Ethephon, Ethylene and Abscission Physiology of *Camellia*.

A thesis presented in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy
in
Horticultural Science
at
Massey University, Palmerston North,
New Zealand.

Allan Brian Woolf

1992

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ABSTRACT

Ethylene application to leaves and floral buds of *Camellia* resulted in abscission with a lag period, the duration of which was dependent on ethylene concentration and cultivar. During this period, cellulase activity doubled in leaf abscission zones, and when abscission commenced, activity increased more rapidly. However, no increase in cellulase activity was observed in floral bud abscission zones. Propylene application revealed that autocatalytic ethylene production increased in leaf abscission zones prior to and decreased after abscission. However, in the leaf blade, no change in endogenous ethylene production was measured, nor were any signs of leaf senescence observed. Application of silver thiosulphate (STS) completely inhibited leaf abscission and delayed and reduced floral bud abscission in response to applied ethylene. This pointed to a similar role for ethylene in both organs, but that the abscission process of floral buds occurred at a faster rate than that of leaves. Application of ethylene for differing durations to floral buds and leaves demonstrated that regardless of ethylene treatment duration, abscission ceased less than 24 hr after ethylene removal indicating that continuous ethylene exposure is required to promote abscission of *Camellia* organs.

Measurement of abscission rate (time to 50% abscission) in response to a range of ethylene concentrations determined that floral buds were more sensitive (that is; responded more rapidly to lower ethylene concentrations) than leaves. Ethylene-sensitivity was influenced by organ maturity. As floral buds matured from initiation to flower opening, the rate of ethylene-promoted abscission increased, indicating greater sensitivity. Leaves were most sensitive to ethylene directly after bud break and sensitivity decreased until 12 weeks after cessation of stem extension; after this time, sensitivity did not change significantly over the next 3 years.

Low temperatures reduced the ethylene-promoted abscission rate of both leaves and floral buds with an exponential relationship. Low temperatures increased the ethylene concentration required to saturate the abscission response. Endogenous ethylene production of *Camellia* leaves increased with higher temperatures and peaked at 20^{o} to 25^{o}C.

Since ethylene release from ethephon may be described in terms of concentration and duration of ethylene exposure, the effect of time, temperature, cultivar, organ type and organ maturity on organ abscission response to ethephon application could be explained in terms of the ethylene-promoted response.

The level of ethylene- and ethephon-promoted abscission were explained in terms of the interaction of ethylene concentration and duration of exposure with organ type, organ maturity and temperature which determined the level of abscission response. Three mechanisms were important in determining the response to ethylene; ethylene-sensitivity, and rate of reaction and reversibility of the abscission process. The rate of the abscission process was determined by ethylene concentration, temperature, organ type and maturity. Since abscission was reversible in *Camellia*, the duration of exposure interacted with the abscission rate to determine the extent of abscission in response to ethylene or ethephon application.

In conclusion, the greatly expanded understanding of the ethylene-promoted abscission process carried out in this study facilitates control (promotion or inhibition) of abscission in *Camellia*. This enhances the possibility for culture and transportation of high quality *Camellia* plants from New Zealand.

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ACKNOWLEDGEMENTS

I am indebted to my supervisors, Dr John Clemens and Dr Julie Plummer, who have provided continual encouragement, challenge and guidance during my PhD. I am very grateful for the cheerful giving of time, ideas and energy to train me in the art of scientific research. I could not have hoped for a better team.

My loving thanks also to Louise, who has helped and encouraged me as a friend, fiancee, and wife over the years of this thesis. I am particularly thankful for her forbearance during a wedding, (short) honeymoon, and the many uncertainties and pressures of the writeup.

I am grateful to the Camellia Society of New Zealand for its generous support and particularly Colonel T. Durrant for literature on the genus *Camellia*. Thanks is also due to the Camellia Memorial Trust for scholarships and interest in the work, and to its members, particularly Dr Rod Bieleski.

I wish to thank those who have helped me climb the research learning curve, particularly Dr Art Cameron, and thanks to Dr Edna Pesis and Dr Mike McManus for patient instruction in cellulase extraction and measurement. Thanks also to Dr Ross E. Lill of Crop and Food Research Institute of New Zealand Ltd, Levin (formally Levin Horticultural Research Center, MAF Levin) for providing unpublished results of trials into storage and ethylene response of *Camellia* and to Margaret A. Scott of Efford Experimental Horticulture Station, Lymington, Hampshire, England for information on flowering and culture of *Camellia*. I am also grateful for the use of the Growth Rooms of the former DSIR Climate Laboratories and the help of the staff there; Dr Ian Warrington, Joselyn, Len and particularly the understanding of Nicki Gardener (nee Seager). I am also in debt to Greg A. Lang (Louisiana State University, Dept of Horticulture, Baton Rouge, LA) for his stimulating and helpful discussion of the role of ethylene in *Olea* abscission.

The support of the staff of the Department of Plant Science, Massey University is gratefully acknowledged. Of these Prof. Errol Hewett Bruce Christie, Dr Nigel Banks, Pam, Hugh, Colin, David, Chris and Lois deserve special thanks. I would particularly like to thank Jonathan Dixon for his training and patience with use of all GC's.

I am also indebted to the Nursery Research Centre for use of facilities for the culture of stock plants and help of its great technicians Franz Ripphausen, Lisa Burton, Andrew Rodgers, and Chris Barnaby.

There are always a key group of people who support you through these times, are great friends and are those who have made it all possible in many and varied ways: Craig Mowatt, Fiona Cayzer, Philippa Thorley, Karen Snoad, Shauna Sylvester, Peter Jeffery, Robyn Symcock, Paul Austin and Millie (The Old Battle-axe) Tetlow.

To all of my flatmates of the "Warriors", "Goodies" and the one and only "Pooh Corner" flats for lots of great times and help in many many ways, I am very thankful guys. Many thanks also to Jeremy Compton who helped extensively with obtaining and sorting references. Also thanks to these people who have counted buds, washed bottles, ground up chemicals etc etc etc etc: Tina, Karen, Carolyn, Stuart, Melony, Liz, Elana, Rick, Howie, Lisa and Toni. I have particularly appreciated the support of my parents over the writeup period in Auckland.

I also acknowledge the technical support of my stereo, nutritional support of Griffins Gingernuts, and narcotic support of Bell Tea and Nescafe Classic Coffee.

I am eternally grateful to my Lord who got me into this project, has strengthened me through it and without whose encouragement I would certainly have ditched it for a less stressful, but much less exciting career.

This thesis is dedicated to my secondary school science teachers, Mr Dryburugh, Mr Khan and Mr Milne.

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CHAPTER 1

GENERAL INTRODUCTION.

Camellia is a genus of hardy evergreen garden plants in the family Theaceae with some 200 species and over 25,000 cultivars (Sealy, 1958; Chang, 1984). Most species originate from China but relatively few are cultivated in other countries, *C. japonica* being the best known of these. Whereas in New Zealand *Camellia* are retailed almost exclusively as garden plants, in Europe, the high-value, potted flowering *Camellia* market is expanding (M.A. Scott, pers. com.). This niche market is similar to that filled by potted *Chrysanthemum* in New Zealand, although it commands a higher price bracket. In 1991, the total ornamental live plant export market from New Zealand was estimated to be worth approximately $3,400,000 (Anon, 1991). The annual *Camellia* export of approximately 150,000 plants accounts for about $900,000 (or 25%) of the total export value .

The favourable growing conditions for *Camellia* in New Zealand (Durrant, 1986) have enabled domestic nurseries to supply these overseas markets. Export is carried out in the southern hemisphere autumn by sea- or air-freight. Plants are then grown-on in the northern hemisphere summer and sold as flowering potted-plants in winter, 6 to 8 months later. General research, although much of it 40 to 50 years old, has been carried out into growth and flowering physiology of *Camellia*. Optimum transport temperature and ethylene-sensitivity has been examined briefly (R.E. Lill, pers. com.). However, pre-export and exportation problems, and pressure from customers in importing countries for a high quality product, necessitates improvements in nursery and transportation technology of *Camellia*.

A large *Camellia* exporting nursery, Duncan and Davies Nurseries Ltd, New Plymouth, N.Z., has identified problems in this production and export chain. Production problems include the high cost of preparing plants for transport which involves manual removal of floral buds and old or damaged leaves with the attendant possibility of fungal infection of wounds. Excessive leaf abscission, vegetative bud death, stem dieback and death of whole plants has occurred after

removal from the transportation environment. Damage symptoms are currently unexplained by factors such as transit temperature or disease incidence and appear to relate to specific issues in *Camellia* physiology.

Ethylene physiology was selected as a key area for study in the export of *Camellia* for a number of reasons. Firstly, the fact that *Camellia* plants are exported in the southern hemisphere autumn and arrive in the northern summer means that the natural flowering period is missed. For this reason, and because floral bud abscission in transit may contribute to disease problems, floral buds must be removed manually. This clearly involves a high labour input when it is considered that approximately 170,000 1-year-old plants (30 cm high) with 4 to 10 floral buds/plant, and 3 to 4,000 3- to 4-year-old plants (1.5 m high) with about 200 floral buds/plant were exported in 1990. It was noted in 1987 that no chemical was known which could effectively disbud *Camellia* (George, 1987). Thus development of a chemical technique for thinning floral buds using an abscission-promoting agent such as ethephon (an ethylene-releasing compound; ERC), would eliminate a labour intensive and costly procedure. Other benefits resulting from ethephon-promoted abscission are that mechanical damage and wounding are minimised because abscission occurs in predetermined and naturally healing abscission zones. Secondly, abscission during transportation may be promoted by exposure to endogenously produced ethylene by plant tissue as a result of wounding or rotting, or exogenous ethylene from other sources. Thirdly, abscission of *Camellia* floral buds occurs under unfavourable indoor conditions and ethylene may mediate this response (Lee and Song, 1992). Ethylene has also been implicated in the promotion or mediation of a wide variety of other important physiological processes including abscission induced by mechanical injury (Wittenbach and Bukovac, 1975), transportation vibration (Auer and McConnell, 1984) or water stress (El-Beltagy and Hall, 1974; Morgan *et al.*, 1977). In addition to this, ethylene is involved in senescence of flowers (Maxie *et al.*, 1973) and leaves (Aharoni *et al.*, 1979a) and plant responses to disease (Spanu and Boller, 1989), pest (Wein and Roesingh, 1980) or viral infection (Ross and Williamson, 1951).

Abscission (Latin: *ab*-from, *scindere*-to cut or sunder) is a key area in the culture and transportation of potted ornamental plants (Reid, 1985), of which *Camellia* is no exception. Abscission of floral buds prior to sale is undesirable as these are the key feature determining saleability and the industry requirement is for a minimum of 6 floral buds on a 30 cm plant at point of sale in England. Vegetative

buds are important as these provide shoots for the subsequent season's growth and flowering, and their death results in poor plant structure. Vegetative bud break requires the presence of leaves at the apex of shoots, hence, excessive leaf abscission may result in reduced vegetative bud break (Funke *et al.*, 1938) and stem dieback. Leaves are clearly also required for aesthetic reasons due to their contribution to the overall plant appearance. Aside from a recent report (Lee and Song, 1992), very little work had been carried out on the ethylene-promoted abscission response of *Camellia* organs.

It was proposed that the physiology of ethylene-promoted abscission and sensitivity of *Camellia* to ethylene and ethephon be examined. To understand the physiological process of *Camellia* abscission, study of abscission kinetics, ethylene sensitivity, autocatalytic ethylene production, ethylene inhibitors and enzyme activity were carried out. The interaction of the environment with ethylene and ethephon application and plant factors were also examined including temperature, organ type, organ maturity, cultivar and genera differences. These factors are important due to their interaction with the duration and concentration of ethylene applied, which modify the abscission response. The involvement of the above factors therefore could be examined in relation to the efficient application of ethephon to remove floral buds, and the effect of ethylene exposure under export conditions. Thus a firm foundation of understanding of abscission physiology of *Camellia* can be built which is also applicable to the horticultural industry for production and export of high quality potted plants.

The research carried out was based within the framework of the culture and export of potted *Camellia* plants. It is therefore pertinent to review firstly the transportation of live *Camellia* plants (Section 1.1), secondly, the often fragmented information of the physiology of *Camellia* growth and flowering (Section 1.2), and finally literature relevant to the study of ethylene-promoted abscission (Section 1.3).

1.1 TRANSPORTATION AND STORAGE OF LIVE *CAMELLIA* PLANTS

1.1.1 INTRODUCTION

Currently, little information is available on the whole-plant postharvest physiology of ornamentals. Most of our understanding is gleaned from information from cut flower, fruit and vegetable research (Conover, 1976). However, there is even less information available on the genus *Camellia*, particularly in comparison with common potted plants such as *Ficus benjamina*, on which extensive research has been carried out into the influence of pre- and post-transportation factors on plant quality (e.g. Conover and Poole, 1986).

Many factors influence the survival and postharvest quality of transported live plants. These factors may be grouped into two areas: plant material and handling, and the transportation environment.

1.1.2 PLANT MATERIAL AND HANDLING

The nature of plant material transported will clearly strongly influence quality and even survival in the transportation environment. Plants of almost any size are surface transported from rooted, or even unrooted cuttings (Conover, 1976) to mature plants. *Camellia* plants ranging in size from liners to specimens 1.5 m high may be successfully transported in bare-rooted (Tompkins, 1950) or potted form as carried out by Duncan and Davies Nurseries Ltd. Tissue hardiness is affected by the season during which plants are shipped and may influence the success of transportation (Hunsinger, 1987a). Thus *Ficus* shipped in the winter are less sensitive to low temperature than those shipped in the summer (Conover and Poole, 1986). Genetic background also affects plant response to transportation. Storage of a range of *Rosa* cultivars revealed that differences exist between cultivars in quality loss after dark storage (Halevy and Kofranek, 1976). In the export of *Camellia* plants from New Zealand to the UK, Duncan and Davies Nurseries Ltd has also observed relatively consistent cultivar differences.

Preparation and packaging of plants has a marked influence on the success of live plants transportation. Because removal of media from roots results in considerable stress (Tompkins, 1950; Phongpreecha, 1992), bare-rooted *Camellia* plants are generally inferior to undisturbed plants after transportation

(Amsler, 1950). For this reason *Camellia* plants are currently exported from New Zealand predominantly in plastic pots utilizing a bark medium. Commercial preparation of plants also involves pruning to uniform height, removal of all floral buds and damaged tissue, a fungicidal dip of the aerial plant parts and subsequent air-drying prior to packing.

Packaging is important in the transportation of plants to avoid physical damage (Hunsinger, 1987a). The crate containing the plants should be strong and light-weight, and restrain the plant to minimise physical damage during transportation (Nuccio and Smith, 1958). Current *Camellia* transportation involves tight bulk packaging of 1-year-old plants in shelved wooden crates (0.75 *x* 0.75 *x* 2.5 m). Crates are then pre-cooled (2^{o}C for 24 hr), wrapped in a large plastic sleeve with slits cut to allow some air change (thus reducing relative humidity (RH) below 100%) and placed in transport containers.

However, correct packaging may not eliminate physiological damage if an incorrect transportation environment is utilized (Conover and Poole, 1984). Factors important to correct environment selection are examined below.

1.1.3 THE TRANSPORTATION ENVIRONMENT

Significant genetic variation exists in the response to temperature and duration of transportation (Poole and Conover, 1983; Sterling and Molenaar, 1986; Hunsinger, 1987a). Although there is no record of *Camellia* tissue suffering chilling injury (temperatures between 0^{o}C and 10^{o}C), temperatures below 10^{o}C cause chilling injury in other genera such as *Euphorbia*, *Ficus* and *Hibiscus* (Staby *et al.*, 1978; Buck and Blessington, 1982; Gibbs *et al.*, 1989). These and many other subtropical and tropical genera store better at 10^{o} to 15^{o}C (Conover and Poole, 1986; Gibbs *et al.*, 1989). However, most temperate genera, including *Camellia*, have lower optimal storage temperatures (Poole and Conover, 1983), such as, 1^{o} to 3^{o}C for *Rosa* and 5^{o}C for *Azalea* (Halevy and Kofranek, 1976; Poole and Conover, 1983). Storage of *Camellia* cultivars 'Moshio' and 'Donation' at a range of temperatures (0^{o}, 2^{o}, 5^{o}, 12^{o}C and 5^{o}, 10^{o}, 20^{o}C) (R.E. Lill, pers. com.; Phongpreecha, 1992, respectively) demonstrates that low temperature storage results in greater plant survival. The best storage/transportation temperature was found by Lill (pers. com.) to be between 2^{o} and 5^{o}C, although the optimum may occur between 0^{o} and 2^{o}C.

Freezing temperatures may occur during low temperature transportation of *Camellia* or in the culture of *Camellia* outdoors. Freezing damage symptoms are observed as tissue browning occurring in all plant parts (Sakai and Hakoda, 1979). The floral bud is the least cold hardy tissue of *Camellia* and leaves and vegetative buds are more hardy with damage thresholds of -5°, -10° and -10°C respectively (Sakai and Hakoda, 1979). These temperatures provide some guidelines for the absolute minimum temperatures for transportation and culture of *Camellia*.

Increased duration of transportation reduces plant survival and quality, mostly by determining the period of darkness (Poole and Conover, 1983; Sterling and Molenaar, 1986). Symptoms may include loss of fresh weight, leaf chlorosis/necrosis, decreased leaf chlorophyll content, and leaf or floral bud abscission (Buck and Blessington, 1982; Batson and Blessington, 1983; Gibbs *et al*., 1989). Extended durations of transportation are possible for foliage species, but require more specific temperature selection to maintain high quality (Poole and Conover, 1983; Hunsinger, 1987a).

Greater duration of simulated transportation decreases survival of *Camellia* 'Moshio' and 'Donation' (R.E. Lill, pers. com.; Phongpreecha, 1992). Although short durations have little influence on plant survival (1 to 3 weeks; Phongpreecha, 1992; R.E. Lill, pers. com.), extended storage duration (up to 11 weeks) results in poor plant quality particularly at higher temperatures (R.E. Lill, pers. com.). Whereas plant transportation from USA to England takes only 10 to 15 days (Hunsinger, 1987b), from New Zealand sea-freight takes a minimum of 6 weeks nursery to nursery. Although *Camellia* plants may survive long periods in the dark without cooling (Amsler, 1950), commercial transportation of *Camellia* by Duncan and Davies Nurseries Ltd is carried out at 4°C. Because of the problems associated with long durations of stress in transit, it may be beneficial in some crops to use air-freight rather than surface-freight (Hunsinger, 1987a). This results in reduction of the transit duration to as little as 1 to 2 weeks and has, at times, been found to be cost-effective for *Camellia*.

Ornamental plants are more susceptible to desiccation due to the high surface to volume ratio of plants than many other horticultural crops such as fruit (Reid and Goszczynska, 1985). Water deficits usually reduce plant quality, particularly if plants are stored for protracted periods (Amsler, 1950; Halevy and Kofranek,

1976; Ben-Jaacov *et al.*, 1982). Plant water status may therefore be a limiting factor in the duration of storage of live plants (Poole and Conover, 1979). If RH is too low and excessive water depletion occurs in the growing medium, plant desiccation and resulting leaf abscission may occur (Conover and Poole, 1984; Hunsinger, 1987a). Conversely, high RH can result in increased disease incidence (Shirazi and Cameron, 1992). Thus although little specific research has been carried out on RH and the transportation of live plants (Conover and Poole, 1984), a RH of 80 to 90% is recommended for live plant transportation (Hunsinger, 1987a).

Ethylene is important in plant transportation as accumulation of endogenously produced ethylene in an enclosed space, or accidental exposure to exogenous ethylene, results in deleterious influences including epinasty, leaf chlorosis/necrosis, flower senescence, leaf, leaflet, stipule, floral bud, flower, petal and fruit abscission (Cunningham and Staby, 1975; Harbaugh *et al.*, 1976; Marousky and Harbaugh, 1979c; Reid and Goszczynska, 1985). The commercial exporter, Duncan and Davies Nurseries Ltd, has suggested that exogenous ethylene during the sea-freight of *Camellia* may provide explanations for some of the damage symptoms observed. Increased ethylene concentration and duration of exposure results in a greater abscission response of plants held under simulated transit conditions (Marousky and Harbaugh, 1979c; Dostal *et al.*, 1991). *Camellia* has also been shown to exhibit a similar response to increased ethylene concentration (R.E. Lill, pers. com.).

Endogenous ethylene has been implicated as the cause of a range of damage symptoms observed in the transportation or storage of ornamental crops other than *Camellia* including ornamental olive (*Olea*), *Capsicum*, *Philodendron*, *Kalanchoe*, *Radermachera* and *Impatiens* (Cunningham and Staby, 1975; Kays *et al.*, 1976; Marousky and Harbaugh, 1979c; Marousky and Harbaugh, 1979b; Wang and Dunlap, 1990; Dostal *et al.*, 1991). For example, the rate of endogenous ethylene production of *Capsicum* plants is so high that under simulated transit conditions (confinement of 40 plants in 3.8 liter for 48 h) an ethylene concentration of 3 $\mu l \cdot liter^{-1}$ results, a level high enough to promote abscission (Kays *et al.*, 1976).

Further evidence for a role of endogenously produced ethylene during transportation other than direct measurement is indicated by the fact that inhibitors of ethylene biosynthesis and action can reduce ethylene response

symptoms. Simulated shipment of *Impatiens* at 25°C for only 72 hr results in 65% flower abscission. Abscission is decreased by application of the ethylene action inhibitor silver thiosulphate (STS; Section 1.3.1.5) and an inhibitor of ethylene biosynthesis aminooxyacetic acid (AOA; Section 1.3.5) (Dostal *et al.*, 1991). Similarly, although plants may respond in similar ways to transportation and storage conditions, one major difference between the two systems is that of vibration during transportation. Application of simulated transit vibrations to *Begonia* and *Schefflera* produces leaf necrosis and leaf and stem tip abscission, symptoms typical of ethylene damage. Ethylene involvement is further implicated because STS application decreases abscission in *Begonia* and *Schefflera*, even though ethylene production could not be detected (Auer and McConnell, 1984). Thus since STS application as a foliar spray inhibits flower and leaf abscission in many species (Cameron and Reid, 1983; Reid, 1985), general use of STS is recommended to inhibit any undesirable ethylene-mediated processes (Veen, 1987).

Decreased temperature minimises the production (Kays *et al.*, 1976; Hunsinger, 1987a) and action of ethylene (Marousky and Harbaugh, 1979b). Thus temperature in storage should be kept to a minimum without causing chilling or freezing damage (Meadows and Richardson 1983; Hunsinger, 1987a). A range of ethylene concentrations applied to *Camellia* 'Moshio' at various temperatures for 1 week results in damage symptoms only at the highest temperature/ethylene concentration combination (10 $ul \cdot liter^{-1}$, 12°C). Symptoms include extensive leaf abscission without chlorosis, and abscission of vegetative buds which are near bud break (R.E. Lill, pers. com.). Thus lower temperatures in the transportation environment will decrease the abscission-promoting effects of exogenous ethylene in *Camellia*.

1.1.4 SUMMARY

It is clear that ethylene plays a role in many areas of the culture and transportation of *Camellia* plants and that abscission is also a key area to be examined. Thus an understanding of the physiology of ethylene-promoted abscission and the influence of environmental and plant factors will be beneficial in the production and export of quality *Camellia* plants from New Zealand.

The physiology of ethylene-promoted abscission within the export framework, requires an understanding of the basic physiology of growth and flowering of *Camellia*. A brief review of the vegetative growth and flowering of *Camellia* was therefore undertaken.

1.2 GROWTH AND FLOWERING IN *CAMELLIA*

1.2.1 INTRODUCTION.

The yearly growth and development cycle of *Camellia* has been summarized by Valder (1978) as follows. Increasing temperatures and photoperiod in spring bring about swelling and subsequent stem extension of vegetative buds. Once shoot extension finishes, floral buds are initiated in leaf axils on current season shoots under conditions of relatively high temperatures, long photoperiods and high light intensities. Both floral and vegetative buds develop over the summer months reaching full size in autumn. Floral buds remain in a resting state until flower opening which occurs between autumn and spring, the timing of which depends on environmental conditions, species and cultivar. Vegetative buds remain dormant until increased photoperiod and temperature promotes bud break.

In the subsequent discussion, the yearly growth cycle will be divided into two basic sections (vegetative growth and flowering) and will follow a near-chronological format. A diagram summarizing the growth cycle of *Camellia* and factors influencing the growth and flowering is presented in Figure 1.1.

1.2.2 VEGETATIVE GROWTH

1.2.2.1 Vegetative Bud Break

Vegetative shoots of most cultivars of *Camellia* have 2 to 3 axillary buds near the terminal buds and these all have the capability of developing into shoots. Because apical buds develop more rapidly than axillary buds, terminal buds are normally larger (length of approximately 1 to 1.5 cm) and give rise to more vigorous shoots than do subtending buds (Anon., 1986). Buds further from the apex are often in a state of paradormancy (dormancy controlled by a structure different from the organ in which dormancy occurs; Lang, 1987) and hence bud break may not occur.

Vegetative buds are pointed and slender (bullet-shaped) in comparison to floral buds which tend to be more barrel-shaped, depending on cultivar (Hume, 1955; Anon., 1986). These shape differences, along with dissection, were used as the means of differentiating between floral buds and vegetative buds in all areas of this study.

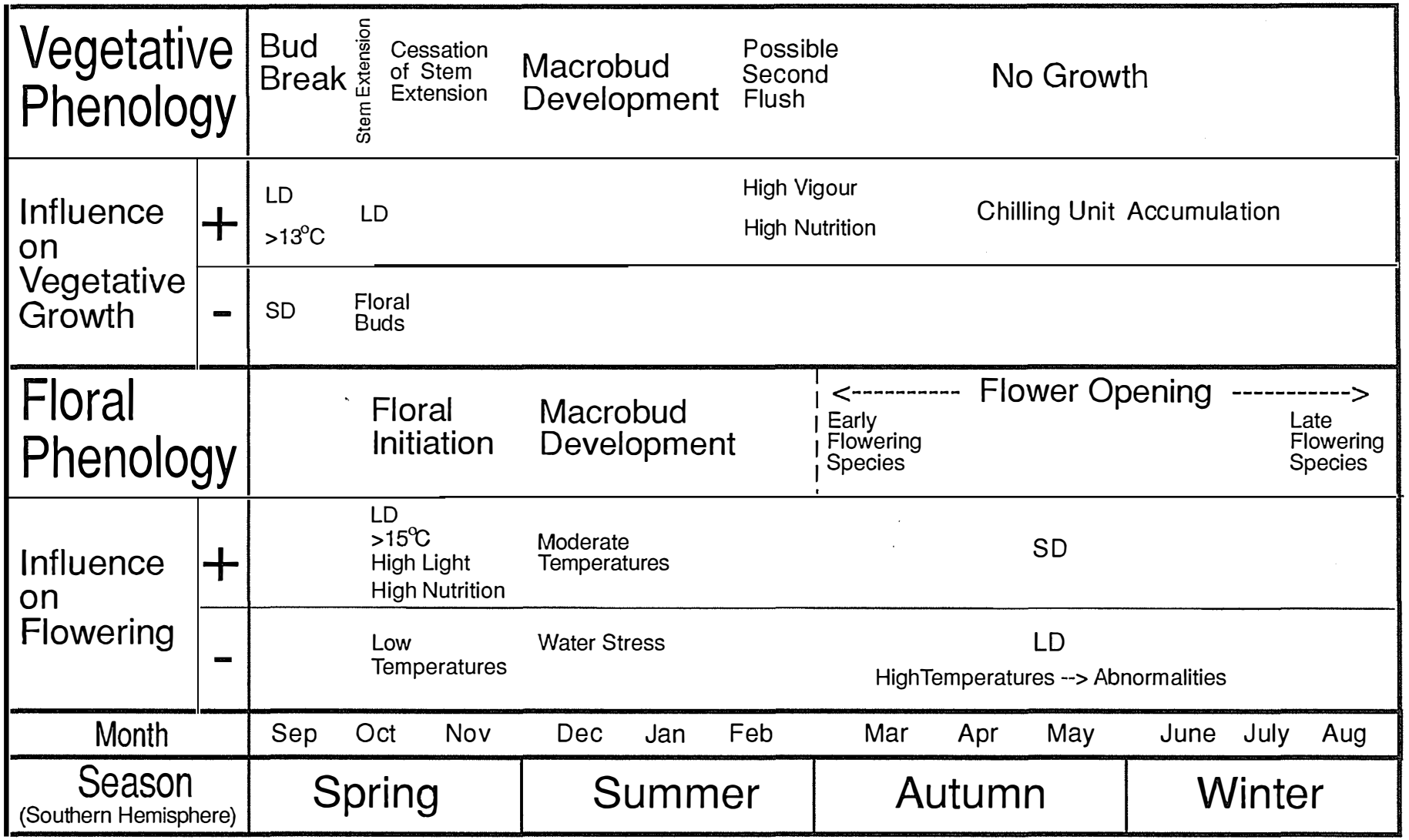


FIGURE 1.1. Yearly growth cycle of *Camellia* and factors influencing vegetative and florall organ development.
Key: SD, short photoperiod; LD, long photoperiod.

In spring or when environmental conditions are favourable, new shoots emerge from apical endodormant vegetative buds (Hume, 1955), that is, from buds that exhibit dormancy arising from perception of an environmental or endogenous signal in the affected structure alone (Lang, 1987). Vegetative bud break involves expansion and abscission of bracts covering the bud, stem extension and subsequent leaf expansion. The period from bud swelling to cessation of shoot growth and leaf expansion is between 6 and 8 weeks depending on species and cultivar (Anon., 1986). Two flushes of vegetative growth can occur in most *Camellia* species, the first in spring and the second in late summer/early autumn (M.A. Scott, pers. com.). Second flushes occur more frequently in situations of high vegetative vigour such as in apical buds and in vigorous (Anon., 1986) or "shy budding" cultivars (Scott, 1977).

Leaves of *Camellia* are borne alternately with 6 to 7 leaves per shoot in most cases, with a minimum of 4 to 5, and maximum of 8 to 9 leaves per shoot (Anon., 1986). Leaves of various ages are borne on the same plant. At vegetative bud break old leaves begin to senesce and abscise, possibly owing to competition for nutrients within the plant (Osborne, 1973; Goldschmidt, 1984). *Camellia* leaves have a maximum lifespan of 3 to 4 years depending on general plant health (Anon., 1986).

Temperature

Vegetative bud break of *Camellia* is strongly influenced by two environmental factors, temperature and photoperiod.

For bud break to occur in dormant vegetative buds of *Camellia*, chilling of 840 hr below 8^{o}C is required prior to the onset of warmer conditions and long photoperiod (Tomita and Uematsu, 1982). After the warm winters of 1948/49 and 1949/50 in USA, a flowering phenomenon known as "clustering" was observed in which many flowers (up to 10 or 12) were borne at the apex of abnormally short shoots. Insufficient winter chilling of vegetative buds was suggested as the reason for inadequate shoot extension (2 to 3 cm compared with 8 to 13 cm in normal shoots) (Hume, 1955). The author has also observed abnormally short internodes but normal node and floral bud number while growing *Camellia* plants continuously out-of-season in high temperatures (15^{o} to 25^{o}C) with natural light and continuous artificial photoperiod.

These results may indicate that chilling of vegetative buds is required for normal bud break once cessation of active growth has occurred. However, it should be noted that constant high temperatures and continuous artificial photoperiods cause continuous vegetative growth (Lammerts, 1958). Also, the author has applied chilling of only 500 hr at 7° ± 2°C to previously unchilled plants and obtained near-normal vegetative bud break under continuous photoperiod and high temperatures (15° to 25°C). It was stated by Lockhart and Bonner (1957) that *Camellia* do not appear to require chilling to break "physiological" dormancy. Thus a possible explanation is that if endodormancy has not been entered (stimulus of lower temperatures and/or shorter photoperiod has not been received), then chilling is not required. Alternatively, chilling may not be required for bud break, but lower temperatures may be required for normal stem extension, possibly through influence on gibberellin levels.

For vegetative bud break to occur, a mean daily temperature of greater than 12.9°C is required (Ee and Uemoto, 1983). Similarly, even under an 8 hr photoperiod (which markedly inhibits bud break) and a day temperature of 21°C, bud break is promoted by a night temperature of 14°C, but not by an 8°C night temperature (Bonner and Lockhart, 1957).

Photoperiod

Short photoperiods inhibit vegetative bud break of *Camellia* such that under a day/night temperature regime of 21°/14°C, a short photoperiod (8 hr) reduces vegetative bud break to result in average shoot lengths of 1.7 cm, compared to 5.6 cm under long (20 hr) photoperiod (Bonner and Lockhart, 1957). Also, vegetative growth of most *Camellia* cultivars does not occur under short photoperiods such as 8 hr (Bonner, 1958; Lammerts, 1958; Valder, 1978). Further, it is suggested that photoperiod rather than temperature is the primary factor controlling vegetative bud break in *Camellia* (Lockhart and Bonner, 1957).

Vegetative bud break occurs in a 3 week period for 82.4% of 68 *Camellia* cultivars (Ee and Uemoto, 1983), suggesting that environmental requirements for bud break vary little between cultivars.

1.2.2.2 Shoot Growth

Shoot growth of *Camellia* is influenced by many environmental, physiological and genetic factors. In general terms, a negative correlation between vegetative growth and flower bud number exists in *Camellia* (Scott, 1977). Cultivars with

many floral buds rarely exhibit a second flush of vegetative growth, whereas cultivars with few floral buds often produce a second flush (Scott, 1977). Similarly, within a plant canopy, shorter or less vigorous shoots have a greater number of floral buds (Anon., 1986), while very vigorous shoots (1.3 m long) which can be stimulated by heavy pruning, produce no floral buds (Durrant, 1986). Since the presence of floral buds inhibits vegetative bud break and growth, floral buds should be removed when competition between floral and vegetative growth is not desired (Anon., 1986). Thus in the export of *Camellia* plants, strong vegetative bud break is required on arrival in the northern hemisphere necessitating floral bud removal to reduce competition.

Vegetative Bud Development

After cessation of stem elongation, vegetative buds develop over the summer and autumn periods reaching maximum size in late autumn. Over the winter period, little change occurs until vegetative bud break in spring.

1.2.3 FLOWERING

Since *Camellia* is grown predominantly for its show of flowers, the area of flowering physiology has of course received much attention. Three areas of flowering will be reviewed briefly in the following discussion; floral initiation, floral development, and flower opening.

1.2.3.1 Floral Initiation

Before reviewing the physiology of flower formation, it should be pointed out that flowering is an exceedingly complex process involving many steps (Zeevaart, 1976). Floral initiation involves the induction and differentiation of floral buds, which occurs many weeks before the difference between vegetative and floral buds becomes visible micro- and macroscopically (Bonner, 1958). However, literature examining flower initiation of *Camellia* does not distinguish between the many steps involved in this process. Thus in this brief review, differentiation between induction, initiation and development to a mature macrobud was not possible.

Floral buds are initiated after shoot stem extension has ceased (Ee and Uemoto, 1983), which occurs approximately 28 days after the start of shoot elongation (McElwee, 1952). Floral initiation occurs at about the same time (within a 3 week period) for most cultivars whether they are early or late flowering cultivars (McElwee, 1952; Ee and Uemoto, 1983).

Floral bud initiation occurs predominantly at the apices of branches and shoots. Buds are typically borne either singly or in pairs, although in some cultivars there may be as many as 3 to 4 at the stem apex. In sub-apical axils rarely are there more than one and never more than two floral buds; where two buds exist in one sub-apical axil, one will be floral and the other vegetative (i.e. sub-apical axils will only occur with; a single vegetative bud, a vegetative bud and floral bud, or a vegetative bud and two floral buds) (Anon., 1986).

Many environmental factors influence floral initiation including temperature, photoperiod, light intensity (Bonner, 1947) and nutrition (Penningsfeld, 1962; Scott, 1977). Scott (1977) described *Camellia* as a facultative long day plant since it is able to initiate and develop floral buds in a wide range of photoperiods (8 to 20 hr). The two major environmental factors influencing floral initiation are the minimum night temperature and photoperiod (Bonner, 1958), temperature being more important than photoperiod (Bonner, 1947; Scott, 1977).

Floral initiation of *Camellia* is promoted strongly by high temperatures. Plants grown under day/night temperatures of 27°/27°C produce over twice as many floral buds as at 27°/15.5°C. No floral buds are produced with lower night temperatures (Bonner, 1947).

As would be expected from examination of the life cycle of *Camellia*, long photoperiods promote flower initiation (Bonner, 1958). There is a photoperiod/temperature interaction such that higher temperatures will further enhance floral initiation under long photoperiods (20 hr; Bonner, 1947). The optimal photoperiod has not been accurately determined, but is between 12 and 16 hr (Bonner, 1958).

1.2.3.2 Floral Bud Development

A period of development of floral parts occurs after vegetative bud break, shoot extension and floral bud initiation has taken place. Floral (and vegetative) buds increase in volume until near-full size is reached by autumn (Hume, 1955).

As found with other facets of *Camellia* growth, environmental factors influence floral bud development. Long photoperiod results in greater floral bud abscission and a larger proportion of abnormally developed flowers (McElwee, 1952). Also, higher levels of nutrition promote early flowering (Penningsfeld, 1962), presumably due to increased rate of bud development. As would be expected, water stress, although it may promote flower initiation, results in slower floral development and increased floral bud abscission (Penningsfeld, 1962).

1.2.3.3 Flower Opening

After the initial developmental stage during summer and early autumn (Section 1.2.3.2), a rest period occurs (coinciding with late autumn to winter) in which relatively little visible change occurs in the floral buds until flower opening. Timing of flower opening of cultivars and species varies significantly, occurring between autumn and spring. Utilizing plants of a range of *Camellia* species and cultivars, a display of flower opening as long as 7 months can be obtained (Ee and Uemoto, 1983).

High temperatures in the winter period can produce abnormal flower opening, such as small size, greenness or pale flower colour and increased floral bud abscission (Bonner, 1947; Ee and Uemoto, 1983). Flower opening is also influenced by photoperiod such that short photoperiod (8 hr) brings about earlier flower opening, whereas, long photoperiod (20 hr) results in a delay in flower opening.

1.2.4 SUMMARY

Vegetative bud break, flower initiation, flower development and flower opening are all influenced by environmental and plant factors. An understanding of the physiology of growth and flowering of *Camellia* allows modification of environmental and plant factors for experimental and commercial purposes.

From an experimental viewpoint, knowledge of the growth and flowering of *Camellia* is beneficial in two ways. Firstly, the literature reviewed elucidates the life cycle and lifespan of *Camellia* organs, important factors in determining sensitivity of natural and ethylene-promoted abscission. Secondly, knowledge of

the growth physiology allows growth modification for experimental purposes, such as artificial promotion of vegetative bud break to produce a range of leaf and floral maturities (Section 4.3).

Ethylene physiology is important commercially in many areas of the culture and transportation of *Camellia*. For example, an understanding of *Camellia* physiology is crucial to successful export because factors involved in the transportation process, e.g. temperature, interact with *Camellia* physiology (such as chilling requirement for vegetative bud break).

Two areas of particular relevance have been examined in the current study. Firstly selective removal of floral buds using ethephon prior to export, and secondly, minimisation of ethylene-promoted abscission during the transportation process. Thus a clear understanding of the ethylene-promoted abscission process and the influence of environment and plant factors on ethephon and ethylene application will result in superior plant quality and more efficient production and export of *Camellia* plants.

1.3 ETHYLENE AND ABSCISSION PHYSIOLOGY

Literature pertinent to the study of ethylene-promoted abscission is herein reviewed.

Sisler and Yang (1987) proposed that control of plant responses by ethylene may be viewed as occurring in three stages: 1) presence of ethylene produced either endogenously or applied exogenously, 2) reception of the ethylene signal and induction of a primary response, and 3) transduction of the "second signal" into mRNA and enzymes synthesis, thus resulting in the physiological response such as abscission.

The mechanism of ethylene action (Stage 2 above) is reviewed here since a clear understanding of the molecular process of ethylene action is important for discussion of possible mechanisms of ethylene-sensitivity in *Camellia* (Section 1.3.1). The third Stage of ethylene action (mRNA and enzyme synthesis and processes involved in abscission) is also reviewed (Section 1.3.2) and since cellulase (ß-1:4-glucanase) activity during abscission was examined in this study, the role of cellulase in the abscission process and its measurement is reviewed (Section 1.3.3). The ethylene biosynthetic pathway is briefly described (Section 1.3.5) since the presence of and process of autocatalytic ethylene production (Section 1.3.6) has a role in both abscission and senescence. Since ethephon was employed in the current research to remove floral buds from *Camellia*, the nature of ethephon is briefly introduced (Section 1.3.7). A key area in this study is the relative sensitivities of organs to ethylene and because confusion exists in the use of this term (Paterson *et al.*, 1990) a definition of sensitivity will be derived (Section 1.3.8). Finally, in the present area of study there is a need to understand the processes involved in application of ethylene at different concentrations and measurement of ethylene production (Stage 1 above) and the assumptions which therefore must be made (Section 1.3.9).

1.3.1 ETHYLENE ACTION

There is debate as to the mode of action of ethylene; whether it acts by binding to a receptor, or via an ethylene metabolite such as ethylene oxide or ethylene glycol (McKeon and Yang, 1987).

1.3.1.1 Ethylene Metabolism

Until recently it had been thought that ethylene metabolism did not occur in higher plants (Abeles, 1973). However, it has become clear that some plants are capable of oxidising ethylene to CO_2 (the process referred to as "OX") and/or incorporating it into tissue (referred to as "TI") (Beyer, 1977; Smith and Hall, 1984b). Metabolism has been found to occur in a wide variety of plant species and tissue types including *Pisum* seedlings (Beyer, 1975a), *Gossypium* leaf abscission zones (Beyer, 1979b), *Vicia* cotyledons (Smith and Hall, 1984b) and cut *Dianthus* (Beyer and Sundin, 1978) and *Ipomoea* flowers (Beyer and Sundin, 1978). Ethylene metabolism in most species is low (Dodds and Hall, 1980) but some species, such as *Medicago* (alfalfa), exhibit extremely rapid metabolism, 30 times that of *Pisum* (Sanders *et al.*, 1989a). The K_m (Michaelis-Menten constant) for OX is $0.9 \cdot 10^{-6}$ M for *Pisum* and $>10^{-9}$ M for *Vicia*, while for TI of *Pisum*, the K_m is $1.6 \cdot 10^{-6}$ M and $4.2 \cdot 10^{-10}$ M for *Vicia* (Smith and Hall, 1984b). The rate of ethylene metabolism also varies between tissues of the same plant. The receptacle of *Dianthus* flowers has a significantly higher TI than petals and peduncle (Beyer, 1977). Similarly, TI of petals is greater than reproductive portions of the flower of *Ipomoea*, and both are greater than that of stem tissue (Beyer and Sundin, 1978).

The final product of ethylene oxidation was thought to be CO_2 (Beyer, 1975a; 1977) but it was later demonstrated that ethylene oxide is a major metabolite of OX (Jerie and Hall, 1978; Sanders *et al.*, 1989a). Ethylene oxide may be further metabolised and retained in the tissue (TI) as ethylene glycol and its glucose conjugate (Blomstrom and Beyer, 1980). However, there are differences in the quantity, or presence, of OX and TI occurring in species and hence differences in the relative amounts of these end-products (Smith and Hall, 1984b).

1.3.1.2 Ethylene Metabolism and Action

Some evidence exists in favour of the role of metabolism in ethylene action. A correlation between the K_m for ethylene metabolism and ethylene levels producing physiological responses has been observed in *Gossypium* leaf abscission zones (Beyer, 1979b) and in seedling (Beyer, 1975c), flower (Beyer, 1977; Beyer and Sundin, 1978) and fruit tissue (Starling *et al.*, 1986). Ethylene OX in *Gossypium* leaf abscission zones correlates with abscission promoted by deblading and does not increase in adjacent petiole tissue as abscission occurs (Beyer, 1979b). The inhibitory effects of Ag^+ on OX and CO_2 on TI also correlates with their influence on ethylene action (Beyer, 1979a).

The above evidence supports a role of ethylene metabolism in ethylene action, however, evidence exists contrary to this hypothesis (Abeles, 1984; Sisler and Yang, 1987). The correlation between ethylene metabolism and action is poor in *Pisum* (Smith and Hall, 1984b) and *Vicia* (Abeles, 1984). Also, there is a low correlation between affinity for propylene and ethylene of *Pisum* and *Vicia* (Smith and Hall, 1984a). Similarly, although a propylene concentration of nearly 100 times that of ethylene is required to reproduce the action of ethylene (Burg and Burg, 1967), propylene is metabolised more rapidly than ethylene (Beyer, 1978). Further, application of products of ethylene metabolism do not result in ethylene-like responses and, conversely, some inorganic compounds that are not metabolised to products similar to those of ethylene metabolism, do produce ethylene-like effects (McKeon and Yang, 1987). Finally, CS_2, a strong inhibitor of ethylene oxidation (>99%) but not ethylene binding (Sanders *et al.*, 1989a), has no effect on ethylene action (Abeles, 1984). These observations cast serious doubt on the physiological role of ethylene metabolism in ethylene action in plant tissue (Dodds and Hall, 1980; Sisler and Goren, 1981).

1.3.1.3 Ethylene Binding

Another possible explanation of the mode of ethylene action is that of binding of ethylene to a receptor, a theory favoured by many researchers (e.g. Bengochea *et al.*, 1980a; Abeles, 1984; Goren *et al.*, 1984; McKeon and Yang, 1987; Sanders *et al.*, 1991).

Ethylene binding has been observed in many plant tissues and species including leaves of *rin* and *nor Lycopersicon* (Sisler, 1982b), *Nicotiana* (Goren *et al.*, 1984; Sisler *et al.*, 1990), *Cucumis* (Goren *et al.*, 1984), *Phaseolus, Citrus* and *Ligustrum* (Goren and Sisler, 1986); cotyledons of *Phaseolus* (Bengochea *et al.*, 1980a); epicotyls of *Medicago* (Sanders *et al.*, 1989a), *Vigna* (Sisler *et al.*, 1990), *Oryza* (rice) (Sanders *et al.*, 1990), and *Pisum* (Sanders *et al.*, 1991); petals of *Dianthus* (Wu *et al.*, 1991b); fruit of *rin* and *nor Lycopersicon* (Sisler, 1982b); roots of *Phaseolus* (Goren and Sisler, 1986); and in cell free preparations of *Phaseolus* cotyledons (Bengochea *et al.*, 1980a), *Vigna* seedlings (Sisler and Isenhour, 1981b) and fruit of *Lycopersicon* (Sisler and Isenhour, 1981a).

Ethylene binding sites have been isolated and incompletely purified from a number of tissues including *Phaseolus* cotyledons (Bengochea *et al.*, 1980b; Thomas *et al.*, 1985) and *Vigna* sprouts (Sisler, 1979). Further to this, an

ethylene binding protein has been purified (Thomas *et al.*, 1985) and a polyclonal antibody raised which was used to probe abscission zones of *Phaseolus* where cross-reacting antigens were found to exist (Connern *et al.*, 1989).

The concentration of binding sites varies between plant species (Sisler, 1990). Variation also exists in the concentration of binding sites within a plant as found in *Oryza* where the concentration ranged from 0.33 pmol·g FWt^{-1} in seeds, to 0.86 and 1.31 pmol·g FWt^{-1} in roots and shoots respectively (Sanders *et al.*, 1990). Similarly, ranking of binding on a fresh weight basis of *Phaseolus* followed from highest to lowest cotyledons, primary leaves, petioles, epicotyls, hypocotyls and roots (Goren and Sisler, 1986). Low concentrations of binding sites have also been found in fruit (Sisler, 1982b).

1.3.1.4 Ethylene Binding and Action

There is reasonably strong evidence supporting the hypothesis that the ethylene binding protein represents a physiological receptor for ethylene. The requirements for a receptor of a growth substance include a high affinity for the growth substance, reversibility, low number of binding sites, specificity for the growth substance and analogues, ability to saturate over a similar range to that of the physiological response obtained (Trewavas and Jones, 1981). Similarly, the dissociation constant should be equal to or less than the concentration required for biological response. After binding, the physiological response should result (Trewavas and Jones, 1981; Starling *et al.*, 1986). Although no direct proof exists that ethylene binding acts as the reception of the ethylene signal (Sisler, 1982b; Goren and Sisler, 1986) ethylene binding fulfills the above criteria for signal reception of a growth substance (Sisler, 1982b; Goren *et al.*, 1984; Goren and Sisler, 1986).

The ethylene receptor is likely to be of proteinaceous nature since it is sensitive to heat and protease activity (Bengochea *et al.*, 1980b). The receptor has been suggested to contain a metal (Burg and Burg, 1967), possibly the copper ion (Beyer, 1976a; Smith and Hall, 1984b; Sisler *et al.*, 1985; Veen, 1986). It is thought that the ethylene binding protein is membrane-bound and, since it is relatively unaffected by enzyme attack, may be buried within the membrane and hence protected by lipids (Dodds and Hall, 1980). Dodds and Hall (1980) suggested that the location of the site of ethylene binding within the membrane of golgi bodies and endoplasmic reticulum would well place it to act as an ethylene receptor since it could control ethylene action by affecting enzyme synthesis and secretion.

If ethylene binding is to represent the reception of the ethylene signal, then the association and dissociation rates must correlate with the physiological response at physiologically active ethylene concentrations (Goren and Sisler, 1986). This has been found to hold true where binding saturates between 0.1 and 0.3 $u l \cdot liter^{-1}$ (in the liquid phase equivalent to 10^{-10} M) (McKeon and Yang, 1987). The binding site affinity for ethylene corresponds well with the concentration of ethylene required for threshold, half maximum and saturation responses (0.01, 0.1 and 1 $u l \cdot liter^{-1}$) (Bengochea *et al.*, 1980b; Goren and Sisler, 1986). It should be noted, however, that in many cases the effect of endogenous ethylene was significantly underestimated, and may have resulted in errors in the determination of ethylene binding (Sanders *et al.*, 1990; 1991).

The discovery of two binding sites with different association rates lead Sanders *et al.* (1991) to propose different physiological roles. In *Pisum* epicotyls the affinities for ethylene of the two binding types were the same (K_D of 6 to $8 \cdot 10^{-11}$ M) and it was suggested that the fast-associating sites represent a functional receptor of the growth response in *Pisum* (rapid response in 6 mins; Warner and Leopold, 1971), while the slower associating binding sites may be the receptor controlling processes with longer induction periods (Sanders *et al.*, 1991) (which could be the case in abscission) or represent a precursor of an ethylene receptor (Sanders *et al.*, 1991).

Further strong support for ethylene binding as the physiological receptor is found in the close parallel between binding site affinity for structural analogues of ethylene and their influence on ethylene response (Dodds and Hall, 1980; McKeon and Yang, 1987). Comparison of K_i values for ethylene analogues and other substances (such as acetylene, propylene, vinyl chloride, carbon monoxide, and 1-butene) indicates a strong correlation with their values for inhibition of *Pisum* epicotyl growth and ethylene binding (Sanders *et al.*, 1991).

Other evidence includes reduced binding activity in an *Arabidopsis* mutant which exhibited ethylene-insensitivity of a variety of ethylene responses including reduction of autocatalytic ethylene production (Bleeker *et al.*, 1988). Also, treatment of tissue with STS reduces ethylene binding in *Dianthus* (Sisler *et al.*, 1986) and the rate of diffusion of ethylene into and out of plant tissue corresponds well to the rate of promotion and reversibility of ethylene action (Goren and Sisler, 1986).

Some results, however, raise doubts as to the role of ethylene binding in ethylene action. Ethylene binding is not inhibited by Ag^+ in *Pisum* epicotyls (Sanders *et al.*, 1991) nor in *Phaseolus* cotyledons *in vitro* (Bengochea *et al.*, 1980b). Also, it would be expected that as tissue senescence progresses with its concurrent increase in sensitivity, ethylene binding would also increase (Wu *et al.*, 1991b). However, contrary to this expectation, increasing senescence results in a decrease in ethylene binding in *Nicotiana* leaves, flowers of *Ipomoea* (Blankenship and Sisler, 1989b), and *Dianthus* petals (Brown *et al.*, 1986). In the examination of *Dianthus* flowers, ethylene binding activity could not consistently explain differences in cultivar sensitivity (Wu *et al.*, 1991b). However, it is well known that a change in sensitivity is not the only factor involved in the response to ethylene because ethylene action is determined by a balance of many factors such as other growth substances (Sexton *et al.*, 1985). Also, lack of inhibition of ethylene binding does not preclude Ag^+ inhibiting transduction of the ethylene signal by producing a conformational change to the receptor such that binding is not inhibited, but the ethylene-receptor complex is rendered inactive (Veen, 1987).

Thus, as noted previously, ethylene binding fulfills many of the requirements of binding of a growth substance to a physiological receptor (Goren and Sisler, 1986) as defined by Trewavas and Jones (1981). Currently, the evidence supporting ethylene binding is greater than that for metabolism, and the former is favoured as the mechanism for ethylene action (Bengochea *et al.*, 1980a; Goren *et al.*, 1984; McKeon and Yang, 1987; Sanders *et al.*, 1991). The current study will follow the assumption that ethylene binding constitutes physiological reception of ethylene, thus, ethylene-binding protein will be viewed as synonymous with the ethylene receptor.

1.3.1.5 Ethylene Inhibitors

Ethylene inhibitors (specifically silver thiosulphate; STS) are important to the horticultural industry to reduce undesirable ethylene responses (Reid, 1985) and the use of STS will be examined subsequently (Section 5.1). However, another use of ethylene inhibitors is in the elucidation of the mechanism of ethylene action (Veen, 1983; Yang, 1987a). Understanding the nature of ethylene inhibitors will help in the development of ethylene action models. Three types of inhibitors of ethylene action exist: CO_2, Ag^+ and 2,5-norbornadiene (2,5-NBD) and analogous cyclic olefins (Yang, 1987a).

The silver ion (Ag^+) has been found to be a powerful inhibitor of ethylene action in plants (Beyer, 1976a). It has been used extensively both horticulturally and in the examination of physiological responses of plants to ethylene. Application of Ag^+ in the form of $AgNO_3$ inhibits the response to ethylene in many systems including abscission of leaves, flowers and fruit (Beyer, 1976a; 1976b), epinasty (Beyer, 1976b), senescence of leaves and flowers (Beyer, 1976b; Goren *et al.*, 1984) and fruit ripening (Saltveit *et al.*, 1978). However, due to low mobility of $AgNO_3$ (Veen and van de Geijn, 1978) and phytotoxic effects (Veen, 1979) the use of the anionic complex of silver, ~~silver thiosulphate~~ STS ($Ag_2S_2O_3$) has become widespread (Sisler *et al.*, 1983). The physiological nature and horticultural use of STS will be reviewed further in Section 5.1.

Silver has been found to inhibit the binding of ethylene in plant tissues such as *Dianthus* petals (Sisler *et al.*, 1985), *Nicotiana* leaves (Goren *et al.*, 1984) and in an extract from *Vigna* seedlings (Sisler, 1982a). However, Ag^+ does not inhibit ethylene binding in cell-free preparations of *Phaseolus* cotyledons (Bengochea *et al.*, 1980b) or *in vivo* in *Pisum* epicotyls (Sanders *et al.*, 1991). Sanders *et al.* (1991) applied Ag^+ while inhibiting endogenous ethylene production with AOA, cobalt, or hypoxia and determined that Ag^+ does not affect ethylene binding directly, but increases the endogenous ethylene production, thus artificially decreasing ethylene binding. They therefore suggested that previous work may have underestimated the quantity of binding sites. The silver ion is thought to act non-competitively with ethylene binding (Goren and Sisler, 1986), although other results indicate a competitive mechanism (Sanders *et al.*, 1989b).

The cyclic olefin 2,5-NBD possesses a strong ability to inhibit ethylene action (Veen, 1987). Many systems of ethylene action have been shown to be inhibited by 2,5-NBD including leaf abscission (Sisler *et al.*, 1985), leaf senescence (Sisler and Pian, 1973), flower senescence (Peiser, 1989; Hyodo *et al.*, 1990) and fruit softening (Blankenship and Sisler, 1989a).

Correlating well with its inhibition of ethylene action is the inhibition of ethylene binding by 2,5-NBD. Reduction, or complete inhibition of ethylene binding has been found in a wide range of tissues and species (Sisler *et al.*, 1985; 1990; Sanders, *et al.*, 1990). Since the effect of 2,5-NBD is reversible by application of ethylene (Sisler *et al.*, 1985), it is suggested that 2,5-NBD acts competitively (Goren and Sisler, 1986; Veen, 1987).

Carbon dioxide is a naturally-produced gaseous product of many biochemical processes within plants. It has been found to inhibit leaf abscission of *Fittonia* (Marousky, 1979) and *Philodendron* (Marousky and Harbaugh, 1979c), fruit abscission of *Prunus cerasus* (Wittenbach and Bukovac, 1973), *Gossypium*, *Carya* and *Hibiscus* (Lipe and Morgan, 1972). Carbon dioxide also inhibits other ethylene responses, including stem elongation (Burg and Burg, 1967), flower senescence (Uota, 1969) and fruit ripening (Arpaia *et al.*, 1985). Commercial horticulture has made extensive use of the ripening inhibiting property of CO_2 (Yang, 1987a). Because its effects are both rapid and reversible (Kao and Yang, 1982), CO_2 is also very useful for examination of physiological processes controlled by ethylene.

Since CO_2 resembles ethylene structurally, reverses the effects of ethylene and produces Lineweaver-Burk plots characteristic of a competitive inhibitor, Burg and Burg (1967) suggested that CO_2 acts as a competitive inhibitor of ethylene by displacing ethylene from the binding site at the receptor. However, CO_2 has been found to have no effect on ethylene binding in a range of tissues and species (Sisler, 1982a; Sisler and Wood, 1988; Sanders *et al.*, 1990; 1991). Low levels of CO_2 (2%) displace 35% of bound ethylene from *Nicotiana* leaves, but high concentrations (10%) do not displace the remaining ethylene (Sisler, 1979). It was therefore suggested by Sisler (1979) that CO_2 acts indirectly with the ethylene receptor. Also, the fact that CO_2 can enhance rather than inhibit ethylene action suggests that CO_2 acts by some mechanism other than binding to the active site of the receptor such as by reducing binding by increasing endogenous ethylene levels (Sanders *et al.*, 1990).

1.3.1.6 Ethylene Action Models

A general model of ethylene action presented by Sisler *et al.* (1983) is presented in Figure 1.2. This shows the theoretical mechanism of ethylene action such that ethylene binding results in the release of a "second message" (or "primary reaction" of Yang (1987a)) a molecule which itself, or possibly through a number of other steps, promotes mRNA transcription from the genome. The new mRNA species result in production of protein molecules, the enzymes which result in the observed symptoms of ethylene action.

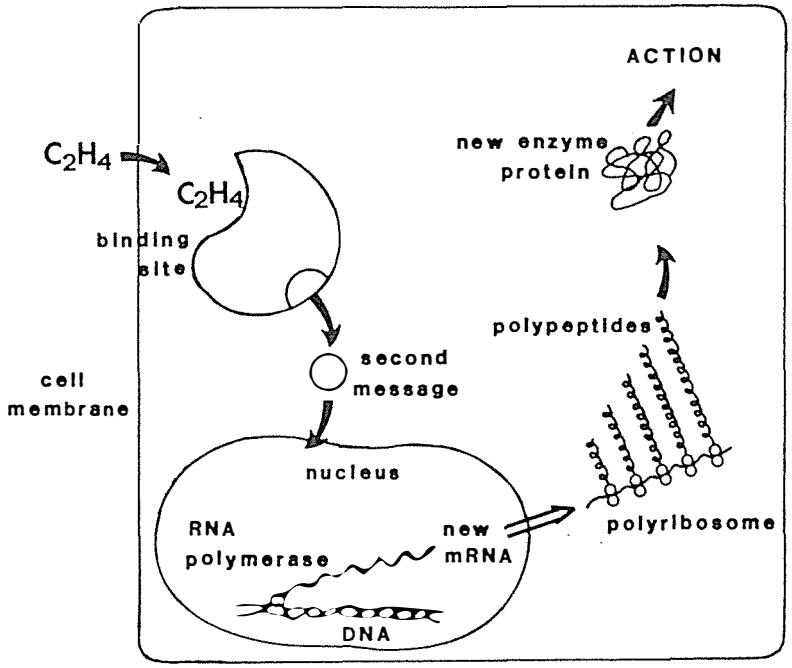


Figure 1.2. Simplified hypothetical model of the mode of action of ethylene in plant cells (Sisler *et al.*, 1983).

A detailed hypothetical model of the nature of the ethylene receptor was presented by Veen (1986) to explain effects of the ethylene inhibitors 2,5-NBD and Ag^+ (Figure 1.3). This author's model proposed a receptor consisting of a regulatory unit (sub-unit A) and one or more sub-units (sub-unit B). Ethylene binding to sub-unit A results in a comformational change allowing sub-unit A to regulate enzymic sub-unit(s) B. 2,5-NBD, binds to sub-unit A at the ethylene binding site, thus acting competitively with ethylene. However, when bound, 2,5-NBD does not produce an allosteric change in the protein, and so inhibits ethylene action. The silver ion, which is of similar structure to copper, competes with copper for the coupling site between sub-units A and B, thus blocking the conformational change of sub-unit B and inhibiting ethylene action.

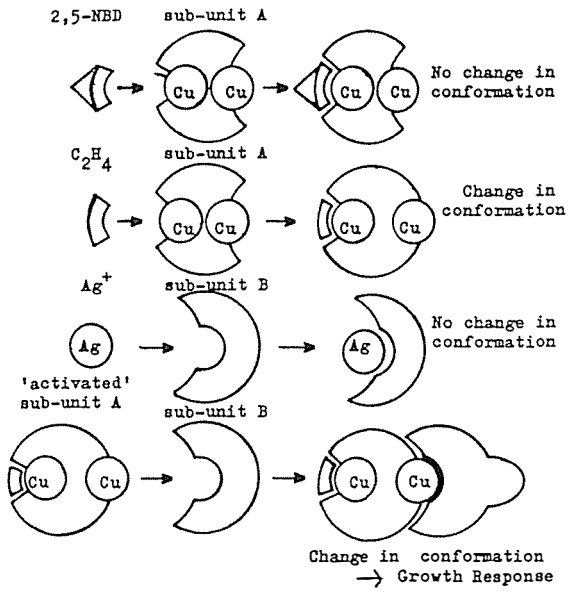


Figure 1.3. Model of mechanism of action of ethylene inhibitors 2,5-NBD and Ag^+ (Veen, 1986).

