Morphological and physiological aspects of flower initiation and development in *Tanacetum cinerariaefolium* L.

by

Phillip Hugh

P.H.Brown, B.Agr.Sc.(Hons.), Tas.

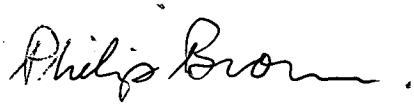
Submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

UNIVERSITY OF TASMANIA

HOBART

JUNE 1992

This thesis contains no material which has been accepted for the award of any other degree or diploma in any other University and, to the best of my knowledge, contains no copy or paraphrase of material previously published or written by any other person except where due reference is made in the text of the thesis.



P.H.Brown
University of Tasmania
Hobart
June 1992

List of abbreviations

ABA	Abscisic acid
a.i.	Active ingredient
BA	6-Benzylaminopurine
DHZ(R)	Dihydrozeatin (riboside)
DNP	Day-neutral plant
FR	Far red light
GA_n	Gibberellic acid A_n
GC-MS	Combined gas chromotography - mass spectrometry
HRC	Horticultural Research Centre
IAA	Indolylacetic acid
IPA	Isopentyladenosine
IRGA	Infra red gas analysis
LD	Long day(s)
LDP	Long-day plant(s)
LSD	Least significant difference
LSR	Least significant range
NB	Night break
PFD	Photon flux density
P_{fr}	Phytochrome in the far red absorbing form
P_r	Phytochrome in the red absorbing form
PGR	Plant growth regulator
ppm	Parts per million
R	Red light
RIA	Radioimmunoassay
SD	Short day(s)
SDP	Short-day plant(s)
SEM	Scanning electron microscope
TIBA	2,3,5-Triiodobenzoic acid
vpm	Volumes per million
Z(R)	Zeatin (riboside)

Table of Contents

Acknowledgements

I wish to sincerely thank my supervisor, Dr R.C.Menary, Reader in Horticultural Science at the University of Tasmania, for his encouragement and guidance throughout this project.

The assistance given by Commonwealth Industrial Gases Ltd in providing and maintaining field plots is gratefully acknowledged. I also wish to thank the staff of CIG and the pyrethrum growers who took the time to discuss with me the details of pyrethrum cultivation in Tasmania and who proved to be an invaluable springboard for ideas during the course of my studies.

The financial contribution provided by an Australian Postgraduate Research Award is gratefully acknowledged.

I wish to thank Dr W.Potts for her help with my field work and experimental design, and for her continued encouragement. I am also deeply endebted to Mrs M.Eldridge for proof reading this thesis. Also I wish to thank Ms R.D'Este, Mrs A.McEldowney, Mr G.Richardson, Mr M.Annandale, Mr W.Peterson and Mr D.Bradford for their technical assistance. The assistance of Mr W.Jablonski of the Central Science Laboratory, University of Tasmania, during the scanning electron microscopy work is gratefully acknowledged.

I would like to thank Dr N.Roberts, Dr R.Mensah, Dr C.Dragar and my fellow postgraduate students for their help and support during this project.

Lastly, I would like to sincerely thank my parents and family for encouraging me to strive for the highest goals, and for giving me all their support during this project.

Summary

This study investigated the morphological and physiological changes associated with flower initiation and development in pyrethrum, *Tanacetum cinerariaefolium* L.

Detailed morphological descriptions of vegetative and floral apices have been given and a scale of reproductive developmental stages based on these descriptions was proposed. It was shown that each stage of apical development was associated with a narrow range of apical diameters. The irreversible commitment to floral development was observed to occur when the first involucral bract was initiated on the apical dome and this point was characterised by a critical apical size. The apical diameter at this stage was always approximately 220 μm.

A juvenile-like condition was described for pyrethrum seedlings, tissue culture explants and vegetatively divided splits. During the period of juvenile-like growth the plants were not competent to respond to normally inductive treatments. The juvenile-like phase lasted until the plants had reached a minimum size or stage of development, but did not depend on chronological age. The attainment of meristem competence was associated with the release of lateral buds from apical dominance. Terminal meristems were never observed to initiate flowers. Axillary meristems became competent to flower a short time after being released from apical dominance, while older axillary meristems were observed to lose their competence. It was noted that the loss of competence to flower of lateral shoot meristems occurred after the release from apical dominance of new axillary buds on each lateral shoot.

