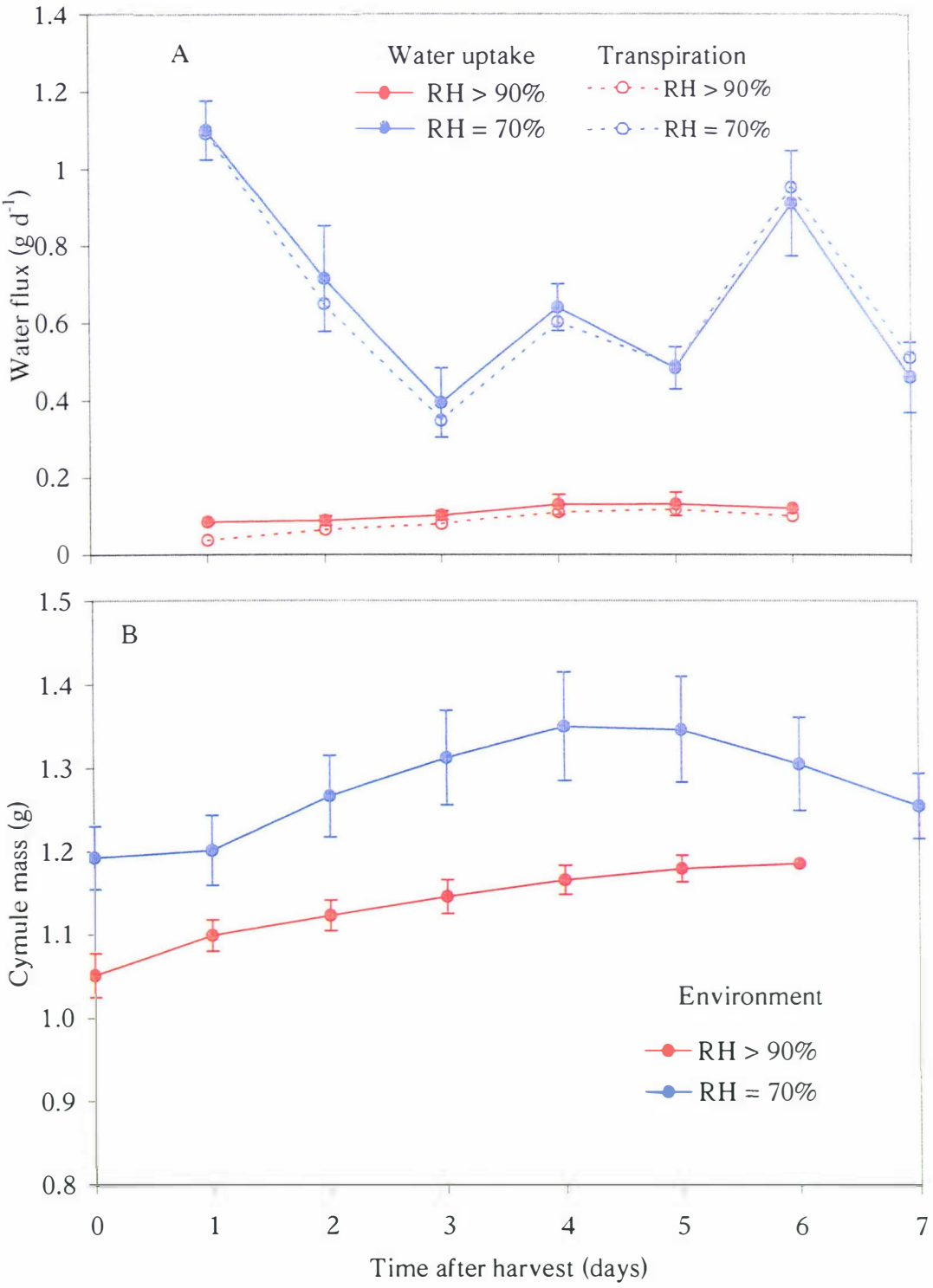


Figure 6.8 Effect of relative humidity on mean (±SE) (A) water flux (water uptake and transpiration) and (B) cymule mass in cut cymules of *Metrosideros excelsa* 'Lighthouse'. For clarity purposes, standard errors bars were omitted for transpiration data although they were similar to those for water uptake.

6.3.4.3 Endogenous ethylene production

Measurements of the amount of ethylene emanated in jar headspaces did not differ for cymules that had been held in the different RH environments ($P>0.05$). Based on data pooled for both environments, the amount of ethylene produced differed across 24 h incubation periods ($P<0.0001$), with the highest levels produced on Days 5-6 (Figure 6.9). Similarly, 48 h incubation periods also differed between each consecutive enclosure days ($P<0.01$) although the highest levels were recorded on Day 4.

Comparisons of the amount of ethylene produced between 24 and 48 h per enclosure date was also statistically significant ($P<0.01$). On enclosure days 4-5, ethylene levels approximately doubled between the 24 and 48 h incubation periods (Figure 6.9). Generally, the rise in ethylene on enclosure day 4 coincided with the shift of cymules from Stage 4 (anthesis) at 24 h to Stage 5 (dehiscence of anthers) by 48 h. On enclosure day 5, cymules at 24 h were at Stage 5 and shifted to Stage 6 (onset of stamen abscission) by 48 h. Therefore, the highest ethylene emanation period occurred either just before or during the time when anthers dehisced.

6.3.5 Experiment 3: Effect of exogenous ethylene on flower quality and endogenous ethylene production.

6.3.5.1 Floral development

Changes in floral characteristics were analysed for cymules pooled for all times of observation after ethylene treatment (0, 24, 48 and 96 h), since responses between these treatments were similar. There was no significant effect of exogenous ethylene treatment (0-10 $\mu l\ l^{-1}$) on the stage of floral development over the 9 day experimental period ($P>0.05$). In all ethylene treatments, cymules reached Stage 3 and 4 on Days 2-3 and Day 4, respectively. Cymules exposed to exogenous ethylene treatments 0 and 0.1 $\mu l\ l^{-1}$ reached Stage 5 on Day 9, whereas those treated with 1.0 and 10 $\mu l\ l^{-1}$ of ethylene failed to reach this stage, and underwent flower abscission instead.