The action of 2,5-NBD in this model is supported by its role in ethylene binding where it has been shown to consistently inhibit binding (Section 1.3.1.5). This supports the notion of 2,5-NBD acting as a competitive inhibitor at the primary receptor site (McKeon and Yang, 1987; Yang, 1987a). This model also agrees with current knowledge of the action of Ag^+ where, in most cases, ethylene binding is not inhibited whereas ethylene action is inhibited (Section 1.3.1.4 and 1.3.1.5). This would be feasible in this model since ethylene binding (to sub-unit A) is not inhibited by Ag^+, but ethylene action is inhibited by competing with the copper molecule in sub-unit B and so blocks coupling of the sub-units to one another. However, the inhibitory effect of Ag^+ can be overcome by application of high ethylene concentrations (Beyer, 1979a), indicating competitive inhibition (Sanders *et al.*, 1989b). This could be explained in terms of availability of sub-unit B such that as the ethylene concentration is increased, formation of an Ag^+-ethylene complex with dissociated Ag^+ from the sub-unit, could result in gradual release of sub-unit B from inhibition resulting in ethylene action at a reduced rate. These problems lead Sanders *et al.* (1991) to note that there is a requirement for greater understanding of the mechanism of Ag^+ action in blocking ethylene response.

1.3.1.7 Transduction of the Ethylene Response

Once ethylene binds to the receptor, how does it bring about a physiological response? It was noted in 1981 that nothing was known about how the ethylene signal was translated into the biochemical products which bring about the

physiological response (Sisler and Goren, 1981) and this has remained the case till the present (Goren and Sisler, 1986; Yang, 1987b; Sanders *et al.*, 1991; Wu *et al.*, 1991b). Thus the extent of our knowledge is hypothetically described as the process of ethylene binding to the receptor, with a "second message" (possibly a ligand; Reid and Wu, 1992) promoting mRNA synthesis and subsequent response (Sisler *et al.*, 1983).

It has been demonstrated experimentally that ethylene action involves synthesis of new genetic information from the genome in the form of mRNA species (Christoffersen and Laties, 1982; Kelly *et al.*, 1987). However, that new species are always synthesized is not yet proven (Sisler and Yang, 1987). The area of induction of enzyme synthesis during abscission will be further examined in Section 1.3.2.

1.3.1.8 The Role of Ethylene Binding in Maturity, Environmental and Hormonal Effects of Ethylene Action

If the above model of ethylene action involving binding to a receptor and subsequent response is accepted, then there are further implications for how both hormone and receptor (ethylene binding) concentrations may control hormone action. Since the hormone-receptor complex is the active state then action may be controlled by changing either the level of hormone or receptor, or by changing the receptor binding affinity (Dodds and Hall, 1980; Sisler and Goren, 1981; Yang, 1985). Much emphasis has been placed on determining changes in the level of hormone, such as correlating ethylene emanation with abscission (e.g. Jackson and Osborne, 1970) or ripening (Burg and Burg, 1965). However, it is clear that a change in the level of the receptor is also of importance in determining plant response and the assumption that receptor concentration does not vary significantly has often been made erroneously (Dodds and Hall, 1980; Trewavas and Jones, 1981; Trewavas, 1982; 1983).

Ethylene sensitivity varies considerably as tissues develop and the influence of maturity on sensitivity is an important area in the current study. Therefore it is of interest to review the possibility that changes in sensitivity with maturity may be related to ethylene binding site activity.

Sensitivity to growth substance varies during plant ontogeny (Trewavas and Jones, 1981). The correlation between an increase in ethylene production and ripening for example, is often poor and it is thus suggested that increased

sensitivity is a better explanation for control of ripening (Trewavas and Jones, 1981; Trewavas, 1981). Increased sensitivity has been suggested to occur during fruit ripening (McGlasson *et al.*, 1978; Trewavas, 1981; Trewavas, 1982; McGlasson, 1985; Yang, 1985) since, as fruit development progresses, the ethylene concentration required to bring about ripening decreases (McGlasson *et al.*, 1978); or, stated another way, the "resistance to ripening" decreases (Yang, 1985). Similarly, in the flower senescence process, as *Dianthus* petals age, the ethylene concentration required to promote senescence decreases (Lawton *et al.*, 1990). Changes in sensitivity during abscission are well-known (Addicott, 1982) and although there are other possible explanations (e.g. control by auxin concentration), this may reflect increased sensitivity (Abeles, 1967).

Although the molecular basis for sensitivity is not understood, a possible explanation is that sensitivity varies as a result of changes in the presence or activity of ethylene receptors (Trewavas, 1982). This has been suggested to be a possible explanation for the greater abscission-sensitivity with age (Dodds and Hall, 1980). Inhibition of the synthesis of ethylene binding protein with cycloheximide in *Citrus* leaves suggests that on a short-term basis, ethylene action is not regulated by binding site level (Goren and Sisler, 1986). The level of ethylene binding sites changes during development of a range of tissues, including cotyledons of *Phaseolus* (Jerie *et al.*, 1979), *Nicotiana* leaves (Goren *et al.*, 1984) and *Dianthus* flowers (Brown *et al.*, 1986; Wu *et al.*, 1991b). However, it remains to be conclusively proven that ethylene binding to a receptor results in, and is correlated with, the ethylene response. Evidence for and against the role of ethylene binding in senescence is briefly examined below. However, ethylene response may be controlled by the effect of hormones or other factors such as environment on the binding site/receptor activity or concentration (Trewavas, 1983; Goren and Sisler, 1986).

Evidence supporting a change in sensitivity reflected by an increase in binding capacity appears inconclusive. Whitehead and Bosse (1991) noted that the peak of ethylene binding occurred before the rise in autocatalytic ethylene in flower petal (Brown *et al.*, 1986) and leaf tissue (Goren *et al.*, 1984). However, a clear lack of correlation of sensitivity and binding has been found in a number of senescence systems. In flowers of *Ipomoea*, in the period where ethylene sensitivity increases markedly, ethylene binding did not change significantly (Blankenship and Sisler, 1989b). After examining the senescence of *Dianthus* petals, it was concluded that changes in binding site number or their affinity could

not account for the pattern of senescence (Brown *et al.*, 1986). Similarly, Wu *et al.* (1991b) examined the correlation of relative sensitivities of *Dianthus* cultivars with ethylene binding, and found that these differences can be only partially explained by binding activity differences.

As senescence progresses, there is a decrease in the level of ethylene binding of *Nicotiana* leaves and *Dianthus* petals (Goren *et al.*, 1984; Brown *et al.*, 1986), and a decrease in binding affinity of *Dianthus* petals (Brown *et al.*, 1986). Since binding is thought to be associated with the membrane (Sisler, 1980), the decrease in binding during senescence may reflect changes in fluidity of the cell membranes during senescence. Thus decreased binding during leaf senescence is more likely a result of senescence itself than a causative factor (Brown *et al.*, 1986).

Other less direct evidence for a role of ethylene binding in ethylene action exists. Application of short-chain saturated fatty acids to *Petunia* flowers (Whitehead and Halevy, 1989) and *Musa* fruit (Whitehead and Bosse, 1991) results in increased ethylene sensitivity. This is suggested to be a result of changes to membrane characteristics resulting in increased ethylene binding and greater sensitivity (Whitehead and Bosse, 1991).

Growth substances other than ethylene have been found to have no direct effect on ethylene binding. This includes application of auxin, cytokinins, ABA and gibberellin *in vitro* (Sisler, 1982a) and auxin *in vivo* (Goren and Sisler, 1986). However, it is suggested that auxin may influence binding site concentration in the long term (Goren and Sisler, 1986).

It is of interest to note that different environmental conditions may also influence ethylene binding characteristics of plant tissue. Leaves of *Phaseolus* grown in a constant environment were less succulent and bound less ethylene than those grown under laboratory conditions (Goren and Sisler, 1986). No explanation for these differences were given, but these results may point to a possible mechanism for control of growth by the environment and may warrant further work.

1.3.2 ETHYLENE AND ABSCISSION

This section examines the final stage in the ethylene-promoted abscission process subsequent to the presence of ethylene and reception of the ethylene signal. Thus, the abscission process itself, the involvement of auxin in the absission process, the specificity of the abscission zone, gene expression, and the role of hydrolytic enzymes is reviewed (Section 1.3.2.1). Since cellulase is a major portion of this study, it is reviewed in more detail in the following section (Section 1.3.3). The role of other growth substances are examined in Section 1.3.4.

1.3.2.1 The Abscission Process

Abscission occurs in a wide range of plant tissues and species (Addicott, 1982) and ethylene is a powerful promoter of this process in many different abscission systems (Sexton *et al.*, 1985). The abscission process involves a highly coordinated sequence of biochemical events resulting in cell wall breakdown. This process, under natural conditions, occurs in predictable positions in a thin layer of cells known as the abscission zone (Sexton and Roberts, 1982). Cell separation is central to abscission and involves synthesis and secretion of enzymes which degrade the cellulose "exoskeleton" and pectin "cement" of the abscission zone cells (Reid, 1985).

During the lag phase (period before break strength decreases) of the abscission process, although no decrease in break strength or abscission is detectable, many processes are occurring which are herein examined. It is therefore pertinent to note that the term lag phase should not be confused with a state of inactivity (Sexton and Roberts, 1982).

The leaf abscission process has been described as occurring in two stages (Chatterjee and Leopold, 1965; Jackson *et al.*, 1973; Addicott, 1982) involving action in the leaf blade and in the abscission zone itself (Goren, 1983). In Stage I, the auxin concentration distal to the abscission zone decreases. This may be promoted by leaf senescence (Mattoo and Aharoni, 1988), removal of the leaf blade (Osborne, 1989a) or by exogenous ethylene application (Goren and Riov, 1989). The interaction of auxin and ethylene in the abscission process are examined in greater detail subsequently (Section 1.3.4.1). Ethylene application therefore has the capacity to decrease the duration of Stage I (Abeles *et al.*,

1971). Once the balance between ethylene promotion and auxin inhibition of abscission is tipped in favour of ethylene, Stage II of the abscission process is entered (Osborne, 1989a).

Although the tissue distal to the abscission zone is important since it supplies growth substances which inhibit the abscission process (particularly auxins), the abscission zone itself is a distinct tissue requiring special consideration since ethylene must be present at the abscission zone for abscission to occur (Beyer, 1975b). The location at which abscission occurs, the abscission zone, contains cells which are often differentiated early in the organ development and are genetically determined such that they have the specific competence to separate, therefore resulting in abscission (Osborne, 1989a; Ramina *et al.*, 1989). Cells of the abscission zone may, even prior to cell separation, be identified from surrounding tissue by a number of characteristics including cell morphology, and cytological and biochemical differences. During the process of cell separation even more distinct changes occur of a physical (Webster, 1968; Huberman *et al.*, 1983), biochemical (McManus and Osborne, 1989; Tucker *et al.*, 1991) and ultrastructural (Gilliland *et al.*, 1976; Baird *et al.*, 1984) nature in both cell walls and cytoplasm which are not found in non-zone cells. This lead Osborne (1989a) to propose a target cell concept: cells in the abscission zone are classified as Type II ethylene-responsive target cells, while adjacent cells that do not respond to ethylene are classified as Type I.

Abscission zone specificity is most strongly indicated by the changes, particularly biochemical, occurring in the abscission zone as abscission proceeds (Reid, 1985). In Stage II of the abscission process where auxin concentration distal to the abscission zone has decreased, ethylene must reach ethylene receptor sites in the specific cells of the abscission zone and initiate processes, such as gene expression, to bring about cell separation (Osborne and McManus, 1984). The reception and transduction of the ethylene signal has been reviewed above (Section 1.3.1) and the results of transduction of the ethylene signal in the abscission zone as ethylene-promoted abscission occurs will be examined herein.

It has been demonstrated that ethylene results in gene expression in ethylene response systems other than abscission, including; root tissue (Christoffersen and Laties 1982), fruit ripening (Christoffersen *et al.*, 1989) and flower senescence (Lawton *et al.*, 1990; Woodson, 1991). Specific to abscission, gene

expression (mRNA) has been examined and found to occur in leaf abscission zones of *Phaseolus* (Abeles and Holm, 1966; Kelly *et al.*, 1987; Tucker *et al.*, 1988), *Lycopersicon* and *Sambucus* (Roberts *et al.*, 1989). Thus Osborne (1989a) stated that there is little doubt that gene expression is involved in the cell separation of abscission. This mRNA translation and protein synthesis (Abeles and Holm, 1966; Pandita and Jindal, 1991) which is localized to the abscission zone (Osborne, 1968) results in *de novo* synthesis of glucanhydrolases, e.g. cellulase (Lewis and Varner, 1970; Durbin *et al.*, 1981) or synthesis/activation of other hydrolytic enzymes such as polygalacturonase (Riov, 1974; Taylor *et al.*, 1990; Lee and Song, 1992), chitinases (Gomez Lim *et al.*, 1987), peroxidases (Gaspar *et al.*, 1978; Pandita and Jindal, 1991) and pectinases (Henry *et al.*, 1974; Huberman and Goren, 1979). Concurrent with increased mRNA and protein synthesis is an increase in respiration rate during either natural or ethylene-promoted abscission (Reid, 1985). Once synthesized, hydrolytic enzymes (such as cellulase) must be transported over the cell membrane to promote cell wall dissolution and thus, abscission. The pathways and sorting signals by which cell wall hydrolases are delivered are not well understood in plants (Morre, 1989). However, it is clear that cell wall hydrolysing enzymes are trafficked over the plasmamembrane and released into the apoplast (Osborne, 1989a), a step, which like enzyme synthesis, is promoted by ethylene (Abeles and Leather, 1971).

Once released from the cytoplasm, enzymes degrade the middle lamella (Sexton and Hall, 1974; Ramina *et al.*, 1989) and cell swelling occurs to provide force to assist cell separation (Wright and Osborne, 1974). The process of cell wall degradation and physical separation of cells does not occur uniformly over the abscission zone plane but progresses from a point source outwards (Durbin *et al.*, 1981; Sexton and Roberts, 1982). Cell separation in the abscission zone is reflected by a relatively linear decrease in break strength with time (Abeles *et al.*, 1971; Sexton and Roberts, 1982) which continues to decrease until organ abscission occurs.

1.3.2.2 Organ Type and the Abscission Process

To examine differences in organ sensitivity in the ethylene-promoted abscission response of *Camellia*, it is important to examine whether the abscission process (morphological, biochemical and hormonal factors) differs between organs of the same plant. Few careful comparisons have been carried out. However, it has been stated that no significant differences have been found between leaf

abscission and abscission of floral buds, flower parts and fruits (Polito and Lavee, 1980; Morgan, 1984). This is also supported by implication since many reviews of abscission do not differentiate between the abscission process of vegetative and reproductive organs (Sexton and Roberts, 1982; Goren, 1983; Reid, 1985; Sexton *et al.*, 1985).

Fruit and Leaf Abscission

That the physiological systems involved in fruit and leaf abscission are similar is supported by the proposed use of leaf abscission as a model abscission system for examination of *Citrus* fruit abscission (Monselise and Goren, 1978). Also, the abscission response pattern to ethylene concentration and ethylene exposure duration of *Olea* fruit and leaves is very similar (Lang and Martin, 1989). The anatomy of the pedicel-peduncle abscission zone of *Olea* fruit has been shown to be nearly identical to that found in leaves (Polito and Lavee, 1980). The biochemistry of hydrolytic enzymes also appears to be similar in organs of the same species. For example, in *Citrus*, which has been examined extensively, cellulase activity increases and correlates with abscission of fruit (Pollard and Biggs, 1970; Goren and Huberman, 1976; Greenberg *et al.*, 1975; Huberman and Goren, 1979) and leaves (Ratner *et al.*, 1969; Huberman *et al.*, 1975). The basipetal movement of auxin from the organ distal to the abscission zone inhibits the promotion of organ abscission (Osborne, 1989a), which has been demonstrated to control leaf abscission (Beyer, 1975b). Similarly, inhibition of auxin transport by ethylene, auxin inhibitors or embryoctomy promote *Prunus* fruit abscission (Ramina *et al.*, 1986). In *Citrus*, leaves exhibit a typical two stage ethylene response while mature fruit show a level of senescence such that ethylene-promoted abscission commences without a lag phase (Ismail, 1970). Since such a response does not occur in immature fruit (Ismail, 1970), the requirement for a level of senescence indicates a role for auxin in fruit abscission. Similarly, the promotion of fruit abscission by mechanical damage to the seed of *Prunus* is suggested to act by destruction of abscission inhibitors (Wittenbach and Bukovac, 1975).

Floral Organs and Leaf Abscission

Although floral organ abscission has been examined less than fruit, it is suggested that, as found in fruit, the leaf and floral bud abscission zones are similar anatomically and in their response to ethylene (Burdon and Sexton, 1989). Strong similarities exist between the concentration-dependent abscission responses of leaves and floral organs of a variety of species (Woltering, 1987),

thus indicating a homologous response. Auxin plays a similar role in floral bud abscission to that in leaves. Application of ethylene or removal of the floral bud accelerates, while IAA inhibits floral bud abscission in *Begonia*. Application of IAA to the pedicel after bud removal substitutes for the presence of the bud (Hanisch ten Cate and Bruinsma, 1973a), a similar result to that obtained with deblading of leaves (Beyer, 1975b). As found for leaves, IAA prolongs the duration of stage I (Hanisch ten Cate and Bruinsma, 1973b). Similar roles of ethylene and auxin in abscission of floral organs of *Glycine* and *Crocosmia x crocosmiiflora* have been proposed (Oberholster *et al.*, 1991; McKenzie and Lovell, 1992).

Exceptions

However, some specific differences in the abscission process of organs do exist. In *Prunus*, differences are evident between the leaf and fruit/peduncle abscission zones. The fruit abscission zone is predifferentiated and exhibits exopolygalacturonase and endocellulase activity where that of the leaf is not predifferentiated and cell wall degradation involves activity of endocellulase and endopolygalacturonase (Rascio *et al.*, 1985; 1987). These differences are also reflected in the nature of the separation process such that only middle lamellae are degraded in fruit abscission zones, while in leaves, cell wall degradation involves digestion of the entire parietal area (Ramina *et al.*, 1987; 1989). The difference in ethylene-sensitivity of fruit and leaves of *Olea* is suggested to be a result of differences in genetic programming of lifespan, the biochemical mechanism of which requires further examination (Lang and Martin, 1985).

1.3.3 THE ROLE OF CELLULASE IN ABSCISSION

Cellulase activity during the abscission process was examined in the current study. The primacy of cellulase in abscission over other enzymes such as pectinases and polygalacturonase has been debated (Goren *et al.*, 1973; Sexton *et al.*, 1980; Osborne, 1989a) so the role of cellulase in the abscission zone will be reviewed here in greater detail.

A dramatic increase in cellulase activity over the abscission process of *Phaseolus* leaves was first reported by Horton and Osborne (1967) and was found to fit the later proposed criteria for involvement of enzymes in the abscission process. According to these criteria, the enzyme should 1) be limited to the separation layer; 2) only be detectable after a period of induction; and 3)

the appearance and quantity of the enzyme should be promoted by ethylene (Abeles, 1968). These criteria were applied to cellulase and found to hold true in many cases (Abeles, 1969; Ratner *et al.*, 1969; Pollard and Biggs, 1970; Rasmussen, 1973; Greenberg *et al.*, 1975; Sexton *et al.*, 1984).

However, inconsistencies have been observed. For example, mature stem-peduncle of *Citrus* fruit abscission zones has been found to lose the ability to abscise in response to ethylene although cellulase activity increases (Huberman and Goren, 1979). This was later explained by increased lignification of outer bark which renders this tissue resistant to cellulase activity (Huberman *et al.*, 1983). Further problems exist which are less easily explained. In pedicel abscission of *Begonia* flower buds, cellulase activity increases subsequent to increased abscission, thus suggesting that cellulase is not causal to abscission (Hanisch ten Cate *et al.*, 1975). Other aberrations include detectable levels of cellulase prior to abscission (Wright and Osborne, 1974) and increases in activity in petiole tissue adjacent to the abscission zone (Lewis and Varner, 1970).

The latter problems in the theory of cellulase involvement in abscission may be explained by the existence of a variety of forms of cellulase (Durbin *et al.*, 1981). As found in the ripening of fruit tissue (Kanellis and Kalaitzis, 1992), a number of enzyme forms of cellulase exist in abscission zone tissue. Different forms of cellulase have been distinguished on the basis of solubility (Lewis and Varner, 1970). There was, however, confusion as to the role of these forms in the abscission process (Durbin and Lewis, 1988). The use of isoelectric focusing later identified two forms of cellulase with isoelectric points of 4.5 and 9.5 (Lewis *et al.*, 1974). With the use of immunological methods and purification of 9.5 cellulase further examination of the role of these isoenzymes was possible (Koehler *et al.*, 1981). It was demonstrated that the isoenzyme 9.5 cellulase was highly correlated with the kinetics of abscission (Sexton *et al.*, 1980) and was thus described as the "abscission cellulase" (del Campillo *et al.*, 1988). The isoenzyme 4.5 cellulase, which is not a precursor of 9.5 cellulase, does not correlate with abscission, but rather declines during abscission (Durbin *et al.*, 1981; del Campillo *et al.*, 1988).

Thus the 9.5 cellulase isoenzyme can be examined with reference to the criteria of Abeles (1968). Firstly, the 9.5 cellulase is limited distinctively to the separation layer (Sexton *et al.*, 1980; Sexton *et al.*, 1981; del Campillo *et al.*, 1988; 1990), while 4.5 cellulase occurs throughout the plant, particularly in young, rapidly

expanding tissue (Durbin *et al.*, 1981; del Campillo *et al.*, 1988). Secondly, if activity of 9.5 cellulase is distinguished from total cellulase activity (the latter determined by viscometry), then 9.5 cellulase is virtually undetectable prior to abscission and the increase in cellulase activity with abscission can be attributed solely to the activity of 9.5 cellulase (Sexton *et al.*, 1980; Durbin *et al.*, 1981; Koehler *et al.*, 1981). Further to this, mRNA species associated with 9.5 cellulase have also been found to be localized in the abscission zone tissue (Tucker *et al.*, 1991). The final criterion, that ethylene should promote quantitatively the level of cellulase during abscission (Abeles, 1968), has also been verified for 9.5 cellulase activity (Sexton *et al.*, 1980; 1981; Durbin *et al.*, 1981; del Campillo *et al.*, 1990) and its associated mRNA species (Tucker *et al.*, 1988).

There are further results supporting the role of cellulase in the abscission process. Firstly, in addition to the correlation of abscission with cellulase (Osborne, 1968; Lewis and Varner, 1970; Wright and Osborne, 1974; Greenberg *et al.*, 1975; Sexton *et al.*, 1980), there has also been a distinct correlation of 9.5 cellulase with break strength decrease (Sexton *et al.*, 1980; Durbin *et al.*, 1981). Secondly, Sexton *et al.* (1980) injected antiserum of the isoenzyme 9.5 cellulase into *Phaseolus* abscission zones and found that 90% of treated tissue failed to break with 200g force, while the break strength of controls was 49g. Also, recoverable cellulase was 24% of that of non-treated controls.

Thus, although there remain some unexplained aberrations (e.g. Hanisch ten Cate *et al.*, 1975), the fulfillment of the criteria of Abeles (1968) and the above results point to a central role of the isoenzyme 9.5 cellulase in the cell wall hydrolysis and subsequent abscission process (Durbin *et al.*, 1981; Osborne and McManus, 1984; Osborne, 1989a).

Measurement of cellulase activity is controversial and somewhat unconventional techniques must be used (Sharrock, 1988). A simple technique is the measurement of decrease in viscosity of a mixture of carboxymethylcellulose (CMC) and tissue extract, one that has been used by many researchers (Ratner *et al.*, 1969; Pollard and Biggs, 1970; Rasmussen, 1973; Huberman *et al.*, 1975; Sexton *et al.*, 1980; 1984; Abeles and Takeda, 1990). The basis for this technique is that cleaving of the long chains of CMC results in decreased viscosity. Because the influence of exocellulases on chain length is negligible in comparison to that of endocellulases which cleave molecules internally, the viscosity technique gives an approximation of endocellulase activity (Sharrock, 1988).

1.3.4 ABSCISSION AND OTHER GROWTH SUBSTANCES

1.3.4.1 The Role of Auxin in Abscission

As previously discussed, auxin plays an important role in the control of abscission where its inhibitory action operates in balance with the promotion of abscission by ethylene (Section 1.3.2.1). The role of auxins in abscission has been studied extensively and reviewed by many (Goren, 1981; Mattoo and Aharoni, 1988; Osborne, 1989a).

The interaction of ethylene with IAA and the control of abscission seems to be more complex than is usually believed (Goren and Riov, 1989) and no one mechanism fully explains the inhibition of auxin transport by ethylene application (Plummer, 1987). In the leaf blade (the first site of ethylene action), ethylene has been demonstrated to increase conjugation (Ernest and Valdovinos, 1971; Riov and Goren, 1979; Riov *et al.*, 1986), reduce synthesis (Ernest and Valdovinos, 1971) and also reduce basipetal auxin transport from the leaf blade to the petiole (Beyer, 1975b; Riov and Goren, 1979). Auxin conjugation has been observed in some cases (Riov and Goren, 1979; Goren, 1983), yet whether or not IAA conjugation is involved in the reduction of IAA level in ethylene-treated leaves is still unclear (Goren and Riov, 1989).

The second important site of ethylene action is in the stem or petiole tissue where auxin concentration is reduced at the abscission zone (Goren and Riov, 1989). Auxin metabolism is increased by ethylene in petiole (Goren, 1983) and stem tissue (Beyer and Morgan, 1969; 1970a). A most important action of ethylene is the reduction of auxin transport demonstrated in petioles (Osborne and Mullins, 1969; Beyer and Morgan, 1971; Beyer, 1973) and stems (Beyer and Morgan, 1970a; Ernest and Valdovinos, 1971). There is a high correlation between the concentration-dependent response of abscission and inhibition of auxin transport (Beyer and Morgan, 1971). Auxin conjugation in the abscission zone may be either promoted (Riov *et al.*, 1986) or not influenced by ethylene (Goren, 1983). It was therefore suggested that no clear conclusions can be drawn on the role of ethylene in auxin conjugation (Goren and Riov, 1989). Also, although ethylene increases auxin degradation and conjugation in *Citrus*, the auxin concentration at the abscission zone may not decrease (Goren and Riov, 1989). This emphasises problems with the current understanding of interaction of auxin and ethylene in the abscission process.

1.3.4.2 The Role of Gibberellins, Cytokinins and ABA in Abscission

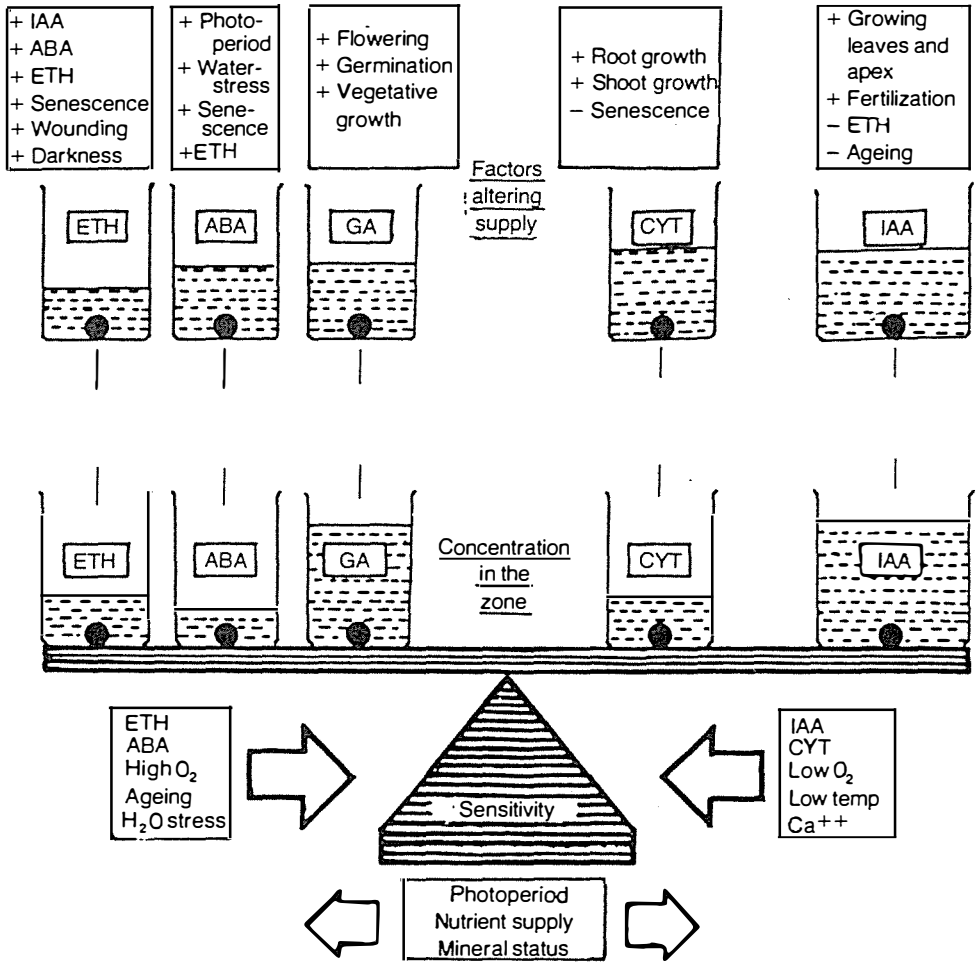
It is important to note that although this study examines the role of exogenous ethylene in abscission, the balance and interactions of other growth substances other than ethylene and auxin, such as gibberellins, cytokinins and ABA, may be involved in the ethylene-promoted abscission process (Sexton and Roberts, 1982; Sexton *et al.*, 1985; Plummer, 1987).

These interactions are illustrated in Figure 1.4 taken from Sexton *et al.* (1985). Generally, abscission is promoted by gibberellins and ABA, while cytokinins inhibit abscission. The level of importance of each of these growth substances is indicated by its distance from the fulcrum of the model presented in Figure 1.4.

Gibberellins may inhibit abscission applied alone (Addicott, 1982) or in the presence of ethylene (Rewinkel-Jansen, 1986), possibly due to their action of increasing sink strength thereby deferring leaf senescence (Addicott, 1982). However, gibberellins may also promote abscission if applied to debladed explants (Rosen and Siegel, 1963; Chatterjee and Leopold, 1964; Bornman *et al.*, 1967; Ratner *et al.*, 1969) or complete explants in the presence of ethylene (Morgan and Durham, 1975). The promotion of abscission by gibberellins during Stage I of the abscission process (Chatterjee and Leopold, 1964) may be due to decreased inhibition of abscission by auxin (Morgan, 1976), increased rate of cell division (Bornman *et al.*, 1967) or increased activity of enzymes involved in abscission (Chatterjee and Leopold, 1964).

Cytokinins act as inhibitors of plant senescence (Mattoo and Aharoni, 1988) and direct the movement of plant assimilates (Kende, 1971; Sexton and Roberts, 1982). At high concentrations, cytokinins inhibit abscission (Chatterjee and Leopold, 1964; Morgan and Durham, 1975) but promote it at low concentrations (Chatterjee and Leopold, 1964) or in some cases promote abscission if not applied directly to the abscission zone (Osborne and Moss, 1963). The inhibition of abscission by cytokinins is due to prolonging the duration of Stage I (Chatterjee and Leopold, 1964) by retarding protein breakdown (Kende, 1971) and therefore leaf senescence (Chatterjee and Leopold, 1964). Conversely, cytokinin application in the second stage of abscission may result in promotion of abscission (Chatterjee and Leopold, 1964).

ABA clearly promotes abscission (Bornman *et al.*, 1967; Cooper *et al.*, 1968; Morgan and Durham, 1975), possibly by stimulating ethylene production (Sagee *et al.*, 1980).

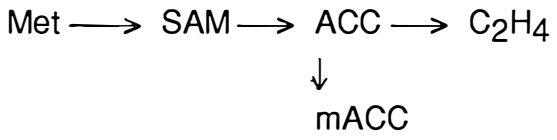


A diagrammatic representation of the possible multifactorial control of abscission. Abscission is envisaged as being determined by the relative concentrations of accelerators and inhibitors and the sensitivity of the zone to them. In this diagram the accelerators are shown on the left hand side (LHS) of a beam balance and they are counterbalanced by inhibitors on the right hand side (RHS). If the LHS of the balance goes down, abscission takes place. Ethylene and IAA have been positioned at the ends of the beam since changes in their concentration (weight) are relatively more effective than fluctuations in the regulators positioned nearer the fulcrum. The concentration of each regulator in the zone is determined by the rates of synthesis, transport and loss and this is depicted as a supply dripping into and out of the containers on the beam. Some of the possible factors that increase (+) and decrease (−) the concentrations of each regulator in the zone are shown above the supply tanks. The balance of the beam can also be affected by changing the sensitivity. This is depicted by being able to move the position of the fulcrum along the beam to the right, making the accelerators more effective and to the left decreasing their efficacy. Some of the factors that influence sensitivity are depicted together with arrows indicating how sensitivity is affected. This model oversimplifies the changes in sensitivity since it assumes that responsiveness to all the accelerators will change together; they may well be able to change independently of one another. ETH = ethylene, ABA = abscisic acid, IAA = auxins, CYT = cytokinins, GA = gibberellic acid

Figure 1.4. Balance model of abscission (Sexton *et al.*, 1985).

1.3.5 THE ETHYLENE BIOSYNTHETIC PATHWAY

The biosynthetic pathway of ethylene has been elucidated, a simplified version of which is presented below.



Methionine (Met) is metabolised to S-adenosylmethionine (SAM) (Adams and Yang, 1977) by the enzyme methionine adenosyltransferase (Konze and Kende, 1979). SAM is subsequently metabolised to 1-aminocyclopropane-1-carboxylic acid (ACC) (Adams and Yang, 1979) by the enzyme ACC synthase (Boller *et al.*, 1979; Yu *et al.*, 1979). The CH_3S group of methionine is recycled via 5'-methylethioadenosine (MTA) (Yang and Hoffman, 1984). AVG (aminoethoxyvinylglycine; Adams and Yang, 1979) and AOA (Yu *et al.*, 1979) inhibit this step, both being inhibitors of pyridoxal phosphate-mediated enzyme reactions. ACC synthase is a key enzyme in ethylene biosynthesis since it regulates the rate controlling step (Cameron *et al.*, 1979; Yang, 1980). The promotion of ethylene production by auxin is thought to act by stimulating the action of ACC synthase (Yang, 1980; Yang and Hoffman, 1984). It was suggested that ACC may be metabolised to products other than ethylene (Apelbaum and Yang, 1981), and it was subsequently established that ACC could be conjugated to 1-(malonylamino)cyclopropane-1-carboxylic acid (mACC) (Hoffman *et al.*, 1982) by ACC-malonyltransferase. Endogenous or exogenously applied ACC is converted to ethylene by "ethylene-forming enzyme" (EFE) (Apelbaum *et al.*, 1981). Due to the lack of success in extracting EFE, it was suggested that the ethylene-synthesis system is highly structured and requires membrane integrity (Lieberman, 1979). However, EFE has now been extracted from *Cucumis melo* (Ververidis and John, 1991) and *Malus* fruit (Kuai *et al.*, 1992) and is referred to as 1-aminocyclopropane-1-carboxylic acid oxidase (Kuai *et al.*, 1992). EFE is inhibited by phosphate and Co^{2+} (Even-Chen *et al.*, 1982) and is the O_2-dependent step in ethylene biosynthesis (Adams and Yang, 1979; Veen, 1987). An important area of ethylene biosynthesis is that of the autocatalytic response which involves increased ethylene production triggered by exposure to ethylene. The autocatalytic response involves an increase in the

synthesis of ACC synthase and EFE (Riov and Yang, 1982; Veen and Kwakkenbos, 1983) and can be inhibited by application of STS which blocks the reception of ethylene signal and therefore also stops the rise in ethylene production (Veen, 1987).

1.3.6 CLIMACTERIC AND NONCLIMACTERIC ETHYLENE PRODUCTION RESPONSES

The autocatalytic promotion of the ethylene biosynthetic pathway is an important process which has been used to categorise ethylene responses. The role of ethylene in fruit ripening has perhaps received the most extensive attention of any of the ethylene responses (Moore, 1979) and models have been proposed to explain genetic differences and variation of ethylene sensitivity with tissue development. An understanding of such models may help to explain differences in the response between tissues of *Camellia* and intergeneric differences of, for example, *Camellia* and *Citrus* (Chapter 5).

Two broad categories, climacteric and nonclimacteric, have been employed for many years to describe fruit ripening (McGlasson, 1985). The two ripening types can be differentiated by their pattern of respiration during ripening (McGlasson, 1985) and by their response to ethylene treatment (Yang, 1987b).

In nonclimacteric fruit, ethylene treatment temporarily increases the respiration rate correlating with the ethylene concentration applied, but no concomitant increase in ethylene production occurs (Yang, 1987b). Ethylene treatment does, however, induce senescence/ripening (McGlasson, 1985), such as found in the degreening of *Citrus* fruit (Young *et al.*, 1970).

For immature climacteric (preclimacteric) fruit, ethylene treatment increases respiration rate but not ethylene production. As found in nonclimacteric fruit, respiration rate returns to basal levels upon ethylene removal. Ethylene treatment hastens the onset of ripening in mature climacteric fruit and is associated with increased respiration rate and ethylene production, the pattern and magnitude of which are not significantly different to that observed during natural ripening (Yang, 1987b). Once autocatalytic ethylene production has commenced, the ripening process continues even if exogenous ethylene is removed (McGlasson, 1985).

During natural ripening of climacteric fruit (ripening in the absence of exogenous ethylene), respiration rate initially decreases, rises as ripening occurs, and subsequently declines during the final stages of senescence (Moore, 1979). During the ripening phase, ethylene production also increases along with changes in texture and composition (McGlasson, 1985).

In nonclimacteric fruit, no correlation between respiration rate and ripening changes can be observed (McGlasson, 1985). Two general patterns can be noted during ripening, either a slow decrease or no change in respiration rate (Moore, 1979).

To explain these differences in ripening behaviour, a model involving two ethylene receptor and control systems has been proposed (McMurchie *et al.*, 1972). System I is present in all fruit tissue, producing ethylene at low rates and regulating the aging process. System II is present only in climacteric fruit and results in the characteristic high levels of autocatalytic ethylene production (McMurchie *et al.*, 1972). This model has been subsequently elaborated on by Yang (1987b). In a climacteric fruit, the System I receptor controls basal ethylene production and respiration via the level of EFE. As fruit mature, ethylene from basal ethylene production complexes with the System I receptor and this complex gradually destroys a "ripening inhibitor". This inhibitor either blocks ethylene binding to, or development of, the System II receptor. Activation of the System II receptor promotes ACC synthase, and, along with the presence of EFE promoted by System I ethylene, results in the autocatalytic ethylene production distinctive to climacteric fruit. Ethylene signal transduction then results in production of the ripening enzymes promoting ripening.

Veen (1987) noted that the System I and II concept proposed by McMurchie *et al.* (1972) for fruit also appears to be valid for *Dianthus* flower senescence. In this ethylene response, application of STS and 2,5-NBD blocks the development of System II ethylene and therefore the postclimacteric autocatalytic ethylene production is also inhibited (Veen, 1987).

1.3.7 ETHEPHON

Ethephon ((2-chloroethyl)phosphonic acid) degrades to release ethylene (Maynard and Swan, 1963). After application, ethephon is absorbed into plant

tissue, a process influenced by temperature, relative humidity, wetting agent, cultivar and a variety of other factors (Klein *et al.*, 1979; Flore and Bukovac, 1982; Bukovac *et al.*, 1986). Ethephon is stable below pH 4, but on entering plant tissue (at a pH generally greater than 4) ethylene is released (de Wilde, 1971).

Although often simply used as "liquid ethylene", a convenient means of applying ethylene to tissue, e.g. as carried out by de Stigler (1980) and Moe and Smith-Eriksen (1986), ethephon application involves a relatively complicated release of ethylene gas as a pulse (Olien and Bukovac, 1982a). This results in unknown ethylene concentrations in abscission zones (Weis *et al.*, 1988), which also vary over time (Beaudry and Kays, 1988a). The peak concentration attained and duration of ethylene release is affected by a wide range of factors, such as temperature, which makes modelling of the response complex (Beaudry and Kays, 1988b; Weis *et al.*, 1988).

The resulting peak of ethylene from ethephon application has been employed to promote differential abscission of organs on plants, a process known as "thinning". This means that one organ may be selectively removed either entirely, as in the early removal of leaves from nursery stock (Larsen, 1970), or partially, as in the removal of some flowers or fruitlets in many fruit crops such as *Malus* (Jones and Koen, 1985) and *Prunus* (Daniell and Wilkinson, 1972).

Although not employed in this study, it should be noted that there is a variety of ethylene-releasing chemicals such as silane ((2-chloroethyl) methyl*bis*(phenylmethoxy)silane), which have ethylene release kinetics different from those of ethephon (Olien and Bukovac, 1982a; Beaudry and Kays, 1988b). They may therefore influence abscission differently from ethephon depending on the tissue's response to the resulting duration of ethylene exposure and concentration attained (Beaudry and Kays, 1987).

1.3.8 DEFINITION OF SENSITIVITY AND RESPONSIVENESS TO ETHYLENE AND ETHEPHON

In the study of ethephon application it is important to determine the relative sensitivities of the target organs since this is part of the basis for efficient chemical thinning (Beaudry and Kays, 1987). Clear knowledge of ethylene sensitivity also allows minimisation of abscission during exportation of live plants.

1.3.8.1 Ethylene-Sensitivity

Although the term "sensitivity" is used frequently, an accurate definition is required since no consensus as to the meaning of sensitivity has been arrived at (Paterson *et al.*, 1990) and a variety of definitions of sensitivity and responsiveness have been used. For example, Trewavas (1981) defines sensitivity as the concentration of receptors and that this may be measured by application of a single, usually saturating growth substance concentration. However, specific to ethylene, Knee *et al.* (1985) define responsiveness as the concentration of receptors and rate of reactions resulting from ethylene reception that may be measured by the level of response to a high (10 ul·liter^{-1}) concentration. This definition is the same as the sensitivity of Trewavas (1981) yet Knee *et al.* (1985) define sensitivity as the concentration bringing about a half maximum response. Another indication of confusion is that the terms sensitivity and responsiveness are used interchangeably (e.g. Sexton *et al.*, 1985; Woodson, 1991). As indicated above, some researchers have viewed ethylene-sensitivity to be the ethylene receptor concentration or their affinity for ethylene (Trewavas, 1982; Paterson *et al.*, 1990). However, many others discuss sensitivity such that it involves the influence of a wide range of factors such as other growth substances or chemicals (Borochov and Woodson, 1989; Woodson, 1991). For example, ethylene binding has not been found to explain ethylene-sensitivity of *Dianthus* flowers (Wu *et al.*, 1991b) and Reid and Wu (1992) even proposed a model which included binding of a "sensitivity factor" to the ethylene receptor as a mechanism of controlling ethylene-promoted senescence. It is pertinent to note therefore that even from the same dose-response curve many sensitivity parameters may be derived, and no single definition is universally accepted (Paterson *et al.*, 1990).

The definitions of sensitivity and responsiveness used in the current study were developed not for differentiating between the possible mechanisms of ethylene sensitivity (such as receptor number) but rather to examine relative sensitivities of *Camellia* organs to ethylene and their changes in sensitivity with maturity.

It has been stated that the absence of a theoretical framework has limited research into hormone sensitivity analysis (Weyers *et al.*, 1987). This problem is reflected even more strongly in the case of definition and analysis of ethylene-promoted abscission. Both ethylene- and ethephon-sensitivity have been analysed subjectively with little generally accepted methodology. To reduce the

subjective nature of interpretation of hormone sensitivity (Firn, 1986) it has been recommended that sensitivity be defined using mathematical parameters derived from sigmoidal dose-response curves (Weyers *et al.*, 1987). However, Weyers *et al.* (1987) warned against use of sigmoidal dose-response plots for qualitative responses such as abscission, since they are best suited to quantitative responses (e.g. growth), and for this reason it was decided not to employ this mathematical analysis in this study. Although no standard analysis protocol has been put forward for the examination of ethylene-promoted abscission-sensitivity, some examples are worth examining. In the study of the influence of maturity on petal abscission of *Pelargonium*, a range of ethylene concentrations was applied and changes to the shape of the dose-response curve with age employed to examine changes in sensitivity or responsiveness (Evensen, 1991). Suttle and Hultstrand (1991) applied ethylene (0 to 50 $\mu l \cdot liter^{-1}$) to leaves of *Gossypium* at two stages of maturity and discussed the differential abscission as a difference in sensitivity by comparison of the percent abscission at a given concentration. Thus the leaf maturity with the highest response for a given concentration was the most sensitive. Similarly, in the examination of differential abscission of *Citrus* organs, time to 50% abscission of fruit and leaves was plotted against ethylene concentration. The higher abscission rate (time to 50% abscission) of fruit compared with leaves, at lower ethylene concentration, was used as the basis for differential abscission of the two organs (Ben-Yehoshua and Eaks, 1970).

Sensitive is defined by the Collins Concise Dictionary Plus (Anon., 1989) as being "capable of registering small differences or changes in amounts" or "quickly responsive to external influences", whereas responsive is defined as "quick or favourable reaction". These definitions along with the above examples lead us to the working definitions employed in the current study.

Sensitivity: greater extent or rate of abscission occurring at lower ethylene concentration.

Responsiveness: comparison of the rate or extent of response occurring at a single, usually high concentration, which may or may not reflect the sensitivity of the response.

The above definitions of abscission "sensitivity" and "responsiveness" can be illustrated using the example of ethylene-promoted abscission of *Citrus* leaves and fruits (Figure 1.5; Ben-Yehoshua and Eaks, 1970). At a concentration of 1 to

100 *u*l·liter^{-1} the abscission rate of leaves is faster (lower time to abscission) than that of fruit and leaves are therefore described as being more responsive. However, at a concentration of 0.1 *u*l·liter^{-1} leaf abscission rate is significantly less than that of fruit and leaves are therefore described as being less sensitive. This definition of sensitivity follows the result that would be expected from application of an ERC such that greater fruit abscission would occur than leaves, and thus fits the concept of greater sensitivity of fruit. It should be noted that greater abscission response of one organ (e.g. leaves) over another (e.g. fruit) at 10 *u*l·liter^{-1} (i.e. greater responsiveness) does not necessarily indicate greater sensitivity of that organ (abscission response at the low ethylene concentration of 0.1 *u*l·liter^{-1}).

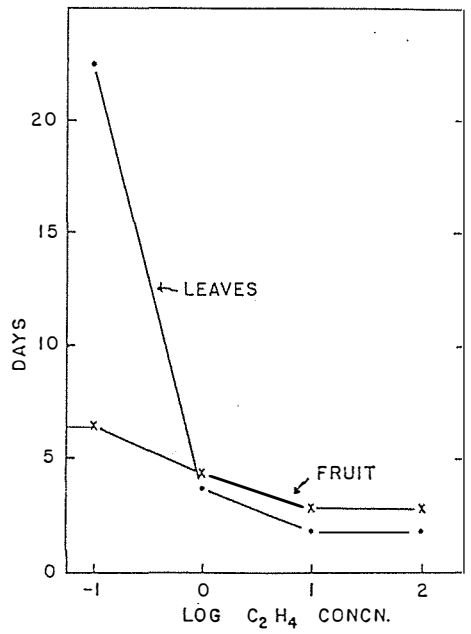


Figure 1.5. Days to 50% abscission of leaves or to complete abscission of fruit in response to 0.1, 1, 10 and 100 *u*l·liter^{-1} ethylene in *Citrus* (Ben-Yehoshua and Eaks, 1970).

1.3.8.2 Ethephon-Sensitivity

The analysis proposed by Firn (1986) is unsuitable for analysis of ethephon application because of the two-step nature of ethephon which involves ethylene release and ethylene action. Also, ethephon levels practical to achieve thinning are not applied with 4 orders of magnitude (e.g. Figure 1.5) and hence lag and saturating responses may not be obtained.

The principle aim of thinning is removal of a target organ. Therefore, description of the target organ as most sensitive to ethephon would require the same definition as employed for ethylene-sensitivity. That is, for one organ to be more sensitive than another, its abscission response (e.g. percent abscission) should be greater at lower ethephon concentrations such as employed by Tripp and Wien (1989). Where no measurement of response to a range of ethylene or ethephon concentrations has been carried out, the term responsiveness will be employed. Thus according to this definition, description of "sensitivity" of *Olea* organs to ethylene and ethephon employed by Weis *et al.* (1988) would be described as a difference in responsiveness rather than sensitivity since only one concentration was employed.

1.3.9 ASSUMPTIONS INVOLVED IN THE APPLICATION OF ETHYLENE CONCENTRATIONS AND MEASUREMENT OF ETHYLENE PRODUCTION

It is important to examine some of the assumptions and factors involved in the application of ethylene and measurement of ethylene production of tissue. A model for the effect of ethylene movement into plant tissue, biosynthetic production, binding and metabolism on ethylene concentration is presented in Figure 1.6.

Ethylene which is physiologically active, is that present within the cell cytosol (Osborne, 1989b) and more specifically, the concentration at the ethylene receptor. Thus the four techniques used for ethylene determination *viz.* measurement of ethylene production from tissue (that is, ethylene emanation) (Ben-Yehoshua and Aloni, 1974; Field, 1981; 1984; Auer and McConnell, 1984), vacuum extraction (Beyer and Morgan, 1970b; Ben-Yehoshua and Aloni, 1974; Saltveit, 1982), attachment of a chamber allowing equilibrium with the internal atmosphere (Banks and Kays, 1988), or sampling from an air space within the tissue (Ben-Yehoshua and Aloni, 1974; Saltveit, 1982) yield at best, estimates of the ethylene concentration of the intercellular spaces (Osborne, 1989b). Also, the concentration within the cell is suggested to be greater than that of the intercellular spaces (Jerie *et al.*, 1978; Solomos, 1989). An example of the problems encountered in the measurement of ethylene is demonstrated by the vacuum extraction technique which yields ethylene concentrations 30% higher than those obtained by sampling the internal air space, the difference possibly

being a result of vacuum removal of dissolved ethylene (Ben-Yehoshua and Aloni, 1974) or compartmented ethylene within tissue structures (Zeroni *et al.*, 1977; Jerie *et al.*, 1978).

Resistance to ethylene movement into or out of tissue will influence the internal gas phase ethylene concentration. The cuticle provides a major barrier to ethylene diffusion through leaves or other plant tissue. Stomata are an important variable factor in ethylene movement since they provide a direct path for diffusion into leaves. Closure of stomata results in a large increase in the resistance to diffusion into and out of leaves (Beaudry and Kays, 1988b). Examination of the literature on the effect of ethylene on stomatal conductance itself shows that although it has been demonstrated to reduce conductance (Pallas and Kays, 1981) it may also increase conductance (Briede *et al.*, 1992), or, in many cases have no effect (Pallaghy and Raschke, 1972; El-Beltagy and Hall, 1974; Aharoni, 1978).

Ethylene does not move within plant tissue in physiological quantities because the rate of emanation and resistance to longitudinal movement are substantial (Zeroni *et al.*, 1977; Jerie *et al.*, 1978). It has been assumed that ethylene moves into and out of tissue by simple diffusion (Burg, 1968), although more recently, it has been suggested that plants may have a greater resistance to ethylene than would be expected from simple laws of diffusion (Hall, 1977).

A further problem generated by our lack of understanding of the mechanisms of ethylene diffusion is that we have no knowledge of the existence or otherwise of differential barriers to ethylene movement in different tissue types (Osborne, 1989b). Thus in the study of differences in abscission responses of inflorescences and leaves (Weis *et al.*, 1988), and fruit and leaves (Lang and Martin, 1989) of *Olea*, it must be assumed that no significant differences exist between ethylene diffusion into the abscission zones.

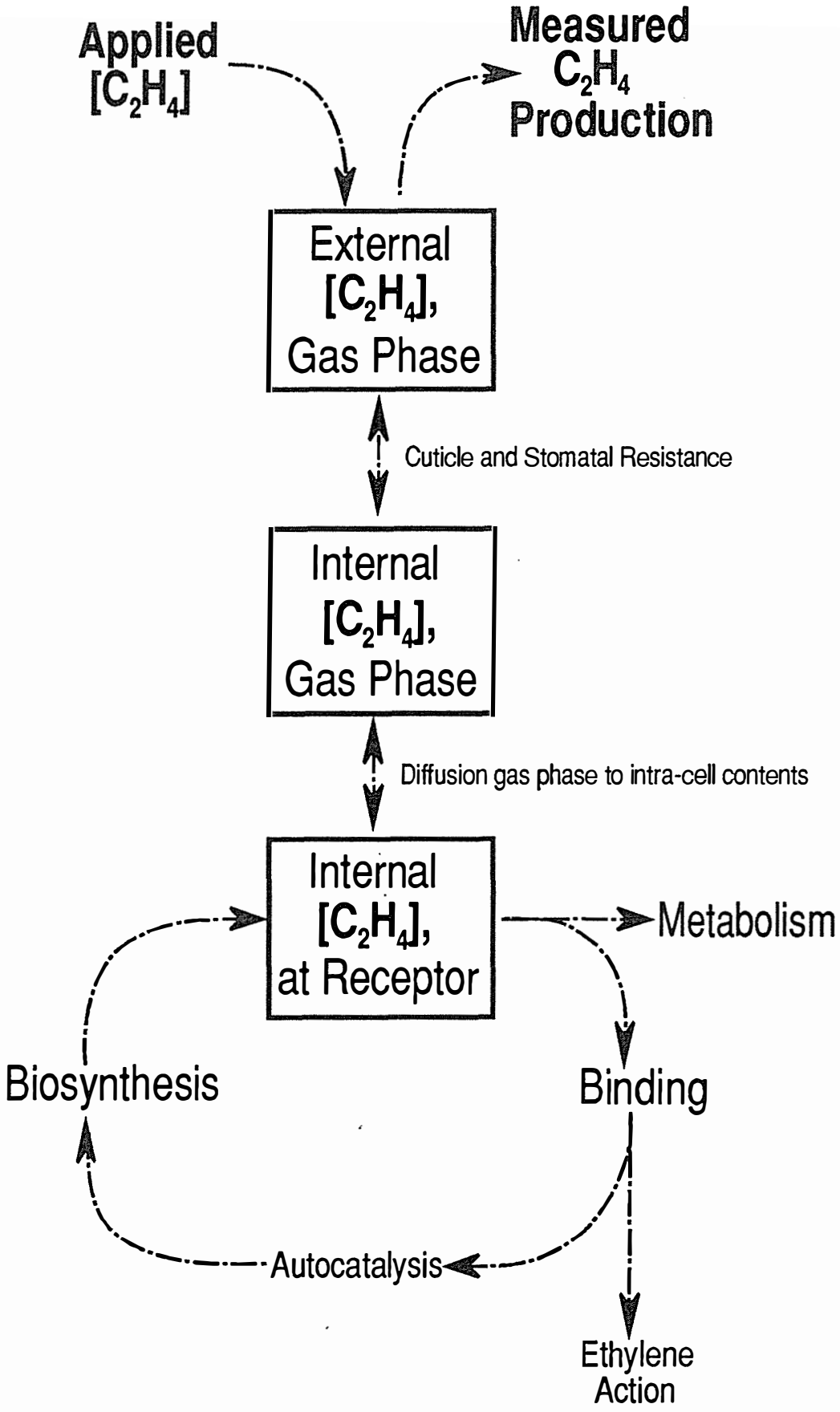


Figure 1.6. Model of factors influencing the application of ethylene, measurement of ethylene production and ethylene concentration at the ethylene receptor.

A further mechanism which may influence the rate of ethylene production or ethylene concentration at the receptor site is that of compartmentation. It was found that in *Vicia*, the ethylene concentration in the lacuna is less than that of the surrounding tissue (Zeroni *et al.*, 1977). This lead to the suggestion that ethylene may be compartmentalized in plant tissue either within anatomical structures such as vascular bundles, or dissolved in certain cell contents. The capacity of tissue to retain ethylene was also found to increase with maturity and it was suggested that this may be a possible control mechanism of ethylene response (Jerie *et al.*, 1978). However, Trewavas (1982) considered this mechanism unlikely and little further experimental evidence or speculation exists in this area.

Both ethylene metabolism (Smith and Hall, 1984b) and ethylene binding (Starling *et al.*, 1986) have been detected in many plant tissues and the action of both decreases the concentration of ethylene internally and at the binding site. There is a low concentration of binding sites in plant tissue (2 to 6.8 $x\ 10^{-9}$ mol binding sites/kg tissue in leaves; Goren and Sisler, 1986). Once ethylene binding sites have reached equilibrium with aqueous ethylene, it is unlikely that such a binding site concentration would significantly reduce ethylene concentration. Once ethylene binds, and promotes action, it dissociates from the receptor, and may either diffuse from the tissue or be destroyed (Goren and Sisler, 1986). In *Musa* fruit it was found that the saturation of ethylene binding was reversible with less than 6 hr ethylene treatment (Whitehead and Bosse, 1991). Similarly to ethylene binding, ethylene metabolism is low in most species (Dodds and Hall, 1980). However, levels of ethylene metabolism have been observed in *Medicago* (Sanders *et al.*, 1989a) and *Vicia* (Jerie *et al.*, 1978) which are sufficiently high to be likely to significantly reduce both the internal gaseous ethylene concentration and the ethylene concentration at the binding sites (Sanders *et al.*, 1989a). The importance of metabolism is also dependent on other factors, such as, photosynthetic rate and external CO_2 concentration which will influence the rate of ethylene metabolism, and therefore its effect on ethylene production (Grodzinski *et al.*, 1982). Ethylene metabolism and binding and their possible role in ethylene action will be examined in greater detail below.

It is clear then, that measurement of ethylene production (emanation) is not a direct measure of the internal concentration (Burg, 1968). Rather, it is a measure of ethylene resulting from biosynthesis diffusing from the plant. Thus by

subtraction, ethylene production is the ethylene that is neither metabolised, nor bound either specifically to sites of high affinity (e.g. ethylene receptors) or non-specifically (to other cellular contents), nor compartmentalized (Zeroni *et al.*, 1977; Jerie *et al.*, 1978; Grodzinski *et al.*, 1982). Although measurement of ethylene production is not a direct measure of the internal gaseous ethylene concentration, a high degree of correlation has been found between the two measurements (Ben-Yehoshua and Eaks, 1970; Beyer and Morgan, 1971; Ben-Yehoshua and Aloni, 1974). Hence, because of the greater technical complexity in measuring internal concentrations, and the current impossibility of measuring ethylene concentration within the cell, ethylene production is used as an approximate measure of tissue ethylene biosynthetic rate since changes in biosynthetic rate will correlate with the rate of ethylene production.

Similarly, in the application of ethylene to tissue and measurement of response, a number of assumptions can be made. Although exogenous ethylene equilibrates rapidly with internal ethylene in leaves (15 min for *Phaseolus*, 30 min for *Citrus*, and 30 to 60 for *Ligustrum*; Goren and Sisler, 1986) which indicates relatively few barriers to ethylene movement into tissue exist, the ethylene concentration at the ethylene receptor is not known. For example, ethylene has a high solubility in lipids and it is therefore possible that the ethylene receptor, which is thought to be membrane bound, may be in a lipid environment with significantly higher ethylene concentrations than that of the external, or internal gaseous environment. Of this, and other possible ethylene compartmentalization mechanisms within the cell, we have little knowledge (Osborne, 1989b). If ethylene concentration is the only factor changed in a system, and ethylene follows diffusion gradients, then it is reasonable to assume that the concentration applied is highly correlated with the concentration at the receptor. Thus in this study, it is assumed that the ethylene concentration applied is not the same as, but correlates well with the internal concentration, and that this in turn correlates with the concentration at the receptor. That these assumptions are valid is supported by the fact that the ethylene concentration required for half maximal, or maximal effect for a range of ethylene responses varies little (with the exception of fruit ripening) for a wide variety of tissue types (Burg, 1968).

CHAPTER 2

SELECTIVE REMOVAL OF FLORAL BUDS FROM *CAMELLIA* WITH ETHEPHON.

I. INFLUENCE OF ETHEPHON CONCENTRATION, ORGAN TYPE AND TIME OF APPLICATION.[1]

2.1 INTRODUCTION

The climate in New Zealand is suited to the commercial production of *Camellia* plants. One-year- to four-year-old container-grown plants may be exported to the northern hemisphere between February and April (late summer to autumn). Floral macrobuds are present on *Camellia* plants at this time. However, vegetative bud break and subsequent shoot extension is required on arrival because floral buds are initiated on shoots produced under long days and high temperatures in the Northern Hemisphere summer (Scott, 1977). Ideally, floral buds should be removed before export because the presence of floral buds inhibits vegetative bud break. In addition, fungal infections may arise from in-transit abscission of floral buds when plants are sea-freighted. A similar problem with *Hydrangea* led to the requirement for defoliation before storage to decrease the incidence of *Botrytis cinerea* Pers. (Bailey, 1990). Floral buds in *Camellia* can be removed manually before transportation, but this has a high labour requirement and resulting wounds provide ideal entry points for fungal diseases.

Ethephon has been used for selective removal of floral buds in *Malus* (Edgerton and Greenhalgh, 1969). Development of a similar procedure for *Camellia* could reduce production costs and disease incidence. The ability of ethephon to promote selective abscission (thinning) is determined both by its ethylene-release kinetics and by the greater sensitivity of the target organ over other plant organs (Beaudry and Kays, 1987).

1 A reprint of this chapter published in HortScience (Woolf *et al.*, 1992) is attached at the rear of this thesis.

The abscission response may be significantly influenced by the time of year at which ethephon is applied (Wittenbach and Bukovac, 1973; Klein *et al.*, 1978; Perry and Gianfagna, 1987). These differences are due to changes in tissue maturity (Jones *et al.*, 1983) and environmental factors such as temperature and relative humidity (RH) (Klein *et al.*, 1978).