The effects of the following environmental conditions on flowering were examined in a series of experiments; vernalisation, daylength, day temperature and photon flux. The major environmental requirement for flower initiation in pyrethrum was found to be a period of low night temperature or vernalisation. While flowering occurred eventually under 'non-inductive' conditions through an autonomous induction process, vernalising conditions were required to stimulate rapid flower initiation and development. Plants displayed a quantitative response to vernalisation as longer periods under vernalising conditions resulted in larger numbers of flowers, longer flower stems and more rapid flower initiation and development. Night temperatures of less than 18 °C were required to provide the vernalisation stimulus, with two weeks at 6 °C or three weeks at 12 °C demonstrated to be the minimum vernalisation requirement under

short days and day temperatures of 20 - 30 °C.

Both day temperature and photon flux density conditions were shown to modify the response to vernalisation. Low photon flux density conditions (350 $\mu mol.m^{-2}.s^{-1}$ or less) retarded flower initiation regardless of day temperature. High day temperatures combined with low photon flux resulted in a devernalisation-like effect where the plants were incapable of responding to otherwise inductive vernalising conditions. A true devernalisation effect was also demonstrated under these conditions with the vernalisation stimulus being reversed by a later high temperature / low photon flux treatment.

Daylength had a quantitative effect on both flower initiation and development, with both processes promoted by long days. The inhibitory effect of short days was thought to be mediated through reduced assimilate supply and not via the phyochrome reactions. It was concluded that pyrethrum is a day-neutral species as its daylength reaction was due to the daily light integral and not to photoperiod.

Autoradiography was used to follow the distribution of ^{14}C photosynthate during flower initiation and development under 'inductive' and 'non-inductive' conditions. This method was also used to study the effect of devernalising conditions on assimilate partitioning. The terminal shoot apex and young developing leaves were the main sinks for assimilates under 'non-inductive' conditions. The sink strength of the axillary shoots in 'inductive' conditions was observed to increase prior to the end of evocation and they became the dominant sink for radiolabelled assimilates as floral development progressed. 'Devernalising' conditions reduced the sink strength of the axillary buds, or prevented the translocation of assimilates to them.

Radioimmunoassays were performed to quantify the changes in plant growth regulator concentrations in mature leaf samples under 'inductive', 'non-inductive' and 'devernalising' conditions. Vernalising conditions stimulated an increase in the concentration of gibberellins while 'devernalising' conditions resulted in a reduction in the concentration of gibberellins to levels below that of unvernalised plants. The concentration of the auxin indolylacetic acid declined under vernalising conditions while 'devernalising' conditions prevented this decline. No evidence was found during this study of a role for the cytokinins or abscisic acid in the flowering of pyrethrum. However a possible role for these hormones could not be ruled out as all the assays were performed on mature leaf samples and as such would not have detected any

localised fluxes of hormones in other plant organs.

Two cultivation techniques for manipulating the flowering behaviour of pyrethrum in the field were examined. The first involved the application of growth retardants to reduce flower stem height and the degree of lodging at harvest. 'EL-500' and 'Cultar' at rates equivalent to 5 Kg active ingredient per hectare or above were shown to significantly reduce flower stem height and lodging. The growth retardants also reduced flower yield if applied during the period of flower initiation. The most effective control of lodging, without reducing flower yield, was found to result from application of the growth retardant immediately prior to the period of maximum stem extension, which under Tasmanian field conditions occurs in October. The growth retardant 'Cultar' reduced the gibberellin concentration in treated plants, and the effects of the growth retardant were reversible by application of gibberellin A_3.

The effect of trimming on the flowering of plants in the field was also examined. Multiple trimming treatments were shown to be the best strategy for promoting vegetative growth during the normal flowering period. Slashing plants to a height of approximately three centimetres above ground level during October and November, when the inductive vernalising conditions no longer prevail, significantly reduced the flower yield and increased the yield of vegetatively divided splits. This is of economic value in field nurseries where multiplication of planting stock is achieved through vegetative division of nursery stock.

The process(es) of flower initiation and development were discussed in light of the experiments detailed above.

I. Introduction

An increasing trend towards environmental awareness and a demand for environmentally friendly consumer products is presently being witnessed in many industrialised countries. A number of these countries have introduced legislation which bans or restricts the use of an ever expanding list of synthetic chemical insecticides which are considered harmful to humans or their environment. Considerable attention is now being focused on safe alternatives and particularly on natural products. One such alternative is the insecticide extracted from the herbaceous perennial plant pyrethrum, *T. cinerariaefolium* L.

Six insecticidally active compounds, collectively called pyrethrins, have been isolated from pyrethrum. The pyrethrins are concentrated in the secretory ducts and oil glands of the ovaries or achenes in the flowers. When the pyrethrin content of the flowers is at its maximum the flowers are harvested and dried before the crude extract is isolated through solvent extraction. Refining of the crude extract yields the active insecticidal components which are mixed with solvents, emulsifiers, propellants and perfumes to make a multitude of different insecticidal formulations. As a natural insecticide which is considered non-toxic to human beings, these pyrethrum formulations have become increasingly attractive to insecticide manufacturers in recent years.