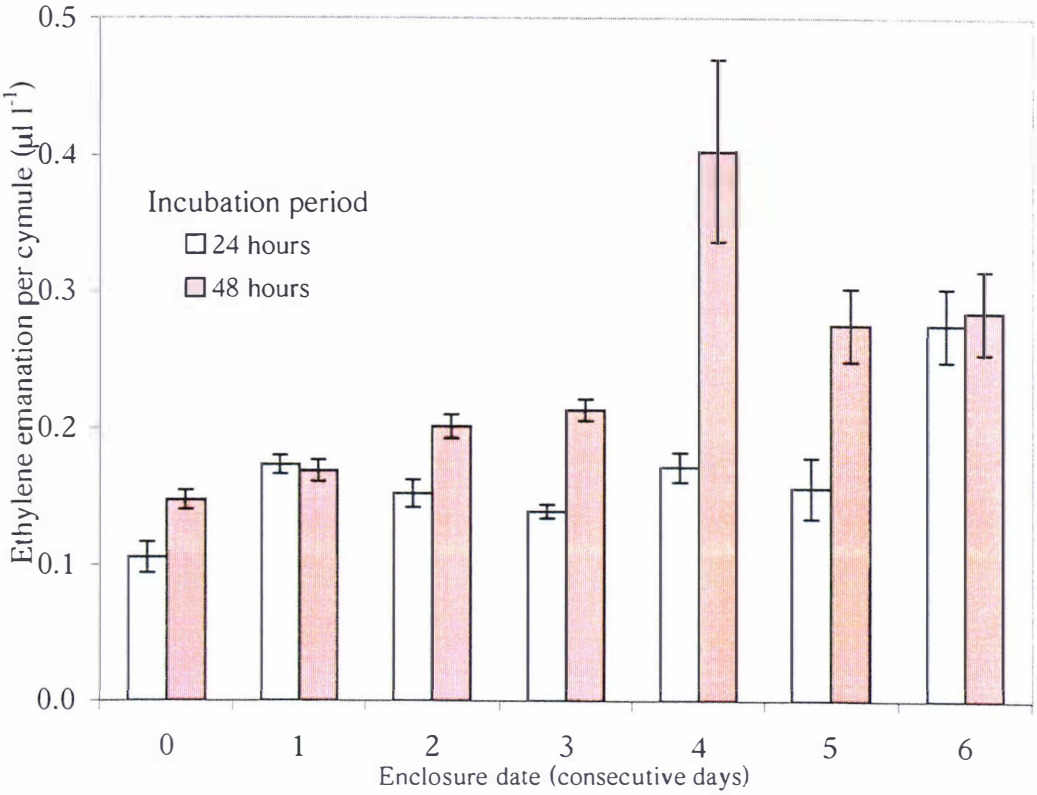


Figure 6.9 Mean (±SE) headspace ethylene concentration above cut cymules of *Metrosideros excelsa* 'Lighthouse' at 24 and 48 h after incubation per enclosure day.

6.3.5.2 Abscission

The amount of flower abscission was associated with the concentration of exogenous ethylene (Table 6.5). However, in the 0 (control) or 0.1 μl l^{-1} of ethylene treatments, flower abscission was relatively low during the 9 day experimental period. Thus, stamen abscission occurred only in the 0 and 0.1 μl l^{-1} treatments, although the level of abscission from both treatments was relatively low (mean 3.1% per cymule on Day 9) and did not differ significantly ($P<0.05$).

There was a significant main effect of ethylene concentration on petal abscission ($P>0.0001$). By Day 6, cymules treated with 10 μl l^{-1} of ethylene incurred the lowest levels of petal abscission (mean 2.5% petals per cymule) compared with 0 (control), and 0.1 and 1 μl l^{-1} treatments (pooled mean 16.7% petals per cymule). The proportion of abscised petals in the 10 μl l^{-1} treatment was low due to the high abscission rate of whole flowers in that treatment.

Table 6.5 Effect of exogenously applied ethylene on percentage of abscised flowers. Mean separation in columns by Least Square Means test, 5% level.

Exogenous ethylene (μl l^{-1})	Days 3	4	5	6	7	8	9
0 (control)	0^a	0^a	0^a	0^a	6.3^a	19^a	19^a
0.1	0^a	0^a	0^a	0^a	0^a	25^a	31^a
1.0	0a	56^b	75^b	75^b	75^b	81^b	81^b
10.0	56^b	88^c	94^c	94^c	100^c	100^c	100^c

6.3.5.3 Wilting

There was a significant interaction of ethylene treatment and time on the level of stamen wilting ($P<0.001$) (Table 6.6). The wilting response in the 0 and 0.1 μl l^{-1} exogenous ethylene treatments were similar, reaching an approximate 51% wilting level by Day 9.

The low levels of wilting in the 1.0 and 10 μl l^{-1} ethylene treatments were due to the high level of flower abscission (≥75%) from Days 5-9 in these treatments.

Table 6.6 Effect of exogenously applied ethylene on percentage of mean stamen wilting. Mean separation in columns by Least Square means test, 5% level.

Exogenous ethylene (μl l^{-1})	Days 5	6	7	8	9
0 (control)	0.6[a]	6.3[a]	19.4[a]	35.0[a]	50.0[a]
0.1	0[a]	8.1[a]	23.1[a]	40.6[a]	52.8[a]
1.0	0[a]	0[a]	1.9[b]	1.9[b]	12.5[b]
10.0	0[a]	1.3[a]	5.6[ab]	-	-

6.3.5.4 Endogenous production of ethylene after exposure to exogenous ethylene

Exposure of cymules to exogenous ethylene had no effect on subsequent ethylene production. There was no significant interaction of sampling time (0, 24, 48 or 96 h) and ethylene level (0.1, 1.0 or 10 μl l^{-1}) on ethylene production ($P>0.05$). Ethylene concentration per 24 h of incubation averaged 0.23±0.1 μl l^{-1} per day.

6.3.6 Experiment 4: Effect of 1-MCP and STS on ethylene-induced responses

6.3.6.1 Water relations

There was significant three-way interaction of time, ethylene, and preventative treatment ($P<0.05$) on both water uptake and transpiration. In the absence of ethylene (0 μl l^{-1} ethylene treatment), water flux (water uptake and transpiration) was higher in the STS treated buds in comparison with all other treatments ($P<0.01$) (Figure 6.10 A). However, in the presence of ethylene (5 μl l^{-1}), water flux was higher in STS treated cymules on all days except Day 4 after treatment (Figure 6.10 B). On this day, water flux did not differ significantly from either the 0 or 150 nl l^{-1} 1-MCP treatments ($P>0.05$), but was higher than the 15 nl l^{-1} 1-MCP treatment ($P<0.05$).