Rate of release of ethylene from ethephon is influenced by a number of environmental factors. Increased air temperature causes a greater rate of ethylene release (Klein *et al.*, 1978). Olien and Bukovac (1978) derived a Q_{10} of 7.0 for ethylene release from ethephon-treated *Prunus* leaves over the range of 10^{o} to 40^{o}C. Relative humidity may influence ethylene release at extremes but does not appear to be responsible for variable field results (Klein *et al.*, 1978).

Many factors influence the sensitivity of plant organs to ethylene. Physiological maturity affects sensitivity to released ethylene. For example, floral buds of *Begonia x cheimantha* (Moe and Smith-Eriksen, 1986) and *Vitis* (Weaver and Pool, 1969) become more sensitive to ethephon as they develop to anthesis. Sensitivity to ethephon is also genetically determined. Cultivars of *Olea* (Hartmann *et al.*, 1970) and *Malus* (Edgerton and Greenhalgh, 1969) differ in abscission-sensitivity to both ethephon concentration and physiological maturity of the plant organ.

Temperature also influences the abscission response of tissue to ethylene gas itself. Ethylene promotion of *Philodendron* leaf and stipule abscission increases with higher temperature (Marousky and Harbaugh, 1979c). It has also been found that higher temperatures promote ethylene-stimulated flower senescence in *Dianthus* (Woltering and Harkema, 1987).

2.2 OBJECTIVES

The objective of this work was to determine whether ethephon could be used for selective removal of floral buds from potted *Camellia* plants with minimal damage to other plant organs.

2.3 MATERIALS AND METHODS

***Camellia* Phenology.** A brief description of the yearly growth cycle of *Camellia* will elucidate the organ morphogenesis, development and organ maturities

present on the plant. Vegetative bud break (spring) involves abscission of bud bracts and subsequent shoot extension. Once extension has ceased, floral buds are then initiated and develop over the summer and autumn. Apical floral and vegetative buds develop fastest and are therefore more mature than subtending buds (Anon., 1986). In late summer, vigorous apical buds may make a second flush of shoot growth. Flower opening occurs between autumn and spring, depending on plant species/cultivar and environmental conditions (Valder, 1978). Thus in late summer, a range of leaf maturities may be present; second flush extending shoots (containing softer, expanding leaves), 3-month-old (current summer shoots), and leaves of 1-year- and 2- to 3-year-old shoots. This is a similar situation to that of *Olea* which are evergreen and retain leaves for 2 or more seasons (Weis *et al*., 1988), and to that of *Citrus* which produce 2 growth flushes and possess a range of leaf maturities (Plummer, 1987). However, *Camellia* shoots are determinant and shorter than those of *Olea*. Vegetative buds are present on current season and previous season shoots only. Natural abscission occurs at different times for each of the organs of *Camellia*; floral buds after flower opening and subsequent senescence, vegetative bud bracts at bud break in spring, and leaves after 2 to 3 years with greatest leaf abscission occurring at vegetative bud break.

Plant Material. Three-year-old *Camellia* plants (15 to 20 floral and vegetative buds/plant) were obtained from Duncan and Davies Nurseries Ltd, New Plymouth N.Z. in 2-liter plastic pots. All plants were sprayed to runoff (approximately 20 ml/plant) using a 2-liter hand-held sprayer. Temperature and RH at time of spraying and minimum/maximum temperature and RH were recorded daily (3:00 pm). Mean minimum/maximum temperature and mean RH were calculated for the period of each experiment (Table 2.1). This, and all subsequent experiments (Chapters 3 to 5) were carried out at Massey University, Palmerston North, N.Z. (latitude 40^{0} 23'S). Abscission proportion data were analysed to obtain means and standard errors, and subsequently converted to percent form for graphing.

Experiment 2.1; Application of Ethephon to *Camellia* 'Anticipation' in Winter.

Camellia 'Anticipation' was used in the first experiment to investigate the effect of ethephon on the abscission of leaves, floral and vegetative buds. Ethephon (Ethrel 48, Rhone-Poulenc Ltd, Wellington, N.Z.) was applied as a foliar spray containing Tween 20 (0.5% v/v) at six concentrations (0, 50, 200, 500, 1000, and 3000 $ul \cdot liter^{-1}$ a.i.) on 1 June 1988. Plants were treated and arranged in the open

in a completely randomised design with 5 whole-plant replicates per treatment. Water was applied to the pot by hand every 4 days as plant water potential has been shown to influence uptake and translocation of ethephon (Klein *et al.*, 1978). After 32 days, the abscission rate had dropped to that of the control plants, and the numbers of leaves, floral and vegetative buds remaining on each plant were recorded.

Effect of leaf maturity on abscission-sensitivity was also examined by determining leaf abscission proportion on 3 shoot maturities (3-month- (current season), 1-year- and 2- to 3-year-old wood). All plants were subsequently placed in conditions promoting vegetative bud break and flower initiation (15^{o} to 25^{o}C and 16 hr photoperiod; Section 1.2.2.1 and 1.2.3.1) to determine the influence of applied ethephon and induced abscission on subsequent growth and flowering.

Experiment 2.2; Two Applications of Ethephon to Two *Camellia* Cultivars in Autumn.

In a second experiment carried out in the autumn of 1989, the effect of ethephon concentration, timing of application, and cultivar were investigated. Ethephon was applied to *Camellia* 'Anticipation' and *Camellia* 'Donation', at six concentrations (0, 500, 1000, 2000, 3000, and 4000 ul·liter^{-1}) on two occasions (3 March, 14 April). A split-plot design pooled over time was employed. Cultivars were randomised in the split-plots and 10 whole-plant replicates used for each cultivar. A second population of untreated plants was used at the second application time. After ethephon application, plants were placed under a plastic covered shelter (13% shade) to eliminate any effect of rain on ethephon-promoted abscission (Hartmann *et al.*, 1970). Hand-watering was carried out every two days. After 14 days the abscission rate had dropped to that of the control plants, and the numbers of leaves, floral and vegetative buds remaining on each plant were recorded. Buds were identified as being either floral or vegetative on the basis of shape, vegetative buds being more pointed and slender (bullet-shaped) than floral buds (Hume, 1955; Anon., 1986). Correct identification was verified by regular sectioning of buds. This criteria was employed to differentiate between floral buds and vegetative buds in all subsequent sections.

2.4 RESULTS

Application of ethephon to *Camellia* 'Anticipation' (Experiment 2.1) caused the abscission of leaves, floral buds and vegetative bud bracts. Increasing

concentration of ethephon promoted ~~greater~~ abscission of all organ types (Experiment 2.1 and 2.2; Figure 2.1 to 2.3). In many cases the response curve was approximately sigmoidal, similar to that found for *Olea* (Lavee and Martin, 1981).

Leaves and floral buds abscised intact at the base of the petiole or peduncle following ethephon application. Vegetative buds in contrast, were either killed or damaged by ethephon. At ethephon concentrations of 1000 to 2000 $\mu l \cdot liter^{-1}$, bud bracts surrounding the small, un-elongated shoot tended to abscise leaving the unexpanded leaves exposed (Figure 2.4). At higher concentrations (3000 to 4000 $\mu l \cdot liter^{-1}$) most of the vegetative buds were killed but did not fall from the plant, as did affected floral buds. Both damaged and killed vegetative buds were regarded as abscised and pooled for the purpose of this work and subsequent experiments (Chapter 3).

Plant organs differed in their sensitivity to ethephon. Floral buds were the most sensitive, vegetative buds were intermediate and leaves the least sensitive (Experiment 2.2; Figure 2.2 and 2.3). In mid-April, application of 2000 $\mu l \cdot liter^{-1}$ ethephon to 'Donation' resulted in 84% of floral buds abscising, whilst only 21% and 11% of vegetative buds and leaves abscised respectively (Figure 2.3). This trend of sensitivity held true in all cultivars and times of application at ethephon concentrations above 1000 $\mu l \cdot liter^{-1}$ in Experiment 2.2. A trend of increasing responsiveness with increasing physiological maturity of leaves (Figure 2.5) similar to that observed in *Olea* (Klein *et al.*, 1978) was also found in *Camellia*. The results were, however, not significant and leaf maturity data were pooled for comparison with floral bud and leaf data (Figure 2.1).

Time of application of ethephon had a significant effect on abscission of the three plant organs. In both cultivars, later application (April) of ethephon (1000 to 4000 $\mu l \cdot liter^{-1}$) resulted in a lower proportion of abscised vegetative buds and leaves (Figure 2.2 and 2.3). However, results suggest that, for some cultivars, later application may result in a higher abscission proportion of floral buds. In *Camellia* 'Anticipation', the proportion of floral buds abscised was greater in April than in March at ethephon concentrations of 500 and 1000 $\mu l \cdot liter^{-1}$ (Figure 2.2). In contrast, in 'Donation', later application resulted in lower floral bud abscission proportion for 1000 and 2000 $\mu l \cdot liter^{-1}$ ethephon (Figure 2.3).

At concentrations of 1000 to 4000 $ul \cdot liter^{-1}$, vegetative buds and leaves of 'Anticipation' were more responsive than 'Donation', except for March application to vegetative buds (Figure 2.2 and 2.3). However, floral bud abscission was only greater in 'Anticipation' at 1000 $ul \cdot liter^{-1}$ for the March application, and at 500 to 1000 $ul \cdot liter^{-1}$ for the April application. Cultivar differences also occur in *Olea* where ethephon-sensitivity of leaves correlates positively with fruit abscission (Hartmann *et al.*, 1970).

Time of application	Temperature (°C) At spraying	Mean min.	Mean max.	Relative humidity (%) At spraying	Mean
1 June, 1988	10	5	13	--z	--z
3 March, 1989	29	15	30	55	51
14 April, 1989	19	12	26	67	62

zRelative humidity not measured in 1988.

Table 2.1. Summary of temperature and relative humidity for three ethephon application times.

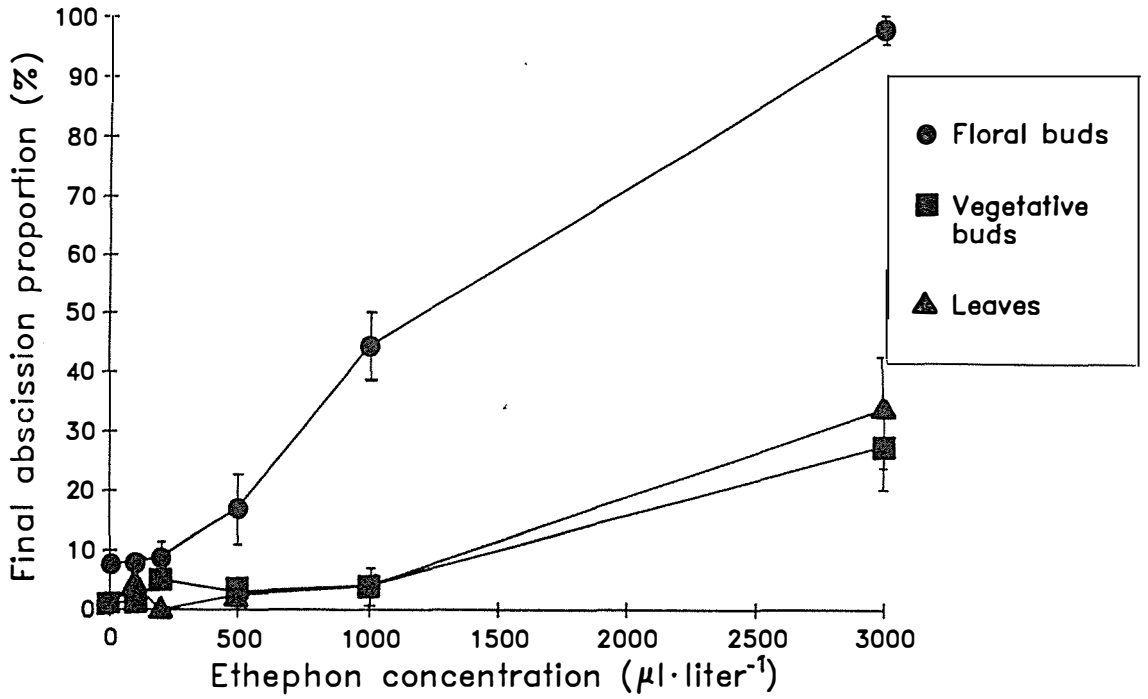


Figure 2.1. Abscission of plant organs from *Camellia* 'Anticipation' 32 days after application of ethephon on 1 June 1988 (winter). Leaf maturity pooled. Vertical bars represent SE of the mean.

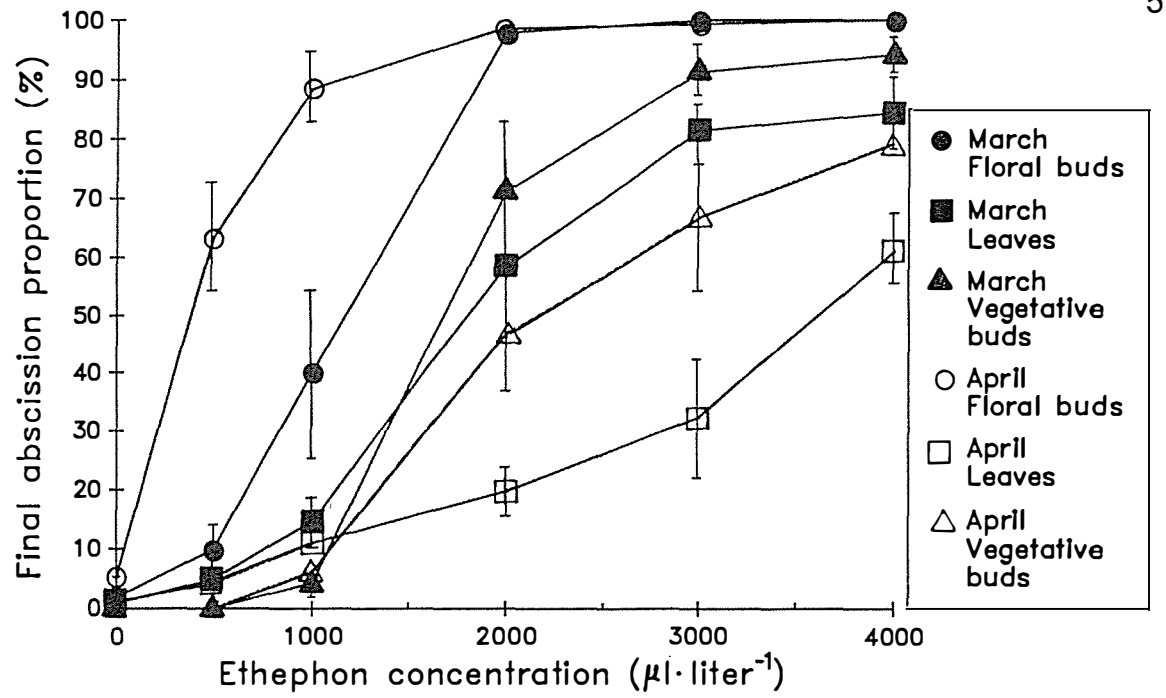


Figure 2.2. Abscission of plant organs from *Camellia* 'Anticipation' 14 days after application of ethephon on 3 March (early autumn) and 14 April (mid autumn) 1989. Vertical bars represent SE of the mean.

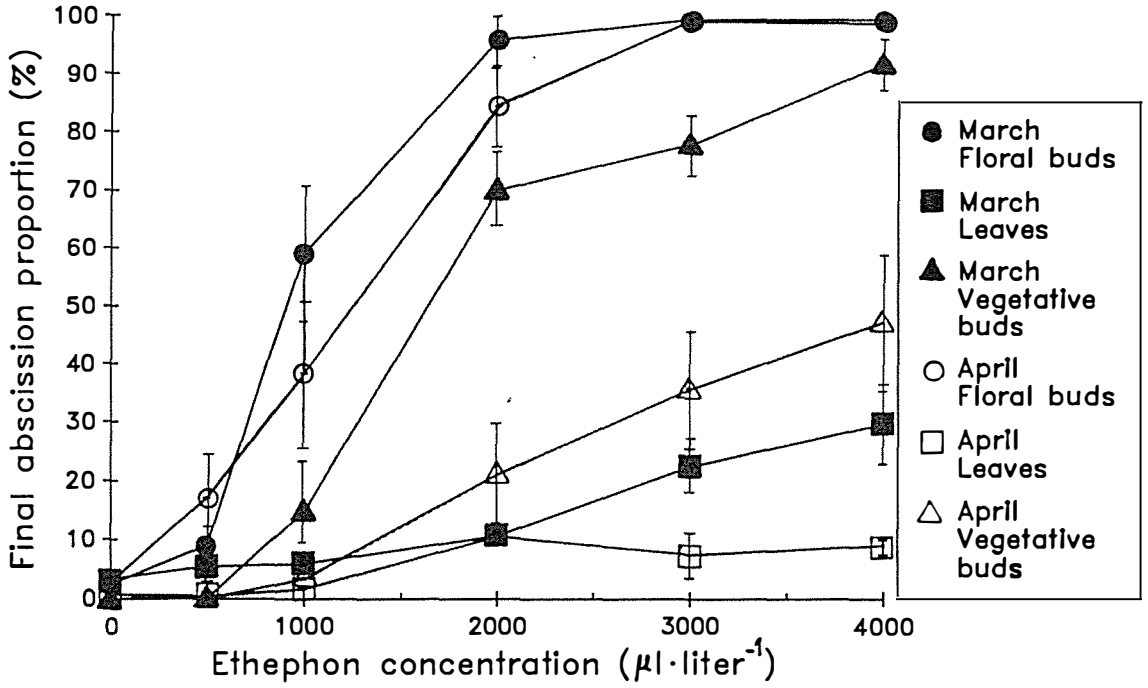


Figure 2.3. Abscission of plant organs from *Camellia* 'Donation' 14 days after application of ethephon on 3 March (early autumn) and 14 April (mid autumn) 1989. Vertical bars represent SE of the mean.

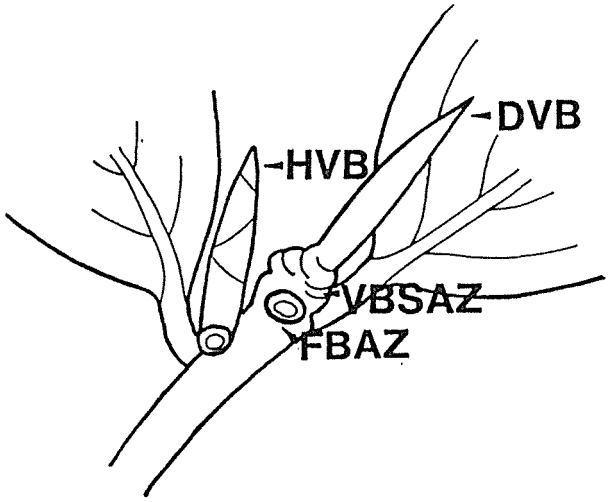


Figure 2.4. Apex of stem of *Camellia* 'Anticipation' with vegetative bud damaged by application of 1000 to 2000 *u*l·liter^{-1} ethephon. HVB, healthy vegetative bud; DVB, damaged vegetative bud; VBSAZ, vegetative bud scale abscission zone; FBAZ, floral bud abscission zone.

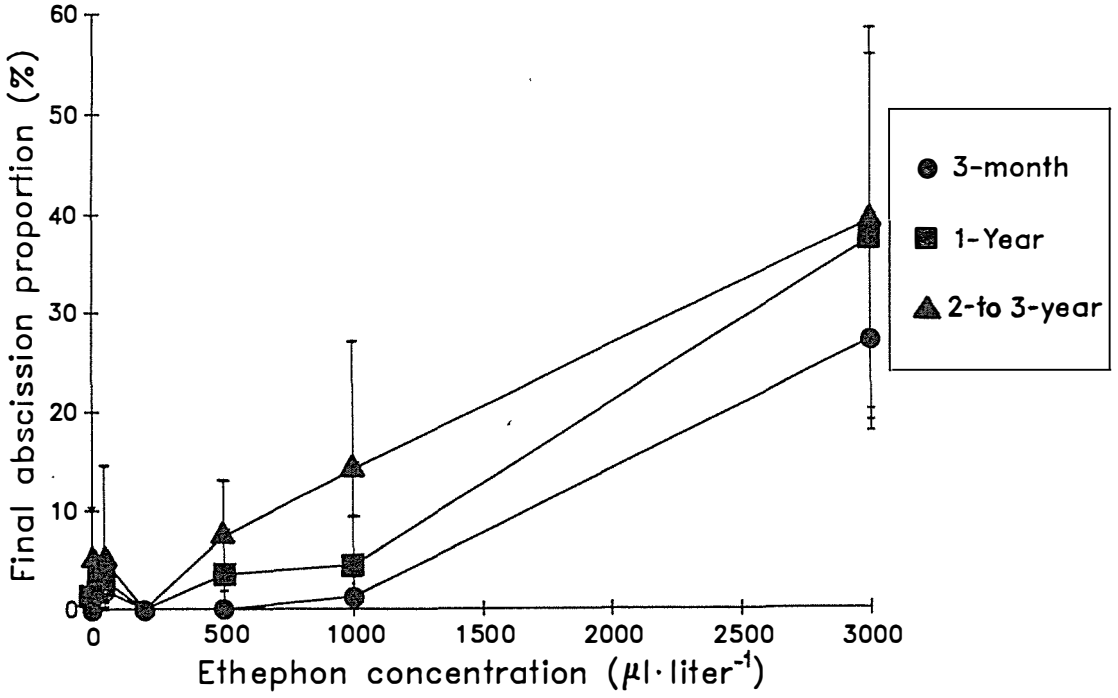


Figure 2.5. Abscission of 3 leaf maturities (3-month-, 1-year- and 2- to 3-year-old) from *Camellia* 'Anticipation' 32 days after application of ethephon on 1 June 1988 (winter). Vertical bars represent SE of the mean.

2.5 DISCUSSION

Sensitivity and responsiveness to ethephon varied markedly among leaves, floral and vegetative buds, floral buds being the most sensitive. This made selective removal of floral buds from *Camellia* with ethephon possible. Differences in ethephon-responsiveness of plant organs in *Camellia* were in agreement with the high abscission of *Camellia japonica* floral buds promoted by ethephon (Lee and Song, 1992) and were comparable to *Prunus* where abscission of 100% of the flowers but only 20% of the leaves occurs after application of 450 $ul \cdot liter^{-1}$ ethephon (Edgerton and Greenhalgh, 1969). The occurrence of differences in ethephon responsiveness of *Camellia* cultivars was consistent with similar differences found in cultivars of *Olea* and *Malus* (Hartmann *et al.*, 1970; Edgerton and Greenhalgh, 1969).

Lang and Martin (1985) suggested that differences in sensitivity between fruits and leaves of *Olea* could be explained in terms of their genetic programming. Fruits are seasonal reproductive organs whereas leaves are photosynthetic organs programmed for a 3 year existence. In the genus *Camellia*, flowers abscise after opening in winter, vegetative bud bracts at bud break in the following spring, and the leaves senesce and abscise after 2 to 3 years. Thus the relative sensitivity to ethephon of these three organs follows the same pattern as their temporal proximity to natural abscission. The trend of greater responsiveness with increasing maturity of *Camellia* leaves (Figure 2.5) also supports this hypothesis.

In contrast to growth after ethephon-promoted defoliation of *Malus* rootstocks (Cummins and Fiorino, 1969), *Camellia* plants grown on after ethephon application showed no decrease in vegetative bud break or subsequent shoot growth (data not shown). Similarly, no delay in timing of flowering was observed in *Camellia* as found for *Prunus persica* (peach) and *Pelargonium* (Crisosto *et al.*, 1989; Semeniuk and Taylor, 1970), nor in the number of flowers subsequently produced as found in *Prunus persica* (nectarine) (Irving, 1987).

Time of application influenced abscission of all plant organs. Later application of ethephon resulted in consistent reduction in abscission of vegetative buds and leaves in both cultivars. This was most probably due to decreased air temperature which would cause less ethylene release to occur from ethephon (Olien and Bukovac, 1982a), and lower response of the plant organ to ethylene

(Beaudry and Kays, 1987). Floral buds of 'Anticipation' showed increased abscission at the later time of application. It is likely that though lower temperature caused lower ethylene release and reduced the abscission response of plant tissue, increase in ethylene-sensitivity as a result of greater maturity was the overriding factor. The effect of time of application could also potentially be due to the influence of RH. However, it seems unlikely that a difference in RH of 11% (Table 2.1) would strongly affect ethylene release as RH is not a major factor in the field application of ethephon (Klein *et al.*, 1978).

2.6 CONCLUSION

It is possible to use ethephon to remove floral buds from potted *Camellia* plants with minimal abscission of leaves and vegetative buds. From the cultivars, concentrations and times tested, mid-April application of 1000 to 1500 $\mu l \cdot liter^{-1}$ ethephon for 'Anticipation' and 1500 to 2000 $\mu l \cdot liter^{-1}$ for 'Donation' are likely to be optimal for the selective removal of floral buds prior to export of *Camellia* in autumn.

CHAPTER 3

SELECTIVE REMOVAL OF FLORAL BUDS FROM *CAMELLIA* WITH ETHEPHON.

II. INFLUENCE OF ETHEPHON CONCENTRATION, TEMPERATURE AND LEAF MATURITY.

3.1 INTRODUCTION

It has been demonstrated that ethephon could selectively remove floral buds from *Camellia* with minimal damage to other plant organs. Use of ethephon to selectively remove a target organ, relies on the greater ethylene-sensitivity of that target organ (Beaudry and Kays, 1987) and this occurs in *Camellia*. The influence of ethephon concentration, cultivar, and time of year of application on thinning of floral buds from *Camellia* has been examined (Woolf *et al.*, 1992; Chapter 2).

The two most important non-environmental factors involved in the selective nature of ethephon are that of ethephon concentration (which will influence the ethylene concentration in the tissue and duration of exposure), and organ-related factors (such as organ type and maturity, which will influence sensitivity to released ethylene). The optimum ethephon concentration for selective removal of floral buds, without excessive leaf or vegetative bud abscission, is between 1000 and 2000 $\mu l \cdot liter^{-1}$, depending on cultivar and environmental conditions (Woolf *et al.*, 1992; Chapter 2). To optimize the selective removal efficiency, the influence of temperature and organ maturity and their interaction with ethephon concentration need to be determined.

As noted previously, abscission promoted by ethephon is affected significantly by the application time in the year which influences tissue maturity and environmental factors, particularly temperature (Woolf *et al.*, 1992; Chapter 2).

Organ Maturity

Tissue maturity has been shown to result in sensitivity differences in a variety of ethylene-promoted responses including fruit ripening (Iwahori *et al.*, 1969; Yang, 1985) flower senescence (Camprubi and Nichols, 1978; Reid and Wu, 1992) and

leaf senescence (Mattoo and Aharoni, 1988). Maturity also influences the sensitivity of ethylene-promoted abscission (examined further in Chapter 4) and ethephon-promoted abscission of a range of tissues. Maturity produces significant differences in ethephon-promoted abscission of floral organs of *Malus* (Edgerton and Greenhalgh, 1969; Jones *et al.*, 1983), *Begonia* (Moe and Smith-Eriksen, 1986), *Vitis* (Weaver and Pool, 1969) and *Olea* (Weis *et al.*, 1988) as well in abscission of fruit in *Prunus cerasus* and *P. avium* in response to ethephon (Wittenbach and Bukovac, 1973) and of *Capsicum* (Beaudry and Kays, 1988a) in response to silane. Leaf tissue maturity also affects the abscission-sensitivity to ethephon of *Olea* (Weis *et al.*, 1988) and *Gossypium* (Morgan, 1969), and of *Prunus persica* to silane (Porpiglia and Barden, 1980).

However, the influence of maturity does not always follow clear patterns in all species. For instance, it is generally accepted that greater leaf maturity increases ethylene-sensitivity (Burg, 1968; Goren *et al.*, 1988). However, there have been exceptions to this rule, such as for young unexpanded leaves of *Gossypium* and *Capsicum* where abscission response to ERCs is high and decreases as leaves cease expanding (Morgan, 1969; Beaudry and Kays, 1988a). The physiological and molecular basis for ethylene-sensitivity differences (due to organ type and maturity) are further reviewed and discussed in Chapter 4.

Temperature

Temperature is one of the most important environmental factors influencing the abscission response to applied ethephon (Olien and Bukovac, 1978). Application of ethephon at lower temperatures results in significantly lower organ abscission (Klein *et al.*, 1978; Sun and Martin, 1982; Jones and Koen, 1985). Temperature has two main influences on ethephon application: lower temperatures decrease ethylene release from ethephon by direct thermal action (Klein *et al.*, 1978) and also decreases tissue response to ethylene (Beaudry and Kays, 1988a).

The quantity of ethylene released from ethephon decreases when applied at lower temperatures to *Lycopersicon* plants (Lougheed and Franklin, 1972). The rate of ethylene evolution from ethephon-treated *Prunus* leaves is strongly dependent on temperature with an E_a value (see Section 3.3 for definition of E_a) of 125.6 $kJ \cdot mol^{-1}$ (Olien and Bukovac, 1978), a value similar to that obtained in a later study (E_a of 100.5 to 108.9 $kJ \cdot mol^{-1}$; Olien and Bukovac, 1982a). Comparable results have been obtained using buffered aqueous solutions of ethephon, E_a of 134.0 $kJ \cdot mol^{-1}$ (Olien and Bukovac, 1978) and 124.7 $kJ \cdot mol^{-1}$

(Biddle *et al.*, 1976). The increased ethylene release from ethephon brought about by increased temperature reduces further ethylene release by depleting the limited pool of applied ethephon (Lougheed and Franklin, 1972). By varying temperature, these authors established that ethylene production is a function of the residual ethephon. Similar work lead Olien and Bukovac (1978) to propose a half-life concept for ethylene release from ethephon, half-life being proportional to the inverse of temperature (5.6 days at 20°C and 26.5 hr at 30°C).

Temperature also influences the tissue response to ethylene and so causes added complications to modelling of the abscission response to ethephon application (Olien and Bukovac, 1978). Abscission at a given ethylene concentration will decrease progressively with lower non-stressing/damaging temperatures (Addicott, 1982). For instance, a temperature decrease from 23.5° to 16°C lowers ethylene-promoted leaf abscission of both *Fittonia* (9% to 0%) and *Philodendron* (28 to 9%) (Marousky, 1979; Marousky and Harbaugh, 1979c). Thus Addicott (1982) suggested a generalised Q_{10} of 2 for abscission, while Olien and Bukovac (1982b) obtained a Q_{10} (20° to 30°C) of 1.2 for ethylene-promoted abscission of *Prunus* fruit.

Relative humidity also has the potential to influence ethylene release after ethephon application. *In vitro*, both high and low RH or vapour pressure (VP) reduce ethylene release from ethephon, optimum RH (producing maximal ethylene release) appears to be between 30 and 70% and the optimal VP doubles with every 10°C increase (Klein *et al.*, 1978; 1979). However, only RH extremes (3 and 93%) reduce the effectiveness of ethephon in the field (Klein *et al.*, 1978). Since temperature is usually inversely related to RH (Porpiglia and Barden, 1980), the influence of temperature on RH should be borne in mind when applying ethephon.

Organ Sensitivity to Ethylene and Ethephon

The relative abscission-sensitivity difference between organs of a given plant is the basis for the practice of chemical thinning (Beaudry and Kays, 1987). Differential organ abscission may result from ethylene (Ismail, 1970; covered further in Chapter 4) or ethephon application. Ethephon application results in greater abscission of fruit than leaves of *Coffea* (Browning and Cannell, 1970), *Carya* (Wood, 1989), *Olea* (Ben-Tal and Lavee, 1976; Klein *et al.*, 1978), *Prunus avium* (Bukovac, 1979), *P. persica* (Daniell and Wilkinson, 1972; Perry and Gianfagna, 1987) and *Vitis* (Clore and Fay, 1970). Flowers exhibit greater

ethephon-sensitivity than leaves in *Malus* (Robitaille *et al.*, 1977; Jones *et al.*, 1989; Irving *et al.*, 1989), *Capsicum* (Tripp and Wien, 1989) and *Prunus persica* (Stembridge and Gambrell, 1971).

After ethephon application, greater abscission of one organ over another can be the result of greater sensitivity to released ethylene or a shorter duration of ethylene exposure required to promote abscission (Lang and Martin, 1989). Hence, relative organ sensitivities may vary with the ethylene source (ethylene or ethephon) (Weis *et al.*, 1988).

3.2 OBJECTIVES

It has been suggested that the time of ethephon application influenced the response of *Camellia* through temperature and tissue maturity effects (Woolf *et al.*, 1992; Chapter 2). Much research has been carried out applying either a range of ethephon or ethylene concentrations at one temperature, or a single concentration at a range of temperatures (Olien and Bukovac, 1978; Jones *et al.*, 1983; Jones and Koen, 1985; Beaudry and Kays, 1988a). However, information is lacking on the interaction between temperature and ethephon concentration and this is required for accurate modelling of ethephon application (Jones and Koen, 1985).

Thus, the influence of temperature, ethephon concentration, leaf maturity and the effect of ethylene gas on abscission was examined for three organs of *Camellia* with the ultimate aim of more efficiently removing floral buds with ethephon.

3.3 MATERIALS AND METHODS

Plant Material. *Camellia* 'Anticipation' plants were obtained mid-winter (1990) as rooted cuttings with one flush of growth completed from Duncan and Davies Nurseries Ltd, New Plymouth, N.Z.. Vegetative bud break was promoted using simulated summer conditions (15^{o} to 25^{o}C and continuous photoperiod obtained using 100W incandescent light bulbs; Section 1.2.2.1). Once stem extension had ceased and matured for 3 weeks, plants were potted into 1.75 liter plastic pots containing a peat and pumice (3:2 v/v) growing medium and amendments of dolomite (3 g·liter^{-1}), 3- to 4- and 8- to 9-month Osmocote^{R} (0.6 and 3 g·liter^{-1}

respectively; Sierra, N.Z.) and Micromax^{R} (0.9 g·liter^{-1}; Sierra, N.Z.). After chilling (3 weeks; 7^{o} ± 2^{o}C), plants were replaced in simulated summer conditions for promotion of a new flush of growth and flower initiation (Section 1.2.2.1 and 1.2.3.1). The resulting plants were approximately 60 cm high typically bearing 10 floral buds, 50 leaves and 15 vegetative buds.

Two concurrent experiments involving treatment of plants with either ethephon or ethylene were carried out at the Climate Laboratory, Horticulture and Crop Research Institute (formerly DSIR Fruit and Trees), Palmerston North, N.Z.. Plants treated with ethephon were placed in controlled environment rooms (for details refer to Warrington *et al.*, 1978). However, because of the possibility of ethylene contamination, ethylene treatment was carried out simultaneously in smaller growth cabinets (with similar environments) separate from the controlled environment rooms. Ethephon application in controlled environment rooms was carried out at four constant air temperatures (10^{o}, 16.7^{o}, 23.3^{o}, and 30^{o} all ± 0.5^{o}C) with RH of 70% ± 5% (corresponding to vapour pressure deficit (VPD) of 0.37, 0.57, 0.87, and 1.27 kPa respectively). Photoperiod consisted of 12 hr full light intensity (photosynthetic photon flux intensity of 700 ± 15 *u*mol·m^{-2}·s^{-1}) and 4 hr photoperiod extension (10 ± 1 *u*mol·m^{-2}·s^{-1}). Carbon dioxide concentration varied between 356 and 576 *u*l·liter^{-1}. Plants were acclimatized in the growth rooms for 10 days prior to ethephon treatment and watered by microtube irrigation once daily at 10^{o}C, twice at 23.3^{o} and 16.7^{o}C, and 3 times at 30^{o}C. Due to the possible effect of released ethylene (from ethephon) on plants in close proximity, ethephon treatments were blocked on trolleys and the trolleys randomly re-located twice-weekly within each growth room (Bussell and Halligan, 1982). Background ethylene was monitored and found to not exceed 0.020 *u*l·liter^{-1} using a Photovac^{R} gas chromatograph (photoionization detector, air carrier gas at 45 ml·min^{-1}, fitted with 15 cm precolumn, 1.8 m main column (type XE60), ambient temperature (approximately 23^{o}C), Alltech Associates NZ Ltd, Auckland, N.Z.).

Ethephon Treatment. Ethephon (Ethrel 48^{R}, Rhone-Poulenc Ltd, Wellington, N.Z.) was applied as an aqueous foliar spray at six concentrations (0, 500, 1000, 2000, 3000, and 4000 *u*l·liter^{-1} a.i.) containing Tween 20 (0.5% v/v). Ethephon was applied to runoff (approximately 20 ml/plant) as a spray using a 2-liter hand-held sprayer to 10 whole plant replicates on May 7 1991 (late autumn). Since temperature and RH influence drying time of sprays (Kays and Beaudry, 1987),

application of ethephon was carried out outside the facility (12^{o}C), foliage dried in the laboratory for 45 mins (21^{o}C, 60% RH) and plants were then placed in the various temperature treatments.

Effect of Leaf Maturity. In previous experiments (Experiment 2.1 and 2.2), a trend of greater ethylene-sensitivity to applied ethephon with increasing leaf maturity was obtained (Figure 2.5). However, differences were not significant, due possibly to lower replication, and did not include second flush shoots consisting of extending stem and young expanding leaves which are often present on *Camellia* plants in late summer/early autumn (M.A. Scott, pers. com.). Thus, at one temperature only (16.7^{o}C), the effect of leaf maturity (within a plant) on ethephon-sensitivity was examined by labelling stem maturities (and thus leaves) in 4 categories; second flush (extending shoots and shoots which had ceased extending in less than 2 weeks), 3-month- (current summer shoots bearing floral buds), 1-year- and 2- to 3-year-old leaves (see Section 2.3).

Flow-through Ethylene Gas Treatment. The effect of temperature on rate of ethylene-promoted abscission was examined by applying ethylene gas in a flow-through system. Ten whole-plant replicates were individually sealed in 15 liter clear polyethylene bags (35 *u*m) and pure ethylene mixed with humidified air to attain 10 *u*l·liter^{-1} at a flow rate of 30 liter·hr^{-1} for each bag (2 air changes/hr). Plants were placed in controlled temperature cabinets to result in air temperature within bags of 10^{o}, 16.7^{o}, 23.3^{o}, and 30^{o} $\pm$ 2^{o}C with continuous light (720 *u*mol·m^{-2}·s^{-1}). Oxygen and CO_2 concentrations were monitored and found to remain at ambient levels in all treatments. Although not measured, RH was maintained at a high level (>90%) by bubbling incoming air through a barostat, thus humidifying the air-flow prior to mixing with ethylene before passage through the treatment bags. Air temperature and ethylene concentration were monitored inside the bags using a datalogger and Photovac^{R} gas chromatograph.

Measurements. The number of floral and vegetative buds and leaves were counted before the application of ethephon and ethylene treatments, and at appropriate intervals depending on abscission rate. Each temperature treatment was terminated when abscission rate of treated plants dropped to that of the controls. As discussed previously (Section 2.4), vegetative buds damaged or killed following abscission of bud bracts were regarded as abscised for the purposes of this experiment.

Second flush shoots were labelled and, with the exception of extending second flush shoots used to analyze the effect of leaf maturity on ethephon-sensitivity (at 16.7^{o}C), were excluded from analysis. This was carried out because such shoots would result in excessive statistical variability since they were present on only one third of the plants, and, because previous experiments (Chapter 4) revealed abscission characteristics greatly differing from those of organs on matured shoots. Low SE values (maximum SE for final abscission of floral and vegetative buds and leaves of 10.1%, 7.7%, and 5.8% respectively) indicated that the presence of extending shoots did not physiologically alter abscission of organs on other shoot maturities.

Statistical Analysis. Final abscission proportion (extent of abscission) was calculated as total abscission over the experimental period expressed as a percentage of the original organ number and was plotted in 3-dimensional form (temperature *x* ethephon concentration *x* final abscission proportion (%)) (Figure 3.1 to 3.3). A Q_{10} value (ratio of final abscission) between 20^{o} and 30^{o}C was calculated and the absolute increase in abscission over the temperature range of 10^{o} to 30^{o}C also included (Table 3.1). Using final abscission proportion data, ethephon concentration was regressed linearly against temperature to obtain slope, slope SE and correlation coefficient (r^2) over the temperature range 10^{o} to 30^{o}C (Table 3.2). Analysis of the interaction effects between ethephon concentration and temperature on final abscission proportion were carried out using SAS. A non-parametric analysis was employed such that final abscission proportion data were ranked and an analysis of variance carried out for each organ.

The rate of abscission was calculated as days to 50% of the final abscission proportion, and converted to 1/time to 50% abscission. Where no abscission occurred, rate was expressed as the experimental end point. Rate was plotted in 3-dimensional form (temperature *x* ethephon concentration *x* abscission rate (1/time to 50% abscission)) (Figure 3.4 to 3.6). The effect of temperature on abscission rate was also expressed by calculation of E_a (activation energy) (Table 3.3). This was carried out by plotting the natural log of 1/rate vs 1/K^{o}) and linear regression fitted to yield slope, slope SE and correlation coefficient (r^2). According to the Arrhenius equation $v = Ae^{Ea/RT}$, where v is the rate of reaction (in this case 1/time to 50% abscission), R is the universal gas constant and T the absolute temperature (K^{o}). Thus activation energy (E_a) and SE can be derived from slope and SE of fitted lines since E_a = slope *x* -R (Field, 1981a).

3.4 RESULTS

Temperature and ethephon concentration had significant effects on abscission of *Camellia* organs, higher temperatures and increased ethephon concentrations resulting in greater extent and rate of abscission (Figure 3.1 to 3.6). Leaf maturity also had significant influence on the abscission response.

For a given temperature, increasing ethephon concentration promoted greater final abscission proportion of all organs of *Camellia* (Figure 3.1 to 3.3). Abscission rate (1/time to 50% abscission) also increased with ethephon concentration (Figures 3.4 to 3.6).

Temperature influenced ethephon- and ethylene-promoted abscission of all organs of *Camellia.* The effect of temperature on natural (i.e. untreated tissue) final abscission proportion of *Camellia* organs was minimal compared to its influence on ethephon and ethylene treatment. No abscission occurred in untreated vegetative buds and over an increase of 20^{o}C (10^{o} to 30^{o}C) natural abscission increased only marginally (2% for floral buds and 3% for leaves) (Table 3.1).

Temperature had a strong influence on final abscission proportion of ethephon treated organs (Figure 3.1 to 3.3). Slope of regression lines fitted to final abscission proportion data (Table 3.2) were positive for all organs at all ethephon concentrations indicating that higher temperatures resulted in increased abscission in response to applied ethephon. At an ethephon concentration of 4000 *u*l·liter^{-1} an increase of 20^{o}C (10^{o} to 30^{o}C) resulted in increased final abscission proportion for floral buds of 9.0% (91.0 to 100%), leaves of 66.8% (11.6% to 78.4%), and vegetative buds of 47.7% (0.7% to 48.4%). Thus maximum Q_{10} (20^{o} to 30^{o}C) values were 1.39, 2.3, and 2.3 for floral buds, leaves and vegetative buds respectively (Table 3.1). Floral buds reached final abscission proportion levels of >90% in 11 of the 24 treatments while leaves only reached a maximum response of 78%, and vegetative buds 48% in the highest temperature/ethephon concentration treatment. Increased temperatures also promoted greater abscission rate of all organs at all ethephon concentrations (Figure 3.4 to 3.6). Abscission rate of floral buds did not reach a response-plateau as found in final abscission proportion of floral buds (Figure 3.1 cf 3.4).

Increased ethephon concentration and temperature resulted in greater final and faster rate of abscission with peak abscission occurring at the highest temperature and ethephon concentration (30°C, 4000 ul·liter^{-1}). The interaction between temperature and ethephon concentration was analysed for each of the three organs of *Camellia*. No interaction was found between temperature and ethephon concentration in any organ.

Efficient thinning requires maximum difference in abscission response between the target and non-target organs (Beaudry and Kays, 1987). The difference between floral buds and leaves (Figure 3.7) and floral buds and vegetative buds (Figure 3.8) were calculated by subtraction of final abscission proportion for each concentration/temperature treatment. Temperature markedly affected the ethephon concentration which brought about maximum abscission difference. Greatest difference was found at the lowest temperature (10°C) and highest ethephon concentration (4000 ul·liter^{-1}) for both floral bud/leaf and floral bud/vegetative bud comparisons. The ethephon concentration resulting in the greatest abscission differential was inversely related to temperature between 10° and 30°C. For each of the temperature treatments applied, the ethephon concentrations which brought about maximal differential abscission were 1000 ul·liter^{-1} at 30°C; 1000 to 2000 ul·liter^{-1} at 23.3°C; 2000 to 4000 ul·liter^{-1} at 16.7°C; and 3000 to 4000 ul·liter^{-1} at 10°C. These ethephon concentrations applied to final abscission proportion differences between both floral buds/leaves (Figure 3.7) and floral buds/vegetative buds (Figure 3.8).

The final abscission proportion trend of organ sensitivity to applied ethephon was found to be that floral buds were most sensitive, leaves intermediate, and vegetative buds least sensitive. This trend held at all concentration and temperature combinations for final abscission proportion (Figure 3.1 to 3.3, 3.7 and 3.8). Rate of abscission (1/time to 50% abscission) of all organs followed a similar trend to that of final abscission proportion (Figure 3.4 to 3.6 cf 3.1 to 3.3). Abscission rate of floral buds and leaves were both greater than vegetative buds (Figure 3.4 to 3.6 and 3.10). However, in contrast to final abscission proportion, abscission rate of floral buds and leaves differed little in response to ethephon and temperature (Figure 3.9 cf 3.7). After application of ethylene gas, leaves and floral buds had a greater abscission rate than vegetative buds at all temperatures. At low temperatures (10°C), abscission rate of leaves and floral buds did not differ, but with increased temperature, the difference in abscission

rate increased (Figure 3.11). This is similar to the results for ethephon application where rate of leaf abscission was slightly greater than that of floral buds at higher temperatures and ethephon concentrations (Figure 3.9).

Higher temperature increased the final abscission response of all organs, but the effect was different for each organ. Slope of regression lines fitted to final abscission proportion data (Table 3.2) revealed that floral buds were influenced more by temperature than were leaves and vegetative buds up to the point where 100% abscission of floral buds occurred (2000 to 4000 $\mu l \cdot liter^{-1}$). This trend of temperature/organ sensitivity was found to hold true at all temperature/ethephon concentration combinations (apart from the above mentioned exception). Higher temperature increased final leaf abscission more than abscission of vegetative buds (Table 3.2).

Measurement of abscission using the parameter of abscission rate likewise indicated differential influence of temperature on ethephon-promoted abscission of organs. However, the influence of temperature on organ response determined by abscission rate was different to that of final abscission proportion data. Abscission rate for leaves was increased more by higher temperatures than that for floral buds after ethephon application (E_a values 91.1 to 96.5 cf 53.2 to 67.0 $kJ \cdot mol^{-1}$ respectively; Table 3.3), whereas the E_a of floral and vegetative buds were similar (53.2 to 67.0 cf 47.3 to 66.7 $kJ \cdot mol^{-1}$ respectively).

The influence of temperature on abscission rate after application of ethylene gas was different from that of ethephon application. In this case, higher temperature increased leaf abscission by the greatest amount, floral buds were intermediate, and vegetative buds least influenced (E_a values 54.2, 45.1, and 24.1 $kJ \cdot mol^{-1}$ respectively; Figure 3.11; Table 3.3).

The effect of temperature on ethylene promoted abscission could be separated from its influence on ethephon-promoted abscission by comparison of the slope of fitted regression lines to Arrhenius plots of abscission rate (E_a). E_a of each organ remained the same for all ethephon concentrations, with the exception of control treatments (0 $\mu l \cdot liter^{-1}$) and the 500 $\mu l \cdot liter^{-1}$ treatment of vegetative buds (Table 3.3). However, for each organ the E_a of ethylene treatment was significantly less than that of ethephon treatment (Table 3.4).

Natural and ethephon-promoted final abscission responses of *Camellia* leaves were influenced by leaf maturity at 16.7°C (Figure 3.12). Natural leaf abscission increased with leaf maturity (final abscission proportion of 0, 1.6, 11.2, and 11.7% for second flush (extending shoots), 3-month-, 1-year- and 2- to 3-year-old leaves respectively) (Figure 3.12). For ethephon-treated shoots, leaves on extending shoots were more sensitive to applied ethephon than all other leaf maturities (3-month-, 1-year- and 2- to 3-year-old) reaching 100% final abscission at 2000 ul·liter^{-1} ethephon. Leaves that had expanded and hardened (3-month-old) were least sensitive to applied ethephon. As leaves subsequently matured from year to year they became progressively more sensitive; 9%, 25% and 63% final abscission proportion for 3-month-, 1-year-, and 2- to 3-year-old respectively (2000 ul·liter^{-1}).

Organ type	Ethephon-concentration (*ul*·liter^{-1})											
	0		500		1000		2000		3000		4000	
	Q_{10}	%Abs	Q_{10}	%Abs	Q_{10}	%Abs	Q_{10}	%Abs	Q_{10}	%Abs	Q_{10}	%Abs
Fl. buds	1.25	2	1.30	14	1.39	55	1.20	40	1.06	13	1.03	9
Leaves	1.2	3	2.3	8	1.86	25	2.11	57	1.85	63	1.83	67
Veg. buds	0.0	0	0.0	0	2.30	13	1.84	30	1.96	45	1.87	48

Table 3.1. Effect of temperature (10^{o} to 30^{o}C) on Q_{10} and absolute change in final abscission proportion of three *Camellia* organs treated with ethephon (0 to 4000 *ul*·liter^{-1}). Q_{10} value (20^{o} to 30^{o}C) calculated from fitted regression lines to temperature range 10^{o} to 30^{o}C. Percent value (%Abs) is the absolute increase in abscission over the temperature range 10^{o} to 30^{o}C. Floral buds (Fl. buds), leaves (Leaves) and vegetative buds (Veg. buds).

Organ type	Ethephon concentration (*ul*·liter^{-1})											
	0		500		1000		2000		3000		4000	
	Slope		Slope		Slope		Slope		Slope		Slope	
	r^2	SE	r^2	SE	r^2	SE	r^2	SE	r^2	SE	r^2	SE
Fl. buds	0.11[1]		1.40		2.72		0.88		0.86		0.41	
	0.65[2]	0.05[3]	0.66	0.71	0.72	1.2	0.79	0.69	0.86	0.23	0.63	0.2
Leaves	0.11		0.84		1.18		2.95		3.25		3.42	
	0.10	0.23	0.74	0.35	0.98	0.13	0.98	0.28	0.98	0.36	0.98	0.3
Veg. buds	0		0.02		0.62		1.58		2.26		2.36	
	1.00	0	0.06	0.05	0.91	0.14	0.88	0.41	0.98	0.22	0.99	0.2

Table 3.2. Effect of temperature (10^{o} to 30^{o}C) on linear regressions produced [1]line slope, [2]slope SE, and [3]correlation coefficient (r^2) of final abscission proportion of three *Camellia* organs; floral buds (Fl. buds), leaves (Leaves) and vegetative buds (Veg. buds) treated with ethephon (0 to 4000 *ul*·liter^{-1}).

Organ type	Ethephon concentration (*ul*·liter^{-1}) 0	500	1000	2000	3000	4000	Ethylene 10 *ul*·liter^{-1}
	E_a r^2 SE	E_a r^2 SE	E_a r^2 SE	E_a r^2 SE	E_a r^2 SE	E_a r^2 SE	E_a r^2 SE
Fl. buds	0[1]	53.2	62.4	60.2	56.9	67.0	45.1
	1.0[2] 0[3]	0.98 5.1	0.94 11.0	0.97 7.9	0.98 6.2	0.98 7.4	0.93 8.5
Leaves	0	94.1	96.5	93.1	91.1	94.6	54.2
	1.0 0	0.93 18.6	0.92 20.2	1.00 3.8	0.96 13.8	0.94 16.2	0.91 12.3
Veg. buds	0	0.00	47.3	62.5	66.7	58.5	24.1
	1.0 0	1.00 0.0	0.89 12.0	0.90 14.9	0.95 11.0	0.97 7.8	0.78 8.9

Table 3.3. E_a (kJ·mol^{-1}) derived from abscission rate (1/time to 50% final abscission) after ethephon (0 to 4000 *ul*·liter^{-1}) and ethylene (10 *ul*·liter^{-1}) treatment at temperatures of 10° to 30°C of three *Camellia* organs, floral buds (Fl. buds), leaves (Leaves) and vegetative buds (Veg. buds). Abscission rate data was graphed as Arrhenius plots and linear regressions used to calculate [1]E_a, (line slope), [3]SE of E_a, and [2]correlation coefficient (r^2).

Organ type	Ethephon E_a	Ethylene E_a	Ethylene/Ethephon *x* 100 %
Fl. buds	59.9	45.1	75
Leaves	93.9	54.2	58
Veg. buds	58.8	24.1	41

Table 3.4. Proportion of temperature effect on ethephon-promoted abscission rate due to influence of temperature on ethylene-promoted abscission rate. Mean E_a (kJ·mol^{-1}) calculated from Table 3.3 (zero values excluded). Floral buds (Fl. buds), leaves (Leaves) and vegetative buds (Veg. buds).

Floral buds
Final abscission proportion

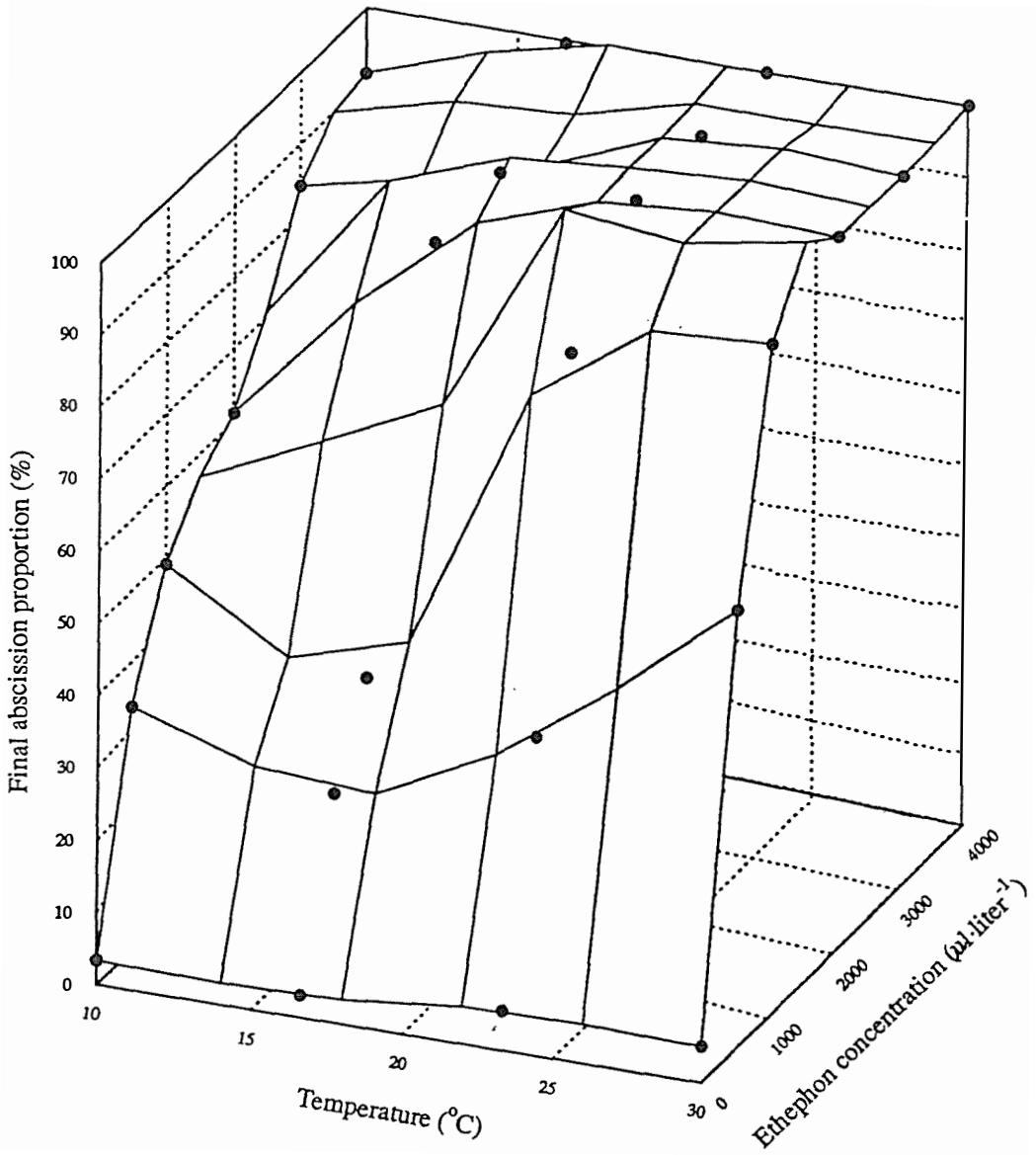


Figure 3.1. Effect of temperature and ethephon concentration on final abscission proportion (%) of floral buds of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991.

Leaves
Final abscission proportion

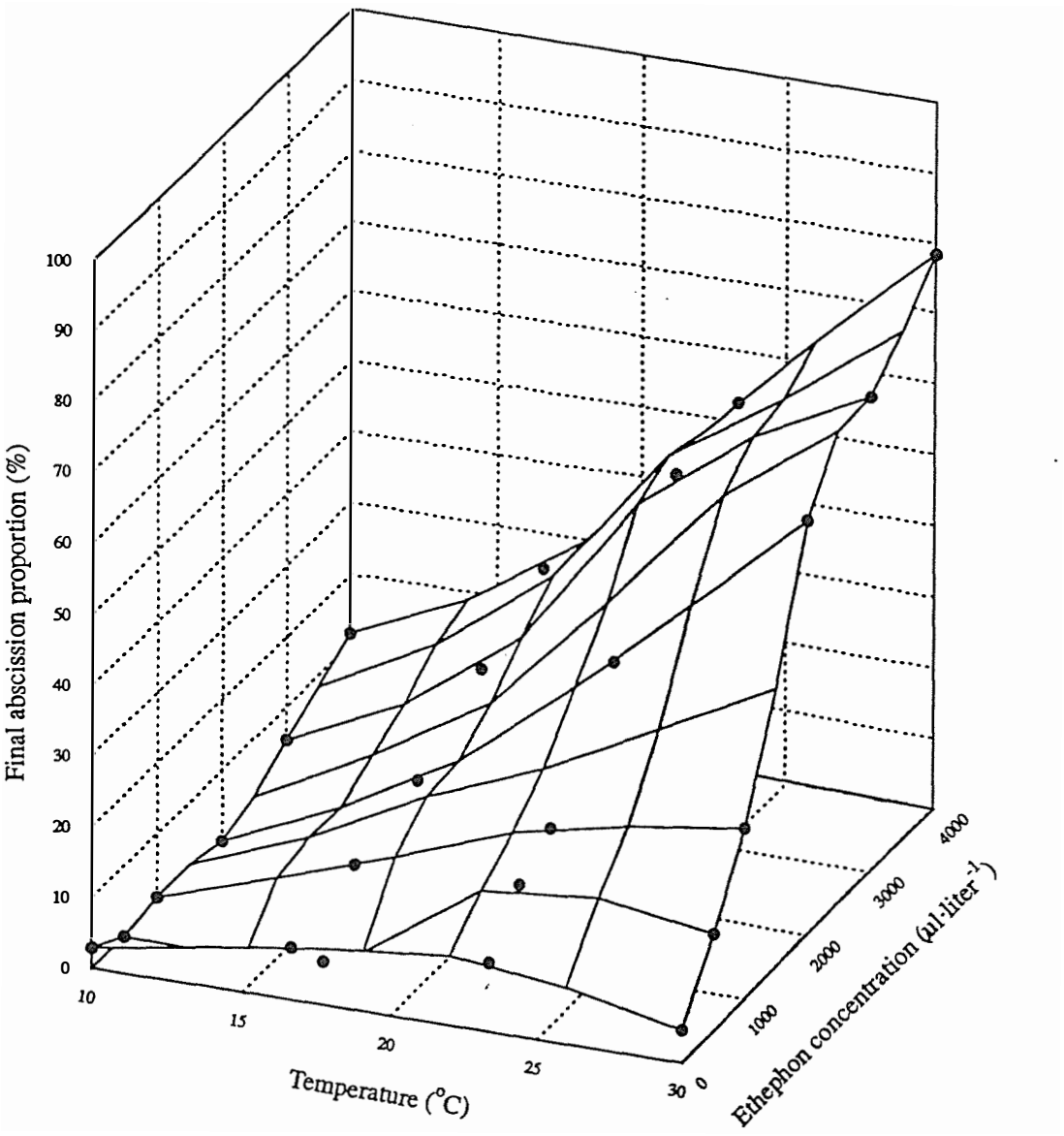


Figure 3.2. Effect of temperature and ethephon concentration on final abscission proportion (%) of leaves of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991.