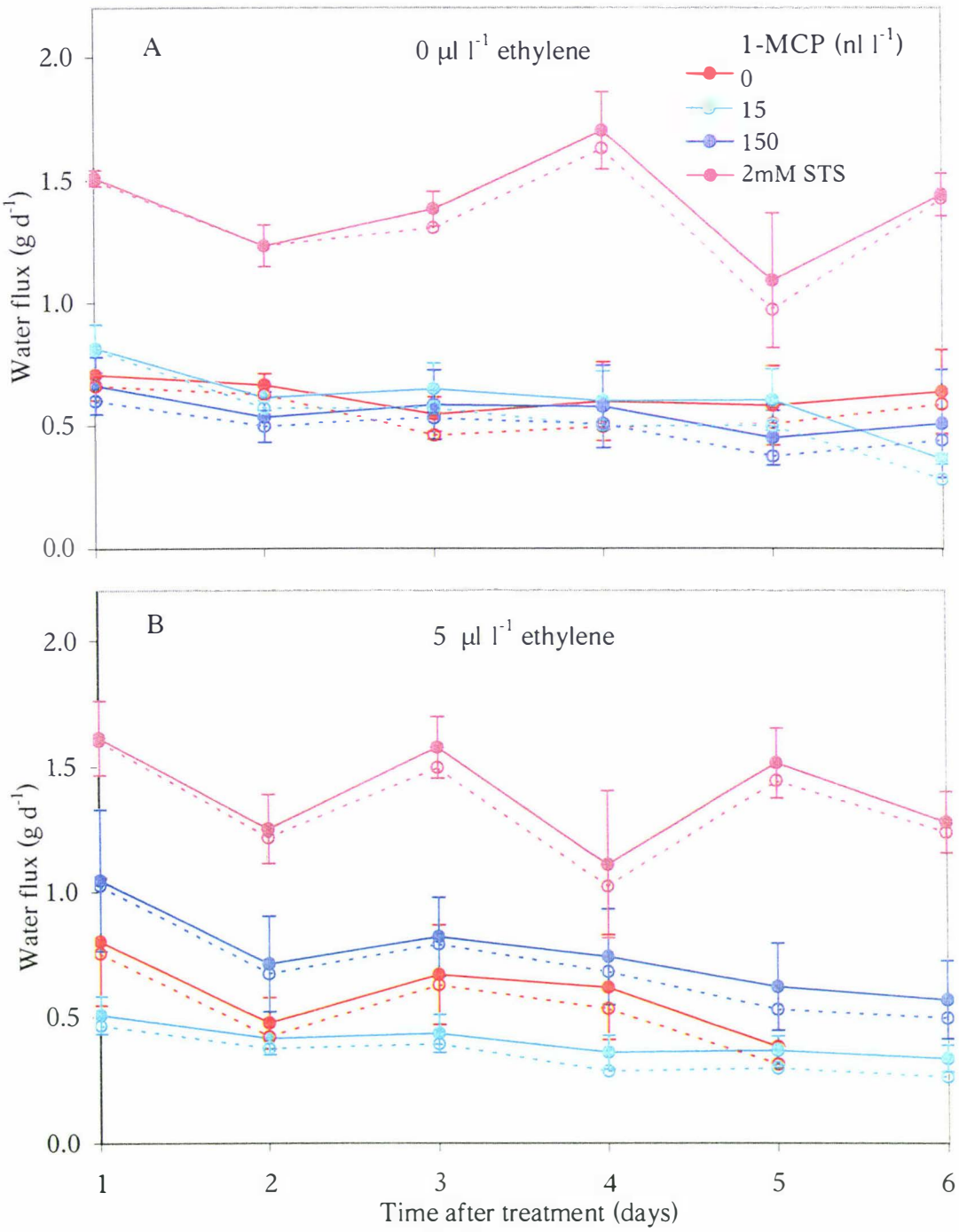


Figure 6.10 Effect of preventative treatment on mean (±SE) water flux (water uptake and transpiration) for cut cymules treated with either (A) 0 or (B) 5 µl l-1exogenous ethylene. For clarity purposes, standard error bars are omitted for transpiration data although they were similar to those for water uptake. Water uptake = solid lines, transpiration = dashed lines.

Treatments with either 0 or 5 nl l^{-1} ethylene did not differ in their effects on the fresh weight of cymules over time ($P>0.05$). The use of a preventative treatment did, however, influence the weight of cymule over time ($P<0.0001$) (Figure 6.11). Generally, cymules that were not treated with a preventative treatment (control) maintained a lower cymule mass over time than treated cymules. Application of STS resulted in a higher cymule mass per day in comparison with cymules treated with either 0 or 15 nl l^{-1} 1-MCP ($P<0.01$). The weight of STS treated cymules was also higher than those treated with 150 nl l^{-1} 1-MCP, but only on Days 5, 7-8 ($P<0.01$).

6.3.6.2 Stamen wilting

There was a significant interaction of the main effects of preventative treatment and time on the level of stamen wilting ($P<0.0001$), although there was no significant interaction of ethylene treatment and time ($P>0.05$) (Figure 6.12). Generally, wilting was inversely correlated with cymule mass and, therefore, treatments in which the mean cymule mass was comparatively higher over time showed the least amount of wilting and the weakest linear fit (Table 6.7). In this case, 2 mM STS and 150 nl l^{-1} 1-MCP treated flowers showed the lowest levels of wilting between days 5 – 8, followed by 15 nl l^{-1} 1-MCP treated flowers, and the highest levels occurred in the 0 nl l^{-1} 1-MCP (control) treatment ($P<0.05$).

Table 6.7 Correlation of stamen wilting and cymule fresh weight of cut cymules of *M. excelsa* pre-treated with preventative treatments of either 1-MCP or STS before application of exogenous ethylene (data pooled for 0 and 5 µl l^{-1} ethylene).

Preventative treatment	Pearson correlation F - value	P - value
0 nl l^{-1} 1-MCP	-0.927	<0.0001
15 nl l^{-1} 1-MCP	-0.792	<0.0001
150 nl l^{-1} 1-MCP	-0.669	<0.0001
2 mM STS	-0.710	<0.0001
Total	-0.777	<0.0001

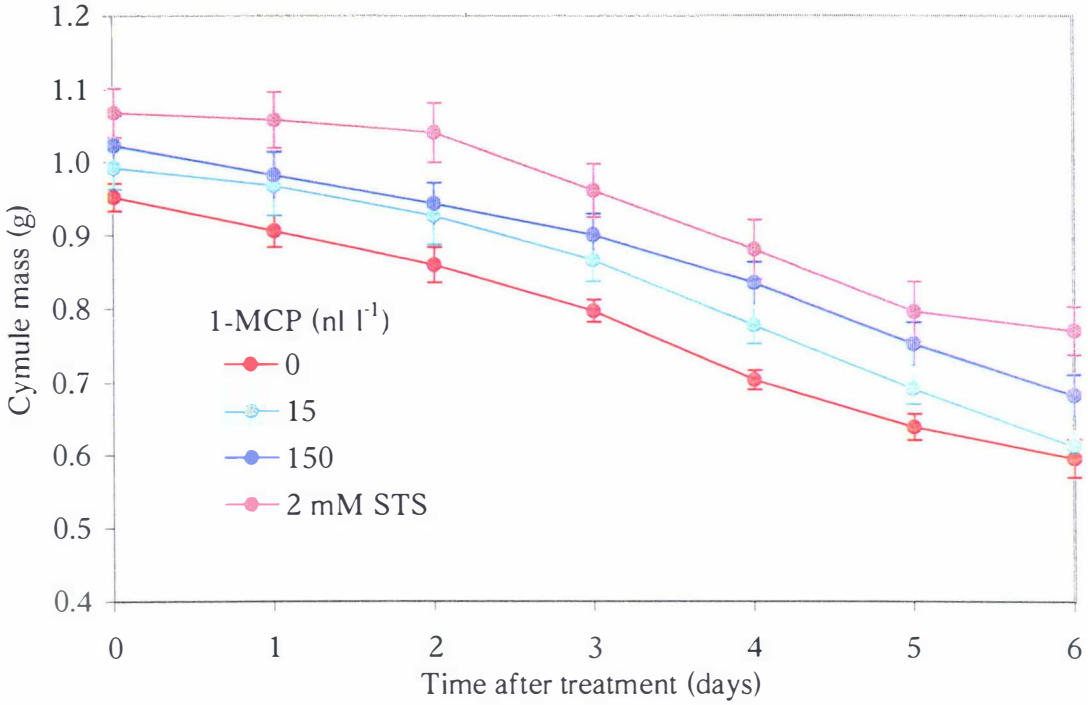


Figure 6.11 Change in mean (±SE) cymule mass following exposure to a preventative treatment and exogenous ethylene (0 and 5 µl l^{-1} ethylene data pooled).

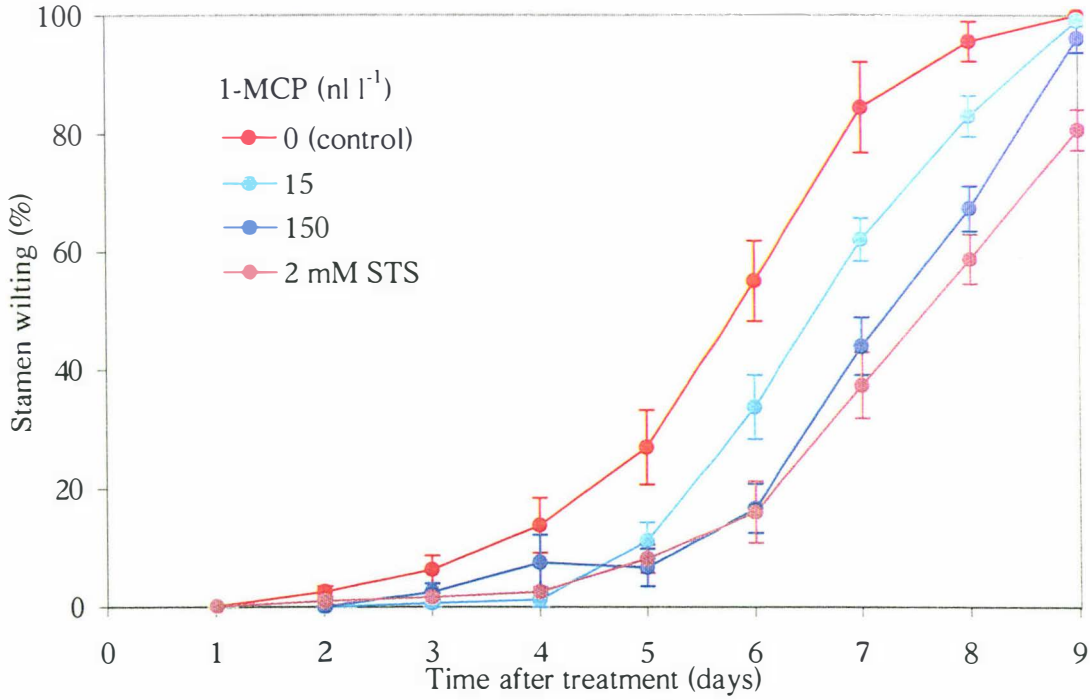


Figure 6.12 Effect of preventative treatment on mean (±SE) stamen wilting. Data pooled for cut cymules treated with 0 and 5 µl l^{-1} ethylene data pooled.

6.3.6.3 Stamen abscission

There was a significant interaction of ethylene treatment, preventative treatment and time on the level of stamen abscission ($P<0.0001$). In the absence of an exogenous ethylene application (0 $\mu l\ l^{-1}$ ethylene), cymules treated with a preventative treatment of STS showed a high level of stamen abscission (mean 29% per cymule) on days 7-9 (Figure 6.13 A). This was followed by intermediate levels in the 0 and 15 $nl\ l^{-1}$ 1-MCP treatments (mean 10% per cymule) ($P<0.05$). The lowest levels of stamen abscission occurred in the 150 $nl\ l^{-1}$ 1-MCP treatments (< 1% stamen abscissions per cymule) ($P<0.05$).

Preventative treatments of 15, 150 $nl\ l^{-1}$ 1-MCP and 2 mM of STS afforded significant protection against stamen abscission following an application of 5 $\mu l\ l^{-1}$ ethylene in comparison with 0 $nl\ l^{-1}$ 1-MCP ($P<0.05$) (Figure 6.13 B). There was, however, no difference between the 2mM STS, 15 and 150 $nl\ l^{-1}$ 1-MCP protective treatments ($P>0.05$). By day 9, only 7% of stamens per cymule had abscissed in protected flowers, whereas 32% of stamens per cymule had abscissed in the controls (0 $nl\ l^{-1}$ 1-MCP).

6.3.6.4 Flower and petal abscission

An exogenous ethylene application of 5 $\mu l\ l^{-1}$ of ethylene was sufficient to cause flower abscission, although only in some treatments (Figure 6.14). By the end of the experimental period, the highest incidence of flower abscission was in the control flowers (mean 88% of cymules in the 0 $nl\ l^{-1}$ 1-MCP treatment), followed by 25% and 13% in the 15 and 150 $nl\ l^{-1}$ 1-MCP treatments, respectively. No flower abscissions were recorded in the 2mM STS treatment throughout the experimental period.

Petal quality was assessed on Day 9 based on a visual score of the severity of petal in-rolling (0-100%) in intact (not abscised) flowers that had been exposed previously to 5 $\mu l\ l^{-1}$ ethylene (Figure 6.15). The highest severity of petal in-rolling was recorded in control plants (mean 95% per cymule) followed by both 1-MCP treatments (mean 50% per cymule). Petals in STS plants remained turgid and showed no indication of in-rolling (mean < 5% per cymule) by Day 9.