Vegetative buds
Final abscission proportion

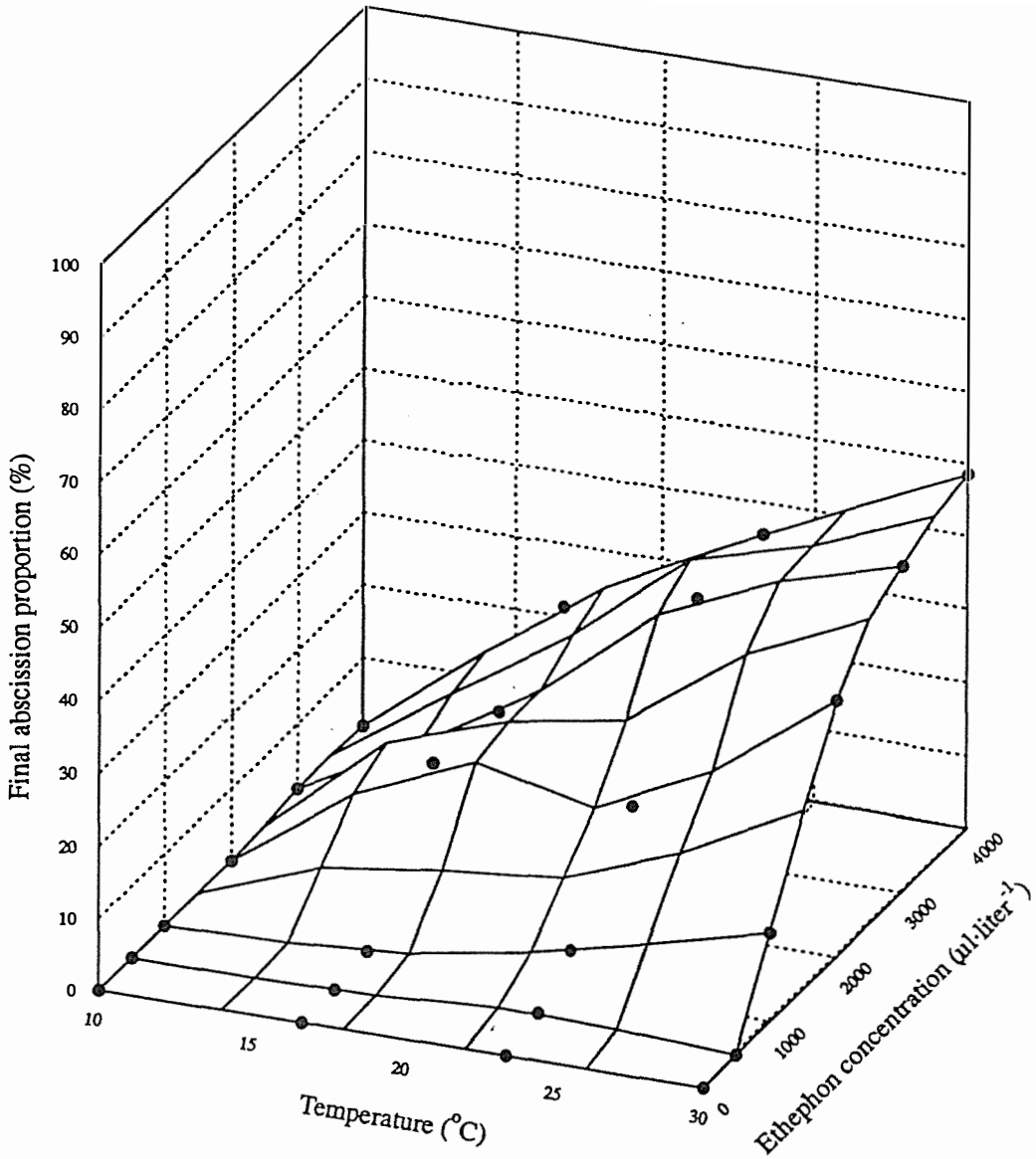


Figure 3.3. Effect of temperature and ethephon concentration on final abscission proportion (%) of vegetative buds of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991.

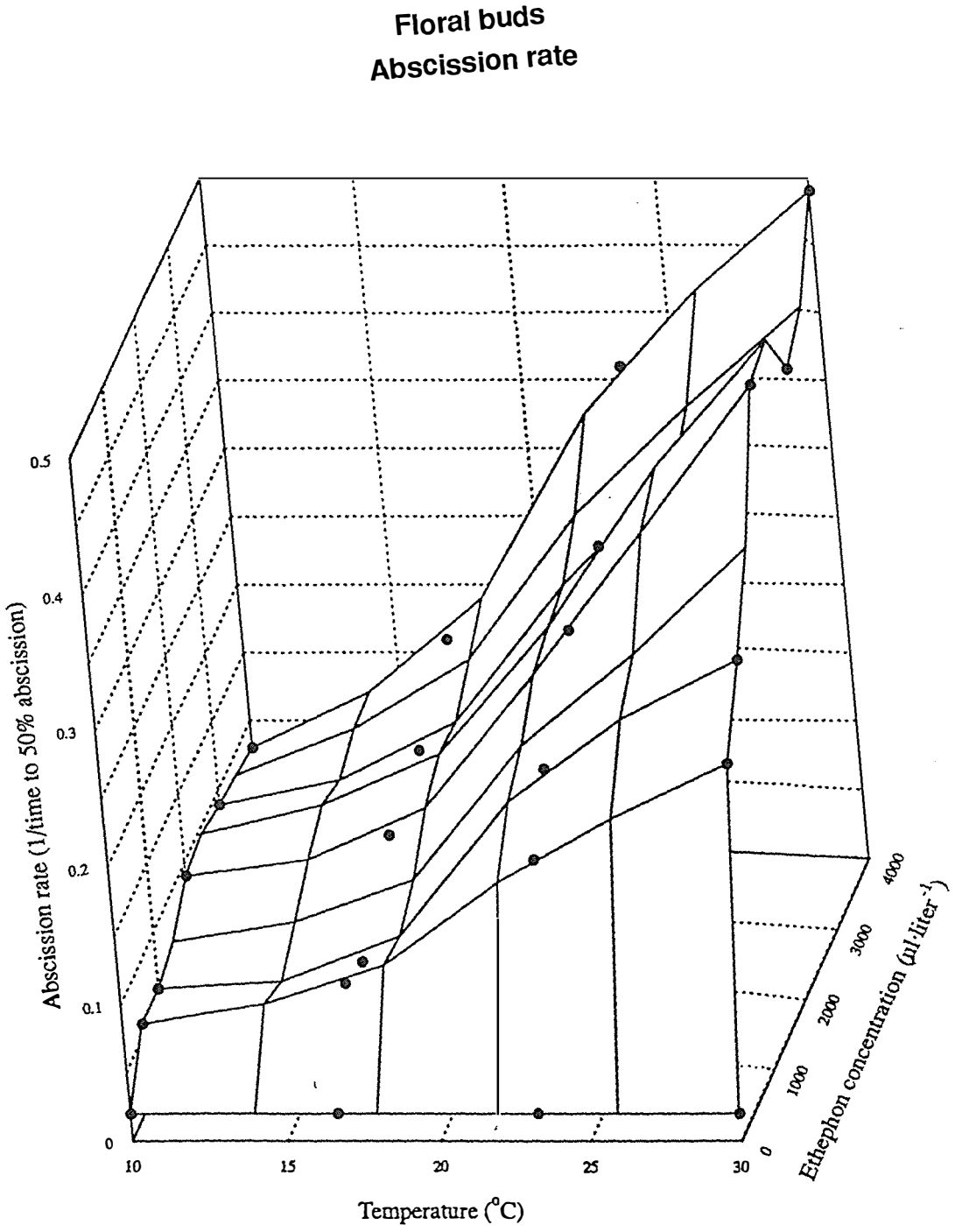


Figure 3.4. Effect of temperature and ethephon concentration on abscission rate (1/days to 50% final abscission) of floral buds of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991.

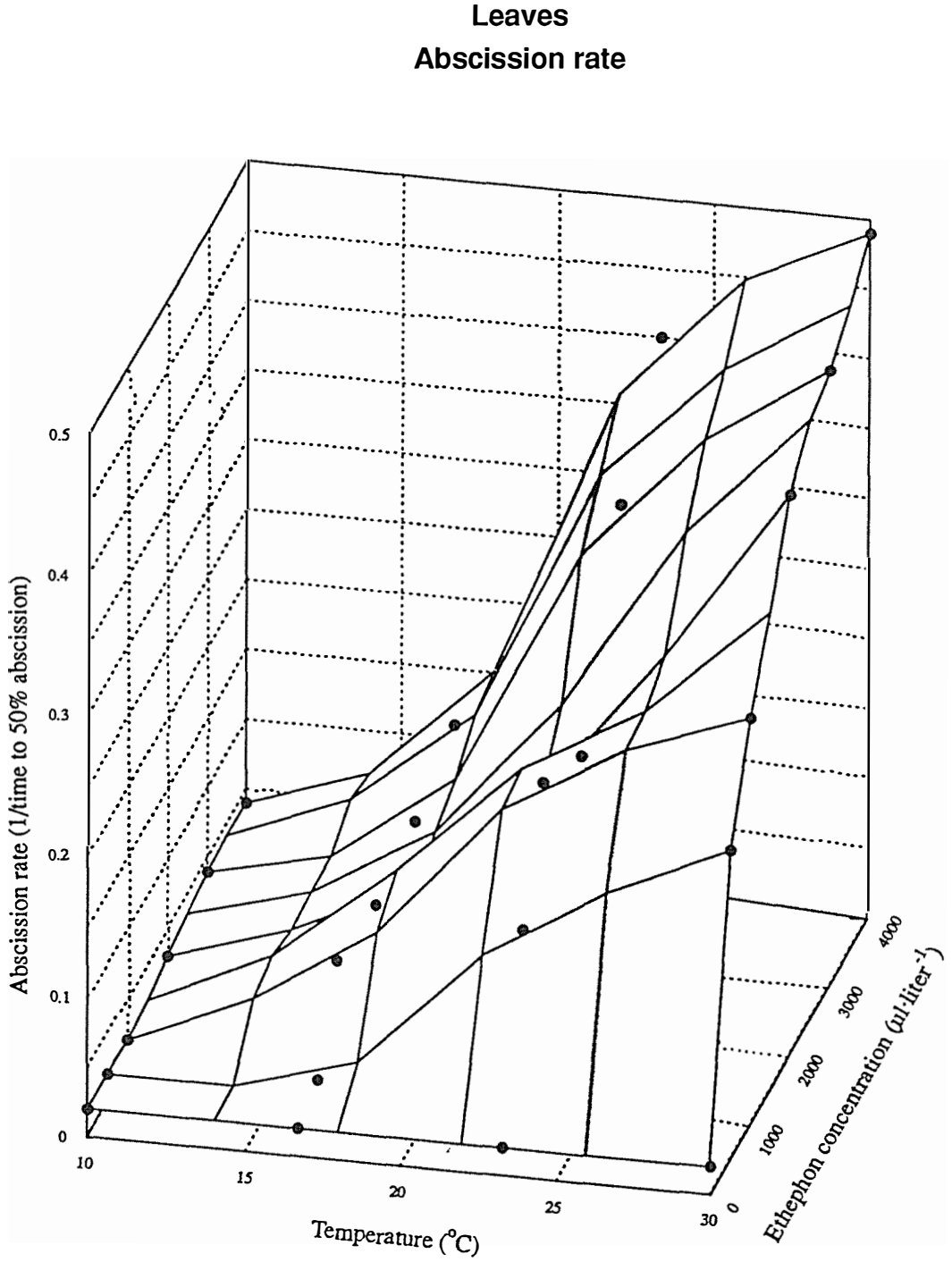


Figure 3.5. Effect of temperature and ethephon concentration on abscission rate (1/days to 50% final abscission) of leaves of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991.

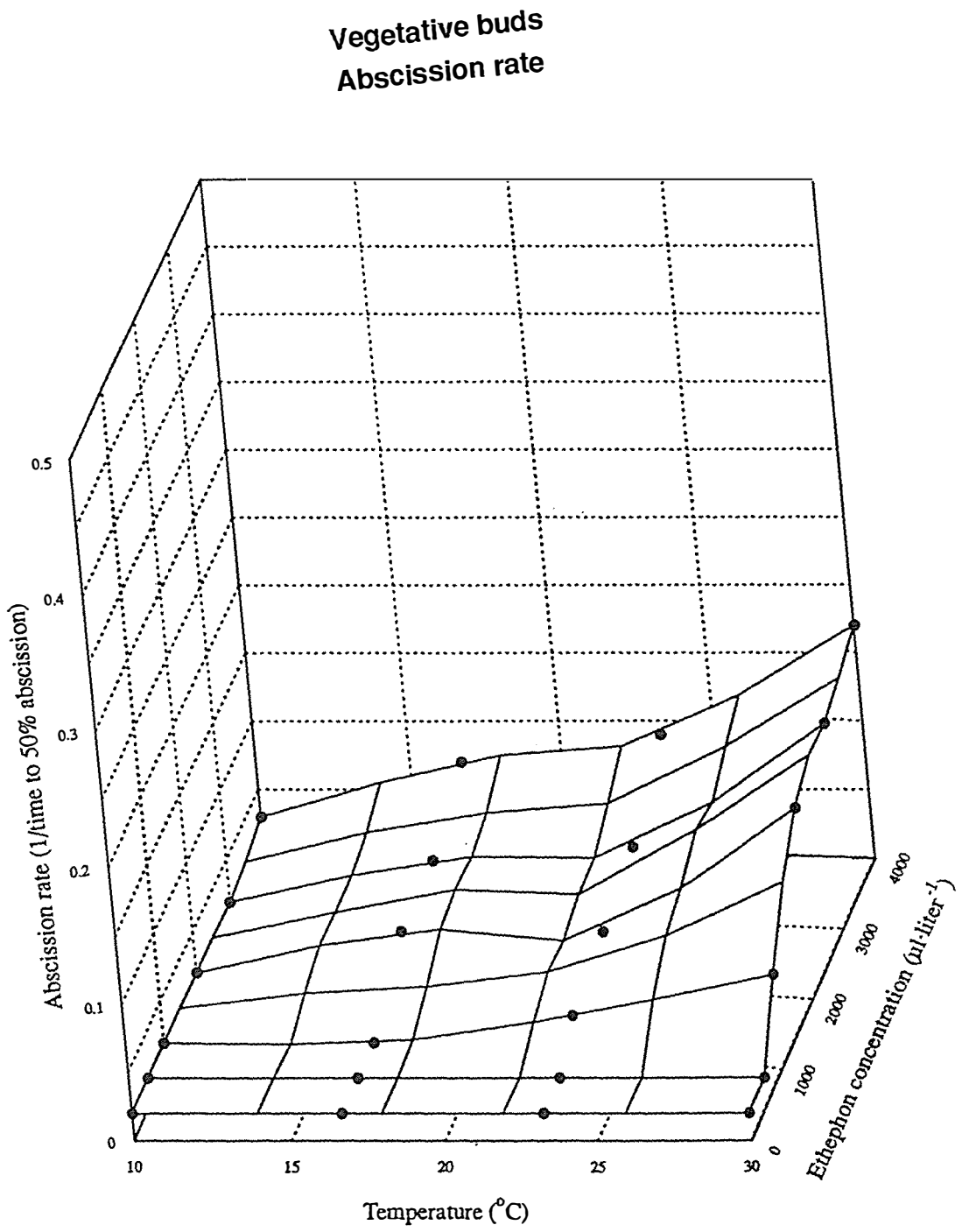


Figure 3.6. Effect of temperature and ethephon concentration on abscission rate (1/days to 50% final abscission) of vegetative buds of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991.

Floral bud/leaf difference
Final abscission proportion

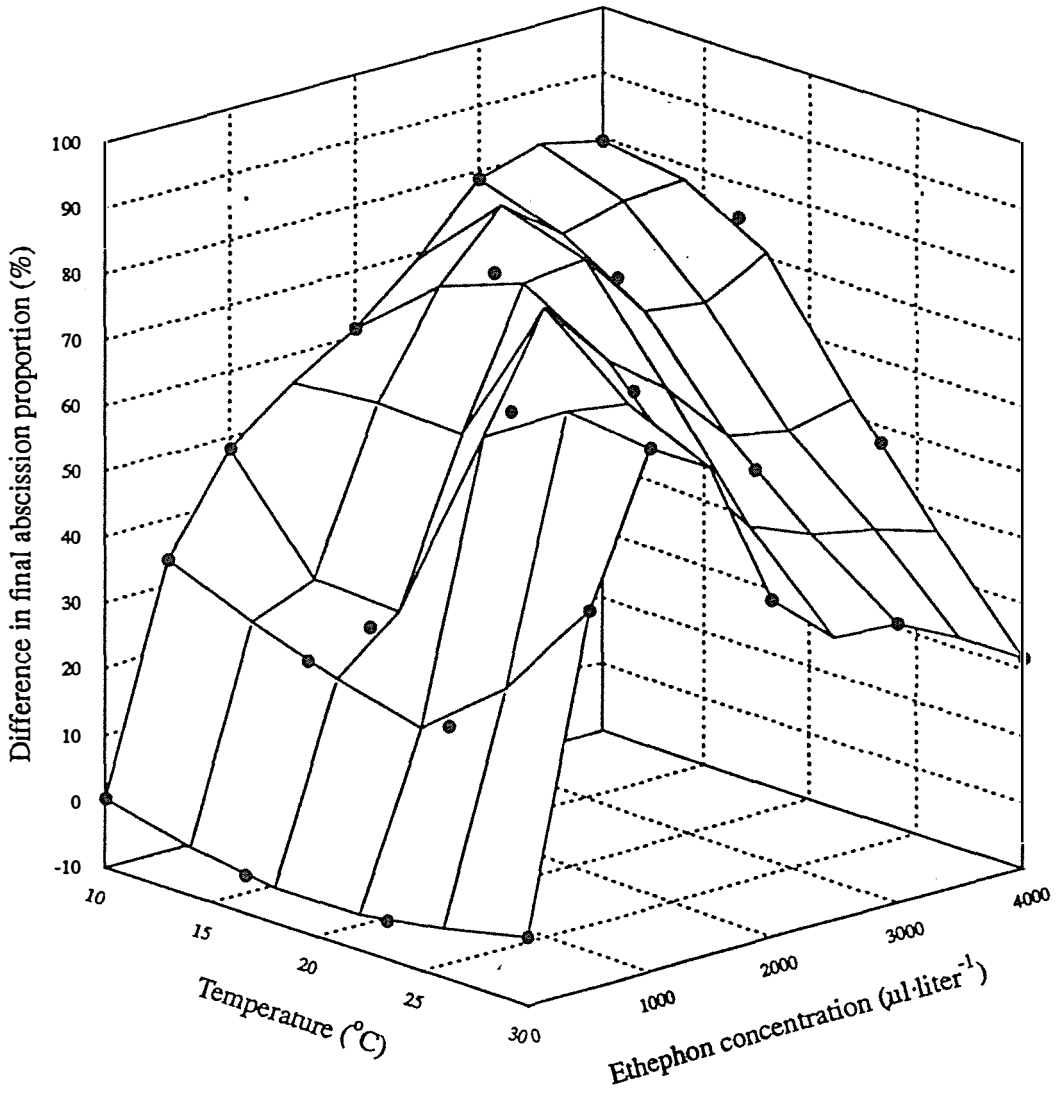


Figure 3.7. Effect of temperature and ethephon concentration on difference between final abscission proportion (%) of floral buds and leaves of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991.

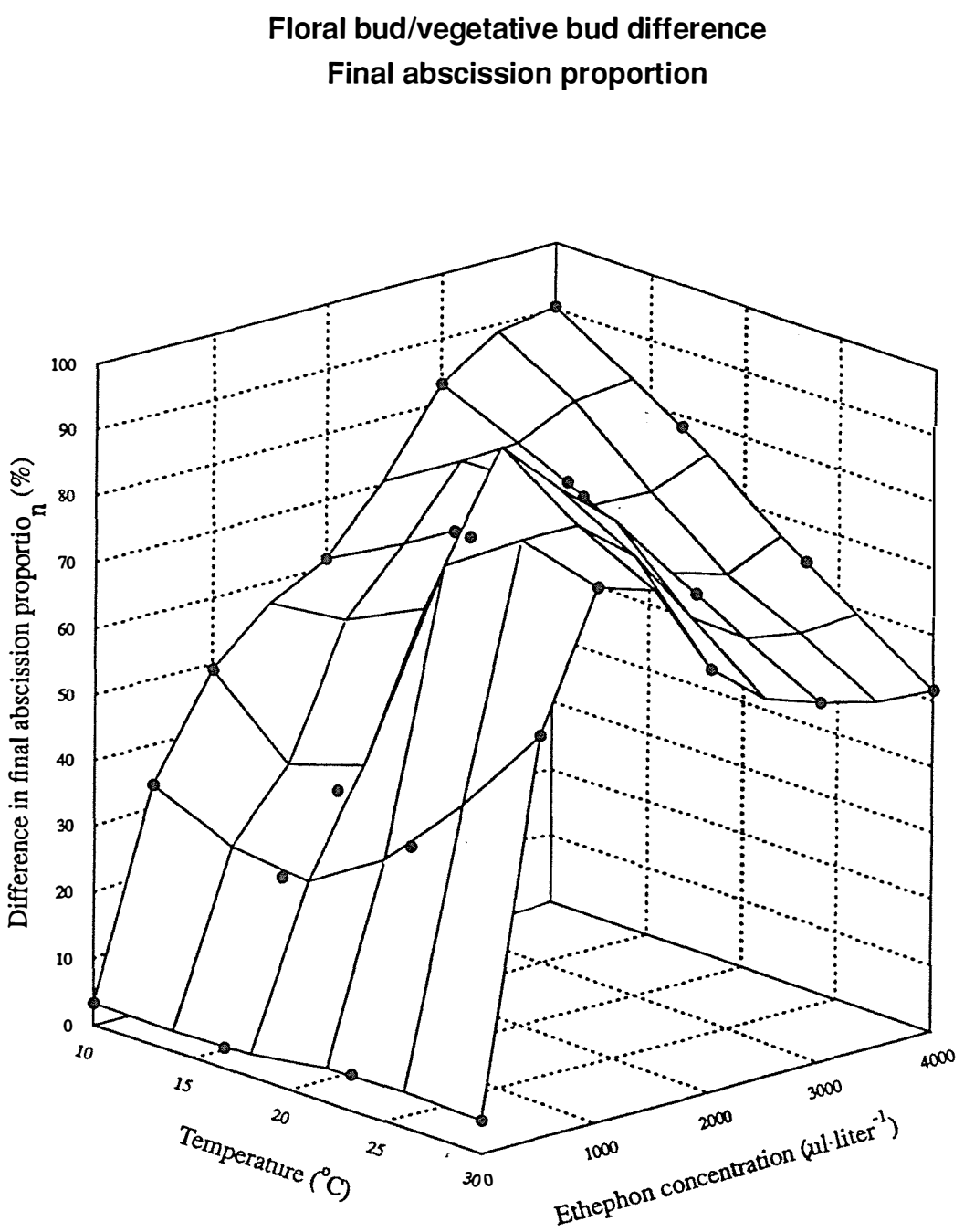


Figure 3.8. Effect of temperature and ethephon concentration on difference between final abscission proportion (%) of floral buds and vegetative buds of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991.

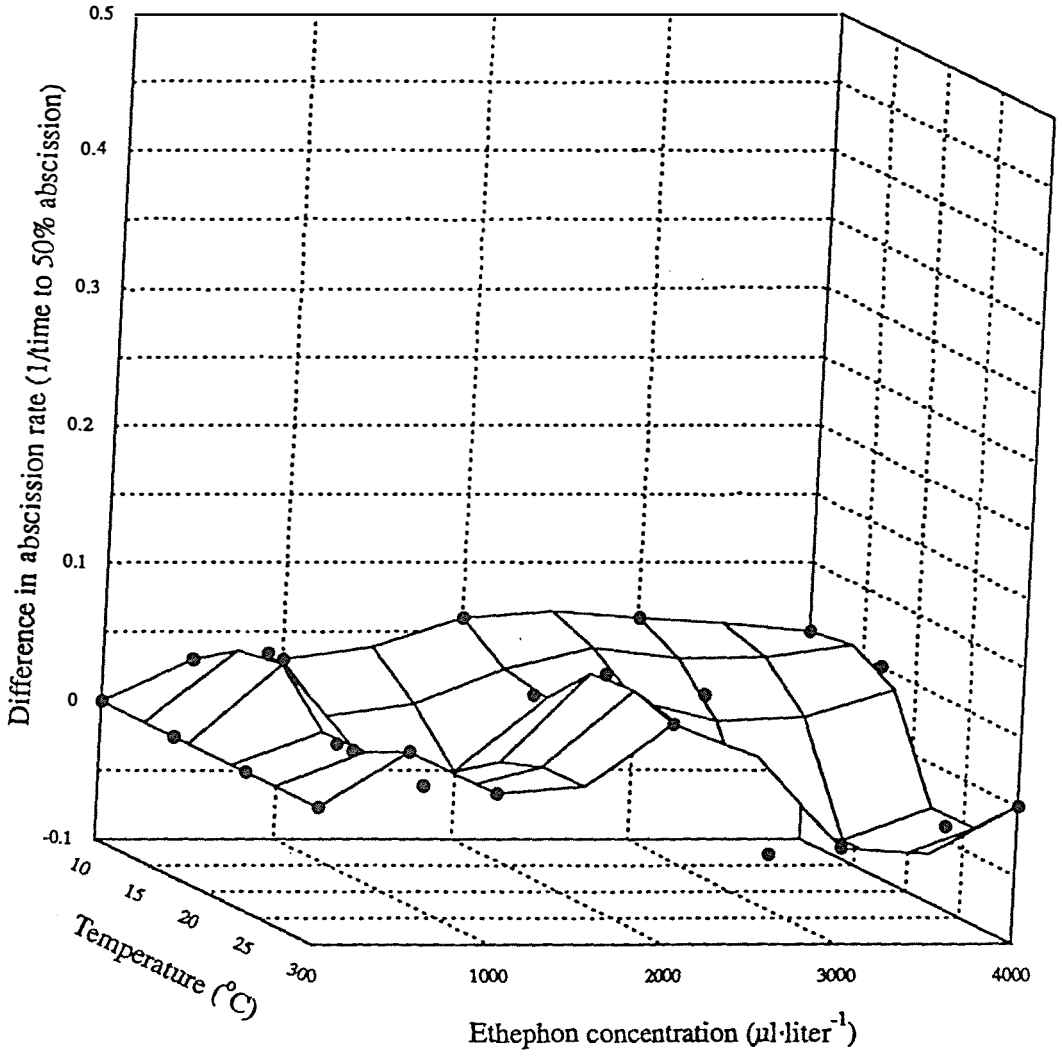


Figure 3.9. Effect of temperature and ethephon concentration on difference between abscission rate (1/days to 50% final abscission) of floral buds and leaves of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991.

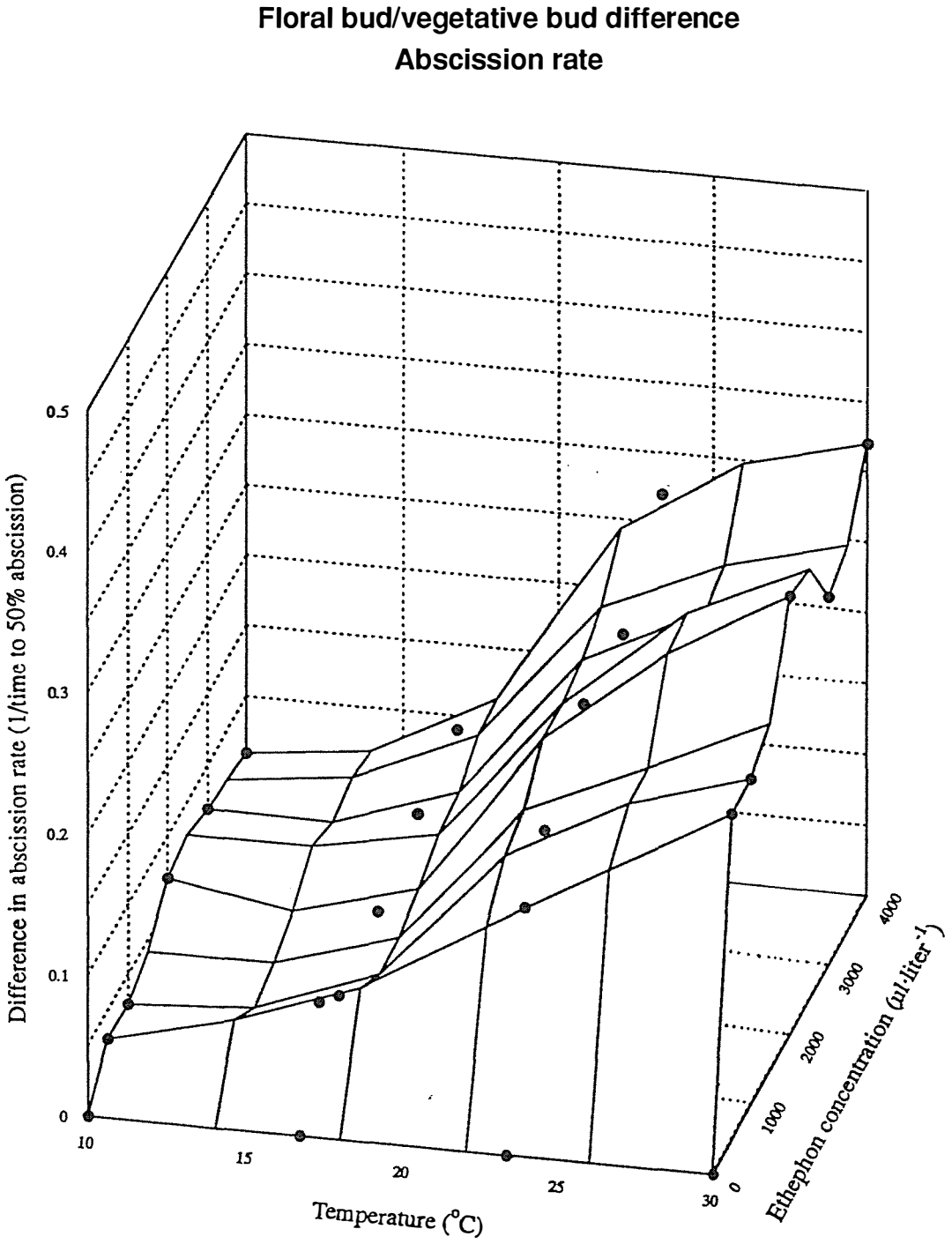


Figure 3.10. Effect of temperature and ethephon concentration on difference between abscission rate (1/days to 50% final abscission) of floral buds and vegetative buds of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991.

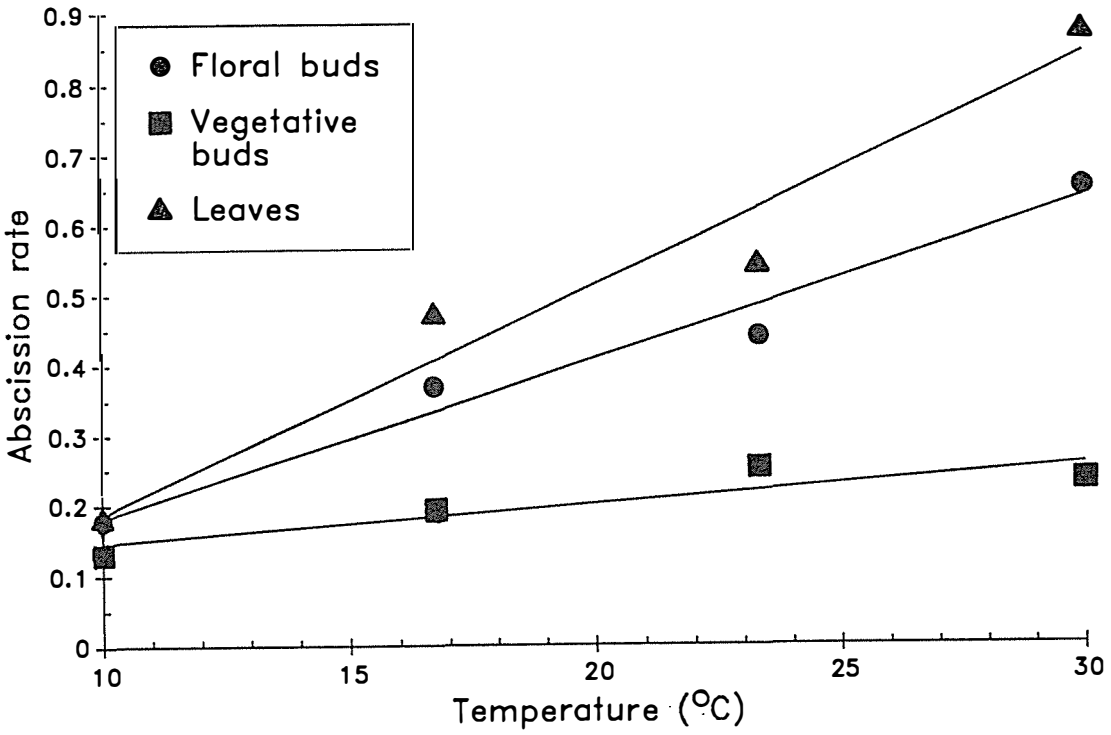


Figure 3.11. Effect of temperature on abscission rate (mean 1/days to 50% final abscission of 10 plants) of 3 organs (floral buds, vegetative buds and leaves) of *Camellia* 'Anticipation' after ethylene application (10 *u*l·liter^{-1}) on 7 May 1991. Effect of temperature on mean abscission rate of floral buds, vegetative buds and leaves described by Y = -0.0469 + 0.0227X, r^2 = 0.97; Y = 0.0920 + 0.0054X, r^2 = 0.66; Y = -0.1396 + 0.0326X, r^2 = 0.95, respectively.

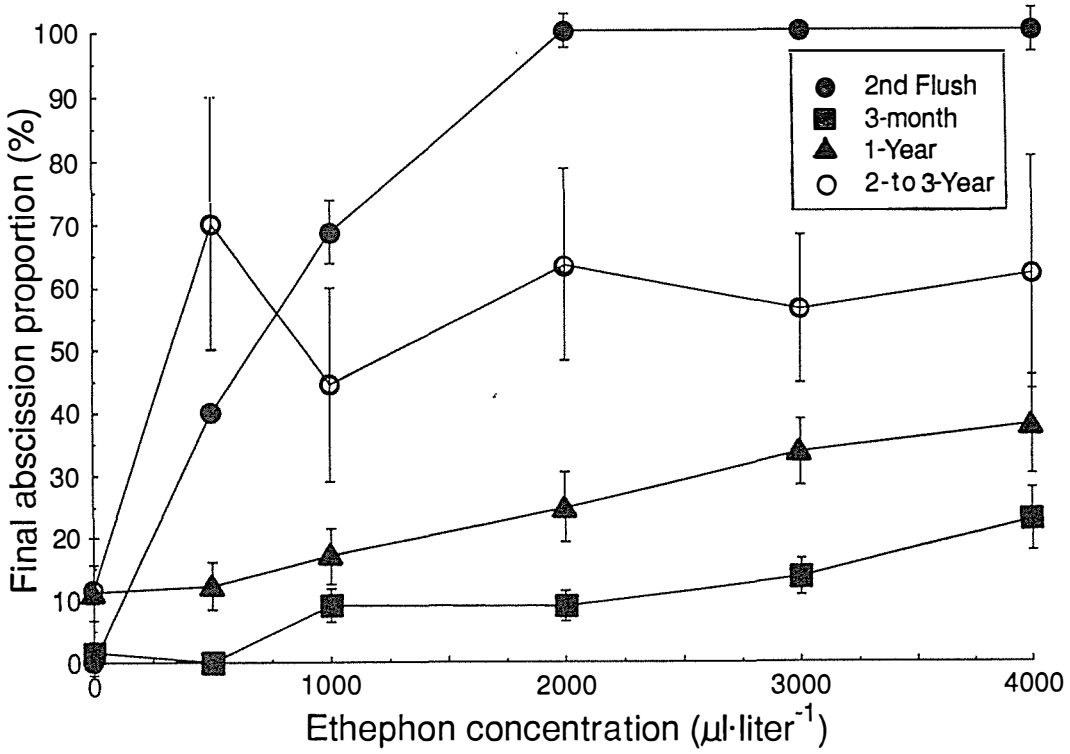


Figure 3.12. Effect of ethephon concentration on final abscission proportion (%) of 4 leaf maturities of *Camellia* 'Anticipation' after ethephon application at 16.7°C on 7 May 1991. Vertical bars represent SE of the mean.

3.5 DISCUSSION

Increased ethephon concentration promoted greater final abscission proportion of all organs of *Camellia* confirming earlier results (Woolf *et al.*, 1992; Chapter 2). Abscission rate also increased with greater ethephon concentration. Both increased rate and extent of floral bud abscission in response to higher ethephon concentrations has also been demonstrated in *Camellia japonica* (Lee and Song, 1992). It is suggested that the mechanism involved in increased abscission response to increased ethephon concentration is that more ethylene is released over a longer time-period and hence ethylene concentrations are elevated and longer durations of ethylene exposure occur at the abscission zones.

Temperature

Temperature influenced all three areas of abscission examined; natural abscission (untreated tissue), ethephon- and ethylene-promoted abscission.

In the absence of ethephon application, the effect of increased temperature (10^{o} to 30^{o}C) on natural abscission was low in floral buds and leaves and non-existent in vegetative buds (Figure 3.1 to 3.3). Abscission was higher in leaves than floral buds at most temperatures, particularly 23.3^{o}C (Figure 3.1 and 3.2) and leaf abscission increased slightly more with higher temperatures than floral buds over the temperature range of 10^{o} to 30^{o}C (Table 3.1). Increased floral bud abscission has also been observed at high temperatures (27^{o}C) in *Camellia* (Bonner, 1947). All plant parts are in competition for limited nutrients, particularly between reproductive and vegetative organs, the numbers of which are balanced to maintain carbohydrate supply to the reproductive organs. As leaves mature on the plant, sink strength decreases and senescence occurs, nutritional reserves are solubilised and exported from the leaf to stronger sinks (Addicott, 1982). Increased leaf age also correlates with a reduction in the concentration of auxin (Street and Opik, 1984) and cytokinins (Kende, 1971). The slightly greater influence of temperature on natural *Camellia* leaf abscission than floral bud abscission may have been because higher temperatures increased respiration and metabolic rate thus placing greater demand for nutrients and carbohydrates on the plant (Evensen and Olson, 1992). This, along with lower sink strength of leaves (lower auxins and cytokinins) resulted in older leaves senescing and abscising more rapidly (0 ul·liter^{-1}, Figure 3.12). Thus abscission of leaves and floral buds of *Camellia* under elevated temperatures was an indication of homeostatic growth control (Addicott, 1982).

In contrast to natural abscission, both ethylene- and ethephon-promoted abscission were markedly influenced by temperature. Ethylene treatment was affected by temperature with higher temperatures resulting in greater abscission rate of all *Camellia* organs (Figure 3.11). Similar results occur with increased temperature of other genera. High temperatures increase abscission of all *Capsicum* organs such that final abscission proportion of largest, fully expanded leaves increases three-fold, and floral bud abscission nearly doubles (Beaudry and Kays, 1988a). It was suggested that temperature has no effect on ethylene-promoted abscission in *Prunus* (Olien and Bukovac, 1982b). However, it is clear that this is not always the case (Figure 3.11; Beaudry and Kays, 1988a) and thus, the effect of temperature on ethylene-promoted abscission should be considered in the modelling of ethephon application.

Ethephon-promoted abscission of *Camellia* was also temperature-dependent which was reflected in the pattern of both abscission parameters final abscission proportion (Figure 3.1 to 3.3), and abscission rate (Figure 3.4 to 3.6). The effect of temperature on ethephon-promoted abscission of *Camellia* is comparable to that obtained in other genera. An increase from 12^{o} to 24^{o}C increases flower thinning of *Malus* by 71% (6% to 77% of control) after application of 400 *u*l·liter^{-1} ethephon (Jones & Koen 1985). Regression analysis produced a slope of 5.93 which is greater than the maximum value obtained for *Camellia* (2.72 at 1000 *u*l·liter^{-1}) and significantly greater than the slope of 1.40 for the nearest equivalent ethephon concentration of 500 *u*l·liter^{-1} (Table 3.2). Thus ethephon-promoted abscission of *Camellia* floral buds is affected by temperature, but is less dependent than are open flowers of *Malus*. Similarly, the abscission parameter of rate (1/time to 50% final abscission) may be used for comparison of *Camellia* results to other genera. Silane (an ERC similar to ethephon) application to *Prunus persica* leaves results in an abscission rate of 2 to 4 days at 16^{o}C and less than 2 days at 27^{o}C (Porpiglia and Barden, 1980). These results are similar to the effect of temperature on ethephon-promoted abscission of *Camellia* leaves treated with ethephon (Figure 3.5).

In other species, application of ethephon at high temperatures has been found to result in ethylene release occurring so rapidly that no abscission resulted (Klein *et al.*, 1978). This was not found in *Camellia*, possibly because as temperature increased, the rate of abscission (and therefore ability to respond to the higher ethylene concentration) also increased (Figure 3.11).

The possibility that RH may have significantly influenced the results obtained was discounted for the following reasons. The optimum VP for decomposition of ethephon approximately doubles for each 10°C increase in temperature (Klein *et al.* 1979). In the current experiment, the four temperatures (10°, 16.7°, 23.3°, and 30°C) and constant RH (70%) correspond to a VP of 0.86, 1.33, 2.00 and 2.70 kPa. Over this 20°C range (10° to 30°C), the VP also increased by a factor of nearly 2 for every 10°C increase (0.86 to 2.70 kPa). Thus the influence of VP would have no significant effect because its effect is relatively constant over the temperature range employed. The results of Klein *et al.* (1979) were obtained by examining the effect of VP on ethylene release from a film of dried buffered ethephon solution on glass, and therefore do not determine the influence of VP on ethephon movement into tissue. Thus, results obtained in the field are more valid to the current system employed and only extremes of RH influence ethephon-promoted abscission (Klein *et al.*, 1978). In this experiment, RH was maintained at a constant 70% and thus would have had little influence on ethylene release under these experimental conditions.

The observed influence of temperature on ethephon-promoted abscission was not due to drying time of the ethephon spray, since after ethephon application all plants were dried at the same temperature. The effect of higher temperatures was therefore most likely due to increased absorption of ethephon (Flore and Bukovac, 1982) and greater thermal decomposition (Olien and Bukovac, 1982a), in addition to the effect of temperature on the abscission response to ethylene.

Organ Type and Ethephon-Sensitivity

Final abscission proportion demonstrated that floral buds were the most sensitive organ to ethephon (Figure 3.1 to 3.3). However, after ethylene application, leaves were more responsive since leaf abscission rate was greater than that of floral buds at all temperatures except 10°C (Figure 3.11). Differences in the ranking of organ responsiveness promoted by ethylene and ethephon were also obtained for *Olea* and the differences were suggested to be due either to differences in sensitivity or to the amount of ethylene in the abscission zone (Weis *et al.*, 1988). Due to problems in the measurement of ethylene within tissue, it is usually assumed that no differences exist between the ethylene concentration within different organs (Weis *et al.*, 1988; Lang and Martin, 1989; Section 1.3.9). It should be noted that differences in tissue response may be a result of differences in the concentration and duration of ethylene present at the abscission zone and

their interaction with the sensitivity of tissues (Lang and Martin, 1989). Ethylene application resulted in a higher abscission rate for leaves than floral buds (Figure 3.11) but little difference occurred between abscission rate of floral buds and leaves after ethephon treatment (Figure 3.9). However, final abscission proportion of floral buds was clearly greater than that of leaves (Figure 3.7). Thus differences between final abscission proportion of the organs was less likely to be a result of the duration of ethylene exposure required for abscission promotion. It is possible that the selective mechanism is the ethylene concentration required to promote abscission (i.e. sensitivity) and hence, the concentration dependent response of floral buds and leaves of *Camellia* is examined in Chapter 4.

Floral buds of *Camellia* have been found to be most sensitive to applied ethephon in this and previous experiments (Figure 3.1 to 3.3; Woolf *et al.*, 1992; Figure 2.1 to 2.3). However, in this experiment, leaves were of intermediate sensitivity and vegetative buds least sensitive; whereas in 1989, vegetative buds were intermediate and leaves the least sensitive. This result may have been caused by the influence of environmental conditions on the physiological stage of development of plant material. Notably, the forcing of plants in a green house at high temperatures and continuous photoperiod rather than culture under natural conditions, may have caused greater demand for nutrients and resulted in greater ethylene-sensitivity. Thus although leaves remained consistently less sensitive than floral buds, their sensitivity to ethephon may have been elevated over that of vegetative buds in the current experiment. This highlights the possible influence that cultural practices and time of application may have on results of ethephon application.

Organ Maturity and Ethephon-Sensitivity

Leaf maturity (extending shoots cf 2- to 3-year-old) influenced response to applied ethephon. Young expanding leaves (of the second flush) were most sensitive, old leaves (2- to 3-year-old) intermediate and fully expanded, matured leaves (3-month- and 1-year-old) were least sensitive to ethephon (Figure 3.12). These results were supported by industry trials carried out in late-summer where second flush shoots (containing expanding leaves) were most sensitive to applied ethephon (data not shown). Similar to *Camellia*, smaller leaves of *Capsicum* plants are more sensitive to an ERC (silane) than larger leaves (Beaudry and Kays, 1988a). The greater ethephon-sensitivity of young (expanding) leaves of *Camellia* is identical to that found in ethephon-promoted abscission of *Gossypium* where young leaves exhibit high sensitivity to ethephon

(Morgan, 1969) and to ethylene (Suttle and Hultstrand, 1991). The possible physiological basis for ethylene-sensitivity of young expanding leaves in *Camellia*, which also parallels that of *Dizygotheca*, *Browallia* and *Capsicum* (Woltering, 1987) is examined and discussed further in Chapter 4.

Apart from young expanding leaves, the increase in ethephon-sensitivity with leaf maturity (3-month- to 2- to 3-year-old; Figure 3.12) of *Camellia* was comparable to that found in *Olea* (Weis *et al.*, 1988) and *Gossypium* (Morgan, 1969) where ethephon-responsiveness increases with leaf age once leaf expansion has ceased. Increasing ethylene-sensitivity with maturity has been observed in abscission of *Melia* leaves (Morgan and Durham, 1980) and *Citrus* fruit (Ismail, 1970). In other genera a similar correlation exists between increasing leaf maturity and greater abscission-sensitivity promoted naturally (Chatterjee and Leopold, 1965; Osborne, 1973) or by deblading (dela Fuente and Leopold, 1968).

These results may be explained in terms of leaf senescence mediated by auxin levels. There exists a balance between ethylene-promotion and auxin-inhibition of abscission, the leaf blade functioning as a source of auxins which are transported to the abscission zone thereby inhibiting abscission (Osborne, 1989a). Exogenous ethylene acts by reducing the transport of auxin from the leaf to the abscission zone to a critical non-inhibiting level at which point abscission may occur (Beyer and Morgan, 1971; Beyer, 1973) (Section 1.3.4.1). That auxins are involved in the maturity response of *Camellia* leaves is supported by the fact that the duration of Stage I of the abscission process decreases with leaf age (Chatterjee and Leopold, 1965; Jackson and Osborne, 1972; Mattoo and Aharoni, 1988), a swing from Stage I to Stage II involves reduced auxin concentration (Chatterjee and Leopold, 1965; Riov *et al.*, 1986) and auxin concentration decreases as leaves senesce naturally (Sexton *et al.*, 1985). Further to this, increased leaf maturity correlates with reduced auxin levels and faster abscission rate in *Coleus* (dela Fuente and Leopold, 1968) (see also Section 1.3.2). Thus, the increasing sensitivity with maturity (3-month- to 2- to 3-year-old) of *Camellia* was most likely a reflection of increased senescence associated with decreasing auxin concentration distal to the abscission zone which rendered the leaves more sensitive to ethylene released from ethephon.

Although the influence of floral bud maturity on ethephon-sensitivity was not examined in this experiment, the effect of maturity on ethylene-sensitivity of floral buds was studied subsequently (Chapter 4).

Efficient Thinning of Floral Buds

The chief aim of thinning is the promotion of target organ abscission with minimal abscission of non-target organs (Beaudry and Kays, 1987). Therefore the greatest abscission difference between target (floral buds) and the non-target organs (leaves and vegetative buds) can be equated with greatest thinning efficiency. Figure 3.7 and 3.8 demonstrate the potential of each ethephon concentration and temperature treatment combination to thin floral buds from *Camellia* at ethephon concentrations greater than 1000 ul·liter^{-1}. The temperature resulting in maximum thinning potential was found to be inversely related to ethephon concentration. Thus the greatest difference at 10^{o}C occurred with highest ethephon concentration (4000 ul·liter^{-1}), while at 30^{o}C, 1000 ul·liter^{-1} was the most efficient floral bud thinning ethephon concentration (Figure 3.7 and 3.8). However, at high ethephon concentrations and high temperatures, sub-optimal thinning occurred because of excessive non-target organ abscission. At low temperatures and ethephon concentrations insufficient thinning occurred due to low abscission of floral buds and non-target organs (Figure 3.1 to 3.3). After applying ethylene to *Capsicum*, Beaudry and Kays (1988a) suggested that most efficient thinning might be achieved at low temperatures. This was found to be true in *Camellia* where, using ethephon, floral bud/vegetative bud and floral bud/leaf differences of 90% and 80% could be achieved at 10^{o}C, whereas at 30^{o}C, the maximum difference achievable was 81% and 70% respectively. The optimal ethephon concentration for thinning was a balance of temperature and ethylene concentration effects on the abscission response of different *Camellia* organs. Optimal thinning was most likely an optimal ethylene concentration/exposure duration which promoted greater abscission of floral buds over the other non-target organs (vegetative buds and leaves) (Olien and Bukovac, 1978).

Use of abscission rate (1/days to 50% abscission) as an abscission parameter provided information similar to that of final abscission proportion since similar trends for each organ were evident (Figure 3.1 to 3.3 cf 3.4 to 3.6). However, two apparent anomalies were observed between abscission rate and final abscission proportion. First, although ethephon-sensitivity of floral buds was greater than that of leaves, i.e. application of ethephon resulted in final abscission proportion of floral buds exceeding that of leaves (Figure 3.7), abscission rate revealed no consistent trend of differences between floral buds and leaves (Figure 3.9). Secondly, at 10^{o}C, the rate of abscission of floral buds and leaves appeared to

be near saturated above an ethephon concentration of 2000 ul·liter^{-1} (Figure 3.4 and 3.5), yet the final abscission response continued to increase markedly with increasing ethephon concentration (Figure 3.1 and 3.2). Thus the abscission parameter, rate of abscission (1/time to 50% abscission), was useful for separating the effect of temperature on the tissue response to ethylene from influence on ethylene release from ethephon. However, it was not of use for prediction of differences in organ sensitivities to ethephon application.

Ethylene gas application allowed separation of the influence of temperature on ethylene release from ethephon from the effect of temperature on the ethylene-promoted abscission response. Although increased ethephon concentration resulted in faster abscission (Figure 3.4 to 3.6), the influence of temperature on ethephon-promoted abscission was the same at all ethephon concentrations. That is, E_a values were unaffected by ethephon concentration (Table 3.3) while E_a values of ethylene treated organs were significantly lower than those of all ethephon treated tissue (Table 3.3). This clearly demonstrated that in all organs of *Camellia*, temperature influenced ethylene-promoted abscission less than ethephon-promoted abscission. The difference was principally due to the influence of temperature on ethylene release from ethephon (Olien and Bukovac, 1978).

The large influence of temperature on ethylene release from ethephon-treated *Prunus* leaves (E_a of 125.6 kJ·mol^{-1}; Q_{10} (10^o to 20^{o}C) of 6.3; Olien and Bukovac, 1978) was not reflected in the abscission rate of *Camellia* (E_a values 47.3 to 96.5 kJ·mol^{-1}, Table 3.3), nor in the Q_{10} values of final abscission proportion (1.3 to 2.3, 20^o to 30^{o}C; Table 3.1). Examination of E_a values of abscission rate in *Camellia* indicated that the influence of temperature on ethylene-promoted abscission accounted for 75%, 58%, and 41% of the influence of temperature on ethephon-promoted abscission of floral buds, leaves, and vegetative buds respectively (Table 3.4). However, due to the poor correlation between abscission rate and final abscission proportion discussed previously, direct translation of the influence of temperature on abscission rate to its influence on final abscission proportion may not be valid. Thus temperature may influence the ethylene-promoted portion of ethephon-promoted abscission less than is indicated by these results.

3.6 CONCLUSION

The first experiment on ethephon-promoted thinning of *Camellia* floral buds (Woolf *et al.*, 1992; Chapter 2) put forward the hypothesis that the time of the year of ethephon application affected the response of *Camellia* by its effect on tissue maturity and on temperature; the former affected tissue ethylene-sensitivity, and the latter influenced ethylene release from ethephon as well as tissue ethylene response. This hypothesis was supported by the results of application of ethephon at a range of temperatures in 1991.

Clearly, increased temperature significantly promoted rate and extent of ethephon-promoted abscission and also increased the rate of ethylene-promoted abscission of all organs of *Camellia*. Application of ethylene and measurement of abscission rate allowed separation of the influence of temperature on ethylene-release from ethephon from the abscission-promoting effect of ethylene. Temperature decreased the abscission rate after ethephon application more than that of ethylene application, presumably due to the influence of temperature on ethylene-release from ethephon (Biddle *et al.*, 1976). However, use of the parameter abscission rate to examine the influence of temperature on each of these processes, may not be an accurate reflection of the percentage of influence on final abscission proportion because of the low correlation of relative organ sensitivities as measured by abscission rate and by final abscission proportion.

The maturity of leaves within each plant significantly influenced the final abscission proportion in response to applied ethephon. Leaves on newly extended shoots were most sensitive, and once shoots extended and leaves hardened, leaves became least sensitive. As leaves subsequently matured year to year, sensitivity to ethephon increased progressively.

Differences in the response of organs to applied ethephon are due either to sensitivity differences of the organs to released ethylene or to differences in exposure time required for abscission. To verify this hypothesis, further research was carried out applying a range of ethylene concentrations (Chapter 4) and different ethylene exposure durations (Chapter 5) to leaves and floral buds of *Camellia*.

Efficient thinning of floral buds of *Camellia* by ethephon followed an inverse relationship between ethephon concentration and temperature. Optimal thinning of floral buds at low temperatures required application of high ethephon concentrations, while at high temperatures low ethephon concentrations could more efficiently selectively remove floral buds.

It is therefore clear that in the field application of ethephon, careful attention must be paid to environmental conditions, particularly that of temperature, and to tissue maturity so that the optimal ethephon concentration can be chosen to efficiently selectively remove floral buds from *Camellia*.

CHAPTER 4

ETHYLENE SENSITIVITY

AS AFFECTED BY ORGAN TYPE, ORGAN MATURITY AND TEMPERATURE, AND ENDOGENOUS ETHYLENE PRODUCTION IN *CAMELLIA*.

4.1 INTRODUCTION

The promotion or inhibition of abscission is important in many areas of horticulture (Reid, 1985). A beneficial use for ethylene is that of selective removal of a given organ with ethylene releasing compounds (ERCs) (Chapter 2 and 3). Abscission physiology of *Olea* in response to both ethephon and ethylene has been extensively examined. Yet Martin (1989) strongly supported further research into ethylene-promoted abscission, since an understanding of the mechanism of action of exogenous and endogenous ethylene is essential for efficient thinning of target organs with ERCs. Similarly, in *Camellia*, ethephon-promoted abscission is complex and detailed research into abscission physiology is required for efficient thinning of unwanted floral buds prior to transportation. Also, in the international trade and culture of potted plants, accumulation of endogenously produced ethylene (at basal[2] levels or as a result of stress or wounding) or accidental exposure to ethylene from other sources (Knee *et al.*, 1985) may result in undesirable abscission. Duncan and Davies Nurseries Ltd, a major exporter of *Camellia* from New Zealand, have suggested that exposure to exogenous ethylene during transportation may be responsible for reduction in plant quality of *Camellia.* Abscission during, or subsequent to *Camellia* export reduces plant quality and therefore requires minimisation of leaf and vegetative bud abscission during the export process.

Thus a central issue to be examined in the promotion and inhibition of abscission is the relative ethylene-sensitivities of *Camellia* organs and how sensitivity varies with organ maturity. The rate of endogenous ethylene production is also important since this may promote abscission during transportation. A major

2 Endogenous ethylene production under normal, non-stressed conditions.

environmental factor is temperature due to its influence on both the abscission response and the rate of endogenous ethylene production. Therefore, relative organ sensitivities to ethylene, the influence of maturity, rate of endogenous ethylene production and the influence of temperature on both abscission and ethylene production is herein reviewed and subsequently examined.

Ethylene Concentration and Abscission

There is debate as to the primacy of the role of ethylene in natural abscission (Morgan, 1984; Osborne, 1989a). Whatever the outcome, it is clear that exogenous ethylene promotes abscission in nearly all species (Addicott, 1982). The concentration of ethylene applied influences both leaf abscission (Kays *et al.*, 1976; Marousky and Harbaugh, 1979c) and floral organ abscission (Hoyer, 1985; Woltering, 1986; Dostal *et al.*, 1991). Higher ethylene concentrations result in greater abscission for a given exposure time (Lang and Martin, 1989; Evensen, 1991) and abscission rate is accelerated as ethylene concentration increases up to a saturation concentration (Ben-Yehoshua and Eaks, 1970). The concentration required to saturate an ethylene response is generally accepted to be 10 ul·liter^{-1} (Abeles, 1973). The abscission response is also saturated at this concentration for most, but not all organs (Ben-Yehoshua and Eaks, 1970; Sexton *et al.*, 1985; Woltering, 1987).

The effect of ethylene concentration may be explained in terms of ethylene binding and subsequent action in the tissue distal to the abscission zone (such as the leaf blade) and the abscission zone itself. The role of ethylene in the abscission process in the leaf blade reduces auxin transport from the leaf blade to the abscission zone (Beyer, 1975b; Section 1.3.4.1). The inhibition of auxin transport in *Gossypium* has been shown to exhibit a concentration dependent response saturating at approximately the same ethylene concentration as the abscission process (10 ul·liter^{-1}) (Beyer and Morgan, 1971). Greater abscission resulting from increased ethylene concentration is due to higher levels of active receptor increasing the formation of the "second message" and hence subsequent biochemical steps leading to expression of the ethylene response (Burdon and Sexton, 1989).

Ethylene-Sensitivity and Organ Type

Differential organ abscission resulting from ethephon application has been found to occur in many species (Section 3.1). However, differences exist in the responsiveness ranking resulting from application of ethephon or ethylene (Weis

et al., 1988). Ethylene released from ethephon produces a peak ethylene concentration and duration of exposure (Olien and Bukovac, 1982a). Therefore the difference in response to ethephon and ethylene may be due to greater sensitivity to released ethylene or a shorter duration of ethylene exposure required to promote abscission (Beaudry and Kays, 1988a; Lang and Martin, 1989). Thus greater responsiveness or sensitivity to ethephon may not necessarily indicate greater sensitivity to ethylene itself.

Significant differences in ethylene-sensitivity between organs of the same species have been found in a range of genera. Floral buds are more sensitive than leaves of *Gossypium* (Lipe and Morgan, 1973), *Begonia* (Hoyer, 1985), *Hibiscus* (Woltering, 1987) and *Capsicum* (Beaudry and Kays, 1988a). Bracts of *Clerodendron* are also more sensitive than leaves (Woltering, 1987). Ethylene-sensitivity of fruit is also greater than leaves of *Gossypium* (Lipe and Morgan, 1973), *Olea* (Lang and Martin, 1987; 1989) and *Citrus* (Ben-Yehoshua and Eaks, 1970). Similarly, shuck dehiscence of *Carya* is more sensitive than leaves, although fruit are less sensitive than leaves (Kays *et al.*, 1975). Hence, it appears that in most cases, reproductive organs have a greater sensitivity to ethylene than leaves. However, there are some genera where little difference in ethylene-sensitivity exists between leaves and inflorescences such as *Pachystachus* and *Beloperone* (Woltering, 1987). However, ethylene concentrations below 3 $ul \cdot liter^{-1}$ where not applied and abscission response differences below this concentration may exist.

Ethylene-Sensitivity and Organ Maturity

Tissue maturity influences ethylene-sensitivity and is therefore, an important variable to be understood for accurate modelling of thinning of target organs with ethephon (Suttle and Hultstrand, 1991). It is generally believed that older leaves abscise more readily than younger leaves in response to ethylene (Burg, 1968; dela Fuente and Leopold, 1968; Osborne, 1973). For example, within *Prunus* shoots, abscission proceeds from the shoot base (oldest leaves) acropetally to younger leaves upon application of silane (Byers, 1978; Porpiglia and Barden, 1980). Similarly, age-dependent leaf abscission is best correlated with greater ethylene-sensitivity of older tissue and not increased ethylene production of *Phaseolus* and *Melia* (Jackson *et al.*, 1973; Morgan and Durham, 1980). Application of apple gas (the active portion of which is ethylene) also results in differing responses of young and old leaves in a number of species. In general, mature leaves are more sensitive than young leaves such as in *Sparmannia*,

Coleus, *Euonymus*, *Rhododendron,* and *Veronica* (Funke *et al.*, 1938). Similarly, this trend also results from ethylene-treatment of a wide variety of potted foliage plants (Woltering, 1987).

Although this appears to be a standard response, exceptions occur in the application of apple gas where only younger leaves abscise in *Camellia* and *Grisellinia* (Funke *et al.*, 1938). Although such exceptions to the generalization of greater sensitivity with increasing leaf maturity are relatively infrequent, additional cases have been reported in more recent years where young leaves of *Capsicum*, *Dizygotheca* and *Browallia* are more sensitive to ethylene (Woltering, 1987). Similarly, Morgan (1969) found that ethephon treatment of *Gossypium* causes abscission of all young terminal leaves and oldest leaves, but no abscission of intermediate age leaves. These findings were also subsequently confirmed with ethylene gas (Morgan and Durham, 1973; 1975; Beyer, 1975b) and Suttle and Hultstrand (1991) observed that terminal *Gossypium* leaves abscise more rapidly and at lower ethylene concentration than lower, older leaves.

Maturity of floral organs also influences ethephon-sensitivity in both ornamental and fruit crops. Increasing sensitivity to ethephon occurs as floral organs mature up to flower opening in *Begonia* (Moe and Smith-Eriksen, 1986), *Malus* (Edgerton and Greenhalgh, 1969) and *Vitis* (Weaver and Pool, 1969). Application of ethylene gas results in similar trends to that of ethephon application. Floral buds of *Begonia* (Hoyer, 1985) and *Hibiscus* (Hoyer, 1986) are less sensitive than open flowers. From the examination of a range of ornamental flowering plants, Woltering (1987) also found that open flowers were more sensitive to ethylene than floral buds. Similarly, petal abscission of *Pelargonium* becomes increasingly sensitive to ethylene as maturity increases (Evensen, 1991). Greater maturity of the floral tissues also increases ethylene-sensitivity of other physiological responses such as ethylene-promoted flower senescence (Camprubi and Nichols, 1978; Marousky and Harbaugh, 1979b; Lawton *et al.*, 1990).

Temperature and Ethylene-Promoted Abscission

It is well known that temperature influences plant response to applied ethylene (Beyer, 1973). Petal abscission of *Pelargonium* has a Q_{10} of 2 between 10^{o} and 20^{o}C (Armitage *et al.*, 1980). Lower temperature slows the rate, or decreases the extent of leaf abscission (Marousky, 1979; Beaudry and Kays, 1988a) and flower and fruit abscission (Marousky and Harbaugh, 1979b; Beaudry and Kays,

1988a). For *Camellia japonica* 'Moshio', a range of ethylene concentrations (0, 0.01, 0.1, 1, and 10 *u*l·liter^{-1}) applied for 1 week at 0°, 2°, 5°, and 12°C results in abscission only at the highest temperature and ethylene concentration (12°C, 10 *u*l·liter^{-1}) (R.E. Lill, pers. com.). Ethylene-promoted abscission of *Capsicum* organs is also influenced differentially by temperature. Increasing temperature promotes flower bud abscission to a greater extent than that of leaves (Beaudry and Kays, 1988a). There exists a dearth of information on the interaction of temperature and ethylene concentration, particularly in the ethylene-promoted abscission response.