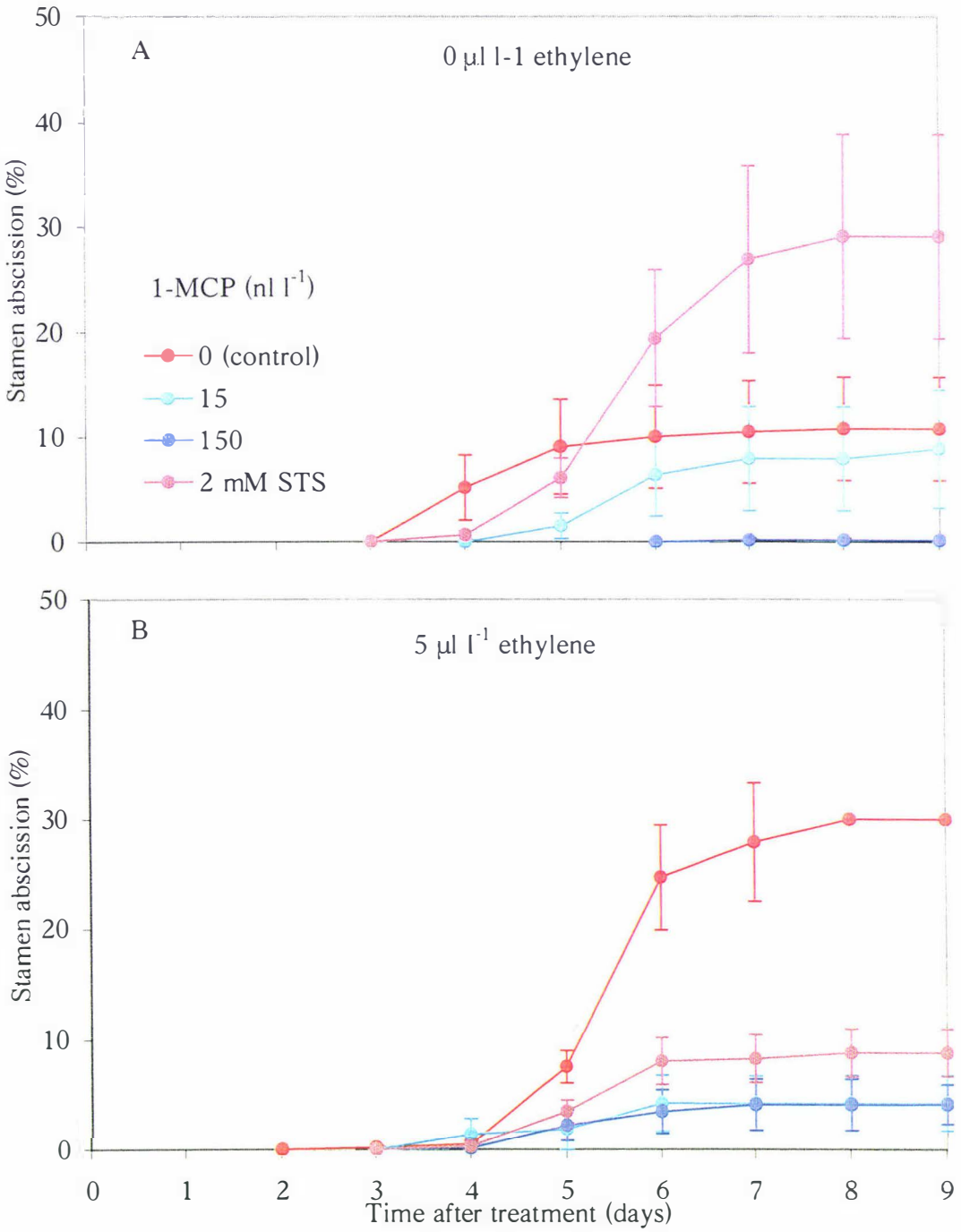


Figure 6.13 Change in mean (±SE) stamen abscission in cut cymules after a preventative pre-treatment of 1-MCP and STS before application of either (A) 0 and (B) 5 µl l^{-1} of exogenous ethylene.

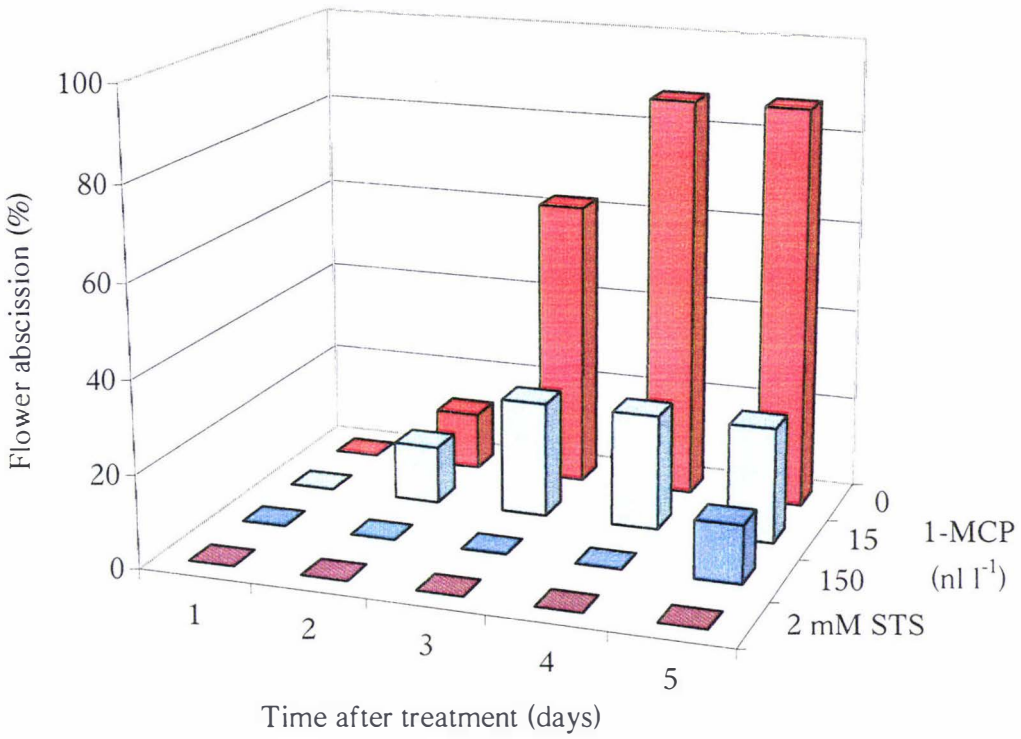


Figure 6.14 Change in mean flower abscission with time following exposure to 5 μl l^{-1} exogenous ethylene in pre-treated cymules of *Metrosideros excelsa* 'Lighthouse' with 1-MCP and STS.

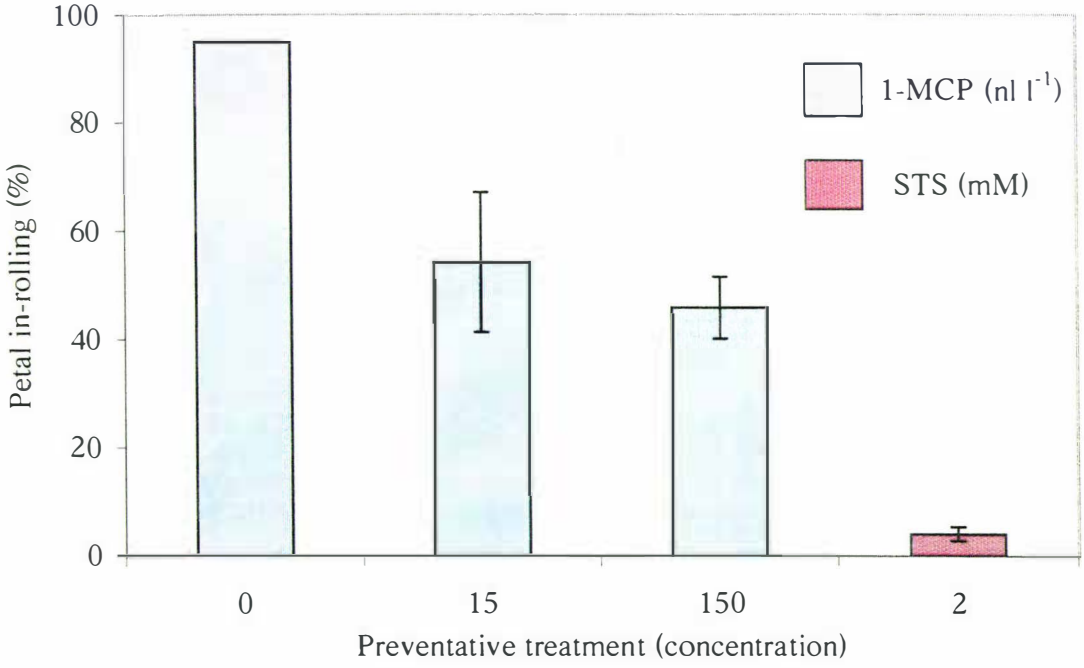


Figure 6.15 Effect on mean (±SE) petal in-rolling on Day 9 following exposur to 5 μl l^{-1} exogenous ethylene in pre-treated cymules of *Metrosideros excelsa* 'Lighthouse' with 1-MCP and STS.

6.4 Discussion

In this study, treatments that induced relatively low levels of stamen abscission in both cultivars of *Metrosideros excelsa* incurred relatively high levels of wilting, and *vice versa*. This study supported the hypothesis that the vase life of *M. excelsa* was limited by both adverse water relations and by ethylene-related abscission of whole flowers and flower organs.

Floral senescence in the family Myrtaceae is strongly related, at least in part, to the water status within cut-flowers (Joyce and Jones 1992, Burge *et al.* 1996, Sun *et al.* 2000). In the closely related *Metrosideros collina*, Sun *et al.* (2000) demonstrated that when stamen abscission did not occur in response to endogenous and/or exogenous ethylene, wilting of stamens ensued. In cut cymules of *M. collina*, the general effect of supplementation of sucrose on stamen wilting was concentration specific, a response that was also inversely and negatively correlated with cymule mass. In the current study, the use of relatively low and/or short-term doses of sucrose (2% supplied continuously or the 10% sucrose pulse 2% holding) did not appreciably increase the level of wilting above that of control water. However, this response may have occurred had higher sucrose concentrations been used, as observed in *M. collina* where concentrations above 10% caused a higher rate of wilting, presumably through a reduction in solution osmotic potential (Sun *et al.* 2000).

Inclusion of HQC in holding solutions can effectively inhibit or depress microbial growth and subsequently improve water relations within the cut flowers (Put and Clerkx 1988). The findings with regards to the addition of HQC in vase solutions of *M. excelsa* were not consistent with those reported for the closely related genera *Chamelaucium* (Joyce 1988), *Leptospermum* (Burge *et al.* 1996) or for *M. collina* (Sun *et al.* 2000). HQC added to holding solutions of *M. excelsa* significantly increased flower wilting, especially when applied continuously and, to a lesser degree, after a 10% sucrose pulse. Specifically, by the end of the experimental period, wilting was highest in 'Lighthouse' following treatment with HQC alone (mean ≥70%) and for 'Vibrance' with both HQC applied continuously and after a 10% sucrose pulse (≥76%). This effect on water relations was most apparent in 'Lighthouse', whereby water uptake and transpiration of cut-cymules treated with HQC was considerably lower than in control water.

Moreover, the rapid decline in fresh weight was strongly and negatively correlated with the degree of wilting, showing the tightest linear fit with HQC solutions. This suggests that the water status of HQC treated cut-cymules in 'Lighthouse' directly affect stamen wilting.

HQC is generally considered non-toxic to most cut flowers even at concentrations effective for controlling microbial growth (Van Doorn 1997). However, cut cymules of *M. excelsa* appear to be very sensitive to this compound when applied alone (irrespective of pH), but less so in the presence of sucrose. Sucrose dissolved in vase water (without a biocide) is conducive to bacterial growth and is subsequently associated with the formation of stem occlusions (Put and Clerkx 1988). Furthermore, solutions with dissolved sugars (as opposed to water) are less easily transported through stems due to a lower osmotic potential of the solution (Van Doorn 1997). Considering that water uptake in the 2% sucrose holding solution (with 200 mg l^{-1} HQC added) was significantly higher than in the solution containing HQC alone, it might be inferred that some level of protection from microbial occlusions resulted from the addition of HQC. Therefore, the wilting response by HQC may have occurred irrespective of its actions in preventing microbial growth in cut cymules of *M. excelsa*. In the woody cut flower *Banksia coccinea*, addition of HQS to a vase solution was considered detrimental, causing a reduction in vase life and acceleration in the opening of florets (Delaporte *et al.* 1997). Similarly, HQ based compounds have also been reported to cause leaf damage or stem browning in *Chrysanthemum* (Gladon and Staby 1976) and *Gypsophila* (Katchansky 1979) and petal discolouration in marguerite daisy (Byrne *et al.* 1979).

High levels of relative humidity (RH) can affect various aspects of flower development (Zieslin and Gottesman 1983, Jones *et al.* 1993a, Burge *et al.* 1998). In *M. excelsa* flowers, high RH not only slowed the rate of floral development (eg. stamen unfolding and expansion) but ultimately induced a flower abscission response at an early stage in development, occurring before the onset of anthesis (Stage 4). It was observed that flowers under the high RH environment remained turgid, even after abscission. Zieslin and Gottesman (1983) also reported a similar response in excised flowering shoots of *Leptospermum scoparium* under comparable conditions. Conditions with high RH (100%) induced whole flower abscission (approximately 25% of fresh weight of stems), although flowers remained turgid. In contrast, at low RH (50-70%) no floral abscission

in *L. scoparium* occurred and flowers eventually shrivelled while petals underwent in-rolling (Zieslin and Gottesman 1983).

Changes in RH may also affect the water balance within cut flowers and subsequent flower qualities (Burge *et al.* 1998). In harvested inflorescences of hybrid *Limonium* 'Chorus Magenta', water uptake was significantly depressed under high RH conditions (98-100%) averaging a 10 ml difference in water uptake per any given day. Fresh weight of inflorescences, however, showed the opposite response being relatively higher at high RH, although no explanation was given for the inverted response (Burge *et al.* 1998). In *M. excelsa*, cut cymules held under high RH also showed lower rates of water flux, but lower cymule fresh weights in comparison with those treated to low RH. This response is likely attributed, at least in part, to the differences in atmospheric and holding solution water potential, whereby the comparatively higher water potential of the atmosphere would depress rates of transpiration and subsequently reduce water uptake (Van Doorn 1997). Consequently, an overall reduction in water uptake and transpiration would account for a low fresh weight as observed in the high RH environment. Furthermore, even though fresh weight was comparatively lower under the high RH conditions, it continued to increase with time and maintained a net positive water balance (transpiration did not exceed water uptake) which would explain the turgidity of stamens at the time of abscission.

It was surprising that there was no significant difference in the amount of endogenous ethylene produced by cymules held in the different RH environments. This is in light of the fact that 100% of cymules had abscised under conditions of high RH, whereas no abscission occurred when RH was low. The lack of maintenance of high humid conditions within the sampling jars held for 48 h may partly account for these observations. Alternatively, high humidity conditions may increase flower susceptibility to ethylene action. Zieslin and Gottesman (1983) suggested that endogenous ethylene was involved in flower abscission in the closely related *L. scoparium*, with higher moisture levels required for the enzymatic activity of the abscission process.

Endogenous ethylene production and/or sensitivity of the plant tissue to ethylene usually increase with tissue age (Høyer 1996b, Woolf *et al.* 1995, 1999). For example,

floral bud abscission in intact pot plants of *Camellia* becomes increasingly sensitive to exogenous ethylene with time, an effect that was also mediated by temperature (Woolf *et al.* 1995, 1999). In cut cymules of *M. excelsa*, endogenous levels of ethylene increased with flower age, showing a rise in production on Day 4 of incubation (0.4 $\mu l\ l^{-1}$ per 48 h) followed by a subsequent decline. Similar peaks in ethylene production have been reported in various woody flowering genera used in the cut-flower industry, such as in *Leptospermum* (Zieslin and Gottesman 1983), *Telopea* (Faragher 1986) and *Boronia* (Macnish *et al.* 1999). In contrast, flower sprigs of *Chamelaucium uncinatum* showed no endogenous ethylene peak during vase life. Research into the respiratory behaviour of flowers during senescence may elucidate as to whether *Metrosideros* is climacteric. It was interesting to note, however, that the highest levels of ethylene production occurred during the time of anther dehiscence (Stage 5), which precedes the stage during which stamens abscise (Stage 6). This contrasts to the response of non-climacteric species in which senescence is not likely to be regulated by ethylene (Olley *et al.* 1996).