Endogenous Ethylene Production, Abscission and Temperature

Endogenous ethylene production of tissue has the capacity to promote abscission. For example, defoliation of *Capsicum* during transit is the result of high endogenous ethylene production (Kays *et al.*, 1976) and high night temperatures promote high endogenous ethylene production which causes fruit abscission in *Malus* (Kondo and Takahashi, 1989). Since the rate of reaction of the ethylene biosynthetic pathway is temperature-dependent (Lyons and Raison, 1970; Olien and Bukovac, 1978; Field, 1985), use of low temperature to avoid endogenous ethylene-promoted abscission may be beneficial (Kays *et al.*, 1976). Olien and Bukovac (1978) examined the endogenous ethylene production of *Prunus cerasus* shoots between 20° and 40°C and obtained an E_a value of 38.5 kJ·mol^{-1} (Q_{10} of 1.8). This was similar to the generalized value of 54.4 kJ·mol^{-1} (Q_{10} of 2.2) which they obtained from the literature on a range of species and tissues (*Malus*, *Pyrus* and *Lycopersicon* fruit, *Pisum* stem sections and *Lycopersicon* foliage).

Temperatures greater than 30°C generally inhibit ethylene production (Lougheed and Franklin, 1972; Abeles, 1973). Others have found that temperatures of 35° to 38°C brought about a plateau and subsequent dramatic drop in basal and wound ethylene production (Saltveit and Dilley, 1978; Field 1981a). The conversion of ACC to ethylene has been isolated as the principal point of the biosynthetic pathway where high temperature inhibition occurs, although the mechanism remains to be established (Field, 1985; Field and Barrowclough, 1989).

Low temperature (<13°C; Wang and Adams, 1982)) may bring about a chilling response in some species (Lyons and Raison, 1970). Field (1985) suggested that a phase-transition occurs in the cell membrane below a critical chilling-sensitive

temperature which increases the E_a of membrane-bound enzymes involved in the ethylene biosynthetic pathway. The temperature at which the change occurs may be identified by examining Arrhenius plots of ethylene production to find the point at which there is a distinct change in slope (Field, 1985). A change in E_a occurs at this temperature and has been found to occur at 10°C in *Malus* fruit (Mattoo *et al.*, 1977), 11.4°C in *Phaseolus* leaf discs (Field, 1981b), 12°C in *Lycopersicon* fruit (Mattoo *et al.*, 1977) and 15°C in *Pisum* stems (Saltveit and Dilley, 1978).

4.2 OBJECTIVES

Three areas of ethylene-promoted abscission physiology were examined in the current study. Firstly, determination of relative abscission-sensitivities of organs to ethylene is important to the selective removal of floral buds with ethephon and in avoidance of abscission during live plant export. A range of ethylene concentrations were employed to examine the hypothesis that floral buds have greater ethylene-sensitivity than leaves.

Secondly, much of our understanding of the effect of maturity on the ethylene-sensitivity of abscission has, with notable exceptions (Suttle and Hultstrand, 1991; Morgan and Durham, 1973), been arrived at incidentally (e.g. Porpiglia and Barden, 1980). Both Trewavas (1983) and Evensen (1991) rued the dearth of systematic examination of sensitivity during ontogeny using dose-response curves. The aim in these experiments was to examine changes in ethylene-sensitivity of floral buds and leaves over a complete season. This would help to address some apparent anomalies of ethylene-sensitivity, particularly of young versus old leaves where examination has involved either the senescing (Jackson and Osborne, 1970) or the young expanding stage of leaf growth (Suttle and Hultstrand, 1991).

Finally, the interaction of temperature with ethylene concentration has received little attention in the abscission process, and yet is of importance to both application of ERCs and in transportation of live plants. In the transportation of plants, ethylene production even at low temperatures can be important (Knee *et al.*, 1985). Along with applied ethylene (e.g. as ERCs), endogenous ethylene production contributes to the ethylene pool within the plant tissue. Thus the

influence of a range of non-freezing temperatures (1° to 30°C) on endogenous ethylene production was examined, as much literature has only determined ethylene production at high temperatures (Knee *et al.*, 1985).

4.3 MATERIALS AND METHODS

Plant Material. In the subsequent experiments, an explant system was utilised so that sufficient replication and accurate control of temperature, ethylene concentration, and tissue maturity could be achieved. To obtain suitable explants, 3- to 4-year-old mother plants of *Camellia* 'Anticipation' and 'Donation' were grown under shade house conditions (13% shading) at ambient temperatures over the 3 years of experimentation (1989 to 1991) at Massey University, Palmerston North, N.Z.. All plants were repotted yearly in late winter in a peat/pumice (3:2 v/v) growing medium with amendments (see Section 3.3). All experiments used explants from apical shoot cuttings which were removed early in the morning and placed directly into moistened plastic bags for rapid transportation to the laboratory. Shoot explants were prepared by re-cutting stems to obtain 4-leaf apical shoots (13 $\pm$ 2 cm) with a minimum of three floral buds. Explant stems were inserted into vials of double distilled water (30 ml) and wound ethylene was dissipated over 24 hr at 25°C (Burg, 1968). Where shoot maturity was not a variable, all shoots were selected for uniform maturity.

Ethylene treatment. Ethylene treatment was carried out by placing explants in glass jars (1.81 liters) sealed with screw-top, air-tight lids fitted with Vacutainer septa (10 ml, Becton Dickinson, New Jersey). Jars were then flushed with ethylene- and CO_2-free air and immediately sealed. Ethylene concentrations were achieved by injecting pure ethylene through the septa using 25 or 10 *u*l Hamilton gas-tight syringes. The resulting concentrations were confirmed by gas chromatography 15 to 45 mins after injection and at the completion of the experiment. Concentrations obtained did not vary by more than 10% over the experimental period. An ethylene concentration of 0 *u*l·liter^{-1} was achieved in control treatments by the use of Purafil^{R}, a commercial granular ethylene scrubber (5 g/jar; Papworth Engineering, Cambridge, N.Z.). Respiration resulted in O_2 depletion and CO_2 accumulation within jars. Since ethylene production (Saltveit and Dilley, 1978) and response is suppressed by increased CO_2 or decreased O_2 (Nichols, 1968), CO_2 concentration of 0 *u*l·liter^{-1} was maintained in all treatments using soda lime (10 g/jar; Ajax Chemicals, Australia). Oxygen concentrations below 10% markedly inhibit leaf abscission of *Gossypium* and

Phaseolus while concentrations between 10 and 21% have little effect (Abeles and Gahagan, 1968; Marynick and Addicott, 1976). In the jar-system employed in this experiment, there was sufficient volume to maintain an O_2 concentration greater than 10% for 38 days in the light (45 $\pm$ 15 $u$mol·m^{-2}·s^{-1}), and 28 days in darkness at 25^{o}C.

Ethylene concentration was measured using a Varian^{R} gas chromatograph (Model 3400, Alltech Associates NZ Ltd, Auckland, N.Z.) equipped with an activated alumina column (3.18 mm x 1.83 m) and a flame ionization detector. The injection port, column, and detector temperatures were 100^{o}C, 100^{o}C and 150^{o}C respectively. Flow rate of N_2 carrier gas was 30 ml·min^{-1}; H_2, 30 ml·min^{-1}; and air, 100 ml·min^{-1}. Oxygen and CO_2 concentrations were measured using a Shimadzu^{R} gas chromatograph (Model 8A, Alltech Associates NZ Ltd, Auckland, N.Z.) equipped with a CTR I column (6.35 mm x 1.83 m; outer column of activated molecular sieve and inner column of porous polymer mixture) and a thermal conductivity detector. The injection port, column and detector temperatures were 60^{o}C, ambient and 60^{o}C respectively. Flow rate of H_2 carrier gas was 30 ml·min^{-1}.

Measurements. Leaf and floral bud abscission were recorded every 6 to 12 hr. To unify jar disturbance, data were collected after tapping the jar once lightly on the bench top. Time to 50% abscission was defined as days to abscission of two leaves or 50% or more floral buds (Ben-Yehoshua and Eaks, 1970). At the completion of the experiment floral buds were collected and bud volume determined by water submersion.

Definition of Explant Maturity. The maturity of explants employed in all experiments was defined as the time from cessation of stem extension. Shoot length of *Camellia* was not uniform and, because explants were excised from the shoot apex, an explant taken from a short shoot was more mature than an explant from a longer shoot. Thus the more common maturity index, time from bud break, was not a reliable indicator of maturity in *Camellia*. Shoot maturity was defined by tagging shoots and measuring stem length weekly (stem base to bottom of apical bud). Explant maturity could therefore be determined as weeks from a zero time of cessation of stem extension. In Experiment 4.1 to 4.4 and 4.6, a representative sample of the shoot population was used to determine cessation of stem extension, however, explant maturity was determined individually in Experiment 4.5.

Experiment 4.1; Ethylene-Sensitivity of *Camellia* Organs. Ethylene sensitivity of two cultivars of *Camellia* were examined in Experiment 4.1. Shoot explants of 'Anticipation' and 'Donation' were collected on 14 March 1990 (21 weeks maturity). A range of ethylene concentrations was applied to explants of 'Anticipation': 0, 0.3, 1.0, 2.5, 10.0, 30.0 ul·liter^{-1}; and of 'Donation': 0, 0.3, 0.6, 1.0, 1.5, 2.5, 4.0, 6.0, 8.0, 10.0, 15.0, 30.0, 100.0 ul·liter^{-1}. The experiment was carried out in a bioassay room maintained at 25^{o} $\pm$ 2^{o}C and fitted with cool white Osram^{R} fluorescent lights providing 45 $\pm$ 15 $u$mol·m^{-2}·s^{-1} at jar height. A randomised complete blocks design with five explant replicates (jars) was employed for each treatment.

Experiment 4.2 and 4.3; Influence of Temperature on Ethylene-Promoted Abscission. The effect of temperature on the abscission response to a range of ethylene concentrations was studied in Experiments 4.2 and 4.3. Shoot explants and opened jars were placed in the respective temperature treatments for 24 hr to allow wound ethylene dissipation and temperature equilibration prior to sealing and ethylene injection. Jars were then flushed with ethylene-free air, ethylene was injected to achieve the appropriate ethylene concentrations, and the resultant concentration was confirmed by gas chromatography. Ethylene treatment was carried out in the dark in a 0 ul·liter^{-1} CO_2 environment. Changes in O_2, CO_2 and ethylene were monitored over the experimental period. Five shoot explants of 'Donation' were used for each temperature/concentration combination. Explants were prepared and ethylene applied as for Experiment 4.1. **Experiment 4.2** was carried out and floral bud abscission results obtained. However, due to the influence of the stage of flower development, leaf abscission data was excessively variable. **Experiment 4.3** was therefore carried out to determine the effect of temperature on leaf abscission. In this experiment, floral buds were removed prior to ethylene treatment.

Experiment 4.2. Ethylene treatment of floral buds was carried out employing explants collected on 23 June 1990 (35 weeks maturity) at 1^{o}, 5^{o}, 10^{o}, 15^{o}, and 25^{o}C. At temperatures of 5^{o}, 15^{o}, and 25^{o}C, ethylene concentrations of 0, 0.5, 1, 2, 3, 4, 6, 8, 10 ul·liter^{-1} were applied. At 1^{o} and 10^{o}C, 0 and 10 ul·liter^{-1} ethylene were applied.

Experiment 4.3. Ethylene treatment of leaves was carried out using explants collected on 19 March 1991 (19 weeks maturity) at 1^{o}, 4^{o}, 8^{o}, 13^{o}, and 25^{o}C. A range of slightly higher ethylene concentrations were used at lower temperatures.

Ethylene concentrations applied at 1°C were: 0, 3, 6.5, 8, 9, 10, 11.5, 13, and 15 *u*l·liter^{-1}: 4°C; 0, 2, 4, 5, 6, 7, 8, 10, 12.5, and 15 *u*l·liter^{-1}: 8°C; 0, 10, and 15 *u*l·liter^{-1}: 13°C; 0, 10, and 15 *u*l·liter^{-1}: and at 25°C; 0, 0.75, 2, 3, 4, 6, 8, 10, and 15 *u*l·liter^{-1}.

Experiment 4.4; Influence of Maturity on Ethylene-Promoted Abscission. Treatment of Explants with Ethylene Through the Natural Season. In Experiment 4.4, different explant maturities (containing floral buds and leaves) were achieved by collecting explants representative of the shoot maturity of stock plants. Five explants were treated with ethylene (10 *u*l·liter^{-1}) at 0.5 to 5 weekly intervals through the period of 5 December 1989 to 25 April 1991 (stem extension ceased on 23 October 1989 and 2 November 1990). Explants of 1-year- and 2- to 3-year-old shoots were removed from 'Anticipation' and 'Donation' on 12 December 1989 to examine abscission of leaves of more than one season of age. No floral buds were present on 1-year- and 2- to 3-year-old wood and all younger shoots were excised leaving 4-leaf-explants. Because of the high respiration rate of open flowers, O_2 levels decreased more rapidly in the flowering period (30 to 45 weeks) than in treatment of unopened floral buds. For this reason, when open flowers were present, experimentation was terminated at 7 days.

Experiment 4.5; Influence of Maturity on Ethylene-Promoted Abscission. Simultaneous Treatment of Explants Produced by Artificial Bud Break. In Experiment 4.5, simultaneous ethylene treatment of a range of explant maturities (containing floral buds and leaves) was carried out following a method similar to Morgan and Durham (1973). Out of season bud break of unchilled 4-year-old plants of 'Anticipation' and 'Donation' was achieved by placing mother plants in simulated summer conditions (18° to 25°C; 16 hr photoperiod Section 1.2.2.1) in mid-April (autumn). Bud break was more variable after such treatment, possibly due to insufficient chilling of vegetative buds (Section 1.2.2.1), and a supply of explants with a wide range of maturities was obtained. Explant maturities for 'Anticipation' were 3, 5, 8, 9, 10, 11, 12, 14, 18, 20, and 21 weeks (after cessation of stem extension); and 1 to 4, 6 to 16 and 18 weeks for 'Donation'. Five explants of each maturity were collected and treated simultaneously with ethylene (10 *u*l·liter^{-1}) on 12 December 1989, by the method outlined above. Control (0 *u*l·liter^{-1}) shoots were of 9 weeks maturity for 'Anticipation' and 2 weeks for 'Donation'.

Experiment 4.6; Influence of Maturity on Ethylene-Sensitivity of *Camellia* Leaves. The assumption that differences in abscission rate in response to a saturating ethylene concentration (10 $\mu l \cdot liter^{-1}$) reflect real differences in the ethylene-sensitivity of tissue maturities was examined in this experiment by application of a range of ethylene concentrations. To obtain explant maturities of -2 and +4.5 weeks (from cessation of stem extension), 'Anticipation' stock plants were placed in simulated summer conditions (as for Experiment 4.5) in March (early autumn). Explants were collected and ethylene concentrations of 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 10.0 $\mu l \cdot liter^{-1}$ were applied on 11 October 1991 in the same manner as for Experiment 4.1. Abscission rate was obtained and analysed along with the response obtained for 'Anticipation' shoots of +21 weeks maturity treated with 0.3, 1.0, 2.5 and 10.0 $\mu l \cdot liter^{-1}$ ethylene of Experiment 4.1.

Experiment 4.7; Influence of Temperature on Endogenous Ethylene Production. Ethylene production without ethylene application was determined in Experiment 4.7 on 4th April 1990 using explants of *Camellia* 'Brian' plants grown in ambient outdoor temperatures (mean daily minimum/maximum 10.2°/18.3°C). Although not accurately measured (as for Experiments 4.1 to 4.6), maturity of 'Brian' shoots were of approximately 20 weeks. Explants were collected and prepared as for shoot explants, however, stem-leaf explants were used which consisted of a single apical-leaf with 1 cm stem, all other organs were excised with a scalpel. Explants were first weighed, then inserted in glass vials (30 ml) with the stem base in small cups (0.5 ml) of double distilled water and soda lime (1 g) was placed in the base of the vial to absorb CO_2. Wound ethylene production was dissipated over 24 hr under ambient laboratory conditions (20°C). Vials were subsequently placed in air temperatures of 1°, 4°, 8°, 13°, 20°, 25° and 30°C in darkness for 4 hr to equilibrate. Vials were then flushed with ethylene- and CO_2-free air, sealed and replaced in the appropriate temperatures. After 18 hr, the ethylene concentration was determined using a Photovac^R gas chromatograph (photoionization detector, details in Section 3.3). Ethylene production was calculated as $nl \cdot g^{-1} \cdot hr^{-1}$ on a fresh weight basis.

4.4 RESULTS

Application of a range of ethylene concentrations to shoots of 'Donation' and 'Anticipation' (Experiment 4.1) revealed greater sensitivity of floral buds over that of leaves of both *Camellia* cultivars (Figure 4.1 and 4.2). At a concentration of

0.25 *u*l·liter^{-1}, abscission rate (time to 50% abscission) of floral buds was 6 days whereas leaf abscission had not occurred by 38 days for 'Donation' (Figure 4.1). At the same concentration, 'Anticipation' responded similarly to 'Donation': 50% floral bud abscission occurred at 15 days whereas leaves took 24 days to reach 50% abscission (Figure 4.2). Increasing ethylene concentration revealed that floral bud abscission maintained an abscission rate greater than, or equal to, leaves up to 10 *u*l·liter^{-1} for both cultivars. At concentrations greater than 10 *u*l·liter^{-1}, leaves of 'Donation' were slightly more responsive than floral buds. Responsiveness of 'Anticipation' leaves and floral buds was the same at 10 and 30 *u*l·liter^{-1} (Figure 4.2). Comparison of cultivars revealed that floral buds of 'Donation' were more sensitive than those of 'Anticipation', whereas 'Anticipation' leaves were sightly more sensitive than 'Donation' since at 0.3 *u*l·liter^{-1}, abscission occurred in 'Anticipation' leaves but not 'Donation'. An increase in ethylene concentration of 1 to 10 *u*l·liter^{-1} resulted in increased abscission rate by 2 days (4 to 2 days) for floral buds and 24 days (26 to 1.75 days) for leaves in 'Donation'. However, between 10 and 100 *u*l·liter^{-1} the increase in response was only 0.25 days (2 to 1.75 days) for floral buds and 0.5 days (1.75 to 1.25 days) for leaves of 'Donation' (Figure 4.1). Similar results were found with 'Anticipation' (Figure 4.2). Thus at 25^{o}C an ethylene concentration of 10 *u*l·liter^{-1} was sufficient to induce near saturation of the abscission response in both cultivars.

Experiments 4.2 and 4.3 examined the effect of temperature on response of 'Donation' to ethylene. The exponential pattern of abscission rate of 'Donation' leaves to 10 and 15 *u*l·liter^{-1} (Experiment 4.3) did not differ over the range 1^{o} to 25^{o}C indicating that the response was saturated at all temperatures (Figure 4.3). Application of a saturating ethylene concentration (10 *u*l·liter^{-1}) to floral buds and leaves of 'Donation' over this temperature range (1^{o} to 25^{o}C; Experiments 4.2 and 4.3) indicated an exponential relationship such that low temperature resulted in a large decrease in abscission rate (Figure 4.4). Response of floral buds and leaves to 10 *u*l·liter^{-1} ethylene differed little between 4^{o} to 25^{o}C (Figure 4.4). However, leaf abscission rate was slower and exhibited much greater variability than floral buds at 1^{o}C.

At 5^{o}, 15^{o} and 25^{o}C, the abscission rate of floral buds of 'Donation' was saturated between concentrations of 3 and 10 *u*l·liter^{-1}, that is, the response curves were parallel (Figure 4.5). However, with decreasing concentration the abscission rate showed a sharp decrease. At 5^{o}C, this occurred between 1 and 2 *u*l·liter^{-1}, at 15^{o}C between 0.5 and 1 *u*l·liter^{-1}, and at 25^{o}C a slight decrease

occurred at 0.5 *u*l·liter^{-1}. Similar results were obtained with leaves of 'Donation' (Figure 4.6). At 25^{o}C, leaves showed a decrease in abscission rate at lower concentrations with a departure from a linear response between 4 and 6 *u*l·liter^{-1}, However, at low temperatures (1^{o} and 4^{o}C) a decrease in abscission rate was not observed.

Treatment of explants of different maturities over 16 months (Experiment 4.4) or simultaneously (Experiment 4.5), revealed that maturity influenced ethylene response of floral buds and leaves of both cultivars of *Camellia*. Leaves of both cultivars underwent a large decrease in rate of abscission-response to a saturating ethylene concentration (10 *u*l·liter^{-1}) between vegetative bud break (-4.5 weeks) and cessation of stem extension (0 weeks) (Figure 4.7 and 4.8). At -4.5 weeks maturity, abscission rate was 0.55 and 0.49 days for 'Anticipation' and 'Donation' respectively (Figure 4.7 and 4.8). In the period from cessation of stem extension (week 0) to +12 weeks, abscission rate decreased to a point of lower ethylene response of 2.5 days to 50% abscission in 'Anticipation'. Similarly, rate of leaf abscission in 'Donation' dropped to 1.75 days by 12 weeks. Abscission rate of leaves remained at this level for the remainder of the treatment period (approximately 1 year) with the exception of the flowering period (weeks 30 to 45) when abscission rate became very variable tending to slow to as long as 5 and 7 days in 'Anticipation' and 'Donation' respectively. One-year- and 2- to 3-year-old leaves responded at a similar rate to that of the current season (6 to 50 weeks) leaves (approximately 1.5 to 2.5 days to 50% abscission).

Floral buds of both cultivars showed a trend of increasing response to applied ethylene as they matured through the summer and autumn seasons (Figure 4.7 and 4.8). The magnitude of this increase in response, as determined by abscission rate (time to 50% abscission), was approximately 2.5 days (3.75 to 1.25 days) and 1.5 days (2.5 to 1.0 days) for 'Anticipation' and 'Donation' respectively.

Simultaneous ethylene-treatment of a range of shoot maturities (Experiment 4.5) yielded remarkably similar results to that of consecutive ethylene-treatment of maturities over 16 months (Experiment 4.4; Figure 4.9 and 4.10 cf Figure 4.7 and 4.8). Simultaneous ethylene treatment of leaves in the maturity range of 1 to 21 weeks showed little difference in the response of leaves of either cultivar (Figure 4.9 and 4.10). Although there was a slight increase in sensitivity of 'Anticipation' leaves (2.5 to 1.75-2 days) over the maturity range 3 to 21 weeks. Greater

ethylene-sensitivity (decreased abscission rate) of younger leaves (0 to 5 weeks), similar to that found in Experiment 4.4, was observed in some 'Donation' leaves (Figure 4.8 cf 4.10).

Simultaneous ethylene-treatment (Experiment 4.5) resulted in a pattern of increasing ethylene-sensitivity (decreasing abscission rate) of floral buds of both cultivars (Figure 4.9 and 4.10) which followed the same pattern as for floral bud maturities treated over 16 months (Experiment 4.4; Figure 4.7 and 4.8). Abscission rate (days to 50% abscission) of floral buds decreased by 1.5 days (4 to 2.5 days) for 'Anticipation', and 1 day (2.5 to 1.5 days) for 'Donation' between maturity ranges 8 to 21 and 6 to 18 weeks ('Anticipation' and 'Donation' respectively). 'Anticipation' had slightly slower abscission rates of leaves and floral buds in both experiments (4.4 and 4.5) compared with 'Donation'. The increased variability of leaf abscission around the time of flower opening found in Experiment 4.4 (30 to 45 weeks), was not found in Experiment 4.5 since the range of maturities employed did not include explants at this stage of development.

Ethylene treatment of three leaf maturities of *Camellia* 'Anticipation' (-2, +4.5 and +21 weeks) with a range of ethylene concentrations revealed greater sensitivity of young expanding leaves (Figure 4.11). Thus a given ethylene concentration of 1 $ul \cdot liter^{-1}$ resulted in abscission rate of 1.3, 5.0 and 13 days for -2, +4.5, and +21 week maturities respectively. Also, at a saturating concentration (10 $ul \cdot liter^{-1}$), greater maturity resulted in a decrease in the fastest abscission rate observed for each maturity; 0.72, 1.71 and 2.5 days to 50% abscission for -2, +4.5, and +21 weeks respectively.

Temperature markedly influenced basal endogenous ethylene production of *Camellia* 'Brian' leaf-stem explants (Figure 4.12). Arrhenius plot (Field, 1985) of data indicated a linear increase between 1°C and 8°C, a distinct change in ethylene production at 8°C, and further linear increase in ethylene production between 8°C and 20°C (Figure 4.13). Linear regression on Arrhenius plot (for details see Section 3.3) was used to obtain E_a values of 73.3 and 22.0 $kJ \cdot mol^{-1}$ for less than 8°C and above 8°C respectively (Figure 4.13). Endogenous ethylene production reached a plateau between 20°C and 25°C and decreased between 25°C and 30°C. Extrapolation of the curve to 0°C allows calculation of Q_{10} values; 0° to 10°C = 3.0, 10° to 20°C = 1.2, and 20° to 30°C = -1.5.

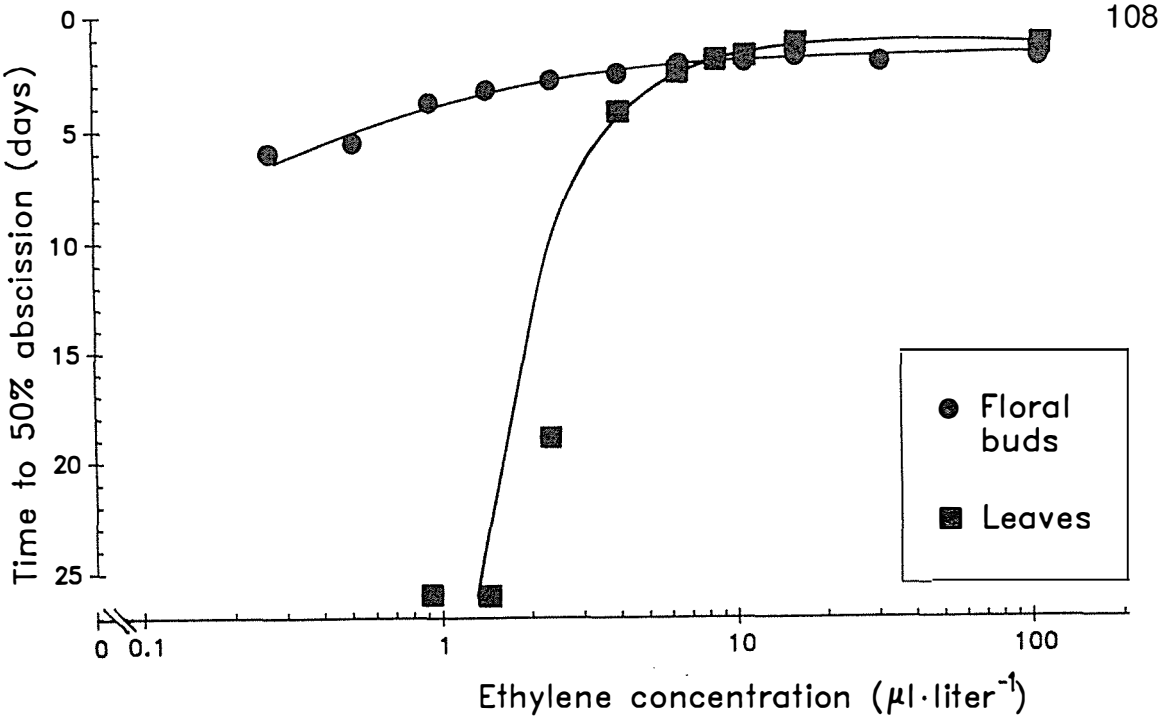


Figure 4.1. Effect of ethylene concentration on abscission rate (time to 50% abscission) of floral buds and leaves of *Camellia* 'Donation'. Lines of best fit. (Experiment 4.1).

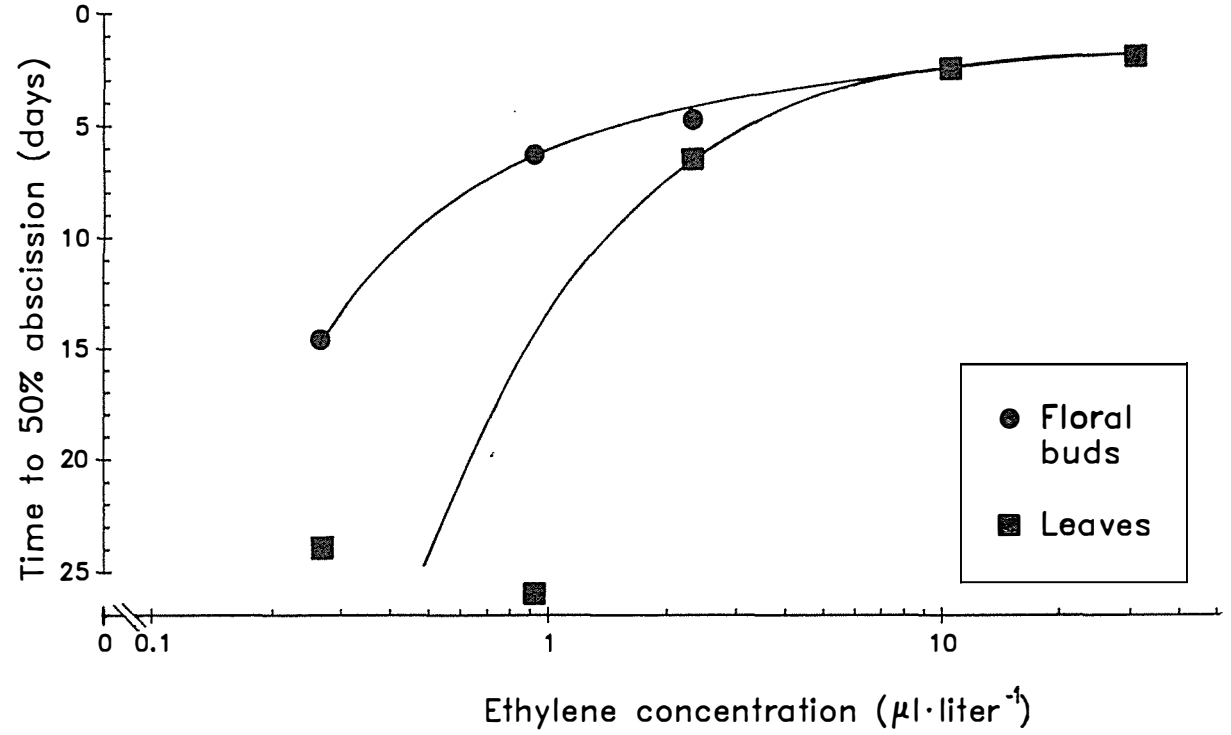


Figure 4.2. Effect of ethylene concentration on abscission rate (time to 50% abscission) of floral buds and leaves of *Camellia* 'Anticipation'. Lines of best fit. (Experiment 4.1).

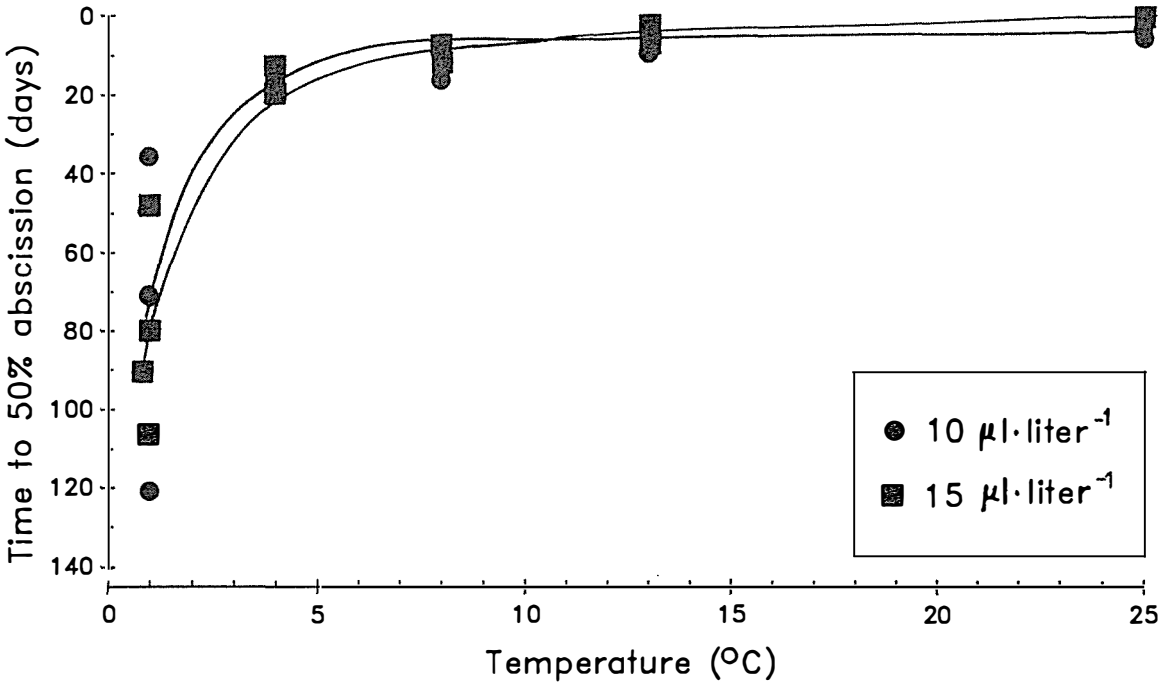


Figure 4.3. Effect of two saturating ethylene concentrations and temperature on abscission rate (time to 50% abscission) of leaves of *Camellia* 'Donation'. Lines of best fit. (Experiment 4.3).

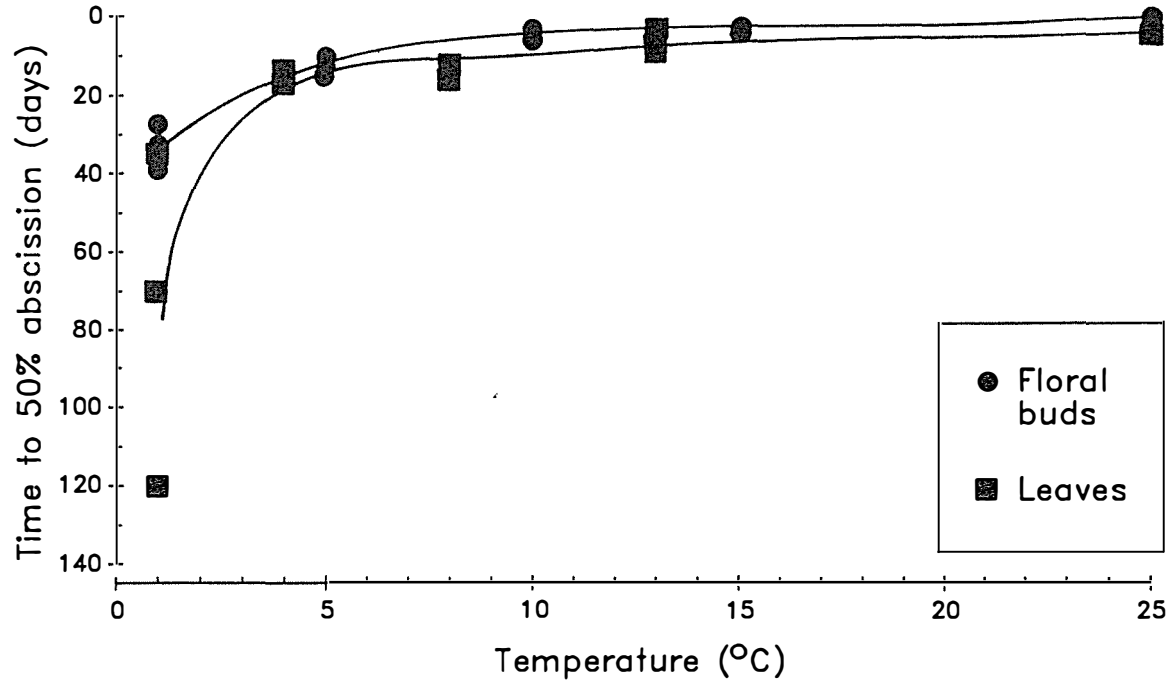


Figure 4.4. Effect of a saturating ethylene concentration and temperature on abscission rate (time to 50% abscission) of floral buds and leaves of *Camellia* 'Donation'. Lines of best fit. (Experiment 4.2 and 4.3).

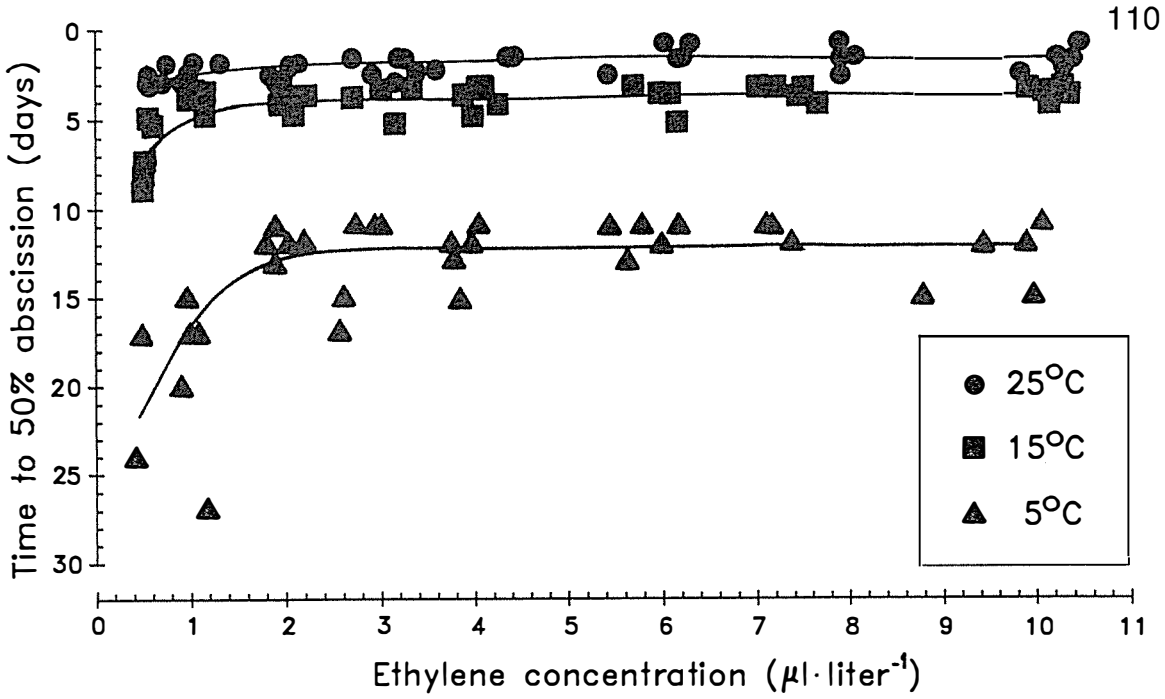


Figure 4.5. Effect of ethylene concentration and temperature on abscission rate (time to 50% abscission) of floral buds of *Camellia* 'Donation'. Lines of best fit. (Experiment 4.2).

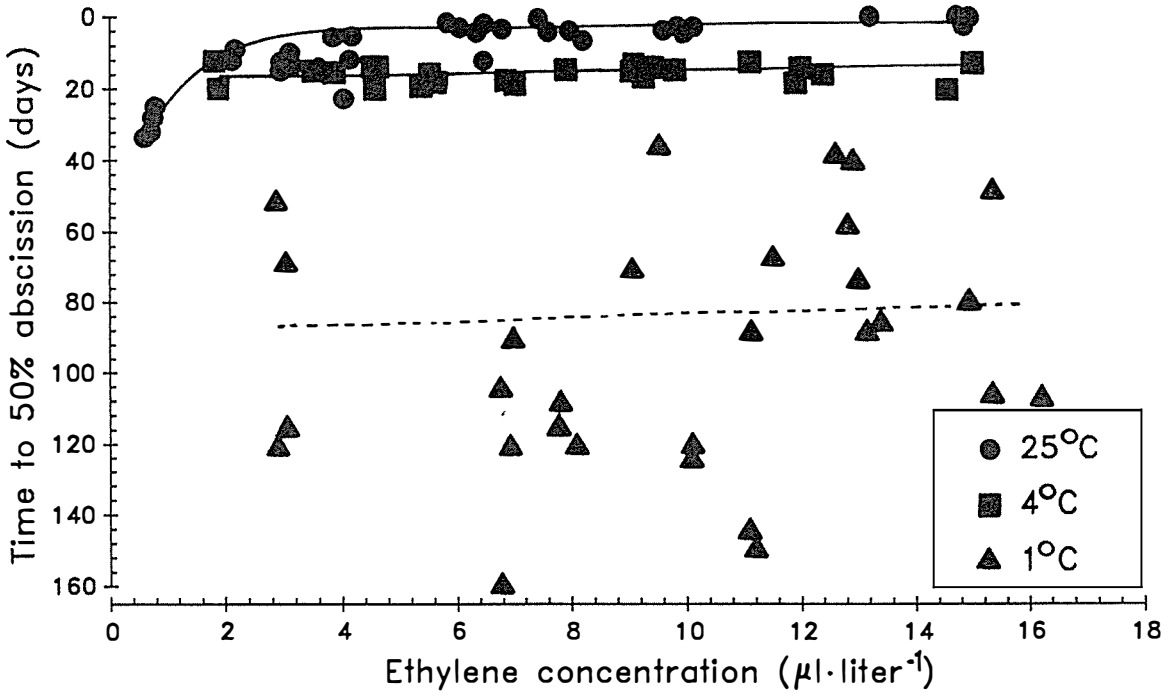


Figure 4.6. Effect of ethylene concentration and temperature on abscission rate (time to 50% abscission) of leaves of *Camellia* 'Donation'. Lines of best fit. (Experiment 4.3).

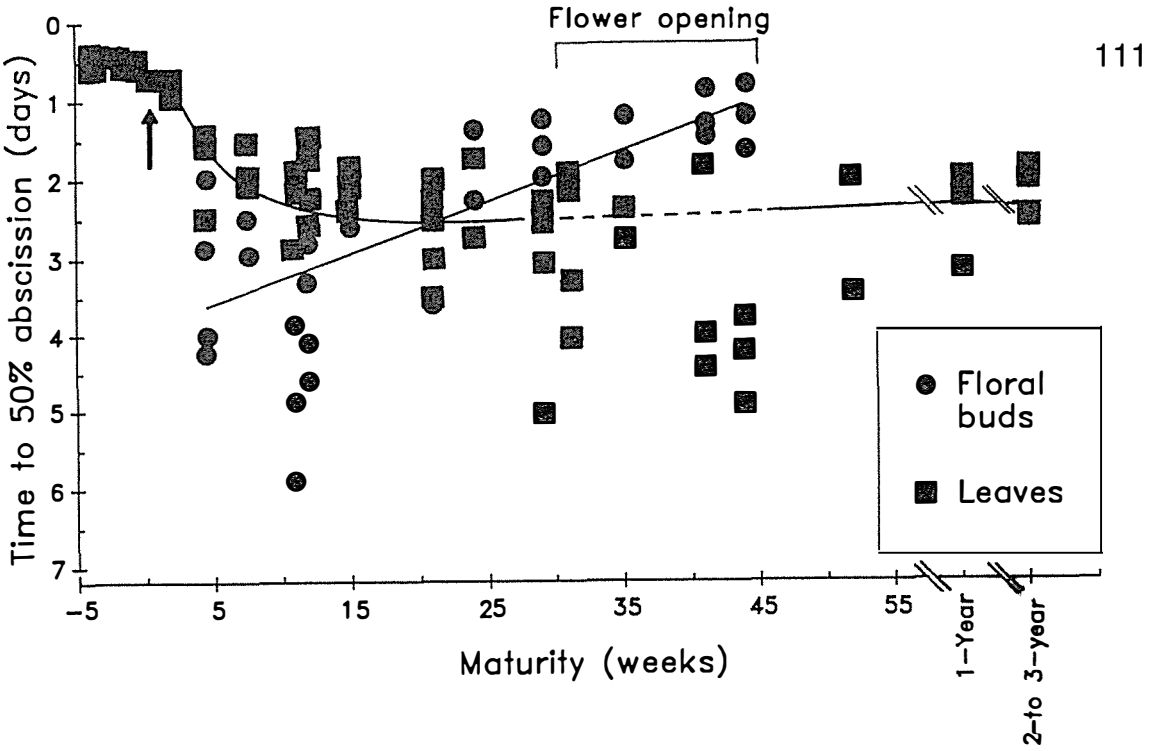


Figure 4.7. Effect of leaf and floral bud maturity (treated over 16 months) on rate (time to 50% abscission) of ethylene-promoted abscission (10 *u*l·liter^{-1}) of *Camellia* 'Anticipation'. Arrow indicates time of cessation of stem extension (0 weeks maturity) and brackets indicate the period of flower opening. Effect of floral bud maturity on abscission rate described by Y = 3.91 - 0.0650X, r^2 = 0.53. Effect of leaf maturity on abscission rate, line of best fit. (Experiment 4.4).

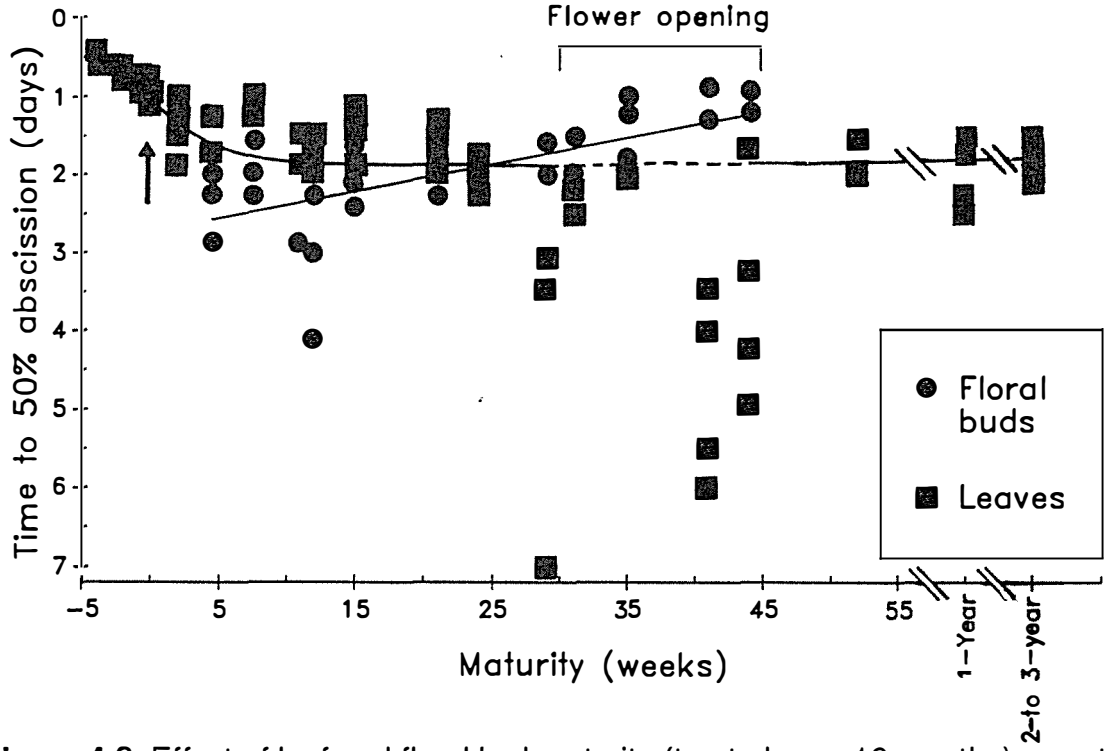


Figure 4.8. Effect of leaf and floral bud maturity (treated over 16 months) on rate (time to 50% abscission) of ethylene-promoted abscission (10 *u*l·liter^{-1}) of *Camellia* 'Donation'. Arrow indicates time of cessation of stem extension (0 weeks maturity) and brackets indicate the period of flower opening. Effect of floral bud maturity on abscission rate described by Y = 2.73 - 0.0340X, r^2 = 0.46. Effect of leaf maturity on abscission rate, line of best fit. (Experiment 4.4).

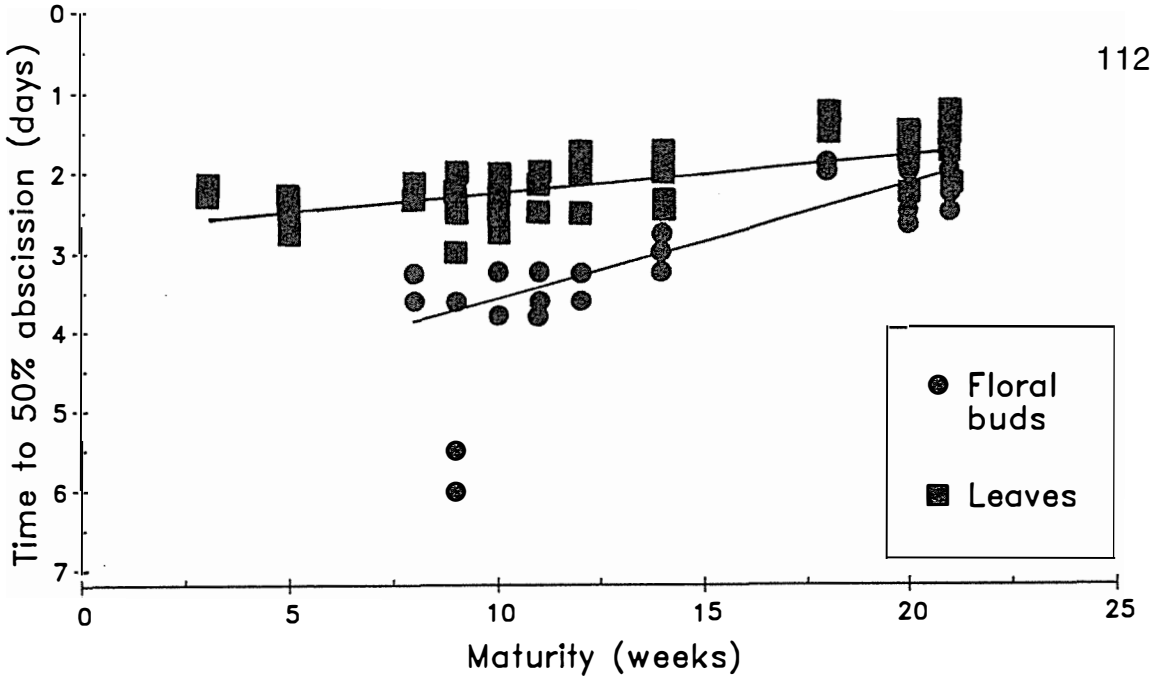


Figure 4.9. Effect of leaf and floral bud maturity (treated simultaneously) on rate (time to 50% abscission) of ethylene-promoted abscission (10 *u*l·liter^{-1}) of *Camellia* 'Anticipation'. Effect of leaf and floral bud maturity on abscission rate described by Y = 2.62 - 0.044X, r^2 = 0.38; Y = 5.03 - 0.144X, r^2 = 0.58, respectively. (Experiment 4.5).

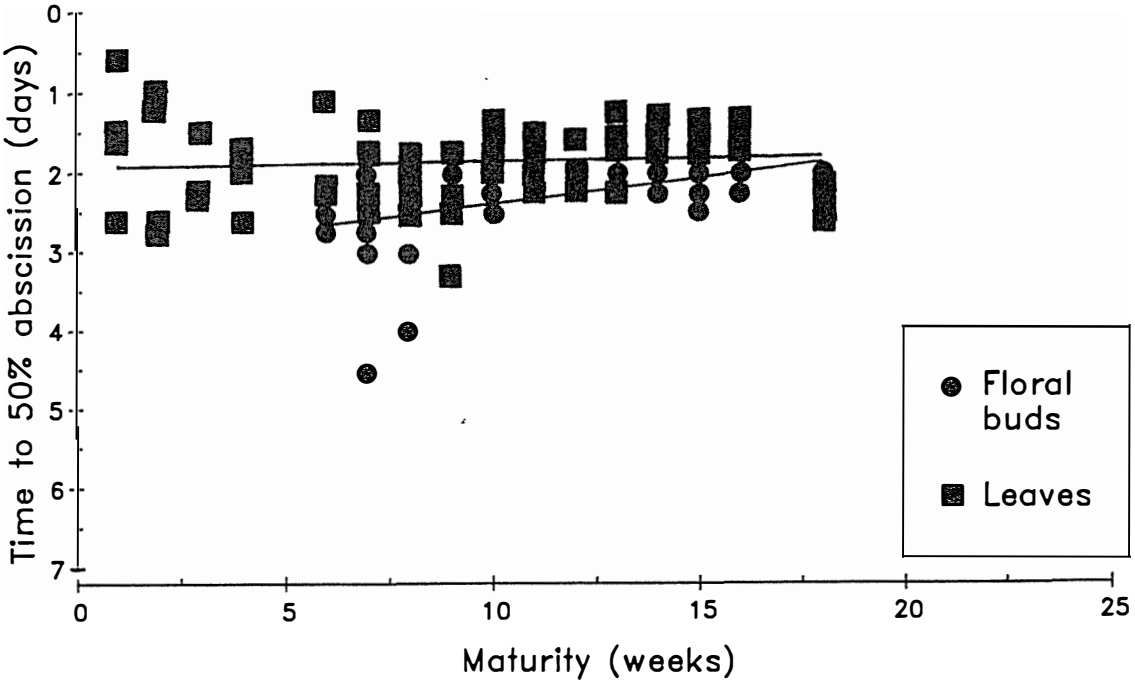


Figure 4.10. Effect of leaf and floral bud maturity (treated simultaneously) on rate (time to 50% abscission) of ethylene-promoted abscission (10 *u*l·liter^{-1}) of *Camellia* 'Donation'. Effect of leaf and floral bud maturity on abscission rate described by Y = 1.94 - 0.006X, r^2 = 0.004; Y = 3.05 - 0.073X, r^2 = 0.27, respectively. (Experiment 4.5).

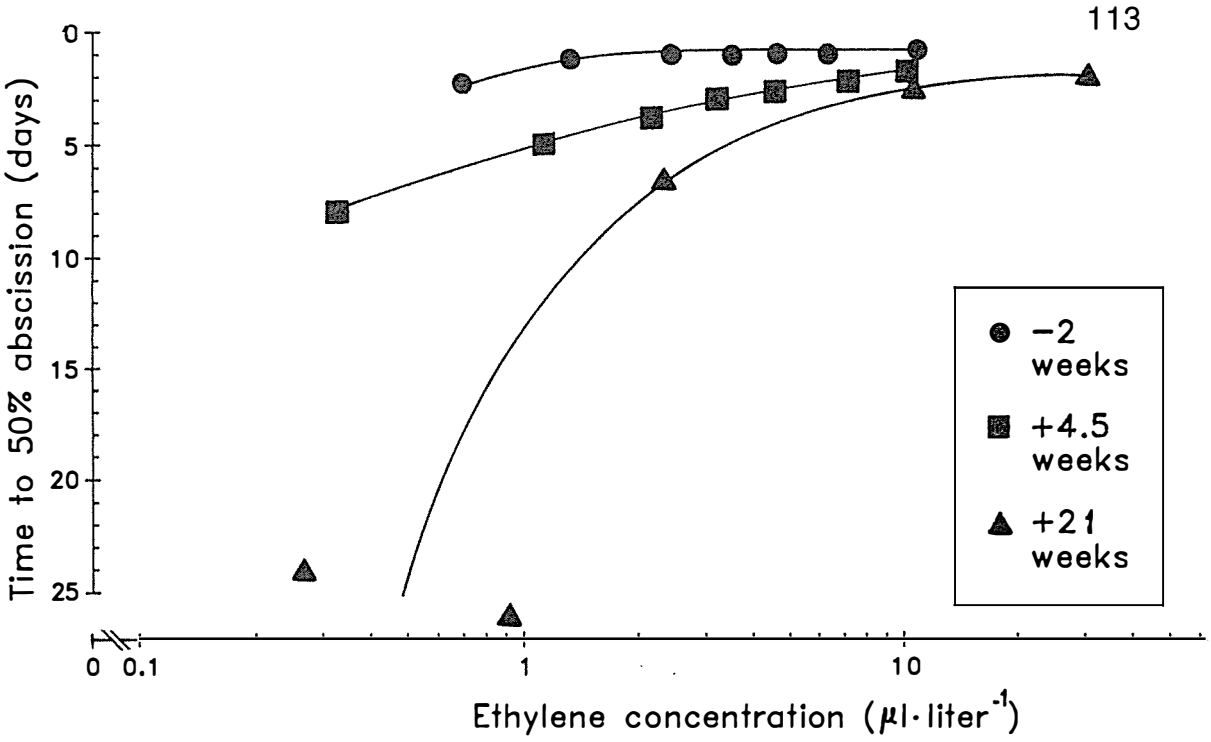


Figure 4.11. Effect of leaf maturity and ethylene concentration on abscission rate (time to 50% abscission) of ethylene-promoted abscission of *Camellia* 'Anticipation'. Lines of best fit. (Experiment 4.6).

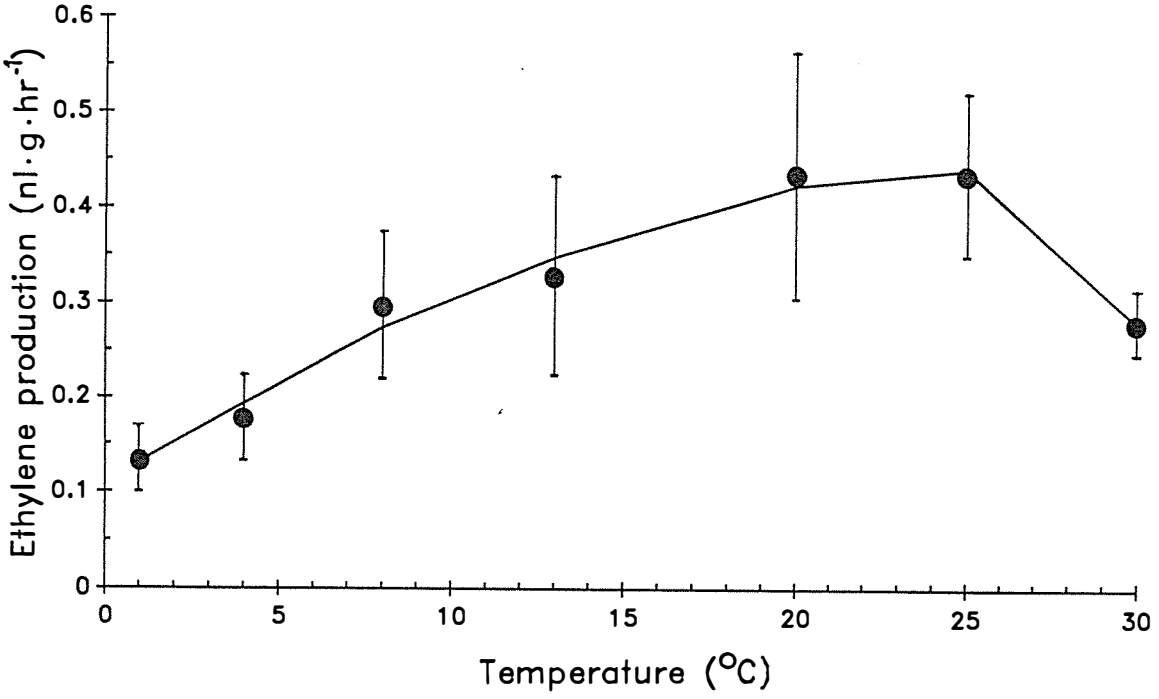


Figure 4.12. Effect of temperature on basal endogenous ethylene production of single-leaf stem explants of *Camellia* 'Brian'. Smoothed fit. (Experiment 4.7).

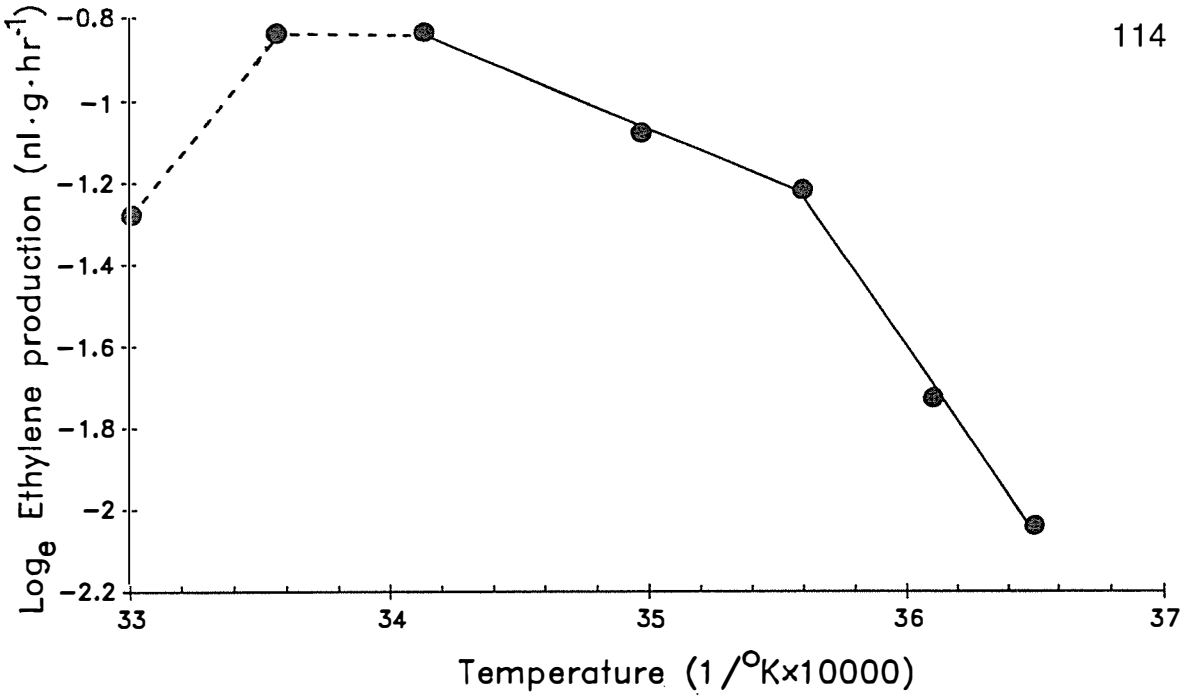


Figure 4.13. Arrhenius plot of temperature vs basal endogenous ethylene production of single-leaf stem explants of *Camellia* 'Brian'. Effect of temperature above and below 8°C described by Y = 8.08 - 0.262X, r^2 = 0.99 and Y = 31.24 - 0.912X, r^2 = 0.99 respectively. (Experiment 4.7).