Exogenous ethylene appears to be involved in flower abscission of various genera within the family Myrtaceae, including *Leptospermum* (*Burge et al.* 1996), *Chamelaucium* (Joyce 1989), and *Metrosideros* (Sun *et al.* 2000), in addition to a number of Australian woody ornamentals from other families (Macnish *et al.* 2000). Cut cymules of *M. excelsa* were extremely sensitive to exogenous sources of ethylene. Ethylene concentrations greater than 0.1 $\mu l\ l^{-1}$ applied for 24 h were sufficient to induce whole flower abscission from Day 3 onwards. A similar response was also effective in hastening floral abscission in cut cymules of *M. collina* (Sun *et al.* 2000). Exposure of cut cymules to exogenous ethylene up to 10 $\mu l\ l^{-1}$ (for 24 h) did not, however, stimulate the production of endogenous ethylene, at least not for cymules pulsed with ethylene at Stage 2 of development (receptacle expansion and start of petal reflexing).

1-Methylcyclopropene (1-MCP) and silver thiosulfate (STS) afforded significant protection from the deleterious effects of ethylene, reducing the level of whole flower and stamen abscission in cut cymules of *M. excelsa*. This is consistent with the efficacy of STS and/or 1-MCP in protecting flowers from ethylene-induced abscission in the closely related genera *Chamelaucium* (Serek *et al.* 1995b), *Verticordia* (Joyce and Poole

1993) and *Leptospermum* (Macnish *et al.* 2000). In this study, the level of protection conferred by 1-MCP was slightly less than that of STS (when treated with exogenous ethylene), but effective up to a maximum dosage of 150 nl l^{-1} of 1-MCP. Concentrations of around 150 nl l^{-1} of 1-MCP in *Metrosideros* are, however, considered to be close to saturation (Sun *et al.* 2000). The effectiveness of STS in reducing the level of stamen abscission was similar for cut cymules of *M. collina* (Sun *et al.* 2000). However, application of 150 nl l^{-1} 1-MCP in the latter species conferred only short-term protection and subsequently enhanced endogenous ethylene production (Sun *et al.* 2000). Adverse ethylene emanation responses have been reported with the use of STS in *L. scoparium*, although protection against ethylene-induced abscission was still conferred (Zieslin and Gottesman 1986). It was surprising that in the present study STS-treated cymules produced a higher level of stamen abscission in the absence rather than presence of an exogenous ethylene treatment, and may reflect an unexplained adverse response to STS in the absence of exogenous ethylene.

Within the scientific literature, the efficacy of STS has primarily been attributed to its role as a potent inhibitor of ethylene action (Serek *et al.* 1995b, Sisler and Serek 1997). However, evidence from this and various other studies suggest that this compound may also play a role in improving water relations within cut-flowers, subsequently improving flower quality and delaying flower senescence (Joyce 1989, Burge *et al.* 1996). In the case of *M. excelsa*, 2mM of STS applied as a spray significantly doubled the level of water flux compared to unprotected cymules. This result was irrespective of whether 0 or 5 µl l^{-1} of exogenous ethylene was applied, although the effect was slightly more pronounced in the former case. Nevertheless, the fresh weight of cymules treated with 2mM STS was higher in comparison with controls (either 0 or 5 µl l^{-1} of ethylene), whilst cymule fresh weight in all treatments was inversely related to the level of wilting. Joyce (1989) also noted a similar improvement in fresh weight of STS-treated cut flowers of *C. uncinatum* in the presence (9.4 µl l^{-1}) or absence of ethylene. Thus, the relative turgidity of petals and low stamen wilting in STS-treated *M. excelsa* cymules can not be accounted for solely by the ethylene-inhibiting action afforded by STS, since the response appears to be coupled by the attainment of a more favourable water balance. A decrease in petal wilting (senescence) in cut flowers of *L. scoparium* was

also attributed to an STS-related improvement in water relations rather than to an anti-ethylene response (Burge *et al.* 1996).

The water relations and flower quality (petal and stamen wilting) of *M. excelsa* flowers appeared to be less affected by 1-MCP than STS treatments. In comparison with unprotected cymules (for ethylene treatments of 0 or 5 $\mu l\ l^{-1}$), 1-MCP treatments showed a marked reduction in the rate of decline of cymule fresh weight and stamen wilting, although water conductivity did not differ from control treatments. In excised miniature potted roses, 1-MCP also improved flower fresh weight despite not conferring protection from ABA-induced senescence (petal in-rolling) (Müller *et al.* 1999). Whether 1-MCP plays a role in water relations in other cut flowers merits further attention.

In conclusion, the hypothesis that the vase life of cut flowers of *Metrosideros excelsa* is limited by the changes in water relations within the cymule, and by the presence of endogenous and exogenously ethylene which are primarily associated with abscission of floral organs, is supported by this study. These findings are consistent with those for closely related genera. A recommendation of the most favourable vase solution varied depending on the flower attribute in question (e.g. wilting or abscission). By the end of the experimental periods, distilled water or a solution with 2% sucrose (with or without a pulse) consistently produced the lowest levels of wilting for both cultivars, approximately less than 50%. The 10%-HQC alone was effective in reducing wilting but only in 'Lighthouse'. Furthermore, applications of 2 mM STS and 150 $nl\ l^{-1}$ of 1-MCP conferred the greatest protection against exogenous and/or endogenous ethylene. Considering the different effects of 1-MCP on the floral vase life of *M. excelsa* and *M. collina*, future studies may wish to investigate its effects in other congeneric species.

Chapter 7

General discussion

Establishment of a floricultural crop within the ornamental industry requires an extensive scientific knowledge of the factors underlying the promotion and maintenance of flowering. Several diverse and novel approaches were undertaken in this study that addressed three factors that potentially limit the successful expansion of *Metrosideros excelsa* as an ornamental container and cut flower crop. The three primary concerns addressed in this study were the lack of scientific information on (a) promoting vegetative phase change, (b) controlling floral induction and subsequent development, and (c) limits to the postharvest potential of *M. excelsa* as a cut flower.

The study of phase change, despite many decades of research in this field, continues to pose a formidable and significant challenge for many biologists studying this phenomenon. One of the primary limitations in this field has been the paucity of information regarding the accurate characterisation of the morphological and physiological processes occurring during phase change, as shown for *Arabidopsis thaliana* (Telfer *et al.* 1997, Orkwiszewski and Poethig 2000). Work on woody plants also poses a challenge given the lengthy period of each phase and the inability to obtain flowering during the juvenile phase in almost all woody plants. This study sought to focus efforts on *M. excelsa* by addressing the fundamental problem in this species, which is the reversion to a juvenile form following micropropagation. The hypothesis that limiting growth to a single stem successfully promotes and accelerates maturation was supported by this study. The use of a novel technique for capturing the optical and dimensional properties of leaves was successfully employed in this study, and provided a tool for accurately characterising the gradual transition in leaves from a juvenile through to an adult form. However, as acknowledged in this and other studies, the attainment of adult leaf characteristics does not imply reproductive competency.

The spatial and temporal changes in individual morphological and anatomical features occurring in leaves of plants of *M. excelsa* undergoing phase change is in accordance with the model proposed by Hackett and Murray (1997), e.g. that phase change is a phenomenon controlled by a multiple, possibly overlapping, set of switches rather than

by a single master-switch. This was evident by differences in the timing of expression of leaf traits, such as size and shape characteristics and the accumulation of a tomentum on the abaxial leaf surface. Support for the multiple-switch regulatory pathway is also in accordance with similar observations in other woody species, such as *Picea sitchensis* (Steele *et al.* 1989) and *Eucalyptus globulus* (James and Bell 2001). Such a conclusion was also reached for the model species, maize (Bongard-Pierce *et al.* 1996, Orkwiszewski and Poethig 2000) and Arabidopsis (Telfer and Poethig 1998). Thus, the search for reliable markers of phase change has been ongoing, and is possibly complicated by the fact that changes in individual phenotypical traits may be independently regulated. The notion that carbon isotope discrimination may be used as a reliable marker of phase change was not borne out of this study. Rather, this attribute appeared to reflect predominantly the physiological status of the plant following a shoot restriction treatment.

Although vegetative phase change in rejuvenated, single-stemmed plants of *M. excelsa* was promoted by limiting shoots to a single stem, this was also accompanied by a number of physiological changes. However, these physiological changes such as the down-regulation of various photosynthetic parameters and the accumulation of starch in source leaves appeared to be symptomatic of a disruption in source and sink relations. These phenomena were also apparent in branched plants, which may have also resulted from changes in source-sink relations. Thus, the confounding of these physiological and phase change effects along the shoot axis of single-stemmed plants complicates the interpretation of processes that relate to either or both phenomena. There have been relatively few studies examining phase change and its relationship to physiological or biochemical factors (e.g. carbohydrate concentrations) during this process. One study, using tobacco mutants with low expression of Rubisco, suggest that attainment of a critical source strength is important in preventing a delayed vegetative phase change response (Tsai *et al.* 1997). However, Tsai *et al.* (1997) note that other factors, other than carbohydrates, may also be important. A further understanding of the role of genetic factors and their relation to various processes associated with phase change would contribute greatly to our understanding in this field.

The roles played by environmental factors such as temperature, daylength and irradiance are critical for the commencement of the flowering process in *Metrosideros.*

Most studies examining flowering suggest that changes in either temperature and/or photoperiod are critical for flowering in myrtaceous species (Shillo *et al.* 1985, Zieslin and Gottesman 1986, Day *et al.* 1994a), although little attention has been given to the role of irradiance during this process. Clearly, the hypothesis suggesting that modification in temperature and daylength during induction were necessary for the commencement of flowering was upheld by this study. Moreover, irradiance also played a significant role in affecting floral development, although prior to this study its effects in a myrtaceous species had not been fully described over a range of irradiance levels. These results are, therefore, not only scientifically interesting but also important from a commercial perspective, given the necessity for the accurate timing, quantity and quality of flowers as demanded by the consumer. In this study, the optimal conditions recommended for increased flowering yields can be obtained by growing plants under inductive cool (mean 15°C), short-days (10 h) for a minimum of 10-20 weeks using an irradiance of 567 μmol m^{-2} s^{-1}. The timing of anthesis can also be accurately predicted, occurring at approximately 11 weeks after transference to a warm (mean 18°C) greenhouse following a 20 week floral induction period.

Interestingly, the size of the bud at the time of induction appeared to be a crucial factor in determining its competency for undergoing floral initiation and subsequent development. This relationship has been investigated in a few other species (Patten and Wang 1994, Ohana and Weiss 1998). As also demonstrated in field-grown plants of *M. excelsa*, larger buds were more likely to become floral, and may have had more developmentally advanced meristems that were better able to respond to environmental signals than those of smaller buds (Sreekantan *et al.* 2001). Thus, the size of the buds may serve as a reliable marker of their competency to respond to florally inductive conditions. Future research in this area would profit from a further understanding of the physiological factors associated with bud size and flowering.

The hypothesis that the vase life of cut flowers of *M. excelsa* is limited by adverse water relations and by the ethylene-related abscission of whole flower and floral organs was supported by this study. It was not surprising that holding solutions that contained sucrose were effective in extending vase life in *M. excelsa*, considering that similar favourable responses have been described in cut flowers of *M. collina* and various other

species within Myrtaceae (Burge *et al.* 1996, Sun *et al.* 2000, 2001). However, the addition of HQC when applied alone or after a 10% sucrose pulse detrimentally affected flower quality, causing premature wilting and subsequently shortening of vase life. Trials into alternative biocides would be strongly recommended given that holding solutions containing sucrose can provide a favourable medium for bacterial growth (Van Doorn 1997). There has been some suggestion, however, that STS may serve as an alternative but effective biocide (Ohkawa *et al.* 1999). Clearly, STS enhanced water uptake in cut flowers of *M. excelsa*, which may have been through the inhibition of intra-vascular microbial activity.

The potency of different ethylene concentrations on flower quality was clearly demonstrated in this study. Exposure to exogenous ethylene levels above >1.0 $\mu l\ l^{-1}$ detrimentally reduced the vase life in cut cymules of *M. excelsa.* Based on an ethylene-response classification scheme proposed by Woltering (1987) for intact flowers from various ornamental species, the results from this study suggests that cut flowers of *M. excelsa* could be classified as being relatively 'sensitive' to the effects of exogenous ethylene. Inhibition of ethylene-mediated damage, however, was possible provided the cut flowers were treated with appropriate inhibitors of ethylene action. In particular, STS clearly afforded significant protection against ethylene-related abscission of floral organs. However, given the recent concerns of the environmental effects of silver, treatment with 1-MCP may provide an effective and safer alternative to STS, despite a slight reduction in flower petal quality.

In conclusion, the objectives as initially stated in this thesis and specified in the sub-contract with Massey University by the Crop & Food Research Institute were addressed and successfully achieved. The current thesis has, therefore, advanced our biological understanding of vegetative phase change, the environmental control of flowering and the postharvest characteristics of cut flowers in *M. excelsa.* These findings are critical for the establishment of *M. excelsa* within the floricultural industry as an ornamental container plant and cut flower.

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Appendix I: Wax Infiltration Procedure

Day 1
Wash out fixative with 70% ethanol.

Day 2
70% OH TBA (for 2 h) – 300 mls distilled water, 500 mls ethanol (95%), 200 mls TBA

transfer,

85% OH TBA (for 2 h) - 150 mls distilled water, 500 mls ethanol (95%), 350 mls TBA

transfer,

95% OH TBA (for 2 h) - 450 mls ethanol (95%), 550 mls TBA

transfer,

100% OH TBA + Erythron. Dye (for 2 h) – 750 mls TBA, 250 mls absol. ethanol, + pinch of Erythrosine dye.

transfer,

100% TBA overnight.

Day 3
At 9am, renew the 100% TBA solution, again at 1 and 4 pm and leave overnight.

Day 4
9-10am TBA/Paraffinol (50:50)

10 am – midday – ½ fill new containers with wax chips. Pour paraffin mix in with samples. Place in oven at 60°C.

12 pm - replace with pure melted wax

4 pm – replace with pure melted wax
At least 4 changes of pure wax. Leave over two days. Store in oven (60°C).

Day 7
Embed samples & section the next day.

Abbreviations: OH = absolute ethanol, TBA = 2-Methylpropan-2-ol tert-Butanol.

Appendix II: Staining Schedule: Safranin/Fast Green