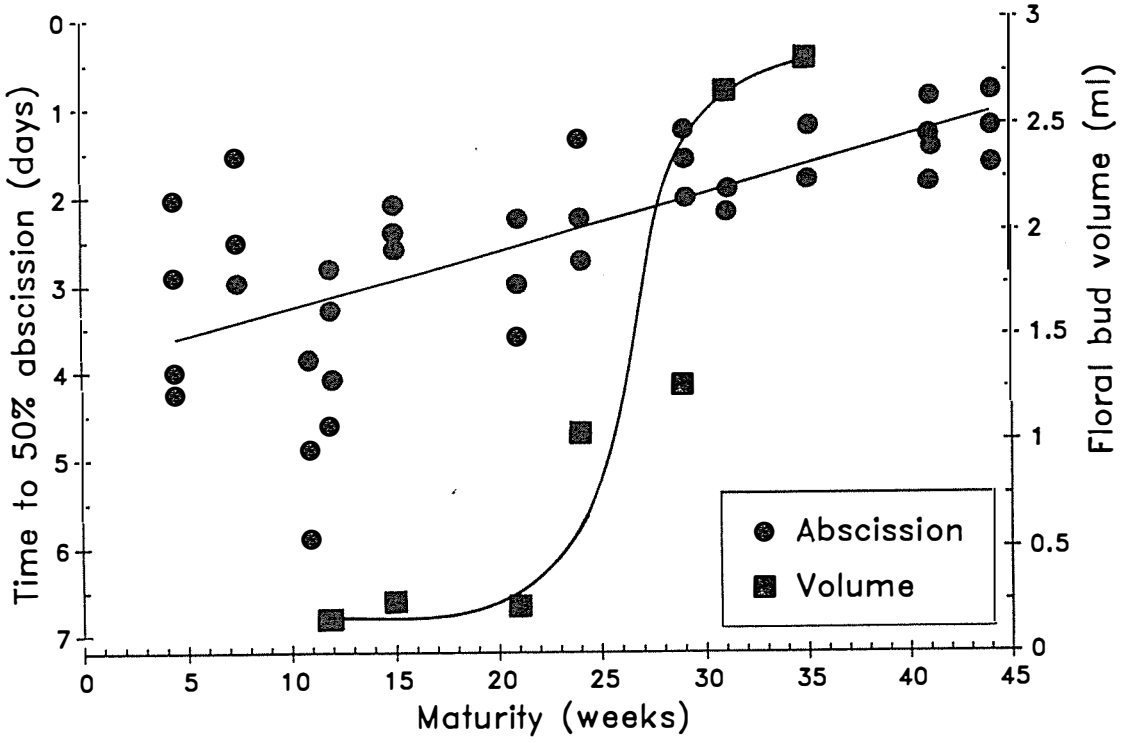


Figure 4.14. Effect of floral bud maturity (treated over 16 months) on rate (time to 50% abscission) of ethylene-promoted abscission (10 *ul*·liter^{-1}) and floral bud volume of *Camellia* 'Anticipation'. Effect of floral bud maturity on abscission rate described by Y = 3.91 - 0.0650X, r^2 = 0.53; floral bud volume, line of best fit. (Experiment 4.4).

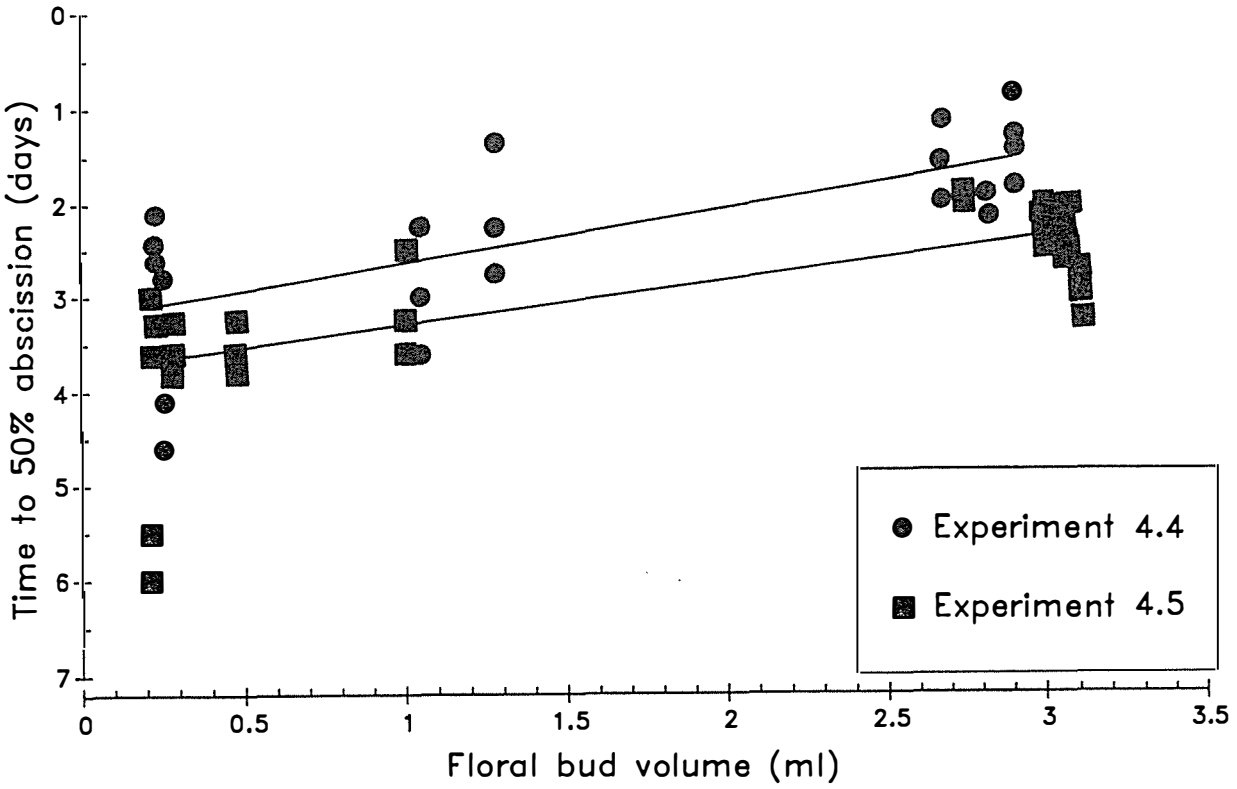


Figure 4.15. Effect of cultural environment (green house (Experiment 4.4) or shade house (Experiment 4.5)) on abscission rate (time to 50% abscission) of floral buds of a given maturity (volume). Abscission promoted by ethylene (10 *u*l·liter^{-1}) of *Camellia* 'Anticipation'. Effect of floral bud maturity of Experiment 4.4 and Experiment 4.5 described by Y = 3.22 - 0.58X, r^2 = 0.52 and Y = 3.76 - 0.46X, r^2 = 0.52 respectively. (Experiment 4.4 and 4.5).

4.5 DISCUSSION

It is clear that the ethylene-sensitivity of abscission in *Camellia* was determined by organ type and organ maturity and that temperature influenced ethylene-promoted abscission as well as the rate of endogenous ethylene production.

Ethylene-Sensitivity and Organ Type

Floral buds were more sensitive to ethylene than leaves in both cultivars of *Camellia* since floral buds consistently responded faster to lower ethylene concentrations than leaves (Figure 4.1 and 4.2; Experiment 4.1). The greater sensitivity of *Camellia* floral buds was comparable to the greater sensitivity of *Citrus* fruit and *Capsicum* floral buds than leaves (Ben-Yehoshua and Eaks, 1970; Beaudry and Kays, 1988a). At high ethylene concentrations (10 and 100 *u*l·liter^{-1}), the abscission rate of *Citrus* leaves is slightly faster than fruit,

however, leaves respond more slowly at 0.1 *u*l·liter^{-1} (Figure 1.5; Ben-Yehoshua and Eaks, 1970). The results for *Citrus* are similar to those for *Camellia* in that at high concentrations the abscission rate of reproductive organs is less rapid than leaves, while the response at low concentrations is more rapid, thus indicating lower sensitivity of leaves than reproductive organs by the definition employed in this study (Section 1.3.9). Similarly, abscission of *Capsicum* floral buds doubles between 0.01 and 1.0 *u*l·liter^{-1}, while a significant increase in abscission of leaves does not occur until ethylene concentration increases from 0.1 to 1 *u*l·liter^{-1} (Beaudry and Kays, 1988a). Floral organs have been found to be more sensitive to ethylene than leaves in genera other than *Camellia*, *Citrus* and *Capsicum* including *Gossypium* (Lipe and Morgan, 1973) and *Hibiscus* (Woltering, 1987). Thus it appears that ethylene-sensitivity of reproductive organs of most genera are greater than that of leaves, with few exceptions (*Pachystachus* and *Beloperone*; Woltering, 1987).

The physiological mechanism which determines differences in abscission of organs of the same species is not known (Woltering, 1987). However, some possible mechanisms for the greater ethylene-sensitivity of floral buds than leaves of *Camellia* may be put forward.

Firstly, one mechanism may be the greater senescence and reduced auxin level of floral buds than leaves. It was suggested that the greater sensitivity of *Olea* fruit than leaves could be explained by the genetically determined life-span of 1 season of reproductive organs, compared to the 3 to 4 years of leaves (Lang and Martin, 1985). *Camellia* floral buds and leaves have similar life-spans to that of *Olea* fruit and leaves and the ethylene-sensitivity differences may be due to the greater physiological age and therefore greater senescence of floral buds over leaves. Progressive aging towards death of leaf tissue results in increased senescence (Osborne, 1973), reduced auxin levels and greater sensitivity to applied ethylene (Riov *et al.*, 1986; Section 1.3.4.1). Auxin plays a similar role in ethylene-promoted abscission of floral buds and leaves (Hanisch ten Cate and Bruinsma, 1973a; Section 3.5). Since *Camellia* floral buds abscised after flower opening, their life-span was significantly shorter than that of leaves (3 to 4 years). It is therefore proposed that since floral buds were in closer proximity to their time to natural senescence and abscission, floral buds were more senescent than leaves and the associated reduction in auxin concentration resulted in greater ethylene-sensitivity, as proposed for other genera (Gilbart and Sink, 1971; Hanisch ten Cate *et al.*, 1975; Woltering, 1987). It is also possible that the greater

physiological maturity of floral buds than leaves may be reflected by greater concentration of ethylene receptors as found during fruit senescence/ripening (McGlasson, 1985; Yang, 1985).

Secondly, aside from the possible influence of relative senescence on receptor number, it is also conceivable that genetic control of abscission zone development of different organs determines the concentration or activity of ethylene receptors (Burdon and Sexton, 1989). Differences in binding site concentration have been noted in tissues from the same plant (Goren and Sisler, 1986; Sanders *et al.*, 1990). Thus floral bud abscission zones may have contained a higher concentration of ethylene binding sites and therefore resulted in a greater abscission response to low ethylene concentrations.

Thirdly, although it has been assumed that there are no differences in the rate of ethylene diffusion into the abscission zones of different organs of *Camellia* (Section 1.3.8) and *Olea* (Lang and Martin, 1989), the existence of differences cannot be ruled out (Osborne, 1989b; Lang and Martin, 1989; Burdon and Sexton, 1989). The rate of ethylene metabolism varies between different tissues of the plant (Beyer, 1977; Beyer and Sundin, 1978) and ethylene metabolism occurs in *Gossypium* leaf abscission zones (Beyer, 1979b; Section 1.3.1.1). Therefore slower rate of ethylene diffusion into *Camellia* leaf abscission zones and/or higher rate of ethylene metabolism in leaf than floral bud abscission zones could have been responsible for reduced ethylene concentration within leaf abscission zones and hence resulted in reduced abscission at low concentrations and lower ethylene-sensitivity.

Fourthly, as noted by Woltering (1987), differences in sensitivity of floral buds may be due to differences in the anatomy of the abscission zone cells as found by Miranda and Carlson (1981).

Finally, although the biochemistry of floral bud and leaf abscission may be the same (Section 1.3.2.2) as found in fruit and leaves of *Citrus* (Ratner *et al.*, 1969; Pollard and Biggs, 1970), differences have been found in *Prunus* (Ramina *et al.*, 1989) and may also exist in *Camellia*. The involvement of cellulase activity in organs of *Camellia* was examined in Chapter 5.

The greater sensitivity of *Camellia* floral buds supports the hypothesis (Chapter 3) that the determining factor in differential leaf and floral bud abscission following ethephon-application was ethylene-sensitivity and not the duration of ethylene exposure required for abscission, which is examined subsequently (Chapter 5).

Ethylene-Sensitivity and Organ Maturity

Maturity significantly influenced ethylene-responsiveness of both floral buds and leaves, although the patterns of response were quite different (Figure 4.7 to 4.10). The influence of maturity on leaf ethylene-responsiveness also correlated with its effect on ethylene-sensitivity (Figure 4.7 cf 4.11). Unexpanded leaves (<0 weeks maturity) were the most responsive to applied ethylene, whereas, the opposite was true of floral buds where responsiveness increased with maturity. The fact that *Camellia* leaves were able to abscise so soon after vegetative bud

break indicates that the abscission zone of leaves in *Camellia* were either already differentiated within the endodormant vegetative bud, or were differentiated very soon after vegetative bud break as found in *Sambucus* (Osborne, 1989a). The influence of maturity on ethylene-responsiveness was similar for both cultivars studied, indicating that the processes involved in determining response were similar in both cultivars and are probably widespread in *Camellia*.

Ethylene-Sensitivity and Leaf Maturity

Young expanding leaves (-5 to 0 week maturity) exhibited high responsiveness as measured by high abscission rate in response to a saturating ethylene concentration (10 ul·liter^{-1}; Figure 4.7 and 4.8; Experiment 4.4 and 4.5). Young leaves also demonstrated greater ethylene-sensitivity as determined by application of a range of ethylene concentrations (Figure 4.11; Experiment 4.6). Similarly, changes in abscission response of *Pelargonium* petals to a single ethylene concentration parallels the changes in response to a range of ethylene concentrations as maturity increases (Evensen, 1991).Thus use of a saturating ethylene concentration to examine changing tissue response (Experiment 4.4 and 4.5) was a valid means for comparison of the influence of maturity on ethylene-sensitivity within a given organ type. However, description of greater abscission of one organ type over another after application of a single ethylene concentration as more "sensitive", as carried out by Weis *et al.* (1988), may be invalid. For instance, floral buds of *Camellia* 'Donation' were more sensitive than

leaves since greater abscission occurred at low ethylene concentrations (Figure 4.1). Using the definition of sensitivity employed by Weis *et al.* (1988), saturated ethylene treatment of *Camellia* floral buds and leaves (Figure 4.8) carried out at maturity of 43 weeks (from cessation of stem extension) would correctly indicate higher floral bud sensitivity. However, treatment at 5 weeks would give the erroneous result that floral buds are less sensitive than leaves. Similarly, in *Citrus*, at an ethylene concentration of 10 ul·liter^{-1}, abscission of leaves occurs faster than that of fruit, yet leaves are clearly less sensitive than fruit since leaf abscission rate decreases significantly below 1 ul·liter^{-1} (Figure 1.5; Ben-Yehoshua and Eaks, 1970). Thus comparison of changing responsiveness due to the influence of maturity (Evensen, 1991) or growth substance application (Reid, 1985), for a given organ can be validly equated with changes in ethylene-sensitivity as determined by response at lower ethylene concentrations. However, examination of differences in organ sensitivity at a single saturating concentration may be erroneous and should best be described as a difference in responsiveness.

The changing responsiveness (Figure 4.7 to 4.10) and sensitivity (Figure 4.11) to ethylene of young leaves is supported by results obtained after ethephon application (Figure 3.12) and in literature examining this and other genera. Greater ethylene-responsiveness of young expanding leaves of *Camellia* found in this study was in agreement with those obtained over 50 years ago, albeit, with a far cruder means of ethylene application ("apple gas") (Funke *et al.*, 1938). Greater ethylene-responsiveness of young expanding leaves also occurs in *Gossypium* (Morgan and Durham, 1975; Beyer, 1975b) and *Grisellinia* (Funke *et al.*, 1938). Similarly, greater ethylene-sensitivity occurs in *Gossypium* (Morgan and Durham, 1973; Suttle and Hultstrand, 1991), *Capsicum*, *Dizygotheca* and *Browallia* (Woltering, 1987). There are several mechanisms which may determine the possibly universal pattern of high ethylene-sensitivity during leaf expansion.

A detailed examination of the role of auxins in the greater ethylene-sensitivity of young leaves of *Gossypium* was carried out by Suttle and Hultstrand (1991). Application of ethylene results in reduction in IAA levels in leaf tissue and reduces IAA transport from the leaves at young and old leaf maturities, yet, the pattern of greater ethylene-sensitivity of young *Gossypium* leaves remains (Suttle and Hultstrand, 1991). Both results are in agreement with existing understanding of the role of ethylene in abscission (Section 1.3.4.1) such that ethylene reduces auxin level in the leaf blade (Beyer, 1975b) and auxin transport to the abscission

zone (Beyer, 1973; Riov and Goren, 1979). Since the auxin concentration of the abscission zone is the most important factor determining sensitivity (Jackson and Osborne, 1972), it was therefore concluded that the mechanism of ethylene-sensitivity must reside in the abscission zone rather than the leaf blade (Suttle and Hultstrand, 1991). Although it was not possible to test the hypothesis due to technical difficulties, it was suggested that the differences may be mediated by accelerated IAA metabolism within the abscission zone or in the ability of the tissue to respond to IAA (Suttle and Hultstrand, 1991). This mechanism is also suggested to be a possible explanation of the greater ethylene-sensitivity of expanding *Camellia* leaves.

Other growth substances may also play a role in the sensitivity of young leaves of *Camellia*. A role for gibberellins in the greater ethylene-sensitivity of young expanding leaves of *Gossypium* was proposed by Morgan and Durham (1975). The level of endogenous gibberellins are high in elongating shoots of *Citrus* (Plummer, 1987) and *Malus* (Taylor *et al.*, 1984) and decreases as elongation ceases (Taylor *et al.*, 1984; Plummer, 1987). Exogenous gibberellins accelerate abscission of *Gossypium* leaves in the absence of ethylene (Chatterjee and Leopold, 1964; Bornman *et al.*, 1967; Ratner *et al.*, 1969) and in the ethylene-promoted abscission process (Morgan and Durham, 1975; Morgan, 1976). The mechanism of gibberellin promotion of abscission was suggested to be a result of decreased abscission-inhibiting influence of auxin (Morgan, 1976; Section 1.3.4.2) and due to the promotory influence of gibberellins on cell division (Bornman *et al.*, 1967) or promoting activity of hydrolysing enzymes (Chatterjee and Leopold, 1964) which therefore increases the rate of the abscission process (Bornman *et al.*, 1967; Morgan and Durham, 1975). Thus the greater ethylene-sensitivity of young expanding leaves of *Camellia* may have been due to high endogenous levels of gibberellins at vegetative bud break which reduced the inhibitory influence of IAA on ethylene-promoted abscission. As shoot extension ceased, endogenous gibberellin levels decreased and the promotory interaction of gibberellin with ethylene-promoted abscission therefore also declined and abscission rate decreased (Figure 4.7 and 4.8).

The response of young expanding leaves of *Gossypium* (Morgan and Durham, 1973; Morgan and Durham, 1975; Beyer, 1975b; Suttle and Hultstrand, 1991) and *Camellia* (Figure 4.7 and 4.8) appeared to be similar. Exogenous cytokinin and ABA produce little promotory or inhibitory interaction with ethylene-promoted abscission of young *Gossypium* leaves (Morgan and Durham, 1975). It is

therefore unlikely that endogenous levels of either cytokinins or ABA interacted with exogenous ethylene to result in the greater ethylene-sensitivity of young leaves in *Camellia*.

A third explanation may be found in the area of sensitivity differences due to changes in receptor concentration or activity (Dodds and Hall, 1980). Treatment of various maturities of *Camellia* leaves with a saturating ethylene concentration (Experiment 4.6) indicated that maximum abscission rate (minimum time to 50% abscission) occurred in youngest leaves (-2 weeks maturity; Figure 4.11). This may indicate that increased maturity resulted in either decreased receptor number (Trewavas, 1982; Paterson *et al.* 1990) or a change in the events subsequent to ethylene binding (Paterson *et al.*, 1990). Ethylene binding decreases on a fresh weight basis as *Nicotiana* leaves mature and expand, although binding site number per cell increases (Goren *et al.*, 1984). Similarly, in developing *Pisum* seedlings, binding site concentration decreases with maturity (Sanders, *et al.*, 1990). Decreased concentration of receptors would be expected to result in a reduction in the ethylene response (Dodds and Hall, 1980; Trewavas and Jones, 1981). Thus it is possible that the loss of high ethylene-sensitivity of young *Camellia* leaves as they matured, was due to decreased concentration of binding sites in the abscission zone such that binding site number was determined prior to cell expansion, and increased maturity involved a decrease in binding site concentration due to increased cell size.

A fourth possibility for a decline in leaf abscission rate with increasing maturity could be that of decreased ethylene permeability of the abscission zone. Ethylene permeability of *Pisum* stem tissue and *Vicia* leaves decrease with plant age (Zeroni *et al.*, 1977), possibly due to increased lignification (Addicott, 1982). At high ethylene concentrations it would be expected that the rate of ethylene diffusion into the tissue would have little effect on the abscission response. However, at low concentrations or if ethylene metabolism is high (Sanders *et al.*, 1989a) and increases with maturity (Beyer, 1977), then internal ethylene concentrations may be significantly reduced (Figure 1.6). Thus the observed differences in *Camellia* may be due to faster movement of ethylene into the abscission zone of soft, young tissue of the petiole, which decreased with maturity. The greater rate of ethylene movement would have resulted in higher internal ethylene concentrations and hence greater abscission, particularly at lower ethylene concentrations and therefore, gave the appearance of higher ethylene-sensitivity (Figure 4.11).

Finally, differences in the abscission response of young leaves may be due to activity of hydrolytic enzymes or their substrate, the cell wall. Jackson *et al.* (1973) suggested that the greater sensitivity of younger leaves may be a result of more efficient production of cell wall degrading enzymes. Such differences could not be examined in *Gossypium* leaves because significant cellulase activity was undetectable (Suttle and Hultstrand, 1991). A preliminary examination of cellulase activity in the abscission zones of *Camellia* leaves indicated that on an abscission zone/g FWt basis, abscission zones of younger leaves had higher cellulase activity than those of older leaves (data not shown). Thus differences may exist in the concentration or activity of hydrolytic enzymes in tissue of different maturities. This area may provide an interesting avenue for examination of differences in the abscission of young leaves, and indeed of many other ethylene-promoted responses influenced by maturity.

It has been suggested that the cell wall of young leaf abscission zone cells may be more readily degraded due to differences in composition or architecture (Jackson *et al.*, 1973; Suttle and Hultstrand, 1991). More specifically, the lignification of tissue is a process known to protect cell walls against degradation by hydrolytic enzymes (Vance *et al.*, 1980). Examples of differential resistance to the action of hydrolytic enzymes have been found in cell wall areas of the same cells (Rascio *et al.*, 1985), cell groups (Rascio *et al.*, 1987) and of walls of different cell types in the same tissue (Huberman *et al.*, 1983; Sexton *et al.*, 1984). Secondary thickening can result in loss of ability for cell separation and subsequent abscission to occur (Sexton and Roberts, 1982). For example, fruit of *Citrus* (Huberman *et al.*, 1983) and *Nicotiana* (Kendall, 1918) lose the capacity to abscise as they mature. The secondary thickening and loss of sensitivity to enzyme degradation is suggested to be due to deposition of lignin which blocks enzyme contact with the microfibril of the cell wall (Huberman *et al.*, 1983). *Camellia* shoots are quite flexible as they extend and as stem extension ceases become more rigid, presumably due to greater secondary thickening. The decreased ethylene-sensitivity of *Camellia* leaves with maturity during shoot extension may have involved increased lignification or modification of the cell wall structure such that the influence of hydrolytic enzymes was slowed or reduced. A means of measuring the ease of degradation of the middle lamellae by hydrolytic enzymes, either physically (e.g. break strength) or biochemically, would provide interesting insights into this mechanism.

In conclusion, the high ethylene-sensitivity of young expanding *Camellia* leaves may have been due to one reversible mechanism as suggested for *Gossypium* (Suttle and Hultstrand, 1991). However, as emphasised by Sexton *et al.* (1985), abscission is controlled by the balance of many factors mediated through a variety of hormonal and biochemical factors. To assume that sensitivity differences resulting from changes in maturity as a reflection of only one factor, as suggested above, is overly simplistic and the results presented here most likely involved two or more mechanisms. For instance, it seems unlikely that changes in receptor number alone would be responsible for first a decrease in sensitivity (as leaves matured from -4.5 to +5 weeks), and a subsequent increase in sensitivity as leaves mature year to year (Chapter 3, Figure 3.12). Although Trewavas (1982) suggested that tissues are continually acquiring and losing their sensitivity during development, it seems unlikely that sensitivity changes in *Camellia* were due to ethylene receptor concentration and/or activity alone. It is proposed that the sensitivity differences which occurred as *Camellia* leaves mature after vegetative bud break were due to changes in biochemical and hormonal factors and their many possible interactions.

Ethylene-Sensitivity and Floral Bud Maturity

The pattern of greater responsiveness or sensitivity to ethylene of floral organs with increasing maturity is supported by results obtained from both ethephon and ethylene application. As found in *Camellia*, the ethylene-sensitivity of many ornamental flowering plants increases as floral buds develop (Woltering, 1987). Similarly, increasing maturity of *Pelargonium* petals results in greater ethylene-sensitivity. Increasing maturity of floral buds up to flower opening also results in greater abscission responsiveness to ethephon in *Begonia* (Moe and Smith-Eriksen, 1986), *Malus* (Edgerton and Greenhalgh, 1969) and *Vitis* (Weaver and Pool, 1969). As fruit maturity increases, ethylene-sensitivity also increases in *Prunus* (Wittenbach and Bukovac, 1974) and *Gossypium* (Lipe and Morgan, 1972).

It is suggested that the increased responsiveness of floral buds with maturity of *Camellia* was not an artifact for the following reason. Floral bud growth followed a sigmoidal increase in volume while the response to applied ethylene exhibited a linear increase in abscission rate with maturity (Figure 4.14). If the influence of maturity on floral bud responsiveness observed was simply due to increased size/mass causing greater force when shaken (and thus an appearance of greater abscission rate), then a corresponding pattern of abscission would be

expected. However, the trend of increasing floral bud sensitivity continued in a linear manner in all treatments (Figure 4.7 to 4.10), and did not reflect the sigmoidal change in mass of floral buds.

The influence of maturity on abscission of floral buds was only examined in terms of ethylene-responsiveness (by applying a single saturating ethylene concentration). However, it is reasonable to assume that the influence of maturity on ethylene-responsiveness correlated with changes in ethylene-sensitivity since this occurred in *Camellia* leaves and has been demonstrated in the abscission of *Pelargonium* petals (Evensen, 1991).

Florai bud abscission could not be measured at less than 5 to 7 weeks maturity (Figure 4.7 to 4.10) because floral initiation in *Camellia* does not occur until after stem extension has ceased (Ee and Uemoto, 1983), approximately 28 days after commencement of shoot elongation (McElwee, 1952; Section 1.2.3.1). Although not examined in detail in this study, the abscission zones of floral buds may not have been differentiated until buds were 2 to 3 mm long, a size at which they had developed sufficiently to be differentiated visually from vegetative buds.

There are a range of possible mechanisms for the increasing ethylene-responsiveness with maturity of *Camellia* floral buds. Since the abscission process of floral buds and leaves is similar (Section 1.3.2.2) perhaps the most likely explanation for the gradual increase in ethylene-responsiveness of floral buds was the role of auxin in aging and senescence. A similar role of depletion of auxin in the abscission process of floral buds and leaves has been suggested (Gilbart and Sink, 1971; Hanisch ten Cate and Bruinsma, 1973a). Thus the greater ethylene-responsiveness of *Camellia* floral buds may have been a result of gradual reduction in auxin concentration with age which resulted in greater sensitivity to applied ethylene.

A second possible explanation for the gradual increase in ethylene-responsiveness of floral buds with maturity lies in the area of the concentration or activity of ethylene receptors (Dodds and Hall, 1980). Although the evidence for correlation of ethylene binding and ethylene-sensitivity is somewhat tenuous (Section 1.3.1.8), some support exists which correlates increasing ethylene binding with leaf (Goren *et al.*, 1984) and flower senescence (Brown *et al.*, 1986). Also, in a proposed model for ethylene-promoted flower senescence, a "sensitivity factor" is included which interacts directly with the receptor molecule

itself to determine sensitivity (Reid and Wu, 1992). A similar mechanism may operate in *Camellia* floral bud abscission zones such that with greater maturity the level of sensitivity factor increased. Thus it is possible that as *Camellia* floral buds matured to the time of flower opening, the concentration or activity of ethylene receptors in the abscission zone increased resulting in increased ethylene-sensitivity.

Further possible mechanisms may involve changes in other growth substances such as gibberellins. Gibberellin levels increase as flowers mature (Street and Opik, 1984) and this may have resulted in a similar interaction with ethylene as observed in young leaves of *Gossypium* (see above; Morgan and Durham, 1975).

Finally, as suggested for maturity differences of young *Camellia* leaves, the ability of tissue to produce cell wall hydrolysing enzymes may have increased with greater maturity of floral bud abscission zones.

Use of the two treatment systems of Experiments 4.4 and 4.5 (ethylene treatments carried out over 16 months or simultaneously) for examination of the influence of floral bud and leaf maturity on abscission, meant that the results obtained could be assured to be maturity effects rather than experimental artifacts. That this was indeed the case is reflected in the strong similarities of the abscission patterns of the two ethylene treatment systems (Figure 4.7 and 4.8 cf Figure 4.9 and 4.10).

However, some differences did exist between the maturity abscission patterns obtained from the two experimental systems. Firstly, greater variability of abscission data of Experiment 4.4 than 4.5 existed, probably because tissue maturity was not determined on an individual explant basis, as carried out in Experiment 4.5. Secondly, the slope of regression lines fitted to floral bud abscission trends in Experiment 4.5 (Figure 4.9 and 4.10) was consistently less than that of Experiment 4.4 (Figure 4.7 and 4.8). This indicated that the rate of floral bud development was faster in plants cultured under green house environmental conditions (18^{o} to 25^{o}C; 16 hr photoperiod) than in the covered shade house (ambient temperatures). The effect of environment on abscission rate at a given maturity was examined by plotting bud volume against abscission rate from Experiments 4.4 and 4.5 and a linear regression line fitted to each data set (Figure 4.15). There was little difference between the slope of the regression

lines of tissues from the two experiments indicating that changes in floral bud maturity had a similar influence on abscission rate under either cultural condition. It is interesting to note that at any given maturity (determined by bud volume), floral buds from plants grown in green house conditions (Experiment 4.5) had a lower abscission rate than tissue from plants grown in ambient temperatures (Experiment 4.4) (Figure 4.15). Forcing of *Pelargonium* at high temperatures results in greater ethylene-sensitivity of petals for a given maturity, than petals of plants forced at low temperatures (Evensen and Olson, 1992). This contrasts with results obtained in *Camellia* where for the same maturity, floral buds grown at high temperatures and continuous light (Experiment 4.5) had a slower abscission rate than floral buds of plants cultured at lower temperatures (Experiment 4.4). Although the nutritional regime of the two populations was similar, it is possible that nutritional stress, which has been shown to increase ethylene-sensitivity in other systems (He *et al.*, 1992), was greater in shade house cultured plants than green house cultured plants. The observed difference may also be due to other physiological factors such as reduced binding site number, since cultural environmental conditions influence ethylene binds site concentration (Goren and Sisler, 1986) and the concentration of other growth substance receptors (Trewavas, 1981).

Decreased ethylene-sensitivity and greater variability of abscission of leaves occurred over the flowering period of both cultivars of *Camellia* (30 to 45 weeks; Figure 4.7 and 4.8). This may have been due to the high respiration rate of open flowers (Coorts, 1973) which would therefore, in the experimental system employed, decrease the O_2 concentration more rapidly than for explants containing only floral buds. This would result in O_2 being limiting and abscission inhibited (Marynick and Addicott, 1976). However, in the 10 weeks prior to flower opening, the time to 50% abscission of floral buds and leaves was approximately the same (Figure 4.7 and 4.8). Since the abscission zones of both organs were in close proximity on the shoot, the abscission zones would be expected to be subject to similar O_2 concentrations. Thus if O_2 was the factor causing increased variability of abscission in leaves, then a similar result in the floral buds would be expected, but this did not occur. A second possibility was that flowering resulted in changes in endogenous levels of growth substances such as auxins (Swamy and Krishnamurthy, 1980) or cytokinins which are known to inhibit abscission (Addicott, 1982; Section 1.3.4.2). For example, flowering of *Perilla* results in increased cytokinin levels which delays leaf senescence and abscission (Beever

and Woolhouse, 1974). Also, differences resulting from detachment of *Citrus* fruit were explained by reduction in auxin supply from leaves to the fruit abscission zone (Ben-Yehoshua and Eaks, 1970).

Temperature and Ethylene-Promoted Abscission

Lower temperatures significantly decreased the abscission response of *Camellia* leaves and floral buds in response to applied ethylene (Figure 4.3 to 4.6). The exponential increase in abscission rate of both *Camellia* organs to increased temperature (Figure 4.4) was a typical temperature response, similar to that found in *Gossypium* leaves (Beyer, 1973). Reduction in abscission with lower temperatures of *Camellia* is also in agreement with that obtained in *Philodendron* leaves (Marousky, 1979) and leaves and floral organs of *Capsicum* (Beaudry and Kays, 1988a). Specific to *Camellia*, the results obtained in these experiments employing the *Camellia* hybrid 'Donation' were supported by those resulting from ethylene treatment of *C. japonica* 'Moshio' (R.E. Lill, pers. com.). With an ethylene exposure duration of 7 days, no abscission of 'Moshio' leaves occurs below 10 $ul \cdot liter^{-1}$ at 12°C, this being in agreement with a 7 day ethylene exposure of 'Donation' at 12°C (Figure 4.3).

The influence of temperature on abscission is an indication of its effect on metabolic activity of the plant (Addicott, 1982). Addicott (1968) suggested that high temperatures promote abscission through increased activity of temperature-sensitive enzymes. An important role of ethylene in its promotion of abscission is in the reduction in auxin transport to the abscission zone (Section 1.3.4.1). A high correlation of the influence of temperature on abscission and auxin transport has been demonstrated in leaves of *Gossypium* (Beyer, 1973). A further important step in ethylene-promoted abscission is reception of the ethylene signal (ethylene binding) (Starling *et al.*, 1986; Burden and Sexton, 1989; Section 1.3.1.4). Ethylene binding is influenced by temperature such that increased temperature (4° to 25°C) increases $^{14}C_2H_4$ binding by 2.4 times (Bengochea *et al.*, 1980b). Thus the effect of lower temperature on *Camellia* abscission may have, in part, involved reduction of the reception of the ethylene signal at the receptor site, slower inhibition of auxin transport, and reduced rate of transduction of the ethylene signal, enzyme translation and activity of resulting hydrolytic enzymes. However, ethylene solubility varies with temperature, being about twice as soluble in water at 0°C as at 25°C (Sisler and Goren, 1981). Assuming that the ethylene concentration within the cell cytosol was the important factor

determining ethylene concentration at the ethylene receptor (Section 1.3.9), then the increased ethylene solubility at lower temperatures would have reduced the influence of temperature on ethylene-promoted abscission.

The change in E_a of endogenous ethylene production observed at 8°C (Figure 4.13) was not observed in the abscission response (Figure 4.3). This may indicate that the ethylene biosynthetic pathway was not utilized in the abscission response of *Camellia*, or that ethylene binding to its receptor was not influenced by the phase state of the cell membrane (in which the ethylene binding protein is thought to be located; Dodds and Hall, 1980; Sisler, 1980). Lower temperature increased the variability of abscission in leaves and floral buds of *Camellia*, the greatest effect observed at 1°C for leaves (Figure 4.6). This is most likely due to decreased rate of reaction of the various biochemical steps involved in the abscission process, and thus also increased variability of time to abscission.

The pattern of influence of temperature on floral bud and leaf abscission was similar between 4° and 25°C (Figure 4.4 to 4.6), indicating a similar effect of temperature on both organs at the stage of maturity employed in this experiment. However, at 1°C, leaves exhibited a large decrease in abscission rate and increase in variability of abscission not observed at the same magnitude in floral buds (Figure 4.4). This sharp decrease in leaf abscission rate at lower temperatures could be suggested to indicate that the ethylene concentration required to saturate the abscission process increased with lower temperature. However, the response to 15 and 10 $ul \cdot liter^{-1}$ did not differ at 1°C (Figure 4.3) suggesting that the response was still saturated. A further explanation for the slower abscission rate of leaves at 1°C may be that since the abscission rate of leaves at higher temperatures was slower than floral buds, the effect of low temperatures simply magnified this difference by reduction in rate of reaction of enzymes involved in the abscission process.

Temperature and Ethylene Biosynthesis

Temperature had a significant influence on basal endogenous ethylene production from *Camellia* (Experiment 4.7; Figure 4.12). The Q_{10} value between 10° and 20°C (1.2) was similar to, but lower than that of *Prunus* shoots (2.2) or the generalized value of 1.8 (Olien and Bukovac, 1978).

Ethylene production of *Camellia* stem-leaf explants at low temperatures was low but detectable (Figure 4.12). Field and Barrowclough (1989) also found strong inhibition of endogenous ethylene production by low temperatures (2° to 5°C) in

Phaseolus leaves and *Dianthus* flowers. Ethylene production of *Camellia* at 2.5°C (Figure 4.12), was similar, although slightly higher than that of *Phaseolus* (Field, 1981b).

An Arrhenius plot was used to determine activation energies (E_a) over the temperature range 1° to 30°C. The critical chilling sensitive temperature (or transition point) reflecting a membrane phase change was observed in *Camellia* at 8°C (Figure 4.13), similar to that of 11.4°C in *Phaseolus* leaves (Field, 1981b). It was possible that the chilling response found at 8°C was an artifact since the number of temperature treatments was relatively few (Field, 1985). However, it should be noted that replication within each temperature was reasonable and the regression fit of the two lines around 8°C was high (0.99 in both cases). It should also be pointed out that a range of chilling response temperatures have been obtained by various researchers which range from 10°C (Mattoo *et al.*, 1977) to 15°C (Saltveit and Dilley, 1978). The differences between results obtained here with *Camellia* and those reported above may be due either to genetic differences or experimental error. It is possible that genetic differences between *Camellia* and other genera employed for examination of temperature influences (*Lycopersicon*, Mattoo *et al.*, 1977; *Pisum*, Saltveit and Dilley, 1978; *Phaseolus*, Field, 1981b) may result in physiological differences in response to varying temperature. Thus as suggested for the abscission response of *Olea* (Lang and Martin, 1985), the genetic and ecological differences between *Camellia* (a hardy woody flowering perennial) compared to *Lycopersicon*, *Pisum*, *Phaseolus* (soft annuals) may explain the lower temperature of apparent chilling response (8°C cf 11.4°C) and the lower high temperature inhibition (20° cf 30+°C). It is also important to note that errors in the determination of the E_a values and the chilling point itself may arise due to limited temperature treatment replication and inaccuracies in the measurement of ethylene production at low temperatures (Field, 1985).

E_a values obtained for *Camellia* (73.3 $kJ \cdot mol^{-1}$ below, and 22.0 $kJ \cdot mol^{-1}$ above the transition point), are in the lower portion of the range derived for *Malus* fruit, *Phaseolus* leaf discs, *Pisum* stem sections and *Lycopersicon* fruit (65.7 to 99.6 $kJ \cdot mol^{-1}$ below and 18.6 to 55.4 kJ·mol-1 above; Field, 1985). However, for the most physiologically comparable tissue type, *Phaseolus* leaf disks, E_a values of *Camellia* leaves were significantly lower, both below (73.3 cf 99.6 $kJ \cdot mol^{-1}$), and above (22.0 cf 55.4 $kJ \cdot mol^{-1}$) the transition point. Similarly, above the transition point, *Camellia* leaves also exhibited a lower E_a value than that of *Prunus*

cerasus shoots (22.0 $kJ \cdot mol^{-1}$ cf 38.5 $kJ \cdot mol^{-1}$). It therefore appears that the influence of temperature on endogenous ethylene production of *Camellia* leaves was similar but less than other genera.

High temperatures (>20°C) resulted in a plateau and decrease in endogenous ethylene production in *Camellia* (Figure 4.12). The plateau (20° to 25°C) and decrease (25° to 30°C) in ethylene production followed a similar pattern to that of basal production of *Phaseolus* (Field, 1981a). However, the inhibition of endogenous ethylene production from *Camellia* at moderate temperatures (20° to 25°C) is at variance with other tissues where inhibition normally occurs at high temperatures (30° to 35°C) (Saltveit and Dilley, 1978; Field, 1985). This result may be due to a number of factors.

The most likely explanation is that the greater respiration rate at high temperatures resulted in partial anaerobosis due to the limited O_2 supply in vials (Field, 1985). Subsequent to Experiment 4.7, a trial was carried out to determine the extent of O_2 depletion which may have occurred in the vials. It was found that after 18 hr at 5°, 15°, 25° and 30°C, the O_2 concentration declined to an average of 20, 18, 14 and 8% respectively (data not shown). The wound ethylene production of stems of *Pisum* seedlings are inhibited below 13 to 14% (Saltveit and Dilley, 1978) and these figures correspond to the temperatures at which a premature reduction in ethylene production was observed in *Camellia*. Thus the decreased ethylene production of *Camellia* leaves at 25° to 30°C, was most likely a result of depletion of O_2 concentrations (to below 14%), the mechanism involved most likely being inhibition of conversion of ACC to ethylene by EFE (Adams and Yang, 1979).

A second explanation could be that the accumulation of ethylene at different temperatures did not follow the same pattern (presumably linear) at different temperatures. The latter case was found to occur in *Phaseolus* leaf discs where the cumulative ethylene production at 25°C was greater than that at 20° and 15°C in the first 2 hr, but after 6 hr fell below even the ethylene concentration produced at 15°C (Field and Barrowclough, 1989).

A third, but less likely possibility was that, as temperature rose and ethylene production increased, autoinhibition of ethylene biosynthesis occurred (Field, 1985) due to reduced activity of ACC synthase and EFE (Riov and Yang, 1982), or because of depletion of metabolic substrates (Field, 1985) such as methionine

or S-adenosylmethionine (Section 1.3.5). Temperatures which brought about apparent high temperature inhibition of endogenous ethylene production (20° to 30°C) were not radically greater than those experienced by *Camellia* tissue in its growing environment (mean daily maximum 18.3°C, highest recorded 31°C) and was significantly lower than temperatures experienced in its country of origin (southern China) where mean daily maximum are as high as 35°C, and highest recorded temperatures exceed 40°C (Pearce and Smith, 1984). It would therefore seem unlikely that temperatures of 20° and 25°C would reflect a stress response, although temperatures of 30°C and greater might be expected to promote stress.

Finally, if the observed lack of increase in ethylene production of *Camellia* at 20° to 25°C was not an artifact, then the suggested mechanism is that of loss of integrity of the ethylene biosynthetic system due to disruption of membrane structure (Field, 1981a).

4.6 CONCLUSION

It was clearly demonstrated that floral buds of *Camellia* were more sensitive to ethylene than leaves, which agreed with trends in most genera where reproductive organs (floral buds, flowers, petals and fruit) are the most sensitive. The maturity of floral buds and leaves of *Camellia* also influenced their ethylene-sensitivity. Leaves were most sensitive during expansion after vegetative bud break, as found in some, but not all other genera. In contrast, ethylene-sensitivity of floral buds gradually increased with maturity.

The use of a single ethylene concentration to determine relative differences in ethylene-promoted abscission was only valid for a given organ, such as employed for examining maturity, but could not be employed for determination of relative ethylene-sensitivities of organs or species. The latter case required use of a range of ethylene concentrations.

Temperature influenced response to ethylene at all concentrations, but had greater effect at lower concentrations and temperatures. Lower temperatures also reduced the rate of endogenous ethylene production in leaves of *Camellia*.

Thus it is clear that many factors influence the abscission-response of an organ to applied ethylene including organ type, maturity, and temperature. These factors should therefore be considered in the application of ethephon for the

removal of floral buds and in the minimisation of abscission due to endogenous or exogenous ethylene during the culture, transportation and sale of potted *Camellia* plants.

CHAPTER 5

THE ETHYLENE-PROMOTED ABSCISSION PROCESS IN *CAMELLIA* AND ITS REVERSIBILITY.

5.1 INTRODUCTION

The ethylene- and ethephon-promoted abscission response of *Camellia* and its interaction with concentration, temperature and maturity have been examined previously (Chapters 2 to 4). This has provided sufficient understanding for a degree of control (promotion or inhibition) of ethylene-promoted abscission. However, there are many physiological and biochemical processes involved in *Camellia* organ abscission which remain unexamined.

The duration of ethylene exposure, a factor which required examination in *Camellia*, is an important determinant of abscission (Beaudry and Kays, 1988a; Lang and Martin, 1989). Continuing abscission after ethylene removal may be influenced by the presence of ethylene autocatalysis within the abscission zone or leaf blade. A subsequent biochemical step in the abscission process is that of production of hydrolytic enzymes involved in cell separation and hence, the presence of autocatalytic ethylene production and changes in cellulase (ß-1:4-glucanase) activity within the abscission zone are important processes for consideration. Also, use of STS, an inhibitor specific to ethylene action, allows further probing of the physiology of ethylene-promoted abscission. These factors have important practical applications since duration of ethylene exposure and STS may be used to avoid and inhibit abscission. Also, knowledge of the abscission process, particularly autocatalytic ethylene production, are important in the minimisation of abscission.

A minimum period of ethylene exposure, sometimes referred to as the induction period, is required for ethylene-promoted abscission to occur (Lang and Martin, 1985). In the process of ethylene-promoted abscission, there exists a period where no abscission occurs and hence, analogous to microbiological growth patterns, may be described as the lag phase (Sexton and Roberts, 1982). In many species, no abscission occurs if ethylene is removed during the lag phase,

a phenomenon which is widespread across genera and plant organs and was also expected to occur in *Camellia*. For example, leaves of *Fittonia* and *Philodendron* require an ethylene exposure duration of at least 2 days for leaf abscission to occur (Marousky, 1979). Similarly, ethylene exposure for 2 days fails to promote *Pelargonium* leaf abscission, while leaf abscission is promoted by exposure of 5 days (Marousky and Harbaugh, 1981). Ethylene exposure for less than 30 hr does not promote leaf abscission of *Dizygotheca* plants (Woltering, 1987). In *Citrus*, a minimum period of 13 hr exposure is required for leaf abscission to take place and removal of ethylene before 13 hr results in no abscission (Sisler *et al.*, 1985). Fruit of *Olea* do not abscise after a 12 hr ethylene exposure period, while 24 hr promotes abscission (Blumenfeld *et al.*, 1978). Finally, petal abscission of *Pelargonium* does not occur with ethylene exposure of less than 40 minutes (Evensen, 1991).

Exposure Duration

The duration of ethylene exposure has a crucial interaction with the ethylene-promoted abscission process. Thus, as indicated in the previous paragraph, an exposure duration shorter than the minimum duration will not result in abscission (Lang and Martin, 1989). However, the abscission-promoting effect of a duration of ethylene exposure is complicated by many factors which are mediated primarily by abscission rate. The rate of abscission is in turn affected by temperature and the ethylene concentration applied. Abscission rate is also influenced by the organ type and genera to which ethylene is applied.

As covered in previous sections, increased ethylene concentration increases the rate of abscission in *Camellia* (Figure 4.1 to 4.2) and in other genera (Cameron and Reid, 1983; Dostal *et al.*, 1991). Similarly, temperature also influences abscission rate (Figure 4.5 and 4.6; Marousky, 1979). Thus for a given ethylene exposure duration and plant organ, increased temperature or ethylene concentration will result in greater abscission due to greater abscission rate. This was observed by Dostal *et al.* (1991) where higher ethylene concentration increases final abscission of *Impatiens* floral buds.

Genetic differences are also of importance since if ethylene exposure duration, ethylene concentration and environmental factors are all maintained at some constant, then differences in time to abscission will result from phenotypic and genotypic effects. For a given plant, phenotypic differences exist between plant organs in their response to various durations of ethylene exposure. The final

abscission of *Hibiscus* leaves is less than that of flowers even after a 3-times-longer ethylene exposure period (Woltering, 1986). Fruits and leaves also differ in their abscission response to the duration of ethylene exposure. Both Blumenfeld *et al.* (1978) and Lang and Martin (1985; 1989) found that olive fruits respond to shorter ethylene exposure durations than do leaves. A similar difference occurs between fruit and leaf explants of *Citrus* where fruit abscission is promoted by 2 to 10 hr ethylene application, whereas leaf abscission requires 48 hr exposure (Ismail, 1970). Woltering (1986) found that in the range of ornamental crops examined, flowers and flower buds abscise after 24 hr, whereas abscission of leaves occurs after a longer period of exposure (72 hr). Similarly, in general, leaves and fruits abscise with 10 to 48 hr ethylene exposure, whereas floral organs respond more rapidly, taking 2.5 to 8 hr (Sexton and Roberts, 1982).

Genotypic variation of the rate of response to ethylene also occurs and therefore interacts with ethylene exposure duration to influence the final abscission response (Hoyer, 1985). As noted in previous paragraphs there are significant differences between genera/species in the time required for abscission to commence. Woltering (1986) sought to obtain an ethylene sensitivity rating for a number of foliage and flowering potted plants and found significant differences between species in the extent of leaf and floral bud abscission resulting from ethylene exposure.

The interaction of duration of ethylene exposure with temperature and ethylene concentration is therefore relatively complicated (Beyer and Morgan, 1971; Hoyer, 1985). However, a factor which further complicates studies of duration of ethylene exposure is that of continuing abscission after ethylene removal. For instance, if, after a given exposure duration, abscission cannot be reversed (as occurs in *Citrus* leaves; Sisler *et al.*, 1985), then modelling of the effect of exposure duration becomes more complicated. Thus the reversibility of abscission after ethylene removal is an important factor for examination.

Reversibility of Abscission

Much research carried out on the effect of ethylene exposure has involved continuous treatment of plants (Ismail, 1970; Ben-Yehoshua and Eaks, 1970; Morgan and Durham, 1973; Beyer, 1975b; Kays *et al.*, 1975; Goren *et al.*, 1988; Weis *et al.*, 1988; Suttle and Hultstrand, 1991). However, relatively little work has been reported on abscission taking place after ethylene removal. Depending on

the plant species, leaf abscission may or may not continue following the removal of ethylene. Abscission occurring a considerable time after the removal of ethylene is observed in *Citrus* where ethylene exposure of 13 to 18 hr results in leaf abscission which does not cease after ethylene removal (Sisler *et al.*, 1985). In *Olea*, an ethylene pulse (12 to 18 hr) results in an increase in leaf abscission 104 hr after commencement of ethylene treatment (Lang and Martin, 1985). Similarly, *Capsicum* plants treated with ethylene for 48 hr and transplanted to the field continue to abscise leaves for several days (Kays *et al.*, 1976). Once the abscission process of *Olea* fruit commences, ethylene removal does not result in reversal of abscission under the conditions examined (Blumenfeld *et al.*, 1978; Lang and Martin, 1985).

Conversely, ethylene removal leads to little or no continuing organ abscission in some cases. Removal of ethylene from *Phaseolus* explants results in cessation of the abscission process as reflected by decreased break strength (force required to separate the organ) (dela Fuente and Leopold, 1968) with reduced abscission and concomitant reduced accumulation of cellulase mRNA (Tucker *et al.*, 1988). The data of Marousky (1979) indicate that ethylene exposure of *Fittonia* for 2 days results in leaf abscission of 1.3, 6.5, and 0%, at 1, 2, and 3 days respectively after the removal of ethylene. A similar trend occurs in *Philodendron* where following a 3 day ethylene exposure, leaf abscission of 14.4% and 7.5% occurs for 0 to 1 and 1 to 3 days respectively after ethylene removal (Marousky and Harbaugh, 1979c). The duration of exposure has little effect on the level of continuing leaf abscission in *Philodendron* after removal of ethylene. During the 24 hr after ethylene removal following 2, 3 and 4 days ethylene exposure, similar levels of abscission resulted (9, 14.4 and 8% respectively) (Marousky and Harbaugh, 1979c). Similarly, flower abscission of *Impatiens* ceases after ethylene removal (Dostal *et al.*, 1991). Thus it is clear that there is a variety of genetic responses of the abscission process to both the duration of ethylene exposure and to its removal.

Mechanism of Abscission Reversibility.

The steps involved in the abscission process (reviewed previously in Section 1.3.2) point to possible mechanisms by which abscission may be reversed (Burdon and Sexton, 1989). A reduction in ethylene concentration near the abscission zone either by removal of ethylene or decrease in ethylene release from ethylene-releasing compounds (ERCs), may result in reversal of the abscission process. If the process of cell wall degradation has already

commenced (with concomitant break strength decrease), then the reversal of the process at this point results in break strength increase, a phenomenon known as retightening (Holm and Wilson, 1977). The duration of the persistence of the effect of ethylene application on break strength of *Phaseolus* leaf abscission zones is relatively short lived (approximately 1 hr) (dela Fuente and Leopold, 1969).

The influence of ethylene is reversible in other ethylene responses such as senescence of leaves (Thomas and Stoddart, 1980) and flowers (Wang and Woodson, 1989). In the abscission response, many steps in the reception and transduction of the ethylene signal have been found to be reversible and the key step in determining reversibility of the abscission response may be that of autocatalytic ethylene production. On removal of exogenous ethylene, rapid diffusion out of the tissue (Goren and Sisler, 1986) results in a rapid decline in internal ethylene concentration and therefore concentration at the receptor, since ethylene binding is also reversible (McKeon and Yang, 1987). Thus after ethylene removal, the ethylene signal can be reversed and the level of "second signal" reduced (Figure 1.2; Sisler *et al.*, 1983). Specific to leaves, removal of ethylene from *Gossypium* and *Phaseolus* results in reversal of ethylene-promoted inhibition of auxin transport, a key point in Stage I of ethylene-promoted leaf abscission (Beyer, 1973). At the translational stage, ethylene removal and replacement with 2,5-NBD (2,5-norbornadiene) to inhibit endogenous ethylene production, reduces cellulase mRNA (Tucker *et al.*, 1988). Similarly, in preclimacteric *Dianthus* petals, it was demonstrated that the expression of senescence-related mRNA requires continual reception of the ethylene signal (Lawton *et al.*, 1990). However, it should be noted that, as yet, no distinction has been made between transcriptional regulation and increased mRNA stability (Sexton *et al.*, 1989). As found for its synthesis, trafficking of cellulase over the cell membrane also requires the constant presence of ethylene (Abeles *et al.*, 1971). Finally, for retightening to occur, Abeles *et al.* (1971) and Leopold (1971) suggested that enzyme systems capable of restructuring the partially degraded cell wall must also be present. Thus, it appears that nearly all steps in the abscission process are reversible, and it is therefore important to establish the mechanism by which abscission continues in some species while not in others.

The variety of genera and tissue responses to exogenous ethylene, climacteric and nonclimacteric (reviewed previously; Section 1.3.6), can be applied to the problem of reversibility of abscission. The hypothesis that the key area in

reversibility of ethylene responses is the autocatalytic ethylene production is supported by evidence in many ethylene response systems. As found in the abscission process, flower senescence of *Dianthus* is reversible in preclimacteric (Lawton *et al.*, 1990) and climacteric ethylene production (Wang and Woodson, 1989). However, in the latter case, the senescence symptoms are only reversible if the autocatalytic ethylene production is blocked using a competitive inhibitor (2,5-NBD; Section 1.3.1.5) resulting in reduction of the ethylene biosynthetic process (Wang and Woodson, 1989). Similarly, the ethylene response of fruit ripening can be reversed by an ethylene inhibitor (Ag^+) (Tucker and Brady, 1987). As for flower senescence, autocatalytic ethylene production also appears to be the key area involved in the reversibility of ethylene action in fruit ripening. For example, ethylene removal from climacteric fruit such as *Musa* after a given duration will not result in reversal of the ethylene response (Whitehead and Bosse, 1991) because ethylene autocatalysis is in progress (Yang and Hoffman, 1984; McGlasson, 1985). The influence of ethylene is reversible on nonclimacteric fruit (which exhibit no autocatalysis), such as *Citrus* fruit.

Species which have been found to have abscission responses which continue after ethylene removal such as leaves of *Citrus* (Sisler *et al.*, 1985), *Olea* (Lang and Martin, 1985) and *Capsicum* (Kays *et al.*, 1976) all exhibit either high levels of autocatalytic ethylene production or high endogenous production levels (Kays *et al.*, 1976; Riov and Yang, 1982; Goren *et al.*, 1988).

Thus, ethylene application is a complicated interaction of factors including the rate of reaction, reversibility of the ethylene response and the presence of autocatalytic ethylene production. Because autocatalysis is central to the reversal of the abscission process and is also a process occurring in the abscission zone, the presence and role of autocatalytic ethylene production is examined in the current study.

Autocatalytic Ethylene Production in Abscission Zones

Ethylene production required for natural abscission (not promoted by exogenous ethylene), shows a climacteric-like pattern. Ethylene production by explants of *Citrus* rises in the 24 hr following excision to a level high enough to bring about leaf abscission. The increased ethylene production precedes abscission which commences the second day following excision and rises to a peak (20 $nl \cdot g^{-1} \cdot hr^{-1}$ by 96 hr) before declining once abscission is complete (Ben-Yehoshua and Aloni, 1974). A similar climacteric pattern of ethylene production, which also correlates

with abscission, occurs in leaves of *Melia* (Morgan and Durham, 1980), *Phaseolus*, *Prunus* and *Parthenocissus* (Jackson and Osborne, 1970). Aharoni *et al.* (1979b) found that autocatalytic ethylene production was located in segments of *Phaseolus* petiole containing the abscission zone. Jackson and Osborne (1970) suggested that the autocatalytic increase in ethylene production is part of the senescence process and ethylene so produced promotes the various biochemical changes required for abscission. Similar to that of fruit, a climacteric rise in respiration has been found in response to applied ethylene in *Coleus* leaf abscission zones (Reid, 1985). Thus although research has been carried out examining the ethylene production during natural abscission, little information is available on endogenous ethylene production occurring during the ethylene-promoted abscission process. This lack of research may be due to the technical problems involved in achieving an ethylene-type abscission response while measuring ethylene production from relatively small tissue (abscission zones). For example, Blumenfeld *et al.* (1978) examined abscission in *Olea* fruits but could not detect autocatalytic ethylene production in response to ethephon.

Autocatalytic Ethylene Production During Leaf Senescence

The leaf blade is also important since autocatalytic ethylene production in senescing leaf tissue has the potential to promote organ abscission after application of ERCs (Goren *et al.*, 1988) or in response to environmental factors which may be present in the transportation of live plants, e.g. darkness, heat stress, chilling temperatures, water stress, or presence of exogenous ethylene (Abeles, 1973; Lieberman, 1979; Morgan, 1984). Leaf senescence involves autocatalytic ethylene production, changes in respiration rate (Thimann, 1980) and chlorophyll content (Fuhrer *et al.*, 1982), and protein and RNA breakdown due to increased activity of hydrolytic enzymes (Mattoo and Aharoni, 1988). It has been suggested that although ethylene may not be the primary controlling agent of natural senescence, it does have a central role in its interactions with other hormones (Morgan, 1984; Bailey *et al.*, 1990). This is supported by the fact that leaf senescence is delayed by application of ethylene inhibitors such as Ag^+ and CO_2 (Aharoni *et al.*, 1979a; Goren *et al.*, 1984), or by hormones such as auxins, gibberellins and cytokinins (Thomas and Stoddart, 1980).

Exogenous ethylene promotes leaf chlorosis in whole plants of many genera. Ethylene treatment results in leaf chlorosis of *Philodendron* in the light (Marousky and Harbaugh, 1979c), of *Pelargonium* in the light or dark (Marousky and Harbaugh, 1979a) and *Kalanchoe* only in darkness (Marousky and Harbaugh,

1980). Ethephon also promotes leaf chlorosis in *Vitis* in the light (Clore and Fay, 1970). Exogenous ethylene results in greatly increased endogenous ethylene production by excised leaves of *Citrus* (Riov and Yang, 1982) and *Olea* (Goren *et al.*, 1988). Exogenous ethylene also promotes chlorophyll degradation and the climacteric-like respiratory rise characteristic of senescence in *Nicotiana* (Aharoni and Lieberman, 1979). Natural senescence results in less pronounced ethylene production in *Beta* and *Nicotiana* (Aharoni *et al.*, 1979b), although in *Nicotiana* leaves, the time to, and peak ethylene production rate, is influenced little by exogenous ethylene (Aharoni and Lieberman, 1979). Similarly, detached *Nymphoides* leaves exhibit no increased chlorophyll or protein degradation in response to exogenous ethylene (Goldney and van Steveninck, 1970). The lack of influence of exogenous ethylene in *Nicotiana* and *Nymphoides* may be due to high endogenous levels of ethylene.

However, exogenous ethylene does not always promote leaf senescence and autocatalytic ethylene production. Of the 27 ornamental foliage species examined by Woltering (1987), only 8 species exhibited leaf chlorosis in response to a 72 hr ethylene-treatment. No chlorosis occurs as *Prunus persica* leaves abscise after application of ERC (Porpiglia and Barden, 1980). Also, Thimann (1980) states that with few exceptions most investigations have found little influence of exogenous ethylene on leaf senescence.

STS and Abscission

As mentioned previously, there are a variety of ethylene inhibitors which may act by inhibition of ethylene biosynthesis or action (Section 1.3.1.5). The silver ion (Ag^+) strongly inhibits ethylene-promoted abscission and other ethylene responses (Beyer, 1976a). It is thought that Ag^+ inhibition acts by preventing ethylene binding through modification of an ethylene receptor molecule (Yang, 1985), or a sub-unit of a receptor complex (Veen, 1986). However, the reduction in ethylene binding found in some experiments (Sisler *et al.*, 1985) is likely to be an artifact (Sanders *et al.*, 1991). It is likely that Ag^+ blocks biological activity of the receptor complex (Veen, 1987), possibly by modification of sub-units of the ethylene receptor (Veen, 1986; Section 1.3.1.5 and 1.3.1.6).

The anionic complex of Ag^+, silver thiosulphate (STS; $Ag_2S_2O_3$) has been used extensively as a research tool in the examination of ethylene physiology (Veen, 1983) and as a commercially significant inhibitor of ethylene action (Cameron and Reid, 1983). After application to plant tissue, STS is freely transported

through the xylem, accumulates laterally by moving apoplastically (Veen, 1987), and subsequently releases Ag^+ (Veen and van de Geijn, 1978). The lower phytotoxicity (Veen, 1979) and persistence in plant material make its use horticulturally more desirable than application of Ag^+ in the form of $AgNO_3$ (Swart, 1980; van Meeteren and de Proft, 1982). In potted plants, STS application inhibits abscission of floral buds or flowers of *Camellia* (Lee and Song, 1992), *Impatiens* (Dostal *et al.*, 1991), *Streptocarpus* (Agnew *et al.*, 1985; Rewinkel-Jansen, 1986), *Hibiscus* (Hoyer, 1986), *Pelargonium*, *Calceolaria* and bracteoles of *Bougainvillea* (Cameron and Reid, 1983). Leaf abscission is also inhibited by STS application in *Begonia* and *Schefflera* (Auer and McConnell, 1984), *Ilex* and *Phoradendron* (Joyce *et al.*, 1990) and *Hibiscus* (Hoyer, 1986).

Propylene

Ethylene action may be promoted by use of other unsaturated aliphatic compounds such as propylene, an ethylene analogue (Burg and Burg, 1967). Thus an important use of propylene is to stimulate an ethylene-like response while allowing simultaneous measurement of endogenous ethylene production (McMurchie *et al.*, 1972). This is not possible using exogenous ethylene since accurate measurement of low levels of endogenous ethylene production may be confounded by slow diffusion of exogenous ethylene out of the plant tissue (Goren and Sisler, 1986). To obtain the same response level, a propylene concentration 130 times that of ethylene is required for the pea straight growth test (Burg and Burg, 1967) and 60 times that of ethylene for promotion of *Phaseolus* leaf abscission (Abeles and Gahagan, 1968). Thus in the current work a propylene concentration of 5000 $ul \cdot liter^{-1}$ was employed to ensure saturation of the abscission response.

5.2 OBJECTIVES

The duration of ethylene exposure, and its removal may be one of the factors determining the selective abscission nature of ethephon application to *Camellia* (Beaudry and Kays, 1988a). Application of ethylene gas allows accurate duration of ethylene exposure to be achieved compared to ethylene released after ethephon application (Blumenfeld *et al.*, 1978). There is an abundance of information on the duration of ethylene exposure required to bring about a senescent or abscission response in other plant species. However, there is much less information on the pattern of abscission prior to and after ethylene removal

for a range of exposure times on any species. There is also little comparison of responses of organs to ethylene removal. Thus the interaction of duration of exposure and the removal of ethylene from floral buds and leaves was examined in *Camellia*.

The ethylene exposure period may either directly promote abscission or induce the autocatalytic synthesis of endogenous ethylene which, in turn, may promote abscission both during and after ethylene exposure (Morgan, 1984; Goren *et al.*, 1988). The endogenous ethylene production of both leaf blades and leaf abscission zones in response to the ethylene analogue propylene was employed to determine whether autocatalysis occurs as ethylene-promoted leaf abscission or leaf senescence occur. Detailed research has been carried out into the physiology of ethylene-promoted leaf senescence of *Citrus* (Riov and Yang, 1982) and therefore *Citrus* leaves were included for comparison with *Camellia* leaf senescence. To further examine the abscission process, the ethylene inhibitor STS was applied.

The possible role of cellulase in the abscission process has been reviewed (Section 1.3.3) and was examined in *Camellia* leaves. Also, since differences in cellulase activity of different organs requires further investigation (Lang and Martin, 1985; Section 4.5), cellulase activity of floral buds and leaves were compared at the time of abscission.

5.3 MATERIALS AND METHODS

Experiment 5.1; Duration of Ethylene Exposure.

Plant Material and Treatment Environment. For examination of the duration of ethylene exposure, four-leaf apical shoots of *Camellia* 'Anticipation' of 21 weeks maturity were excised from stock plants in the early morning of 3 April 1990. Stem bases were placed in vials of double distilled water (30 ml), wound ethylene was dissipated for 24 hr, and explants were then placed in jars (1.81 liter). Five (one shoot per jar) replicates were used for each treatment period. This, and all subsequent experiments, were carried out in a randomised complete block design in a constant environment ($25^{o} \pm 2^{o}C$, continuous light (45 ± 15 $u mol \cdot m^{-2} s^{-1}$) provided by cool-white Osram^{R} fluorescent tubes) unless otherwise stated.

Ethylene Application and Removal. Explants were treated with a saturating ethylene concentration (10 *u*l·liter^{-1}) for 0, 18, 27, 38, 50 or 74 hr, or continuously treated for 168 hr. Ethylene was removed by flushing the jars with ethylene-free air, placing an ethylene scrubber Purafil^{R} (5 g) in the base of the jar and resealing (except for continuous treatment). After flushing and at completion of the experiment, the efficacy of ethylene scrubbing was verified. Floral bud and leaf abscission were recorded every 6 to 12 hr and the experiment was discontinued after 7 days (168 hr).

Experiment 5.2; Ethylene Production From the Leaf Blade and Abscission Zone.

Plant Material. Endogenous ethylene production was examined from leaf tissue or explants containing abscission zones in response to propylene application. This allowed measurement of endogenous ethylene production during ethylene-like promotion of leaf abscission or leaf senescence. Single-leaf stem explants (consisting of 1 cm of stem and a single apical leaf with all other abscission zones removed) and leaf-blade explants (consisting of a leaf blade and petiole with no abscission zone) of *Camellia* 'Brian' were collected as for Experiment 5.1 on 20 June 1991 (approximately 30 weeks maturity). Leaf-blade explants of *Citrus limon* 'Meyer' were included for comparison with *Camellia* and were prepared similarly to leaf-blade explants of *Camellia* except that the petiole was excised at the base of the leaf blade so as to remove the abscission zone at the petiole/leaf blade junction since this was not present in *Camellia*. Explants were weighed, inserted into vials with 1 ml double distilled water in the base and placed in humidified flowing air for 24 hr to dissipate wound ethylene prior to treatment.

Propylene Treatment. The bases of explants were placed in double distilled water (1 ml) in glass vials (30 ml). Ten explants of each explant type (leaf-blade, and single-leaf stem explants) were treated with propylene while control explants were placed in flowing-air. A flow-through propylene concentration of 5000 *u*l·liter^{-1} was obtained by mixing pure propylene with flowing air using a capillary system similar to that of Knee *et al.* (1988). No ethylene was detectable in the resulting propylene mixture. A capillary tube (150 *u*m) mounted with a hypodermic needle was inserted through the top of each vial with a flow rate of 1.5 ml·min^{-1} (3 air changes/hr). Ethylene production was determined at intervals over the following 125 hr by removing the flow-through system, flushing vials with ethylene-free air (to ensure <0.005 *u*l·liter^{-1} background ethylene concentration),

and re-injection of propylene (<5 min.after flushing). Ethylene production was then determined by sealing vials for 2 hr, measuring the resulting ethylene concentration, and re-establishing the flow-through system by re-insertion of the needles. Ethylene concentration was determined for samples (1 ml) injected into a Photovac^{R} gas chromatograph (see Section 4.3). Abscission, background ethylene at the time of removal of the flow-through system, propylene, CO_2 and O_2 concentrations were monitored periodically throughout the experiment which was carried out at $29^{o} \pm 0.5^{o}C$ with a light intensity 45 ± 15 *u*mol·m^{-2}·s^{-1}.

Experiment 5.3; STS Inhibition of Ethylene-Promoted Abscission. Four-leaf apical shoots of *Camellia* 'Anticipation' were excised from stock plants and prepared as for Experiment 5.1 on 3 May 1990 (24 weeks maturity). Half of the shoots were pulsed with STS (0.2 mmol·liter^{-1}) for 24 hr in the light while controls were placed in distilled water (Veen, 1979). Ethylene was applied to 5 explants per treatment by injection of pure ethylene into jars to achieve concentrations of 0, 1 and 10 *u*l·liter^{-1} (see Section 4.3). Abscission was examined daily over a 21 day period.

Experiment 5.4 and 5.5; Cellulase and Abscission in *Camellia*.
Plant Material. In both experiments, cellulase activity was determined in abscission zones cut from 3-leaf apical shoots containing 2 to 3 floral buds obtained from a uniform hedge of *Camellia* 'Brian'. These were collected on 17 December 1991 (approximately 4 weeks maturity) and randomly allocated to ethylene or no ethylene (control) treatments.

Ethylene Treatment. Shoots treated with ethylene were inserted into vials of double distilled water and placed in clear 30 liter perspex cases. Pure ethylene was mixed with humidified air to result in ambient O_2 and CO_2 levels and an ethylene concentration of 15 *u*l·liter^{-1} (sufficient to promote a saturated abscission response; Section 4.4). Flow rate through the cases was selected to maintain three air changes/hr, thus ensuring no CO_2 accumulation or O_2 depletion. Temperature in the cases was maintained at $29^{o} \pm 1^{o}C$ and light intensity 45 ± 15 *u*mol·m^{-2}·s^{-1}.

Experiment 5.4; Cellulase Activity at Time of Abscission of Leaves and Floral Buds. Comparisons of cellulase activity of leaf and floral bud abscission zones were carried out using a population of shoots bearing both floral buds and leaves. For ethylene treated tissue, abscission zones of leaves and floral buds

were collected at the time of abscission, all samples were obtained within a 10 hr period. Abscission zone sections (1 mm either side of the abscission layer) were excised with a scalpel, weighed, frozen by immersion in liquid air and stored frozen (-70^{o}C) for subsequent cellulase extraction and analysis. Abscission zone sections of control shoots (no ethylene, not abscised) were excised at the time of commencement of ethylene treatment and prepared in the same manner. Triplicate samples each of 30 abscission zones were collected for each treatment.

Experiment 5.5; Cellulase Activity over the Leaf Abscission Process. An examination was made of the change in cellulase activity over the leaf abscission process. A population of shoots was placed in four 30 liter perspex cases, treated with ethylene, and triplicate samples collected at 10 sampling times over the 80 hr abscission process. Leaf abscission zones were excised and prepared in the same manner as for Experiment 5.4.

Cellulase Extraction. Cellulase was extracted and activity determined at one time for samples of both Experiments 5.4 and 5.5. Cellulase was extracted from abscission zones by removing samples from frozen storage and grinding each replicate with acid washed sand in a mortar and pestle using 1.5 ml of extraction solution. The latter consisted of sodium phosphate buffer (0.2 M; pH7), macaptoethanol (0.01% v/v) and NaCl (1 M). The mortar and pestle were rinsed with extraction solution after each replicate. Ground samples were centrifuged (Epindorf^{R} desk-top centrifuge, 13,000 rpm, 5 min.), and the supernatant collected and stored (-70^{o}C) for subsequent determination of cellulase activity by viscometry.

Cellulase Activity. The sodium salt of carboxymethylcellulose (CMC, 2% w/v; BDH, Poole, England) was used as a substrate with sodium benzylpenicillin (0.01%, w/v, Glaxo Laboratories, Greenford, England) included in the stock solution as an antibiotic (Kanellis *et al.*, 1989). Cellulase activity was determined by incubation (25^{o}C, 8 hr) of a combination of enzyme extract (250 *u*l) with CMC (750 *u*l) in a shaking water bath. The time for movement of 100 *u*l of the reaction mixture through pipettes (200 *u*l) was used to measure percent decrease in viscosity hourly over an 8 hr period (Durbin *et al.*, 1981). The reaction solution was mixed prior to each run and the same pipette used for each sample. Cellulase activity was examined simultaneously for 8 tissue samples of each run to minimise variability. Percent decrease in viscosity was converted to a relative

unit/g fresh weight/30 abscission zone basis (not the strict unit term employed by Durbin *et al.*, 1981), each chemical replicate run plotted over time, and average cellulase activity at 2.5 hr used for treatment comparison.[3]

5.4 RESULTS

Both leaves and floral buds followed a sigmoidal abscission response to continuous ethylene exposure (Figure 5.1). The major differences between the response of the leaves and floral buds were observed in the time to commencement of, and variability of abscission. The lag phase for leaf abscission was longer than that for floral buds (by approximately 10 hr). Leaf abscission increased in a similar manner to that of floral buds reaching the stationary phase by 60 hr although complete leaf abscission did not occur until 140 hr.

A minimum period of ethylene exposure was required for abscission (cell separation) to commence. Discontinuous ethylene exposure in Experiment 5.1 indicated that neither floral buds nor leaves abscised with less than 18 hr ethylene exposure period, and that abscission commenced between 18 and 31 hr after exposure (Figure 5.2 and 5.3). In support of this, the continuous exposure treatment of Experiment 5.1 indicated that no abscission occurred in floral buds until 24 to 31 hr after commencement of ethylene application, and between 31 and 37 hr for leaves (Figure 5.1).

The sigmoidal abscission response of leaves to ethylene continued until ethylene removal. Following ethylene removal, some abscission (residual abscission) continued to occur (Figure 5.3). Only 13.5% (SE=3.0) leaf abscission occurred after the removal of ethylene. The mean time to final abscission after removal of ethylene was 7.0 hr (SE=1.5) with the latest abscission occurring 23 hr after ethylene removal. After the completion of the residual abscission phase, no further abscission occurred before termination of the experiment (up to 96 hr later).

Floral buds responded similarly to leaves to the removal of ethylene (Figure 5.2). The amount of residual floral bud abscission was 23.9% (SE = 8.1) and mean time to completion of residual abscission was 7.9 hr (SE = 2.5) with the latest abscission occurring 17 hr after ethylene removal. As with leaves, after the completion of the phase of residual abscission, no further abscission occurred.

[3] Activity at 2.5 hr was in the linear portion of the cellulase assay time course.

The cellulase activity of abscission zones of floral buds differed from that of leaves before ethylene application (control) and at completion of ethylene-promoted abscission (abscised) in Experiment 5.4 (Table 5.1). Cellulase activity of control floral bud abscission zones was similar and slightly higher than that of leaves. In abscised leaf abscission zones, cellulase activity was 355% greater than that of control abscission zones. However, floral bud abscission zones exhibited no increase in cellulase activity between control and abscised abscission zones.

Experiment 5.5 demonstrated that cellulase activity increased by 840% (0.13 to 1.09 units·g^{-1}/30 abscission zones) over the ethylene-promoted leaf abscission process in *Camellia* 'Brian' (Figure 5.4). Leaf abscission commenced after 58 hr from the start of ethylene treatment, rose rapidly to 83% (74 hr), and peaked at 95% by 88 hr (Figure 5.4). Cellulase activity did not change between 0 and 20 hr, but doubled in activity between 20 and 58 hr, and increased nearly five-fold with a linear response after 58 hr showing no decline in activity up to 88 hr. The rise in cellulase activity (20 to 58 hr) preceded the onset of leaf abscission (58 hr).

Increase in ethylene production (0.25 to 0.42 nl·g^{-1}·hr^{-1}) of *Citrus limon* 'Meyer' leaf tissue (containing no abscission zones) was apparent after only 6 hr of propylene exposure. A climacteric like pattern was observed such that a steady increase occurred up to 78 hr (62.2 nl·g^{-1}·hr^{-1}), after which, ethylene production declined (Figure 5.5). In stark contrast, leaf-blade explants of *Camellia* did not exhibit any leaf senescence as there was no increase in endogenous ethylene production after application of exogenous propylene to the leaf tissue. Both the level of ethylene production from control (air) and propylene-treated *Camellia* leaves were similar to control leaves of *Citrus* (approximately 0.1 to 0.2 nl·g^{-1}·hr^{-1}). *Citrus* leaves exhibited leaf chlorosis and general loss of integrity, while no chlorosis of *Camellia* leaf blades was observed after 124 hr exposure to propylene (5000 *u*l·$liter^{-1}$).

Application of propylene to single-leaf stem explants of *Camellia* resulted in abscission which was accompanied by a climacteric pattern of endogenous ethylene production compared to relatively constant ethylene production of control (air) tissue which did not abscise (Figure 5.6). Ethylene production was more strongly promoted prior to the large increase in abscission (20 to 60 hr). To further examine ethylene production and the abscission process, ethylene

production was plotted around the point of abscission for each explant, the ethylene production at 5 hr intervals determined and the mean and SE calculated (Figure 5.7). Ethylene production increased prior to abscission and declined rapidly after separation of the leaf blade.

Propylene treatment (5000 ul·liter^{-1}) of *Camellia* 'Brian' in Experiment 5.2 resulted in very similar abscission kinetics to that of ethylene treatment of 'Brian' (15 ul·liter^{-1}) in Experiment 5.5 (Figure 5.6 cf Figure 5.4). With ethylene treatment, abscission increased most significantly between 55 hr and 80 hr (Figure 5.6), and after propylene treatment between 58 hr and 78 hr (Figure 5.4). Maximum abscission proportion attained was 90% and 95% for propylene and ethylene treatment respectively.

Ethylene-promoted abscission of *Camellia* 'Anticipation' leaves showed a concentration dependent response (Figure 5.8). Abscission commenced after between 1 to 2 days and attained 50% and 100% abscission by day 2 and day 5 respectively at an ethylene concentration of 10 ul·liter^{-1}. For 1 ul·liter^{-1} ethylene, abscission started 3 to 4 days after commencement of treatment and 50% and 100% abscission occurred at day 7.5 and day 16 respectively. In the same experiment (Experiment 5.3) an STS pulse (0.2 mmol·liter^{-1}, 24 hr) completely inhibited leaf abscission in shoots exposed to either 1 or 10 ul·liter^{-1} ethylene over the 21 day experimental period.

Ethylene treatment of floral buds resulted in similar abscission to that of leaves except that floral buds were less influenced by ethylene concentration reaching 100% abscission by day 7 instead of day 16 as for leaves at 1 ul·liter^{-1} (Figure 5.9 cf Figure 5.8). Fifty percent abscission of floral buds occurred between 1 to 2 days and 3 to 4 days for 1 and 10 ul·liter^{-1}. STS at the concentration used was not able to completely inhibit ethylene promoted abscission of floral buds (Figure 5.9). At ethylene concentrations of 1 and 10 ul·liter^{-1}, commencement of floral bud abscission was delayed by day 4 and day 7 respectively over that of untreated explants, and final abscission decreased from 100% to 40% and 77% respectively. No phytotoxic symptoms were observed in any organs of *Camellia* in response to applied STS.

Organ type	Control Activity	SE	Abscised Activity	SE
Floral buds	0.19	0.06	0.19	0.03
Leaves	0.11	0.01	0.39	0.03

Table 5.1. Cellulase activity of abscission zones of floral buds and leaves of *Camellia* 'Brian' before ethylene application (control) and at completion of ethylene-promoted abscission (abscised) (Experiment 5.4).

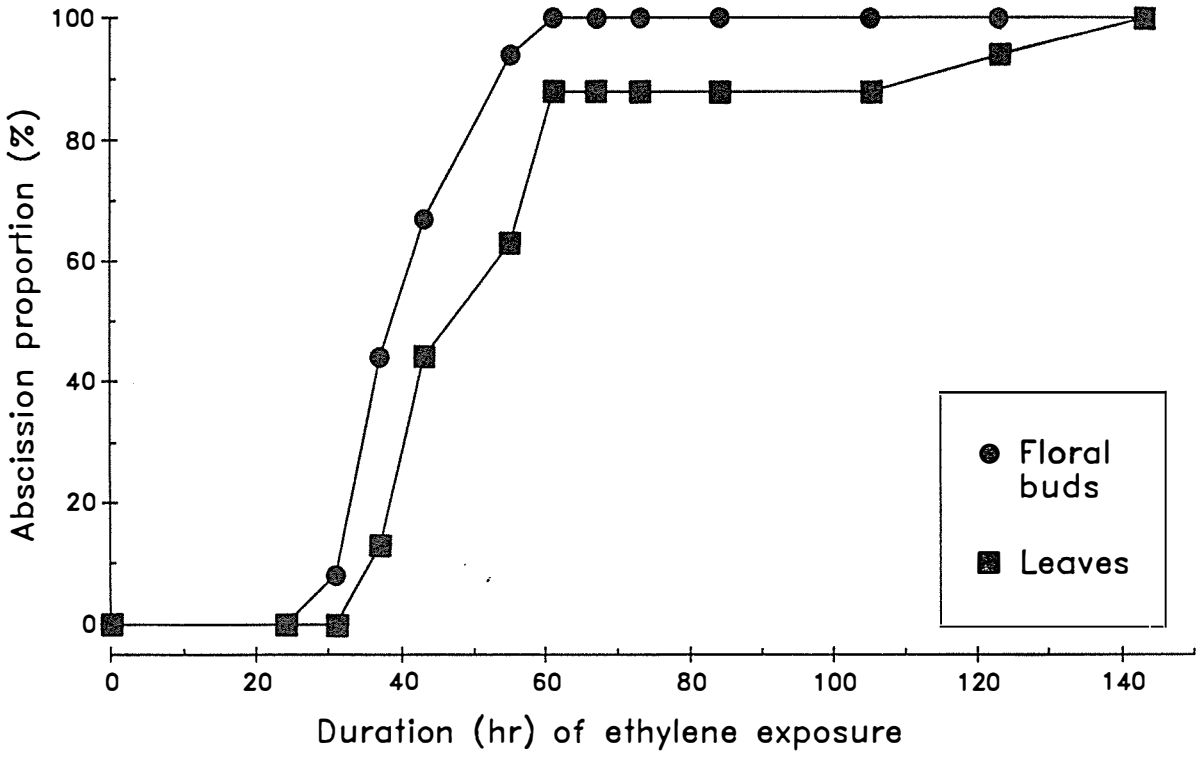


Figure 5.1. Abscission proportion of floral buds and leaves of *Camellia* 'Anticipation' treated with 10 *u*l·liter-1 ethylene continuously (Experiment 5.1).

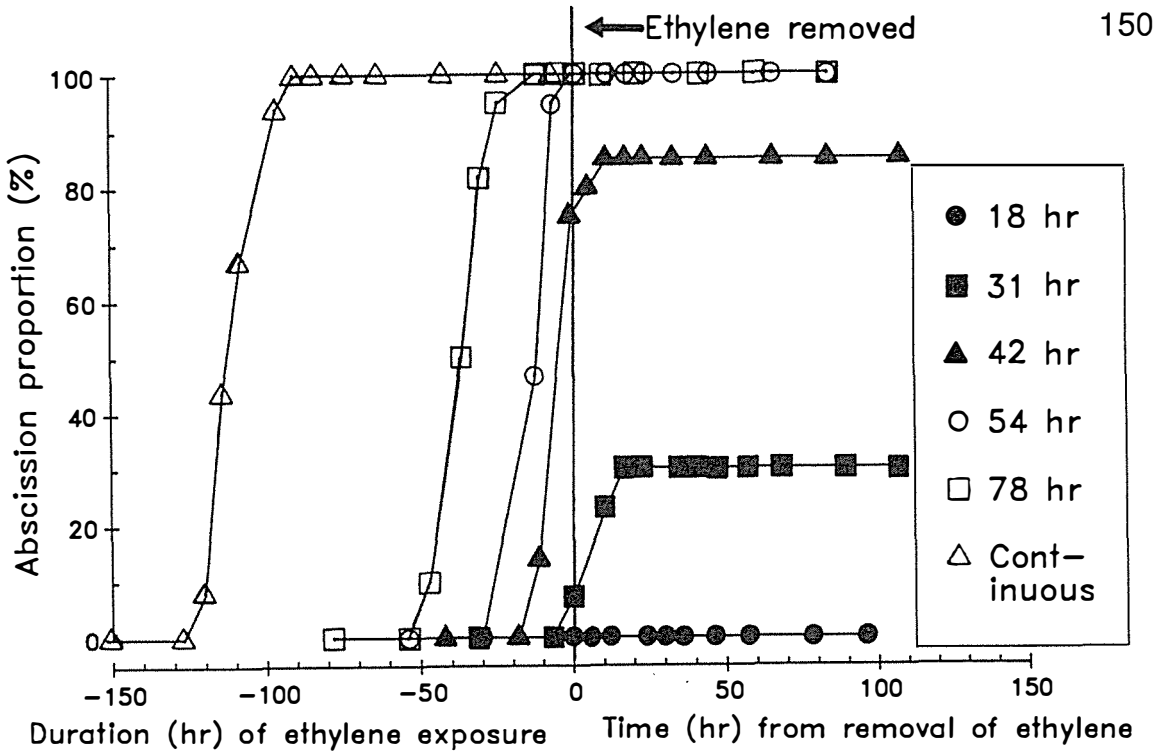


Figure 5.2. Abscission proportion of floral buds of *Camellia* 'Anticipation' treated with 10 *u*l·liter-1 ethylene for 18, 31, 42, 54, 74 hr, and continuously (Experiment 5.1). Ethylene removed at time 0 hr (arrow).

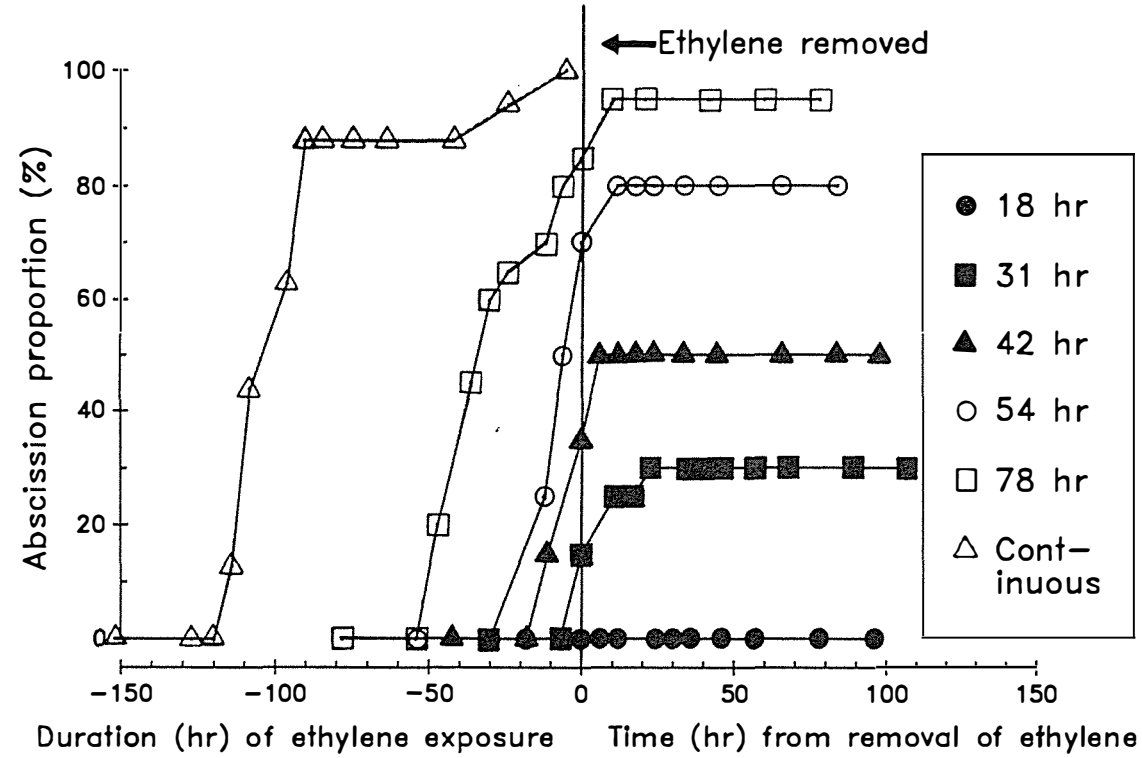


Figure 5.3. Abscission proportion of leaves of *Camellia* 'Anticipation' treated with 10 *u*l·liter-1 ethylene for 18, 31, 42, 54, 74 hr, and continuously (Experiment 5.1). Ethylene removed at time 0 hr (arrow).

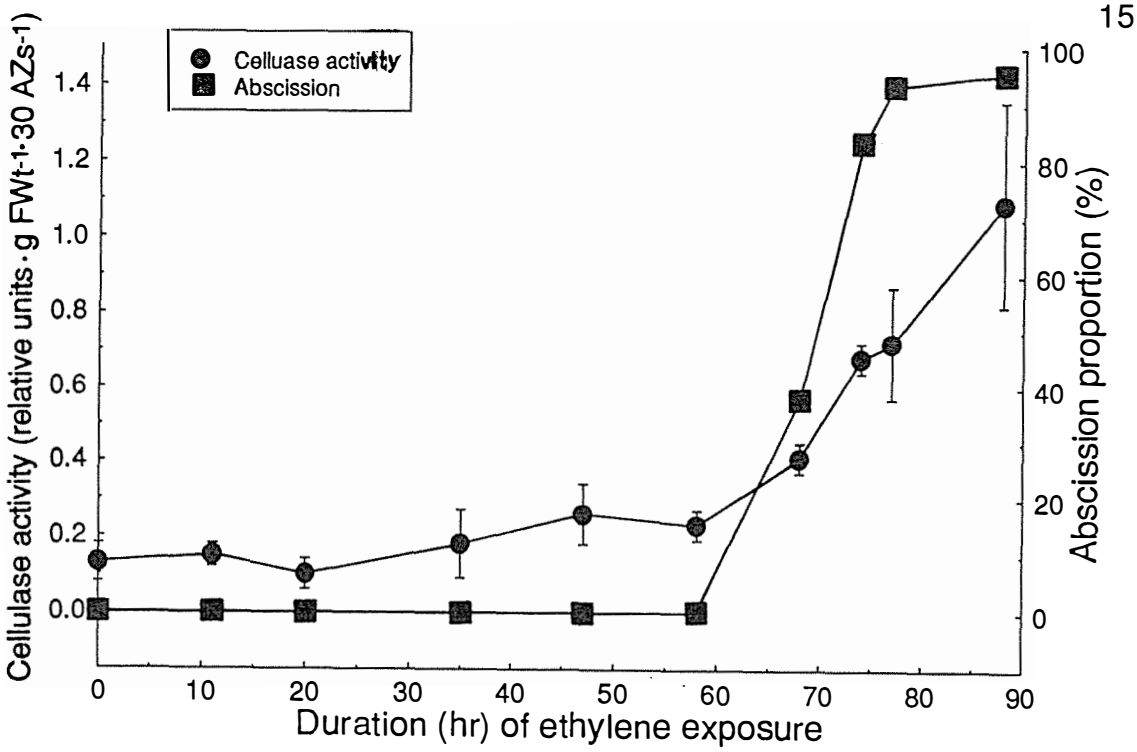


Figure 5.4. Cellulase activity of 30 leaf abscission zones over the ethylene-promoted abscission process of *Camellia* 'Brian'. Vertical bars represent SE of the mean. (Experiment 5.5).

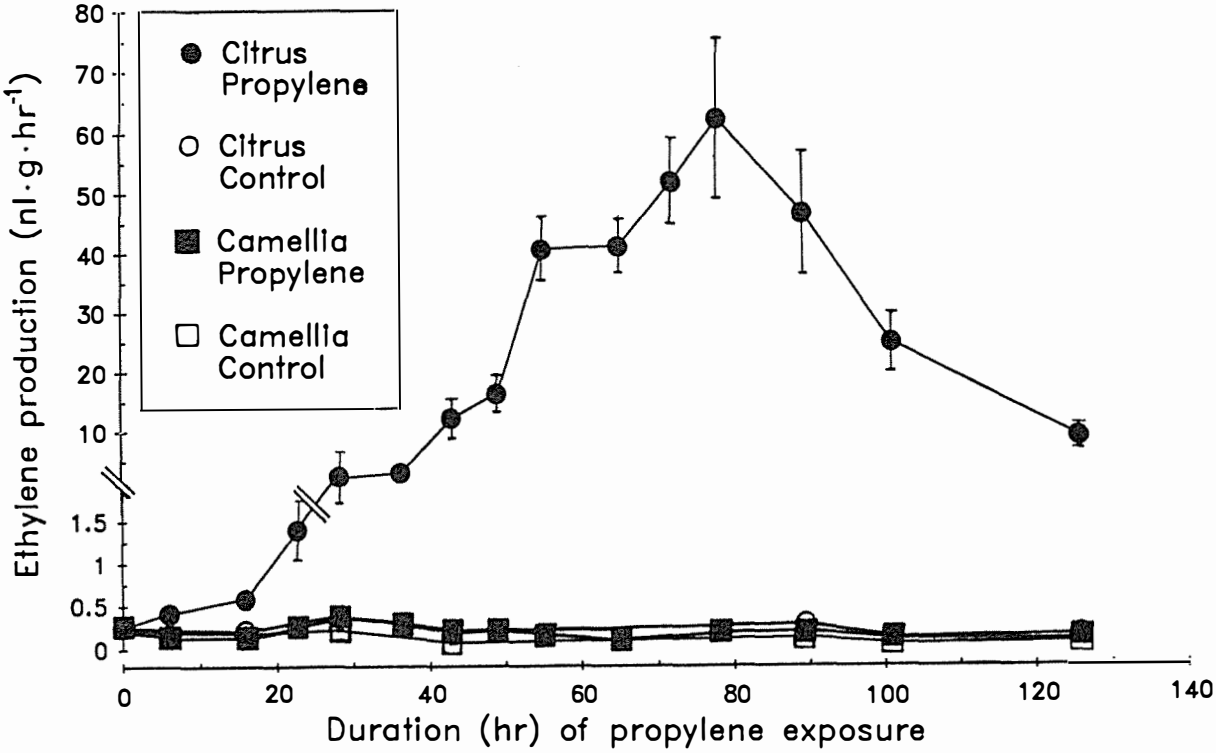


Figure 5.5. Ethylene production of leaf-blade explants of *Camellia* 'Brian' and *Citrus limon* 'Meyer' treated with 5000 *ul*·liter^{-1} propylene and air (control). Vertical bars represent SE of the mean. (Experiment 5.2).

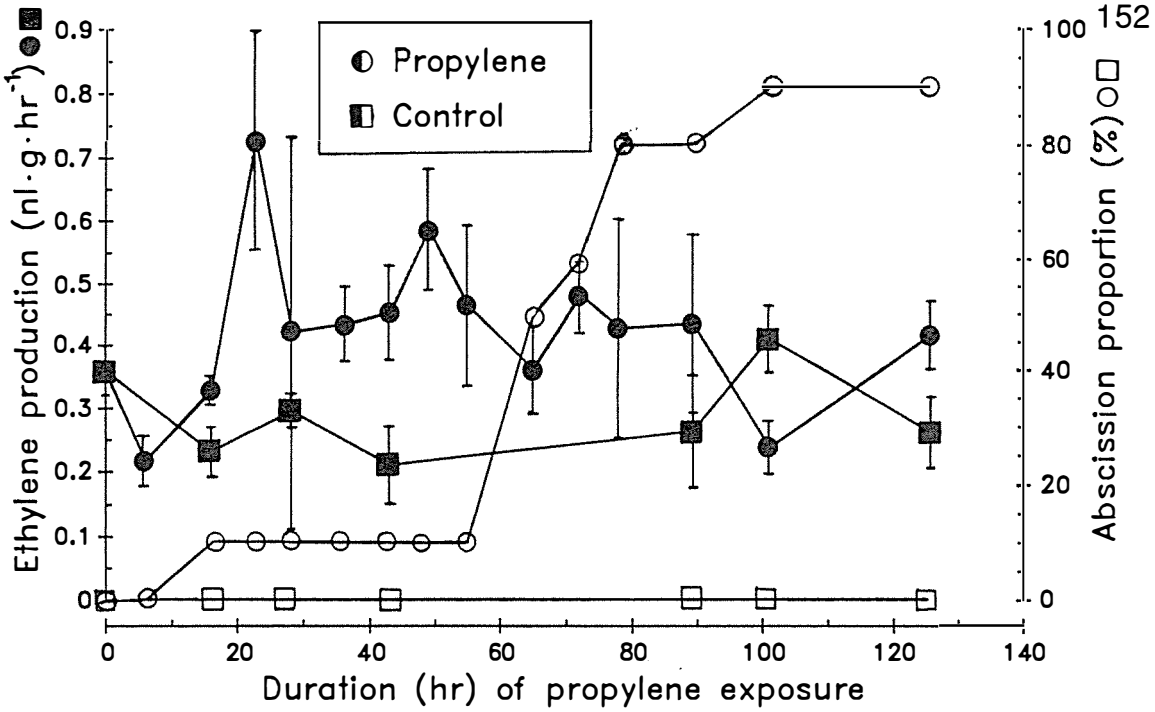


Figure 5.6. Effect of duration of exposure on mean ethylene production (filled symbols) and abscission (empty symbols) in response to propylene (5000 *u*l·liter^{-1}) and air (control) treatment of single-leaf stem explants of *Camellia* 'Brian'. Vertical bars represent SE of the mean. (Experiment 5.2).

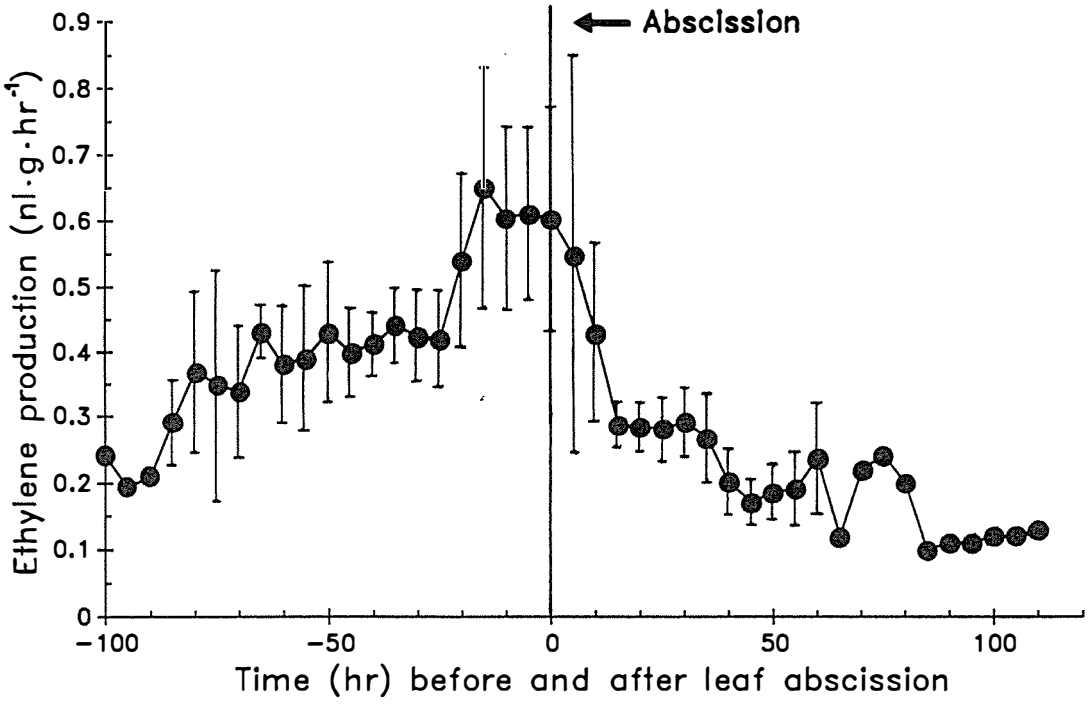


Figure 5.7. Effect of duration of exposure to propylene on mean ethylene production around the time of abscission. Single-leaf stem explants of *Camellia* 'Brian' were exposed to 5000 *u*l·liter^{-1} propylene. Vertical bars represent SE of the mean. (Experiment 5.2).

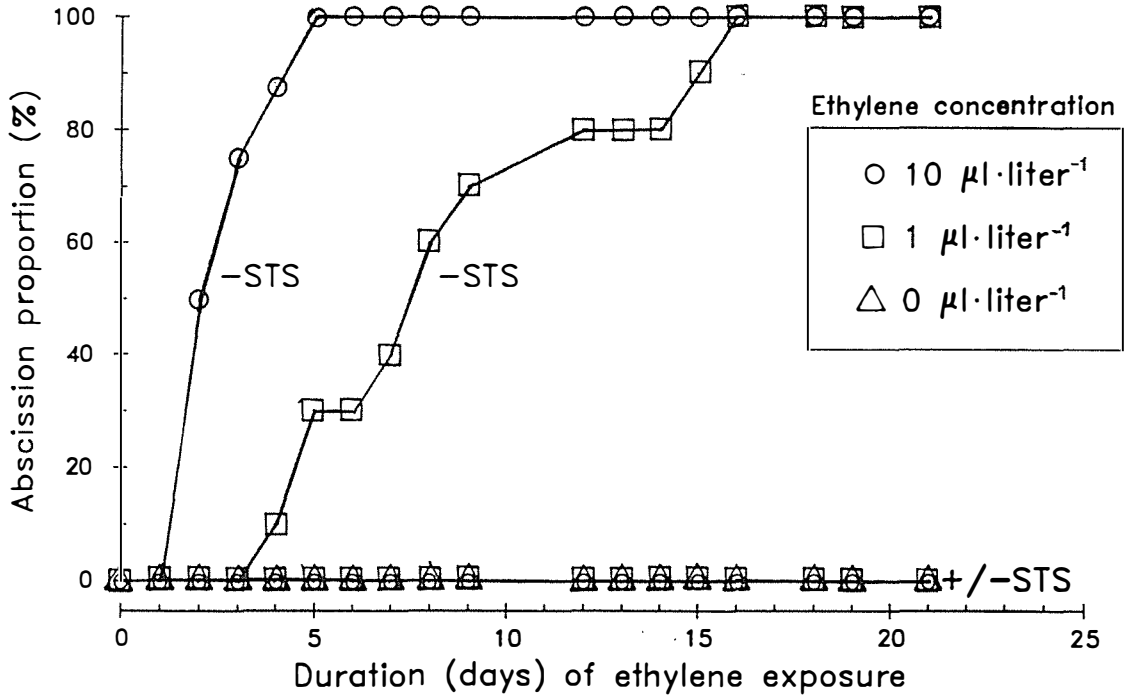


Figure 5.8. Abscission proportion of leaves of *Camellia* 'Anticipation' treated with silver thiosulphate (STS; 0.2 mmol·liter^{-1}, 24 hr) or double distilled water and subsequently exposed to 0, 1, or 10 *u*l·liter^{-1} ethylene. (Experiment 5.3).

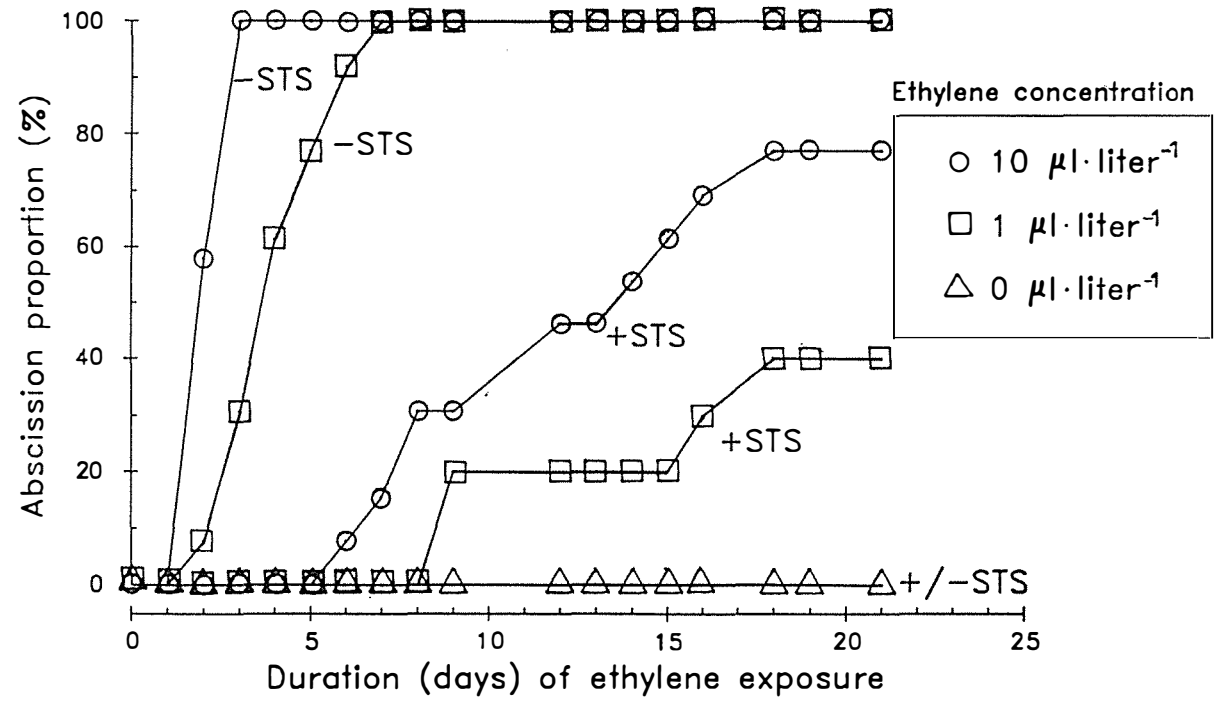


Figure 5.9. Abscission proportion of floral buds of *Camellia* 'Anticipation' treated with silver thiosulphate (STS; 0.2 mmol·liter^{-1}, 24 hr) or double distilled water and subsequently exposed to 0, 1, or 10 *u*l·liter^{-1} ethylene. (Experiment 5.3).

5.5 DISCUSSION

Differences in the result of ethylene and STS application were obtained between genera and organ/tissue types of *Camellia* as reflected by abscission, endogenous ethylene production and cellulase activity.

Autocatalytic Ethylene Production of the Leaf Blade

The classic senescence response of the leaf blade with corresponding increase in endogenous ethylene production observed in *Citrus sinensis* 'Washington Navel' on application of exogenous ethylene (Riov and Yang, 1982) was also observed in *Citrus limon* 'Meyer' tissue treated with propylene (Experiment 5.2; Figure 5.5). The magnitude of the peak endogenous ethylene production of *C. limon* (62 $nl \cdot g^{-1} \cdot hr^{-1}$) was less than that of *C. sinensis* and *Olea europaea* 'Manzanillo' leaves (145 and 95 $nl \cdot g^{-1} \cdot hr^{-1}$ respectively) (Riov and Yang, 1982; Goren *et al.*, 1988). Time to peak production of ethylene in *C. sinensis* is 48 hr (Riov and Yang, 1982); *C. limon* (80 hr) is intermediate between *C. sinensis* and that of *O. europaea* (122 hr) (Goren *et al.*, 1988). Autoinhibition of endogenous ethylene production observed in *O. europaea* leaf tissue over the first 60 hr (Goren *et al.*, 1988), was not observed in *C. limon*, this being in agreement with the response of *C. sinensis* (Riov and Yang, 1982).

However, propylene treatment of *Camellia* leaf-blade explants (containing no abscission zones) carried out concurrently with those of *C. limon* 'Meyer', exhibited no increase in ethylene production, nor were any visible signs of chlorophyll degradation apparent. It was clear from the results obtained using *Citrus limon*, that the lack of ethylene production of *Camellia* was not due to failure of the experimental system as results for *C. limon* were comparable to those obtained by Riov and Yang (1982). The system employed was capable of measuring ethylene production rates as low as 0.05 $nl \cdot g^{-1} \cdot hr^{-1}$ (0.002 $ul \cdot liter^{-1}$ over a collection duration of 3 hr in a 30 ml vial), thus making it unlikely that autocatalytic ethylene production was undetected. *Camellia* response to air and propylene in the dark was also checked and no increase in endogenous ethylene production or leaf chlorosis was observed (data not shown). A commercial exporter of *Camellia* from New Zealand, Duncan and Davies Nurseries Ltd, currently export plants at $4^{o}C$ for extended periods of time (6 to 10 weeks) without observing widespread leaf chlorosis. *Camellia* leaves treated with ethylene abscised without chlorophyll degradation (R.E. Lill, pers. com.). The results of this experiment were also verified by observations of research in

Camellia over 4 years in which the response to applied ethylene and ethephon in a wide range of ethylene concentrations, temperatures and in the dark and the light to leaves of different maturities have been examined. In no case has leaf chlorosis been observed in response to applied ethylene and/or darkness.

The results of Experiment 5.2 and the above observations strongly support the hypothesis that leaf blades of *Camellia* do not share the senescent response to dark treatment of *Kalanchoe* and *Nymphoides* (Goldney and van Steveninck, 1970; Marousky and Harbaugh 1980), nor to exogenous ethylene treatment as found for *Philodendron*, *Citrus*, and *Olea* (Marousky and Harbaugh, 1979c; Riov and Yang, 1982; Goren *et al.*, 1988). Although a climacteric-like rise in ethylene production occurs in *Nicotiana* leaves, this is not significantly accelerated by application of exogenous ethylene (Aharoni and Lieberman, 1979). Thus the response of *Camellia* tissue was in agreement with the suggestions of Thimann (1980) that, in general, exogenous ethylene has little influence on endogenous ethylene production of leaves. It was noted in reference to the study of flower senescence, that ethylene-sensitive systems have received much attention while the ethylene-insensitive systems remain, in the main, unstudied (Reid and Wu, 1992). Due to the lack of literature on ethylene-insensitive leaf senescence systems, this also appeared to be the case in leaf tissue. The same implication of the requirement for greater research into systems which do not respond to ethylene also applies to *Camellia*. *Camellia* appeared to be a useful model of an unresponsive system due to its lack of senescence under a wide range of environmental and exogenous ethylene treatments and its general availability and significance world-wide.

Ethylene-promoted leaf senescence and resulting autocatalytic ethylene production of *Olea* leaves is considered to be of importance in application of ERCs or ethylene since the autocatalytic ethylene production could itself promote fruit (Goren *et al.*, 1988) or leaf abscission (Aharoni *et al.*, 1979b). Leaves of *Camellia* cannot play a similar role in abscission after application of ERCs or exogenous ethylene from other sources, since no ethylene-promoted autocatalytic senescence response was observed.

The physiological basis for the dramatic difference in response to ethylene of the leaf blade of *Camellia* and *Citrus* (Figure 5.5) and other genera may be explained in terms of the climacteric- or nonclimacteric-type responses. The latter terms were developed for explanation of differences between genera in the process of

fruit ripening (Moore, 1979) and flower senescence (Veen, 1987; Section 1.3.6). Ethylene application to *Citrus* leaf tissue resulted in senescence and autocatalytic ethylene production indicating a climacteric-type response, although there were differences between this and climacteric fruit where continuous ethylene exposure is not required (Riov and Yang, 1982). Contrary to this, the *Camellia* leaf blade exhibited no increase in ethylene production, a characteristic of a nonclimacteric system (McGlasson, 1985) and hence suggests that the leaf blade possesses only the System I mechanism. A possible problem in categorisation of *Camellia* leaf blades as nonclimacteric is that in some ethylene responses described as nonclimacteric, an ethylene response is observed in response to ethylene application (McGlasson, 1985), such as *Citrus* fruit ripening (degreening; Young *et al.*, 1970), where a temporary increase in respiration rate although no increase ethylene production occurs. In *Camellia*, no increase in ethylene production or leaf senescence (as measured by visual assessment of chlorosis) was observed. Although the lack of ethylene production of *Camellia* fits the nonclimacteric model, the lack of ethylene response (in this case leaf senescence) did not appear to fit the model. However, the ripening of *Fragaria* fruit is very similar to the pattern of leaf senescence of *Camellia* since it is described as nonclimacteric and ripening is not associated with increased respiration or autocatalytic ethylene production, nor do exogenous ethylene or ethylene inhibitors influence ripening (Venis *et al.*, 1992). Also, the lack of ethylene response of *Camellia* leaves did not prove that the tissue was definitely nonclimacteric since other factors such as endogenous hormone levels may provide other explanations for a lack of response at this stage of maturity. It should also be noted that the respiratory response to ethylene application was not examined in the current study and that a temporary rise in respiration rate is characteristic of nonclimacteric system (McGlasson, 1985). Therefore to elucidate the climacteric or nonclimacteric nature of *Camellia* leaf senescence, further research into the respiratory pattern of ethylene treated leaves, and the pattern of ethylene production and respiration rate during natural senescence may be beneficial.

The physiological basis for the nonclimacteric type response of *Camellia* leaves may be explained in terms of genetic effects. Ethylene regulates many aspects of plant growth and development by selective expression of families of genes, this being reflected by mRNA expression which precedes ethylene responses such as senescence (Borochov and Woodson, 1989; Woodson, 1991). The genetic background of a plant tissue has a marked effect on the ethylene response

observed because significant variation in ethylene sensitivity and production exists between, for example, flowers of a given genus (Woltering and van Doorn, 1988), even those which are closely related (Wu *et al.*, 1991a). Differences in, not only the magnitude, but even the presence, of a given ethylene response exist between cultivars of the same species. Although *Dianthus* flowers are widely used as the model for examination of ethylene response and biosynthesis (possessing the classic autocatalytic ethylene production system), a cultivar 'Sandra', exhibits a non-climacteric type senescence response showing no autocatalytic ethylene production at all which results in longer vase life than other cultivars (Wu *et al.*, 1991a). Similarly, ethylene treatment of flowers of a range of *Rosa* cultivars show that flower opening rate may be inhibited, not affected, or even accelerated depending on cultivar (Reid *et al.*, 1989). Thus since such dramatic differences in ethylene response exist within a given genus, it is less surprising that *Camellia* leaves exhibit no autocatalytic ethylene production or senescence, while those of *Citrus* do.

Genetic differences are reflected in a number of mechanisms which may provide possible explanations for the lack of response of the leaf blade. A simple explanation for the lack of an ethylene response is absence of ethylene receptors (Trewavas and Jones, 1981) and this could be the situation for *Camellia* leaf tissue. However, this seems unlikely since ethylene binding sites have been found in a wide range of tissues and species (Section 1.3.1.3) and the abscission zones, which must possess receptors due to their abscission response, are present on the same organ (leaves). It is also possible that the ethylene receptor, which is thought to be membrane bound (Dodds and Hall, 1980; Sisler, 1980), may be in some way "veiled or protected" (Knee, 1989) in the leaf until late in the senescence process. *Camellia* leaves possess polyphenol (or tannin) concentrations as high as 10-25% and since this chemical group is known to bind to proteins (Haslam, 1989), they may be a possible candidate for the "veiling" of the ethylene receptor protein.

A second possible explanation could be that of hormone levels, particularly those known to inhibit senescence. It could be suggested that *Camellia* leaf blades may possess high levels of cytokinins and/or gibberellins which are known to inhibit leaf senescence (Thomas and Stoddart, 1980; van Doorn *et al.*, 1992). However, it would be expected that under some of the varied conditions to which leaves were exposed, including high ethylene concentrations/temperatures and range of maturities, these high levels of inhibition would be overcome. Also, if the leaf

blades of *Camellia* were to possess unusually high plant growth substance levels, it would be expected that this would be reflected in the abscission response of *Camellia* by either particularly rapid or slow abscission. However, this was not observed and so may point to other mechanisms.

A final explanation for the lack of senescence of *Camellia* leaf blades, which may be the most likely, is simply that the senescence process of *Camellia* is not under the control of ethylene receptors, as suggested for other genera (Thimann, 1980). For example, although *Dianthus* petal senescence is promoted by ethylene with concomitant increase in mRNA species, no senescence occurs in leaves on the same stem (Lawton *et al.*, 1990). Similarly, some genes promoted by ethylene in *Lycopersicon* are specific to fruit tissue and do not occur in leaves (Lincoln and Fischer, 1988). Also, senescence not controlled by ethylene occurs in many flower species (Reid and Wu, 1992).

Autocatalytic Ethylene Production of Leaf Abscission Zones

Although leaf-blade explants of *Camellia* exhibited no autocatalytic production in response to exogenous propylene (see above), single-leaf stem explants (containing the leaf abscission zone) did show increased endogenous ethylene production as abscission occurred. Since autocatalytic ethylene is unlikely to emanate from the stem tissue (from which abscission zones were excised) and autocatalysis was not found in the leaf blade (Figure 5.5), then, by subtraction, the ethylene production observed in single-leaf stem explants is due to production from the leaf abscission zone itself. The ethylene production of *Camellia* in single-leaf stem explants followed a climacteric pattern rising from 0.2 $nl \cdot g^{-1} \cdot hr^{-1}$ to a peak production rate of 0.6 to 0.65 $nl \cdot g^{-1} \cdot hr^{-1}$, 15 hr prior to the point of abscission and declined subsequently (Figure 5.7). A similar climacteric ethylene production pattern has been observed during natural abscission of *Melia* (Morgan and Durham, 1980), *Phaseolus* (Jackson and Osborne, 1970; Jackson *et al.*, 1973), *Prunus* and *Parthenocissus* (Jackson and Osborne, 1970).

Although not examined in *Camellia* abscission zones, two possible mechanisms for the increase in ethylene production observed in *Camellia* may be suggested. Firstly, increased ethylene production has been argued to be a result of senescence such that breakdown in vacuole membrane permeability releases methionine into the cytoplasm where it enters the ethylene biosynthetic pathway and is converted to ethylene (Kende and Hanson, 1977; Section 1.3.5). However, abscission is a highly controlled process that does not exhibit senescence with

concomitant organelle disintegration or autolysis (Sexton and Hall, 1974; Gilliland *et al.*, 1976; Sexton and Roberts, 1982) and in most cases, abscission does not involve cell fracture at the separation layer (Osborne, 1989a). The second, and most probable mechanism, is that reception of ethylene results in autocatalysis of the ethylene biosynthetic pathway. This may involve promotion of a System II pathway (McMurchie *et al.*, 1972; Yang, 1987b) and results in increased ACC synthase and EFE activity (Riov and Yang, 1982; Veen and Kwakkenbos, 1983; Section 1.3.5). The rapid decrease in ethylene production after abscission was similar to that found in *Citrus* leaves (Ben-Yehoshua and Aloni, 1974) and *Melia* (Morgan and Durham, 1980). It is suggested that the decreased ethylene production following abscission was due either to "turning off" of the ethylene biosynthetic system after separation, or loss of membrane integrity (a requirement for ethylene biosynthesis; Lieberman, 1979) in the abscission zone as tissue drying occurred.

Of what significance is this rise in ethylene production? The internal ethylene concentration of the abscission zones is the determining factor in the abscission process (Lang and Martin, 1989). However, the ethylene concentration within the abscission zone of *Camellia* could not be determined accurately from the data presented here (Ben-Yehoshua and Aloni, 1974). Ethylene production from *Camellia* over the abscission process increased by a factor of 2 to 3 (0.2 to 0.3 $nl \cdot g^{-1} \cdot hr^{-1}$ to the peak of 0.6 to 0.65 $nl \cdot g^{-1} \cdot hr^{-1}$). Since the rate of movement of ethylene out of the abscission zone can be assumed to remain constant, this increase in ethylene production was taken to represent a similar increase in internal ethylene concentration. The ethylene production of natural abscission of *Citrus* from the minimum to peak rate involved a ten-fold increase for ethylene extracted from the internal atmosphere or 8.6 times for ethylene production measured by enclosing the tissue (Ben-Yehoshua and Aloni, 1974). The increased ethylene synthesis in *Citrus* is far greater than that observed in *Camellia* and thus, autocatalytic ethylene production may have no significant role in ethylene-promoted abscission of *Camellia* leaves. It is also pertinent to note that Marynick (1977) found a lack of a statistical correlation between ethylene production and leaf abscission in *Gossypium*. This, along with results obtained in other species, lead him to question the assumption that one biochemical process (ethylene production) is responsible for promotion of abscission in all species. It would be of interest in future work into *Camellia* abscission to determine the ethylene production from smaller tissue sections containing the abscission zone (Aharoni *et al.*, 1979b) and determination of the internal ethylene concentration

(Ben-Yehoshua and Aloni, 1974). However, reducing tissue size promotes greater wounding and poses problems in obtaining sufficient tissue for accurate determination of internal ethylene concentration.

Clearly, there are significant differences in response to exogenous ethylene of *Camellia* leaf blade and leaf abscission zone tissues. As discussed above, the response of *Camellia* leaves to ethylene can be described as nonclimacteric. Climacteric response to ethylene characteristically involves autocatalytic ethylene production and a response to ethylene (Section 1.3.6). Thus the abscission zone tissue of *Camellia* may be characterised as climacteric since autocatalysis occurred and a response (abscission) was observed. A similar example of climacteric and nonclimacteric systems occurring in the same plant is found in *Citrus* where fruit tissue is nonclimacteric (Young *et al.*, 1970) while leaf tissue exhibits a climacteric type response (Riov and Yang, 1982). Similarly, *Olea* leaves exhibit a climacteric type response to exogenous ethylene while fruit show no autocatalytic ethylene-promotion (Goren *et al.*, 1988). This supports the characterisation of *Camellia* abscission zones as a climacteric tissue, while the leaf blades are nonclimacteric. Hence, in terms of the model proposed by Yang (1987b) abscission zones of *Camellia* could be said to be climacteric, possessing both System I and II mechanisms since application of ethylene resulted in promotion of autocatalytic ethylene production and an ethylene response (i.e. abscission) similar to that of *Citrus* (Ben-Yehoshua and Aloni, 1974).

The use of the ethylene analogue propylene in Experiment 5.2 produced near-identical abscission kinetics to that of saturating ethylene concentration of Experiment 5.5 (Figure 5.6 cf Figure 5.4). This validates the assumption that the propylene concentration (5000 $u l \cdot liter^{-1}$) was saturating and simulated the effect of ethylene on abscission of *Camellia* and senescence of *Citrus* (Experiment 5.2). Use of propylene allowed simultaneous measurement of endogenous ethylene production while promoting an ethylene-like abscission process.

Residual Abscission.

Times to completion of residual abscission of *Camellia* leaves and floral buds were similar. The pattern of non-reversible or residual leaf abscission in *Camellia* was similar to that of *Fittonia* where abscission reduces dramatically after ethylene removal and ceases 2 days after ethylene removal (Marousky, 1979). As found in *Camellia*, leaf abscission of *Philodendron* also decreases after ethylene removal but continues between 1 and 3 days (Marousky and Harbaugh,

1979c). The abscission response of floral buds of *Camellia* was similar to *Impatiens*, where ethylene removal also results in cessation of abscission (Dostal *et al.*, 1991). However, the response of *Camellia* was different from that of *Olea* fruit (Lang and Martin, 1985) and leaves of *Citrus* (Sisler *et al.*, 1985), *Olea* (Lang and Martin, 1985) and *Capsicum* (Kays *et al.*, 1976) where abscission continues long after ethylene is removed. The level of continuing abscission after ethylene removal of the latter species complicates modelling of abscission response in comparison to *Camellia*. Since ethylene removal from *Camellia* results in relatively rapid cessation, exposure duration can be approximated as time required for abscission and this therefore simplifies modelling of *Camellia* abscission.

Although the biochemistry of cessation of abscission of *Camellia* was not examined, suggestions for the mechanisms may be put forward on the basis of results of other genera. Exogenous ethylene diffused rapidly from leaf and abscission zone tissue (less than 1 hr; Goren and Sisler, 1986). The decreased ethylene concentration within tissue and dissociation from the ethylene binding site or receptor (McKeon and Yang, 1987), resulted in reduction of the level of activated ethylene receptor and therefore reversal of its promotory influence. Removal of ethylene also would have reduced the level of cellulase mRNA (Tucker *et al.*, 1988), cellulase activity (Osborne and McManus, 1984) and trafficking of cellulase over the cell membrane (Abeles *et al.*, 1971). These steps would also have been influenced similarly for other hydrolytic enzymes. Although only the completion of the abscission process was examined, break strength decrease may also be reversible in *Camellia*, as was found in *Phaseolus* and *Citrus* (dela Fuente and Leopold, 1969; Holm and Wilson, 1977).

The length of the residual abscission of *Camellia* provides an indication of either the duration of processes not requiring the presence of ethylene, or, the time taken to turn off the autocatalytic ethylene production process in the abscission zone. Promotion of break strength decrease by ethylene in *Phaseolus* was reversed 1 hr after rapid ethylene removal by evacuation (dela Fuente and Leopold, 1969). Although break strength was not measured, the duration of ethylene-promotion of the abscission process in *Camellia* (maximum of 17 and 23 hr for floral buds and leaves respectively) is of much longer duration than that of *Phaseolus*. In *Citrus* leaf tissue, autocatalysis continued at significant levels for longer than 12 hr after ethylene removal (Riov and Yang, 1982). Thus the residual abscission of *Camellia* may be due to gradual reduction of autocatalytic

ethylene production in abscission zones, although it remained at a level high enough to continue the abscission process for a duration of 17 to 23 hr (Figure 5.2 to 5.3).

It is proposed that genera differences observed between *Camellia* and other species may be explained by the presence or absence of ethylene autocatalysis of leaf tissue. *Olea*, *Capsicum* and *Citrus* are genera which have been found to possess abscission responses which continue after ethylene removal.

That leaf abscission may continue long after ethylene removal was implicated by results in *Olea* in 1985 where a relatively short ethylene pulse (12 or 18 hr) resulted in leaf abscission occurring 104 hr after ethylene was removed (Lang and Martin, 1985). However, it was later noted that this response (observed in both leaves and fruit, the latter examined below) may have been due to frost damage (Lang and Martin, 1989). Subsequent examination of the abscission response of *Olea* leaves to ethylene (Lang and Martin, 1989) supported this suggestion since abscission was measured 96 hr after commencement of treatment and little further abscission occurred at this period (G.A. Lang, pers. com.). Autocatalytic ethylene production of *Olea* leaves occurs after exposure of more than 72 hr and demonstrates a concentration-dependent response (Goren *et al.*, 1988). Goren *et al.* (1988) therefore concluded that the leaves have the capacity to produce ethylene in response to exogenous ethylene or ethephon and so further contribute to fruit, and presumably leaf, abscission. Ethylene release from ethephon continues at elevated levels for periods between 3 and 4 days (Lougheed and Franklin, 1972; Perry and Gianfagna, 1987; Daniell and Wilkinson, 1972) and as long as 10 days (Olien and Bukovac, 1982a). Therefore it seems possible that autocatalytic ethylene production from leaf tissue could be promoted by ethephon and abscission also subsequently promoted. However, the initial promotion of abscission by ethylene or ethephon application would not seem to involve autocatalysis, as would be the case for short durations of ethylene exposure (G.A. Lang, pers. com.). Lang (pers. com.) therefore suggests that the response to ethylene in the abscission zone (cell separation) and the leaf blade (senescence with concomitant autocatalysis) can be independent processes with distinctive dose responses.

Fruit removal force (FRF) continued to decrease in *Olea* fruit 31 hr after an ethylene pulse (18 hr) (Lang and Martin, 1985), but this is most likely due to frost damage since a similar result was not observed in later experiments (Lang and

Martin, 1989). FRF of *Olea* continue to decrease for up to 12 hr after an ethylene exposure duration of 24 hr (Blumenfeld *et al.*, 1978). Neither Blumenfeld *et al.* (1978) nor Goren *et al.* (1988) could detect endogenous ethylene production from *Olea* fruit and it is therefore suggested that autocatalytic ethylene production was not involved in abscission of *Olea* fruit. It is proposed that the duration of continued abscission observed by Blumenfeld *et al.* (1978) is not likely to constitute a significant response resulting from ethylene production from the fruit or leaf since it occurs over a relatively short duration.

Although no detailed data were reported, leaf abscission of *Capsicum* appears to continue for several days after ethylene treatment (Kays *et al.*, 1976). This may be a result of a high level of endogenous ethylene production (Kays *et al.*, 1976) and/or due to autocatalysis in leaves as a response to exogenous ethylene.

The abscission response of *Citrus* leaves is of interest since replacement of ethylene with air makes little difference to the abscission response. It appears that ethylene acts irreversibly between 10 and 30 hr and application of 2,5-NBD makes little difference in the abscission response observed (Sisler *et al.*, 1985). However, retightening (break strength increase) of fruit abscission zones does occur in *Citrus* (Holm and Wilson, 1977). Although *Citrus* leaves do not exhibit a significant increase in autocatalytic ethylene production until 24 hr (Riov and Yang, 1982), results obtained in this study (*Citrus limon*; Figure 5.5) demonstrated promotion of ethylene autocatalysis in only 6 hr. This could point to a possible role in the further promotion of abscission after ethylene removal, since, although ethylene production decreases after ethylene removal (Riov and Yang, 1982), ethylene production does continue at significant levels for as long as 12 hr. However, the fact that 2,5-NBD (which inhibits autocatalysis; Section 1.3.1.5) does not influence the abscission response (Sisler *et al.*, 1985) places doubt on this explanation and indicates that the irreversibility of *Citrus* abscission may operate at another point in the ethylene transduction pathway. It has been previously noted that the results of Sisler *et al.* (1985) obtained with *Citrus* are in contrast to those of *Phaseolus* (Tucker *et al.*, 1988).

It is clear from the above discussion that the area of reversibility of abscission and possible mechanism(s) requires further examination in a range of genera. For example, careful study of the kinetics of *Olea* abscission after ethylene removal may help us to understand apparent anomalies occurring in the literature (i.e. Blumenfeld *et al.*, 1978; Lang and Martin, 1985; 1989). Also, the possible

mechanism for continuing abscission in *Olea*, autocatalytic ethylene production, has only been examined in *Olea* leaves directly after ethylene removal (Goren *et al.*, 1988). The reversibility of this system after ethylene removal has not been examined in *Olea* (as carried out for *Citrus*; Riov and Yang, 1982).

In summary, although this area requires more careful and systematic examination, it appears that from results of dela Fuente and Leopold (1969) and other literature, the ethylene-promoted abscission response is reversible in a wide range of genera (with the possible exception of *Citrus*). It is possible that the autocatalytic ethylene production system may be the step which continues to promote abscission after ethylene removal in the few species in which abscission does continue.

Cellulase Activity and Leaf Abscission

Cellulase activity increased during the ethylene-promoted leaf abscission process of *Camellia* (Experiment 5.5). After application of ethylene to *Camellia* leaves, there was a lag phase (0 to 20 hr) where no increase in cellulase activity was observed which was similar, though longer than that of *Citrus* (Goren *et al.*, 1973). For cellulase to promote abscission it must increase in activity prior to abscission (Goren *et al.*, 1973). This was found to occur in *Camellia* where increased cellulase activity (20 to 58 hr) occurred well before commencement of leaf abscission (58 hr) (Figure 5.4), as in *Citrus* fruit (Huberman and Goren, 1979) and in leaves of *Phaseolus* (Horton and Osborne, 1967), *Citrus* (Goren *et al.*. 1973) and *Impatiens* (Sexton *et al.*, 1984). Cellulase activity continued to rise after abscission rate had slowed, a result also obtained in *Phaseolus* where cellulase activity increases as long as tissue desiccation is prevented (Durbin *et al.*, 1981).

However, it is pertinent to ask whether the relatively small increase in cellulase activity of *Camellia* leaves prior to the commencement of abscission (20 to 58 hr; Figure 5.4) could explain the promotion of abscission. The isoenzyme 4.5 cellulase has been found to be responsible for the cellulase activity present prior to abscission (Durbin *et al.*, 1981) and as abscission proceeds, the activity of this isoenzyme remains constant or decreases (Durbin *et al.*, 1981; del Campillo *et al.*, 1988). In contrast, activity of the 9.5 cellulase isoenzyme correlates highly with break strength decrease of *Phaseolus* leaves, and the increase in cellulase activity determined using viscometry found to be attributable to increased activity of 9.5 cellulase (Sexton *et al.*, 1980; Section 1.3.3). Thus although determination

of the activity of isoenzymes of cellulase were not made, it is suggested that the increased activity of cellulase in *Camellia* leaves was due to *de novo* synthesis of 9.5 cellulase which resulted in break strength decrease and subsequent abscission. Therefore the increase in cellulase activity over the period 20 to 58 hr, although not as large as the increase after 58 hr (Figure 5.4), is suggested to reflect an increase in 9.5 cellulase alone. A similar small increase in cellulase activity has been shown to occur prior to break strength decrease as determined by viscometry (Lewis and Varner, 1970) and radioimmuno-assay (Sexton *et al.*, 1980). The correlation of cellulase activity and abscission, points to a possible role for cellulase in the abscission process of *Camellia*, as suggested for leaves of *Gossypium*, *Coleus* (Abeles, 1969), *Phaseolus* (Sexton *et al.*, 1980; Durbin *et al.*, 1981) and *Citrus* (Goren *et al.*, 1973).

Cellulase Activity and Organ Type

The cellulase activity of leaf abscission zones before ethylene application (control) and at completion of ethylene-promoted abscission (abscised) in Experiment 5.4 (Table 5.1) were similar to the results in Experiment 5.5 (Figure 5.4). Contrary to this, the cellulase activity in floral bud abscission zones of *Camellia* did not increase as abscission occurred (Table 5.1). This result is at variance with that found in *Citrus* where cellulase activity increases and correlates with the abscission of both leaves (Ratner *et al.*, 1969) and fruit (Pollard and Biggs, 1970; Huberman and Goren, 1979). However, differences in activity of hydrolytic enzymes have been found in *Prunus*, where polygalacturonase exhibits exo-activity in fruit but endo-activity in leaf abscission zones (Rascio *et al.*, 1985; 1987). It is possible that other hydrolytic enzymes such as polygalacturonases (Riov, 1974; Huberman and Goren, 1979), pectinases (Henry *et al.*, 1974), or peroxidases (Gaspar *et al.*, 1978) are involved in abscission of floral buds. Polygalacturonase activity has recently been found to correlate with floral bud abscission of *Camellia* (Lee and Song, 1992) and therefore points to a possible role of polygalacturonase in both floral bud and leaf abscission (although polygalacturonase activity in leaf abscission zones was not examined). The higher cellulase activity of floral buds compared with leaves in untreated tissue may be a reflection of the temporal proximity of the organ to time of natural abscission. Thus floral buds which abscise at flowering, had greater activity than leaves which abscise at 3 to 4 years of age. An unlikely possibility is that enzyme synthesis is not involved in the abscission of floral buds as suggested for petal abscission of *Geranium* (Sexton *et al.*, 1983). It should also be noted that these results are based on one point in the abscission process of

floral buds. Examination of activity of cellulase and polygalacturonase over the entire abscission process, as carried out for leaves (Experiment 5.5), would clarify this situation.

Abscission Kinetics and Organ Type

Ethylene promoted abscission of leaves and floral buds of *Camellia* at both low (1 ul·liter^{-1}) and high ethylene concentrations (10 ul·liter^{-1}). The time to 50% abscission of floral buds was less than that of leaves (Figure 5.8 and 5.9) indicating greater floral bud sensitivity, which correlated with the lower concentration dependency of floral buds than leaves (Figure 5.9 cf 5.8). The results obtained with *Camellia* were similar to that of *Olea* where fruit have a shorter lag phase than leaves (Blumenfeld *et al.*, 1978; Lang and Martin, 1989). Increased ethylene concentration (10 cf 1 ul·liter^{-1}) decreased the duration of the lag phase of *Camellia* leaves. Decrease in time to 50% abscission was found with floral buds of *Schlumbergera* where higher ethylene concentration also decreases the duration of lag phase (Cameron and Reid, 1981) as found in leaves of *Camellia*, but, because abscission measurement was carried out daily, a possible decrease in lag phase of floral buds could not be detected. The effects of higher concentration and more rapid abscission of floral buds, are suggested to be a result of increased rate of processes which bring about abscission, such as reception and transduction of the ethylene signal and, timing and rate of initiation of cell wall hydrolytic enzymes (Weis *et al.*, 1988).

Leaves had a more variable abscission pattern than floral buds (Figure 5.1) and required presentation of ethylene production as time around the point of abscission (Figure 5.7 cf 5.6). Leaves emerged and expanded sequentially on each shoot and variation also exists between *Camellia* shoots. Differences in leaf maturity result in differences in auxin concentration and this may explain the variation in leaf abscission rate (Suttle and Hultstrand, 1991). Less variable floral bud abscission may be a result of the position of floral buds on the explants. Floral buds were predominantly at the apex of shoots and would exhibit less variation in maturity and therefore of abscission.

The lag period, or time required for abscission to commence after ethylene application, was similar for both leaves and floral buds (Figure 5.1, 5.2, and 5.3). Goren and Sisler (1986) noted that removal of ethylene will result in complete diffusion of ethylene from leaf tissue in 10 to 60 min, depending on species. Similarly, it was found in preliminary experiments, that less than 30 min was

required after ethylene treatment (10 ul·liter^{-1}) of *Camellia* explants for complete dissipation of applied ethylene (data not shown). Thus removal of ethylene can legitimately be taken as the point of cessation of influence of external ethylene on the plant tissue. A minimum period (18 to 31 hr) of ethylene exposure was required for leaf and floral bud abscission to occur in *Camellia*. This was consistent with ethylene-promoted abscission in *Olea* where Lang and Martin (1989) found leaf abscission commenced after 18 hr and fruit abscission after 12 hr. Minimum ethylene exposure periods have also been found for other crops such as *Fittonia*, *Philodendron* and *Citrus* (Marousky, 1979; Marousky and Harbaugh, 1979c; Sisler *et al.*, 1985). For leaves of *Camellia* 'Donation' and 'Anticipation' the duration of the lag phase was similar (between 24 hr and 31 hr; Figure 5.1, 5.8) but it was considerably longer (58 hr; Figure 5.4) for 'Brian'. Thus both genotype and phenotype (organ type) influenced the duration of the lag phase.

STS Application

STS inhibited ethylene-promoted abscission of leaves and floral buds from *Camellia* (Experiment 5.3) similarly to other flowering and foliage species (Joyce, 1989; Wang and Dunlap, 1990). The Ag^+ ion released by STS is a potent inhibitor of ethylene action (Beyer, 1976a). The fact that abscission proportion of both leaves and floral buds were decreased by STS application indicated that in both organs of *Camellia*, the same point in the process of ethylene-promoted abscission was inhibited. The delay in onset of floral bud abscission in *Camellia* was similar to that of *Schlumbergera* where 4 mM STS delays abscission of floral buds by 2 days in response to 0.5 ul·liter^{-1} ethylene (Cameron and Reid, 1981). Abscission of *Camellia* floral buds at the completion of the experiment decreased substantially in plants treated with low ethylene concentrations and to a lesser extent in those treated with high concentrations. Similarly, application of STS to *Calceolaria* substantially decreases flower abscission in response to low levels of ethylene exposure (Cameron and Reid, 1983). As found in leaf abscission of *Camellia* (Figure 5.8), complete inhibition of ethylene-promoted leaf abscission occurs with basal STS application to *Phoradendron* (Joyce *et al.*, 1990) and after spray application to *Radermachera* (Wang and Dunlap, 1990). Similarity of *Camellia* results to that of other species mentioned above indicates that the role of ethylene in abscission of *Camellia* was similar to that of other higher plants. It is suggested that in *Camellia*, STS inhibited the action of ethylene by blocking production of the activated receptor (Yang, 1985; Veen, 1986; 1987) thus inhibited subsequent steps of the abscission process such as synthesis of cell

wall hydrolysing enzymes. STS also blocks the development of System II ethylene production (Veen, 1987) and it is therefore proposed that autocatalytic ethylene production which occurred in abscission zones of *Camellia* (Figure 5.7) was inhibited.

STS did not completely inhibit floral bud abscission in response to ethylene (1 or 10 $ul \cdot liter^{-1}$). This was most probably due either to differences in the ethylene sensitivity of the organs, or differences in distribution of STS in the plant tissue. It was unlikely that differences in distribution of STS accounted for the partial inhibition of floral bud abscission. The rate of movement of STS in cut *Dianthus* stems was calculated to be 2 $m \cdot hr^{-1}$ to over 3 $m \cdot hr^{-1}$ depending on transpiration rate (1 $ml \cdot hr^{-1}$ and 0.3 to 1.4 $ml \cdot hr^{-1}$, respectively) (Veen and van de Geijn, 1978; Reid *et al.*, 1980). Explants of *Camellia* employed in Experiment 3 were only 13 cm long (compared to 45 to 50 cm of *Dianthus*) with a transpiration rate of 0.24 ± 0.03 $ml \cdot hr^{-1}$ under the experimental conditions employed. It would therefore be very unlikely that STS had not moved throughout the whole explant after 24 hr treatment. Alternatively, it could be suggested that STS had moved to leaves along the transpiration stream but that floral buds, with a lower transpirational rate, received lower levels of STS than leaves. However, reproductive organs (e.g. floral buds) have greater sink strength than leaves (Addicott, 1982), and for *Olea* shoots treated basally, inflorescences accumulate 465% more ethephon than leaves (Weis *et al.*, 1988). Also, developing inflorescences of *Pelargonium*, not visible at time of spray application of STS, are protected from the effects of ethylene (Cameron and Reid, 1983) indicating that substantial STS movement occurs within the plant tissue. Therefore, it would appear very unlikely that the abscission zones of *Camellia* floral buds, which are in close proximity with those of leaves, would have received substantially less STS than leaves.

The fact that floral bud abscission was not completely inhibited over the experimental period may be due to the greater sensitivity of floral buds over that of leaves of *Camellia* (Figure 5.8 and 5.9; Chapter 4). Because a range of STS concentrations was not applied it was not possible to determine whether the abscission response to STS was saturated. However, it may be of use to compare the amount of STS and response of *Camellia* to the work in *Dianthus* (Reid *et al.*, 1980). STS is transported up stems in solution and the concentration of STS in the treatment solution does not vary over time as solution volume decreases. Hence, if the transpiration rate is known, the approximate quantity of

silver in the tissue may be estimated from the STS concentration and duration of pulsing. In *Dianthus*, the amount of STS present in the tissue correlates well with its effectiveness and is saturated between 0.5 and 5 *u*M/flower (Reid *et al.*, 1980). The quantity of STS in *Camellia* explants treated with 0.2 mM STS for 24 hr was estimated to be 1.15 $\pm$ 0.14 *u*M/explant. Thus it could be said that, since *Dianthus* is more sensitive to ethylene than *Camellia* (exhibiting a response to as little as 0.01 *u*l·liter^{-1} ethylene; Woodson, 1991), and the amount of STS in the *Camellia* stem is more than that in *Dianthus* on a size basis, then the response of floral buds should be inhibited maximally. However, it should also be noted that the response of Reid *et al.* (1980) was not determined for application of exogenous ethylene, and hence direct comparison can not be made to that of *Camellia*. Also, a basal pulse of STS (4 mM for 24 hr) *Chamelaucium* (Geraldon Wax) completely inhibited floral bud abscission with application of 5.6 *u*l·liter^{-1} ethylene, however, abscission was recorded for only 1 day after ethylene treatment (Joyce, 1989).

Thus, it seems most likely that the greater ethylene-sensitivity of floral buds was responsible for the inability of 0.2 mM STS to completely inhibit abscission over the experimental period rather than the distribution of STS in the explant tissue. STS treatment of *Camellia* for 24 hr may have achieved maximal inhibition, but it was possible that for complete inhibition of ethylene-promoted floral bud abscission, higher levels of STS may be required. Application of STS as either a spray or medium drench might prove to be a useful tool to inhibit leaf abscission of *Camellia* during export, similar to treatments employed with *Streptocarpus* (Agnew *et al.*, 1985). Also, STS may be employed to inhibit possible post-sale floral bud or flower abscission as already proposed for *Camellia* (Lee and Song, 1992) and *Schlumbergera* (Cameron and Reid, 1981).

5.6 CONCLUSION

In conclusion, application of ethylene to *Camellia* resulted in promotion of abscission of both floral buds and leaves with a concentration dependent response. Floral buds were more sensitive than leaves as indicated by shorter time to 50% abscission. An ethylene exposure period of 24 to 31 hr was required for reception of the ethylene signal and commencement of the abscission process.

The abscission process involved increased ethylene production within the abscission zone. Cellulase activity also increased during abscission and correlated well with leaf abscission. Although other hydrolytic enzymes may be involved, it is suggested that subsequent dissolution of cell wall middle lamellae was a result of enzyme activity leading to organ abscission (Sexton and Hall, 1974). No increase in cellulase activity was observed in floral buds, and other enzyme species may be involved in the cell separation process of floral buds and leaves of *Camellia*.

Removal of ethylene resulted in cessation of abscission in a minimum of 7 hr with latest abscission occurring 23 hr later. After completion of this residual abscission, no further abscission occurred. Residual abscission was most likely due to completion of processes initiated during the ethylene exposure period (Kays *et al.*, 1976) such as activity of cellulase already outside the cell membrane (Abeles *et al.*, 1971) or due to gradual decrease in autocatalytic ethylene production from the abscission zone. Because leaf tissue of *Camellia* exhibited no senescence in response to applied ethylene, the continuing abscission after ethylene removal was not a result of autocatalytic ethylene production from the leaf blade. This may be the mechanism by which abscission ceases after ethylene removal, whereas in other genera, abscission may continue.

As found in many other plant species, application of STS inhibited the abscission-promoting effect of ethylene on floral buds and leaves of *Camellia*. The results indicate a typical central role of ethylene in ethylene-promoted abscission. STS did not completely inhibit abscission of floral buds, possibly as a result of greater ethylene-sensitivity.

CHAPTER 6

GENERAL DISCUSSION.

The concentration and duration of ethylene exposure interacted with organ type, organ maturity and temperature to determine the final level of ethylene-promoted abscission of *Camellia*. The mechanisms determining the level of response involved sensitivity to ethylene, rate of reaction and reversibility of the abscission process. These complicated interactions of ethylene with physiological and biochemical plant factors and environmental factors, were aptly demonstrated in the elucidation of mechanisms involved in selective removal of floral buds from *Camellia* using ethephon.

The Abscission Process

Application of ethylene either directly or via ethephon promoted abscission of all *Camellia* organs; floral buds, leaves and vegetative buds (Figure 2.1 and 4.1). The biochemistry of the ethylene-promoted leaf abscission process involved a lag phase (Figure 5.1) during which cellulase activity (Figure 5.4) and autocatalytic ethylene production (Figure 5.7) increased in abscission zones. A correlation of cellulase activity with leaf abscission has also been observed in *Phaseolus* (Sexton *et al.*, 1980), *Citrus* (Goren *et al.*, 1973), *Gossypium* and *Coleus* (Abeles, 1969). Similarly, although autocatalytic ethylene production in the abscission zone during the natural abscission process has been observed in *Citrus* (Ben-Yehoshua and Aloni, 1974), *Melia* (Morgan and Durham, 1980), *Phaseolus*, *Prunus* and *Parthenocissus* (Jackson and Osborne, 1970), this report appears to be the first examining autocatalysis occurring during ethylene-promoted abscission. STS application inhibited ethylene-promoted abscission of *Camellia* organs (Figure 5.8 and 5.9) and indicated that ethylene action required an activated ethylene receptor and that, as in other systems, the Ag^+ ion released from STS had blocked production of the activated ethylene receptor. No senescence or ethylene autocatalysis was observed in the leaf blade of *Camellia* as found in *Citrus* (Riov and Yang, 1982) and *Olea* (Goren *et al.*, 1988). Therefore ethylene autocatalysis of the leaf blade could have had no abscission-promoting influence on abscission as proposed by Goren *et al.* (1988).

It is pertinent at this point to discuss the similarity or otherwise of the abscission process in the two organs examined in most detail in this study, floral buds and leaves. The abscission zones of floral buds and leaves of *Camellia* exhibited many similarities in response to applied ethylene. This was reflected in nearly all responses including the sigmoidal shape of abscission kinetics (Figure 5.1), pattern of concentration-dependent response to ethylene (Figure 4.1) and ethephon (Figure 2.2 and 2.3), reduced abscission at lower temperatures promoted by ethylene (Figure 4.5 and 4.6) and ethephon (Figure 3.4 to 3.6) reversibility upon removal of ethylene (Figure 5.2 and 5.3), and inhibition of abscission by STS (Figure 5.8 and 5.9). Differences that existed in the magnitude of response to these treatments may be explained in terms of differences in sensitivity or rate of response (examined subsequently). The abundance of similarities indicates that the abscission processes of floral buds and leaves were regulated by the same ethylene biosynthetic and action pathway and lends support to the hypothesis of Woltering (1987) and others (Polito and Lavee, 1980; Morgan, 1984) that the abscission process of floral and vegetative organs are similar (Section 1.3.2.2). There is a general absence of equivalent research into response of different organs of the same plant to duration of ethylene exposure, although some comparable research is available. Residual abscission of *Philodendron* stipules appears to respond similarly to that of leaves after removal of ethylene (Marousky and Harbaugh, 1979c). In contrast, *Citrus* fruit and leaves have significantly different patterns of abscission. *Citrus* fruit commence abscission in less than 10 hr and do not exhibit a two-stage abscission process whereas leaves take more than 48 hr with a two-stage response. Differences observed in *Citrus* may be due to differences in maturity and/or degree of senescence between leaves and fruit (Ismail, 1970). This difference does not appear to be present in floral buds and leaves of *Camellia*.

Although the above results support the hypothesis of similarity of the abscission process in floral bud and leaf organs of *Camellia*, there were some observed differences in the biochemistry of abscission (Section 5.5). Although cellulase activity increased over the abscission process of leaves (Figure 5.4), activity of floral buds did not (Table 5.1). As discussed previously (Section 5.5), this may indicate that cellulase is not involved in abscission of floral buds or leaves. Examination of cellulase activity over the abscission process of floral buds and other enzyme species, notably polygalacturonase which has recently been shown to increase in *Camellia* floral bud abscission zones (Lee and Song, 1992), is recommended to address this issue.

Interaction of Ethylene Concentration and Exposure Duration with the Mechanisms of Abscission

The reaction rate of the abscission process was determined by a number of environmental and plant factors. In the application of ethylene, two key factors determining action were the concentration and duration of exposure (Figure 4.1 and 5.2; Lang and Martin, 1989). It is clear that ethylene concentration was important in determining ethylene-promoted abscission of *Camellia* (Figure 4.1 and 4.2) and ethylene responses in general (Trewavas and Jones, 1981). A key effect was that of ethylene concentration and the rate of abscission. Higher concentrations increased the rate of abscission of floral buds and leaves (Figure 4.1 to 4.2). The increase in the instantaneous measure of abscission rate used in these experiments (time to 50% abscission) was due partially to a decrease in the lag phase (observed in leaves; Figure 5.8) and to an increase in the maximum rate (slope of the ethylene concentration vs time curve) of abscission (Figure 5.8 and 5.9). Similarly, increased concentration increases abscission rate and decreases the duration of the lag phase of abscission of other species of flowers and floral buds, such as *Schlumbergera* (Cameron and Reid, 1981).

The mechanism by which ethylene concentration influenced abscission rate may be explained in terms of ethylene binding and subsequent action. The factor determining ethylene action is the formation of the ethylene-receptor complex (Trewavas, 1982), the mechanism of which has been proposed to be ethylene binding (Dodds and Hall, 1980; McKeon and Yang, 1987; Section 1.3.1.4). It would be expected that increased ethylene concentration resulted in a greater rate of formation of the active state of the receptor, i.e. greater rate of ethylene association with the ethylene binding protein. Therefore the greater extent and rate of abscission resulting from increased ethylene concentration can be explained in terms of higher levels of active ethylene-receptor complex, greater formation of the "second message" (Sisler *et al.*, 1983; Figure 1.2; Section 1.3.1.6) and hence, greater activation of subsequent biochemical steps resulting in abscission, *viz.* synthesis of mRNA, proteins and hydrolysing enzymes such as cellulase.

This hypothesis is also supported by results obtained from application of STS to *Camellia* floral buds where STS increased the duration of the lag phase and slowed the rate of abscission (Figure 5.9). As discussed previously, Ag^+ may not inhibit ethylene binding but rather the formation of an activated ethylene receptor

by modifying a receptor sub-unit (Section 1.3.1.5 and 1.3.1.6). Whatever the details of the inhibition of ethylene action by Ag^+ at the receptor, it is clear that the flow of signals following ethylene reception were inhibited. Thus the results observed in *Camellia* indicated that formation of the active state of the ethylene receptor or subsequent steps was slowed, but not completely inhibited by STS. Therefore, the reduced rate of transduction signal resulted in a slower rate of abscission of floral buds at low and high ethylene concentrations (1 and 10 $u l \cdot liter^{-1}$; Figure 5.9).

According to the definition employed in this thesis, comparisons of ethylene sensitivity were carried out by determining the rate or extent of abscission at low ethylene concentrations (Section 1.3.8). Because abscission was a concentration dependent response (such that increased concentration resulted in increased rate), then the concentration applied had an important interaction with the concentration-dependent response of abscission of organs and therefore determined whether abscission occurred or not. For example, application of low ethylene concentrations (<0.3 $u l \cdot liter^{-1}$) to 'Donation' for 10 days, resulted in no leaf abscission but floral bud abscission did occur. However, application of high concentrations (>2 $u l \cdot liter^{-1}$) for the same period resulted in abscission of both floral buds and leaves (Figure 4.1).

The mechanisms involved in determining ethylene-sensitivity have been discussed previously (Sections 4.5). Abscission may be regulated by the balance of many hormonal and biochemical factors as shown aptly in the balance model of Sexton *et al.* (1985; Figure 1.4). Each of the steps involved in determining abscission; concentration of ethylene reaching the ethylene receptor, other growth substances, anatomy, biochemistry and nature of the cell wall, were all suggested to be possible mechanisms involved in determining ethylene-sensitivity (Section 4.5).

Whatever the mechanism(s) involved in determining ethylene-sensitivity, the primary underlying mechanism active at low ethylene concentrations is suggested to have been the rate of ethylene reception and signal transduction. The steps involved in the abscission process were reversible (Section 5.1). Thus in *Camellia*, the rate of formation of the active state of the ethylene receptor and subsequent stream of signals must have occurred at a rate fast enough to overcome the reversible reactions. Therefore it is suggested that failure of low ethylene concentrations to promote abscission of *Camellia* was because the

balance of ethylene association/dissociation from the ethylene receptor resulted in insufficient levels of the active ethylene-receptor complex to promote the biochemical steps required to bring about abscission, principally, decreased auxin concentration, and promotion of hydrolysing enzyme action. The reduced effect of STS at high ethylene concentrations (Figure 5.9) indicated that inhibition of signal strength was less at high than low ethylene concentrations. The total inhibition of leaf abscission after STS application were also consistent with the above hypothesis since presumably, the level of the transduction signal was not sufficient to promote the abscission of the less ethylene-sensitive organ (leaves), whereas it was sufficient for the more sensitive organ (floral buds) (Figure 5.9 and 5.8 respectively).

The duration of ethylene exposure was also important in ethylene application to *Camellia* since the time of removal of ethylene determined the extent or even occurrence of abscission. Removal of ethylene prior to commencement of abscission (i.e. during the lag phase) resulted in no abscission. Also, once abscission had commenced, ethylene removal resulted in cessation of abscission such that only an average of 13 to 23% abscission occurred after ethylene removal (Figure 5.2 and 5.3). Important interactions with duration of ethylene exposure were the reversibility of the abscission process and rate of abscission.

As discussed in Section 5.1 and 5.5, most steps of the ethylene-promoted abscission process appear to be reversible. Therefore continual ethylene treatment is required to maintain the stream of biochemical signals that results in abscission (Sexton and Roberts, 1982). In *Camellia*, limited exposure duration resulted in either no abscission or cessation of abscission due to reversal of the abscission process. Thus, from results obtained in other genera it is suggested that reversal of the ethylene-promoted abscission process after removal of ethylene treatment involved diffusion of ethylene from the tissue (Goren and Sisler, 1986), reversal of auxin transportation inhibition (Beyer, 1973), decreased autocatalytic ethylene production in the abscission zone (shown to occur in the leaf blade; Riov and Yang, 1982), turning off of mRNA and enzyme synthesis (Tucker *et al.*, 1988) and, if break strength had decreased, retightening occurred (Abeles *et al.*, 1971).

The importance of the sensitivity to ethylene is also reflected in the interactions which may have occurred with the duration of exposure at low concentrations. Low ethylene-sensitivity was equated with reduced rate of abscission at low

ethylene concentrations. Therefore application of a low ethylene concentration for an exposure duration less than that required for abscission resulted in lower extent of final abscission. For example, an ethylene exposure duration of 10 days at any concentration greater than 0.3 ul·liter^{-1} resulted in abscission of floral buds, but only promoted leaf abscission at a concentration higher than 2 ul·liter^{-1} (Figure 4.1). Stated another way, although floral buds had a greater ethylene-sensitivity than leaves, if a low ethylene concentration (1 ul·liter^{-1}) was maintained for a long duration (26 days), then the final abscission outcome was the same for both organs.

Interaction of Temperature, Phenotype and Genotype with the Abscission Process

Temperature and Ethylene-Promoted Abscission

Examination of the influence of temperature (10^{o} and 30^{o}C) on the rate of ethylene-promoted abscission of whole *Camellia* plants at a saturating concentration found an E_a of 45.1, 24.1 and 54.2 kJ·mol^{-1} for floral buds, vegetative buds and leaves respectively (Table 3.3; Figure 3.11). This contrasts markedly with the results obtained by Olien and Bukovac (1982b) in *Prunus* fruit, who suggest that the action of ethylene has limited dependence on temperature. It is recommended that the influence of temperature on the ethylene-promoted abscission process should not be underestimated in examination of abscission promoted by either ethylene or ERCs, such as ethephon.

Application of ethylene at a lower temperature range (1^{o} to 25^{o}C) to shoot explants demonstrated that temperature influenced ethylene-promoted abscission in an exponential fashion with the greatest reduction in abscission rate occurring at temperatures below 5^{o}C (Figure 4.4). Low temperatures reduced abscission by reducing the rate of the abscission process. Similarly, the rate of flower senescence also decreases with reduced temperature such that longer duration of exposure is required to elicit the same response (Woltering and Harkema, 1987). This also supported the concept of rate of signal transduction from an ethylene treatment.

Thus, an important interaction of ethylene concentration and duration of exposure was with temperature mediated by abscission rate. Low temperatures resulted in reduced rate of abscission, and because the abscission response was reversible, for a given ethylene concentration and duration of exposure, the extent of abscission was lower. For a constant duration of ethylene exposure, the

interaction of ethylene concentration and temperature was such that at lower temperatures the influence of lower concentrations on abscission rate was increased. That is, the marked reduction in abscission rate occurred at higher concentrations at lower temperatures (Figure 4.5 and 4.6).

Phenotype

The definition of sensitivity employed in this thesis (extent or rate of abscission at lower ethylene concentration; Section 1.3.8) was important in the examination of differences in abscission response for the following reasons. Weis *et al.* (1988) applied a single high concentration of ethylene and ethephon to *Olea* to determine sensitivities of leaves and inflorescences. They found that this resulted in differences in the ranking of organ sensitivities depending on the ethylene source; ethylene or ethephon. A similar problem would have arisen in *Camellia* if sensitivity had been defined as the abscission rate, or extent of abscission, at a single high ethylene concentration. For example, at concentrations of 10 to 100 ul·liter^{-1}, the rate of abscission of floral buds was less than leaves (Figure 4.1), yet at all ethephon concentrations examined the extent of floral bud abscission was greater than leaves (Figure 2.1 to 2.3). If Weis *et al.* (1988) had applied a range of ethylene concentrations as found by Lang and Martin (1989), they would have observed the greater response of fruit (and so presumably inflorescences) to low ethylene concentrations. Thus according to the definition employed in this study, inflorescences and fruit of *Olea* would be defined as more sensitive than leaves, whereas the greater response at a single high ethylene concentration would be simply described as greater responsiveness. Similarly, the higher abscission rate of leaves than floral buds at high concentrations (>10 ul·liter^{-1}), was described as greater responsiveness rather than greater sensitivity that it would have been described as by Weis *et al.* (1988). In *Camellia*, floral buds were clearly more sensitive than leaves since their abscission rate at low ethylene concentrations (1 ul·liter^{-1}; Figure 4.1) was much greater than leaves, a very similar result to that obtained in *Citrus* fruit and leaves (Figure 1.5; Ben-Yehoshua and Eaks, 1970).

Greater sensitivity (rate or extent of abscission at low concentrations) is a more useful parameter than simply the rate or extent at higher concentrations, since differential response at low ethylene concentration is central to removal of target organs using ethephon (Beaudry and Kays, 1987) and in minimising abscission during transportation. The importance of the sensitivity to ethylene was also reflected in its interaction with the duration of exposure. Ethylene-sensitivity was

more important than duration since the effect of a given duration of exposure may have been influenced by sensitivity changes with maturity such that an ethylene application of a given duration had different effects on the extent of abscission depending on the time of the year (Figure 4.7 to 4.10).

As found in many other genera, maturity of all organs of *Camellia* had a significant influence on sensitivity to applied ethylene and ethephon. The high sensitivity of leaves on extending shoots (e.g. at vegetative bud break in spring or second flush in late summer) was found to occur in response to both ethephon (Figure 3.12) and ethylene (Figure 4.7 and 4.8). Thus the greater rate of abscission at low ethylene concentrations was also reflected in the greater final abscission in response to ethephon application. The suggested mechanisms involved in sensitivity of young leaves has been discussed previously (Section 4.5).

An anomaly appeared to exist in the response of leaf maturities greater than 1-year-old to ethylene and ethephon. Leaves of *Camellia* naturally senesce and abscise after 3 to 4 years (Anon., 1986). Application of ethylene to *Camellia* leaves of 1-year- and 2- to 3-years-old did not exhibit significantly greater ethylene-responsiveness than current season leaves (2- to 5-months-old; Figure 4.7 and 4.8). However, application of ethephon to whole *Camellia* plants did reveal greater sensitivity to ethylene released from ethephon of 1-year- and 2- to 3-year-old than 3-month-old leaves (Figure 3.12). Jackson and Osborne (1970) found greater ethylene-sensitivity of senescing (chlorotic) leaves of *Phaseolus* compared with green leaves. In this study 2- to 3-year-old leaves treated with ethylene were green and exhibited no visible signs of senescence. Hence, it is suggested that for *Camellia* leaves, sensitivity to ethylene only increased near the time of natural senescence, such as vegetative bud break when nutrient demand was greatest in the plant. Ethephon application to whole plants (Section 3) was able to detect differences in ethylene-sensitivity presumably due to higher replication and wider range of leaf maturities which would have resulted in the presence of leaves closer to natural senescence. Thus although the role of ethylene in natural abscission was not examined in this study, it is suggested that prior to natural abscission (at 2 to 3 years of age), there is a second peak in endogenous ethylene production and/or ethylene-sensitivity (Morgan and Durham, 1980) resulting in abscission. The increase in sensitivity may be due to either decreased auxin concentration which decreases with increasing leaf senescence (dela Fuente and Leopold, 1968; Street and Opik, 1984) or

increased receptor number, as found in fruit tissue (Yang, 1985). However, binding site concentration does not correlate with increasing senescence of *Nicotiana* leaves and *Ipomoea* and *Dianthus* flowers (Blankenship and Sisler, 1989b; Brown *et al.*, 1986) indicating that increased ethylene, or decreased auxin concentration, were more likely candidates for increased sensitivity with annual leaf maturation of *Camellia*.

That ethylene-sensitivity increased with greater floral bud maturity (Figure 4.7 and 4.8) supports the hypothesis of Section 2.5 that increased abscission of 'Anticipation' floral buds with later ethephon application (Figure 2.2) was due to increased floral bud maturity resulting in greater sensitivity to ethylene released from ethephon. Less research has been conducted into the mechanism of ethylene sensitivity of floral buds compared to that carried out in leaves. This may be due to the more complicated nature of the floral ontological process which involves flower initiation, development of the macrobud, flower opening and petal senescence and abscission. Potential also exists for pollination and fruit set with subsequent delay in ovary senescence. The nature of this process clearly results in a more complicated model than the relatively simple process of leaf senescence and subsequent abscission. Nevertheless, the process of abscission in flower and fruit tissue is of vital importance to horticulture and further work in this area is warranted.

Genotype

Differences between cultivars of *Camellia* were observed in response to ethylene and ethephon application. Application of ethephon to two cultivars of *Camellia* exhibited differences in the response of all organs of both cultivars. Of the two cultivars examined in detail, all organs of 'Anticipation' appeared to be more sensitive to applied ethephon than 'Donation' (Figure 2.2 and 2.3). Industry trials carried out by Duncan and Davies Nurseries Ltd, New Plymouth, N.Z. on 19th February, 1990 (21^{o}C at spraying) determined that the optimum ethephon concentration for removal of floral buds varied between 1500 and 3000 $\mu l \cdot liter^{-1}$. No clear correlation existed between cultivar or species and the optimum ethephon concentration, although *C. japonica* cultivars tended to be less sensitive than hybrid cultivars.

Differences in the presence or absence of continuing abscission after ethylene removal between genera (e.g. *Camellia* vs *Citrus* and *Olea*) were suggested to be a result of differences in the autocatalytic ethylene production of the leaf blade

after ethylene application (Section 5.5). *Camellia* abscission was reversible in a relatively short period (Figure 5.2 and 5.3) and this indicated that either the autocatalytic ethylene production in the abscission zones was not sufficient to promote abscission, or that it decreased after ethylene removal. The fact that species where autocatalytic ethylene production from the leaf blade are those in which abscission continues after ethylene removal (*Citrus*, *Olea* and *Capsicum*), supports the hypothesis that this was the mechanism which continued to promote abscission after ethylene was removed (Section 5.5).

The greater ethylene-sensitivity of floral buds of *Camellia* agrees with that found in most genera where reproductive organs such as floral buds, flowers and fruit exhibit greater ethylene-sensitivity than leaves (Section 4.1). It is of interest to compare the sensitivity of floral buds and leaves of *Camellia* to the wide range of potted ornamental plants examined by Woltering (1986). Because abscission of both *Camellia* organs ceased after ethylene removal (Figure 5.2 and 5.3), the level of abscission at a given time approximated the final abscission proportion (extent of abscission). Thus to rank *Camellia* in the (relative) sensitivity rating of Woltering (1986) the extent of abscission of *Camellia* 'Donation' floral buds and leaves at 24 and 72 hr ethylene exposure respectively were calculated. The results indicated that leaves of *Camellia* were relatively insensitive to ethylene, ranking between *Hedera* and *Aralia* (Rating 1; least susceptible), while floral buds were more sensitive, ranking between *Vinca* and *Streptocarpus* (Rating 7; second most susceptible).

Ethephon Application

The mechanism involved in the selective removal of floral buds of *Camellia* with ethephon can be explained in terms of the responses obtained from application of ethylene as discussed above. Ethylene concentration and duration of exposure resulting from ethylene release from ethephon interacted with organ type, organ maturity and temperature. Temperature had an added effect on ethephon application as it influenced ethylene release from ethephon as well as the rate of response to ethylene.

Ethephon application and its subsequent degradation to ethylene results in a pulse of ethylene consisting of a combination of peak ethylene concentration and duration of ethylene exposure (Section 1.3.7). This differs significantly from ethylene gas application where the concentration is either continuous, or more delineated due to the rapid rate of diffusion into and out of plant tissue (Goren

and Sisler, 1986). Due to the complicated nature of ethylene release from ethephon, prediction of responses to ethephon using ethylene application is difficult (Beaudry and Kays, 1988a). However, application of ethylene provided a means of elucidating the mechanisms involved in ethephon-promoted abscission of *Camellia*.

Ethephon Concentration and Temperature

As found with ethylene, ethephon concentration also strongly influenced the abscission response of *Camellia* organs (Figure 2.1 to 2.4) as did the temperature at application (Figure 3.1 to 3.10). It is suggested that increased ethephon concentration resulted in greater ethylene release, and thus both higher concentration and longer exposure duration. As discussed previously, higher ethylene concentration and duration had an important interaction mediated through rate of the abscission process. The response observed after application of higher ethephon concentrations is suggested to involve increased ethylene concentration and duration of ethylene exposure which resulted in increased abscission rate (Figure 3.4 to 3.6). Since abscission was reversible, increased rate of the abscission process and longer exposure duration would have resulted in a greater level of final abscission. Since the rate of abscission did not exhibit a plateau (Figure 3.4 to 3.6), it appears that the rate of response to ethephon was not saturated in *Camellia* at the highest concentration employed (4000 $ul \cdot liter^{-1}$).

Similarly, reduction in temperature reduces the rate of ethylene release from ethephon (Olien and Bukovac, 1978), and has a similar effect to reducing ethephon concentration, except that at low temperatures the release of ethylene will occur for a longer duration but at a slower rate and therefore at a lower peak concentration (Beaudry and Kays, 1987). This therefore reduced the resulting level of abscission. A similar example of the interaction of ethylene concentration and duration of ethylene exposure are found in the abscission of *Olea* fruit such that increased ethylene concentration results in increased rate of promotion of fruit abscission (decreased FRF) which interacts with the duration of exposure (Blumenfeld *et al*., 1978).

Ethephon and Selective Removal of Floral Buds

It was clear that choice of the appropriate ethephon concentration is crucial in selective removal of floral buds from *Camellia* (Figure 2.1 to 2.4, 3.7 to 3.8). The mechanism involved in the selective removal of floral buds from *Camellia* may have been due to organ abscission response differences to the duration of

ethylene exposure or to the ethylene concentration (Daniell and Wilkinson, 1972; Perry and Gianfagna, 1987; Beaudry and Kays, 1988a; Lang and Martin, 1989).

Continuous ethylene exposure was required for abscission to occur in both floral buds and leaves of *Camellia* (Chapter 5). Hence, for a given ethylene concentration and temperature, the time required for abscission to occur with continuous ethylene treatment could be approximately equated with the exposure duration required for promotion of abscission. If the duration of ethylene exposure was the dominant factor determining the abscission differences between *Camellia* organs after ethephon application, then floral buds would be expected to require significantly shorter durations of ethylene exposure than both leaves and vegetative buds. This was however only found to be true at low concentrations (Figure 3.11 cf Figures 4.1 and 4.2). Thus selective removal of floral buds from *Camellia* by ethephon was not likely to be due to the duration of ethylene exposure required for abscission alone. In Chapter 4, it was clearly demonstrated that floral buds of *Camellia* were far more sensitive than leaves, i.e. floral bud abscission rate is higher at low ethylene concentrations than that of leaves (Figure 4.1 and 4.2). This indicates that at high ethylene concentrations, duration of ethylene exposure is not one of the factors determining differential abscission of *Camellia* organs. However, at low concentrations, duration did interact with the rate of abscission to produce lower abscission of less sensitive organs such as leaves.

Temperature and Ethephon-Promoted Abscission

Temperature is the environmental factor with most influence on ethylene-, and particularly ethephon-promoted abscission (Chapters 3 and 4). Reduced temperature was found to decrease ethylene- and ethephon-promoted abscission, endogenous ethylene production, and is known to decrease ethylene release from ethephon (Olien and Bukovac, 1978). It is clear then that the influence of temperature involved a complex web of effects on ethylene-, and particularly ethephon-promoted abscission.

Lower temperatures strongly inhibited ethephon-promoted abscission with E_a of 59.9, 58.8 and 93.9 $kJ \cdot mol^{-1}$ for floral buds, vegetative buds and leaves respectively (Table 3.3). Lower temperatures resulted in lower peak ethylene concentration but a longer duration of ethylene exposure. The superior thinning efficiency which occurred at low temperatures and high ethephon concentrations is suggested to be a result of the ethylene release kinetics interacting with the

greater ethylene-sensitivity of *Camellia* floral buds. Thus greater floral bud abscission at low temperatures (Figure 3.7 and 3.8) is proposed to be a result of higher ethylene-sensitivity of floral buds (Figure 4.1), while less sensitive tissue (e.g. leaves) failed to respond. Conversely, ethephon application at high temperatures (or environmental conditions which promote greater ethylene release) resulted in less effective selective removal of floral buds due to high peak ethylene concentration which failed to differentiate between floral buds and non-target organs abscission. In this case, the peak ethylene concentration and duration of ethylene exposure were sufficiently high and long that leaf abscission was promoted significantly and less difference in abscission level of the organs resulted.

It is clear then, that high temperature increased ethylene- and ethephon-promoted abscission as well as endogenous ethylene production. The question arises as to the relative importance of each of these areas. Increased temperature increased the endogenous ethylene production in *Camellia* (Figure 4.12) as found in many other species. The chilling response observed at 8^{o}C in *Camellia* indicated that as temperature rises above 8^{o}C the influence of temperature on ethylene biosynthesis was reduced. Since endogenous ethylene production alone was unable to promote abscission, even at high temperatures (Experiment 4.2 and 4.3), then it was unlikely that the influence of temperature on this area was important except for its role of increasing the ethylene concentration within the tissue, albeit by a small amount (Field, 1985; Section 1.3.9; Figure 1.6). As discussed in Section 3.5, the effect of temperature on the ethylene-promoted abscission process appeared to be responsible for 41 to 75% of the effect of temperature on abscission promoted by ethylene, depending on the organ (Table 3.4). This indicated that temperature influenced the response of the tissue to ethylene as much as, or more than its influence on ethylene release from ethephon. This was a somewhat surprising result considering the E_a for ethylene release from ethephon of 125.6 kJ·mol^{-1} (Olien and Bukovac, 1978). However, the correlation of abscission rate (1/time to 50% abscission) used to separate the influence of temperature on ethylene- or ethephon-promoted abscission, did not correlate well with the final abscission proportion. Thus the effect of temperature may have had a greater effect on ethephon-promoted, and a lesser effect on ethylene-promoted abscission than indicated (Section 3.5).

Time of Ethephon Application

Perhaps the most complex interactions influencing *Camellia* abscission are those involving time of the year, because of its overriding influence on many environmental and plant tissue factors.

Ethephon application carried out in 1988, 1989 and 1990 (Chapters 2 and 3) demonstrated this fact in that, although floral buds were consistently more sensitive to ethephon than all other organs, the sensitivity and responsiveness of leaves and vegetative buds varied between application times. The different ranking between experiments may be explained by cultural differences resulting in greater tissue maturity which influenced sensitivity to ethylene released from ethephon.

Both temperature and organ maturity have been isolated and had a strong influence on the abscission response to ethylene of *Camellia*. For example, the greater ethephon-sensitivity of expanding leaves obtained in field trials were verified by ethephon application to whole plants (Figure 3.12) and in response to ethylene (expanded vs unexpanded leaves; Figure 4.11). Thus the time of ethephon application is suggested to be the result of difference in temperature at time of application and the effect of time on organ maturity.

Clearly there are a large number of interactions involved in the application of ethephon to *Camellia*. The results obtained have demonstrated the importance of ethephon concentration, temperature, maturity of organs and the organ type. These factors were mediated by the interaction of ethylene concentration and duration of exposure with the rate, sensitivity and reversibility of the ethylene-promoted abscission process.

Future Directions for Research

The current study has revealed some further areas for examination of abscission physiology.

It was beyond the constructs of this thesis to examine the biosynthetic pathway of ethylene in *Camellia*. Examination of ethylene biosynthesis in vegetative or floral tissue would be beneficial for both understanding and control of the abscission process. Application of ACC, methionine, and inhibitors of the biosynthetic pathway such as AOA, AVG and Co^{2+} would demonstrate the presence of the ethylene biosynthetic pathway in *Camellia* as carried out in other species such as *Citrus* leaf tissue (Riov and Yang, 1982) and leaf explants (Sisler *et al.*, 1985). Also, examination of the respiration rate during natural and ethylene-promoted abscission could further define the climacteric- or nonclimacteric-type patterns of abscission and senescence of *Camellia*.

The biochemistry of the abscission process in *Camellia* is an interesting area for further examination. As noted, the fact that cellulase activity did not increase in floral bud abscission zones over the abscission process places some doubt into its role in floral bud abscission, and therefore also in leaf abscission. Determination of activities of cellulase, polygalacturonase (recently shown to increase in *Camellia* floral buds; Lee and Song, 1992) and possibly other hydrolytic enzyme species such as pectinases (Henry *et al.*, 1974) in abscission zones of both organs of *Camellia* may help to determine which enzyme(s) are involved in the hydrolysis of cell walls during *Camellia* abscission. Although there was a correlation of cellulase activity with leaf abscission in *Camellia*, it should be remembered that abscission is the final event due to middle lamellae hydrolysis (Sexton and Hall, 1974). Measurement of decreasing break strength is the first physically detectable sign of middle lamellae degradation (Sexton and Roberts, 1982). Increased cellulase activity preceding a decrease in break strength has been found in a range of species and tissues (Craker and Abeles, 1969; Ratner *et al.*, 1969; Huberman and Goren, 1979). Use of decreased break strength as an abscission parameter and examination of activities of cellulase isoenzymes, as carried out by Durbin *et al.* (1981), would provide further insight into the role of cellulase in the abscission process of *Camellia*. Similarities of enzyme species occur in *Citrus* leaves (Ratner *et al.*, 1969) and fruit (Pollard and Biggs, 1970), however, differences have been observed between organs of *Prunus* (Rascio *et al.*, 1985; Rascio *et al.*, 1987). An examination of enzyme species and activities in floral bud and leaf abscission zones might also reveal differences to provide a biochemical basis for differential ethylene-sensitivities of *Camellia* organs (Blumenfeld *et al.*, 1978).

As discussed in Section 4.5, there are many possible mechanisms determining ethylene-sensitivity differences between organs and maturities including growth substance concentration, anatomical, ethylene diffusion characteristics, ethylene receptor concentration/activity, enzyme species and abscission zone cell wall digestibility. The possible roles of auxin (Suttle and Hultstrand, 1991) and gibberellins (Morgan and Durham, 1975) in the high ethylene-sensitivity of young leaves has been examined in *Gossypium*. However, further examination of these and other factors involved will increase our understanding of the mechanisms of ethylene-sensitivity, the ethylene-promoted abscission process, and so also improve our control of abscission. The rapid change in sensitivity of *Camellia* and *Gossypium* leaves during the expanding leaves (which can be artificially

promoted in *Camellia*; Section 1.2.2.1) provides a useful mechanism for studying the influence of maturity on ethylene-sensitivity in an abscission system which, due to the rapid changes, is a superior model system than studying the influence of senescence (aging year to year) on ethylene-sensitivity and abscission.

A fascinating, and important area for further examination is that of the reversibility of the abscission process. It has been proposed that genera differences are a result of the presence or absence of autocatalytic ethylene production by the leaf blade (Section 5.5). However, detailed examination of the level of abscission occurring after ethylene removal is rare and careful study of the response of a range of species is needed to verify this hypothesis. This should involve examination of the abscission kinetics prior to and after ethylene removal, determination of the presence and reversibility of autocatalytic ethylene production of leaves and abscission zones, and application of inhibitors of ethylene biosynthesis and action which would prove useful in elucidating the mechanism(s) involved. For instance, if AOA or AVG were to be applied prior to propylene treatment and the abscission response determined, the role of autocatalytic ethylene production in the abscission process could be elucidated.

Industry Application

Selective removal of floral buds prior to export

To selectively remove floral buds from *Camellia*, either ethephon or ethylene could be employed. This thesis has examined a wide range of factors involved in the application of ethephon and shows that in the practical application of ethephon these factors should be considered if the desired promotion of floral bud abscission is to be achieved. The two most important factors for consideration were ethephon concentration and temperature such that ethephon concentrations between 1000 and 3000 $ul \cdot liter^{-1}$ resulted in efficient thinning (Figure 2.1 to 2.3). However, an inverse relationship between temperature and optimum ethephon concentration was found to exist such that optimum thinning occurred with low ethephon concentrations at high temperatures and *vice-versa* (Figure 3.7 and 3.8). Other factors such as the cultivar and maturity of target and non-target organs were of importance also. The overriding influence of time of application should be considered since it influenced organ maturity and temperature.

Although not tested on a commercial level, it is possible that ethylene gas could also be employed to remove floral buds. A low ethylene concentration (0.3

$\mu l \cdot liter^{-1}$) could be applied to plants for the duration of time required to promote floral bud abscission (6 to 14 days; Figure 4.1 and 4.2) and the plants then removed from ethylene treatment. Because floral buds had greater ethylene-sensitivity than leaves, floral buds would abscise and leaves not respond. Also, since abscission was reversible (Figure 5.8 and 5.9) then significant levels of abscission would not occur after ethylene removal. To treat plants in this way would require a sealed environment with relatively constant temperature, constant ethylene concentration and CO_2 scrubbing. Although this practice is more expensive and technically more complicated than spray application of ethephon, the results would be less variable. Also, ethylene release from ethephon treated tissue may continue for up to two weeks after application (Olien and Bukovac, 1982a), whereas no such problem could result with ethylene treatment since complete diffusion of ethylene out of tissue occurs in approximately 1 hr (Goren and Sisler, 1986). Thus plants could be packed for export directly after ethylene treatment.

Minimisation of abscission during export

During the export of live *Camellia* plants a key aim is that of minimisation of abscission of leaves and vegetative buds. The results obtained point to two means of minimising abscission of these *Camellia* organs during export. For a given ethylene concentration, the time to abscission can be taken to be the maximum duration of ethylene exposure, since durations longer than this will bring about abscission. Thus the results obtained for a variety of ethylene concentrations and temperatures (Figure 4.3 and 4.4) can be employed to determine the maximum duration of ethylene exposure for any ethylene concentration at different temperatures since they were also carried out in the dark (as in the transportation environment). As a general rule, export at temperatures between 0^{o} and $5^{o}C$ appear to be optimal for minimisation of the influence of endogenous and exogenous ethylene and for optimal plant quality.

A second means of minimising abscission would be to apply the ethylene action inhibitor STS. Since the effects of STS are prolonged (Cameron and Reid, 1981), STS could be applied either as a spray or pot-drench to *Camellia* plants to provide protection from endogenous and exogenous ethylene-promoted abscission during and after transportation. This would also be of use in decreasing ethylene damage in the post-sale environment (Lee and Song, 1992).

Finally, as proposed for *Dianthus* (Reid and Wu, 1992) the variation in ethylene-sensitivity observed between cultivars and species of *Camellia* provides a genetic pool from which further screening and breeding could be carried out to select less abscission-sensitive cultivars.

Conclusion

Both ethylene and ethephon promoted abscission of all organs of *Camellia* with a concentration dependent response.

The ethylene-promoted process of *Camellia* leaves involved a lag period during which cellulase activity and autocatalytic ethylene production increased in the abscission zone. Cellulase activity did not however, increase in floral buds. Ethylene failed to promote senescence and concomitant autocatalytic ethylene production in the leaf blade as found in other genera, and so cannot be involved in promotion of abscission in *Camellia*. STS inhibited ethylene-promoted leaf and floral bud abscission suggesting that Ag^+ was involved in blocking the same point in the reception or transduction of the ethylene signal. *Camellia* leaves and floral buds required constant ethylene exposure for abscission to occur indicating that all steps of the abscission process were reversible.

The interactions of ethylene concentration and duration of ethylene exposure with abscission rate, ethylene-sensitivity and the reversibility of the abscission process are suggested to be useful in the explanation of the observed abscission responses to a variety of factors such as temperature, phenotype and genotype.

Sensitivity to ethylene and ethephon was a key factor determining the abscission response and was affected by the phenotype (organ type, organ maturity and tissue type) and genotype (*Camellia* species, cultivar, or genera). The environment, particularly temperature, influenced both ethephon- and ethylene-promoted abscission, with ethephon-promoted abscission being affected by the greatest amount. The influence of time of year on the effectiveness of thinning of floral buds may be explained principally by the effect of tissue maturity and temperature on the abscission process.

Practically, abscission could be promoted in floral organs (using ethephon or ethylene) or inhibited (using STS or limited ethylene exposure) and so result in production and transportation of high quality *Camellia* plants. All of the factors

mentioned above must be taken into account if effective thinning of floral buds by ethephon, or minimisation of ethylene-promoted abscission during transportation, is to be achieved.

The research carried out in this thesis has provided a firm foundation of knowledge of the sensitivity of *Camellia* organs to ethylene and ethephon, and how environmental and plant factors influence the resulting extent of abscission. Further research may be carried out with clear understanding of the role of exogenous ethylene in the abscission of *Camellia*. This study has greatly extended the previously very limited understanding of ethylene-promoted abscission physiology of *Camellia*.

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