Pyrethrum has been cultivated commercially since the early 1800's. The major producers today are located in East Africa where pyrethrum is grown as a highland crop in equatorial regions. In 1978 pyrethrum was introduced into Tasmania and a plant improvement programme was commenced. By comparison with the major producing countries, pyrethrum is grown in Tasmania in arable regions close to or a few hundred meters above sea level, and the prevalent growing conditions produce only one main flush of flowers rather than year round production. To facilitate mechanical harvesting only clonal material generated through tissue culture and vegetatively divided or 'split' is planted in the state.

The differences in environmental conditions, cultivation practices and harvesting techniques between Tasmania and the traditional growing regions have presented many unique hurdles to the establishment of pyrethrum as a successful commercial crop in the state.

This study has addressed one of the areas necessary to ensure the continued

viability of commercial pyrethrum production in Tasmania, the factors influencing the flowering behaviour of pyrethrum. There is currently a lack of scientific knowledge on the flowering of pyrethrum despite the fact that the crops economic success depends on the yield of flowers. Hence it is desirable to have an understanding of the physiological and morphological changes associated with flowering and their interactions with the environment in order to provide management for the large scale production of flowers.

Aims of the Present Study

(1) Describe in detail the morphological changes associated with flowering in *T. cinerariaefolium.*

(2) Measure and characterise the changes associated with the transition from juvenile growth to meristem competence.

(3) Measure the effects of temperature and photon flux density on photosynthesis in order to more fully interpret the influence of environmental variables.

(4) Investigate the effects of environmental variables on the initiation and subsequent development of the inflorescence.

(5) Examine the changes in endogenous plant growth regulator concentrations during floral initiation and development.

(6) Examine the partitioning of photosynthetic assimilates within the plant during floral initiation and development.

(7) Investigate various cultural practices with a view to manipulating the flowering behaviour of *T. cinerariaefolium* to aid in the commercial exploitation of the crop.

II Literature Review

II.1 Introduction

1.1 The family Asteraceae

The pyrethrum of commerce (*Tanacetum cinerariaefolium*) is a member of the family Asteraceae, the largest family of angiosperms.The family also contains the genera *Dendranthema*, *Aster*, *Xanthium*, *Rudbeckia*, *Helianthus*, *Dahlia* and *Cosmos* that include species which have been extensively studied in flowering research. The flowering behaviour of the Asteraceae is characterised by the production of 'capitula' or 'heads', in which all florets are inserted on the more or less flattened axis (receptacle) and are surrounded by bracts. The capitulum is a racemose inflorescence because the florets are initiated acropetally on the broadened axis (Kinet, Sachs and Bernier, 1985). The family Asteraceae contains around nine hundred genera and about thirteen thousand known species (Weier *et al*, 1982). Of these, several species belonging to the genus *Tanacetum* are known to possess insecticidal properties. *T. coccineum* and *T. cinerariaefolium* are the two principal species possessing active insecticidal constituents.

1.2 The history of pyrethrum cultivation

T. coccineum originated in the Caucasus while *T. cinerariaefolium* is a native of the Adriatic coast of Yugoslavia (Contant, 1976). Commercial production of *T. coccineum* began in the early 1800's with the development of a lively trade in ground flowers ('Persian powder'). Production has declined since 1840 when the superior yield of insecticidal constituents from *T. cinerariaefolium* was discovered, and today *T. coccineum* is restricted to homestead cropping in its native area. Yugoslavia remained the dominant producer of *T. cinerariaefolium* up until the First World War when Japan siezed the world market. After the war the crop spread, with the African countries Kenya, Tanzania, Rwanda and Congo proving particularly suitable for the cultivation of pyrethrum. Japanese exports ceased during the Second World War and the East African countries took over as the world's major suppliers and have remained so until the present time (van Rijn, 1974). Kenya is currently the

world's major producer with over two thirds of the world market (Bhat, Menary and Pandita, 1985).

Several attempts have been made to grow pyrethrum as a commercial crop in Australia, with the earliest reported plantings being around 1895. Prior to 1978, trial cultivations had been attempted in Victoria, New South Wales and Tasmania and had all proved unsuccessful (Bhat and Menary, 1984a). In 1978, pyrethrum was introduced into Tasmania and a plant improvement programme was commenced. Commercial production using the selected clonal material from the plant improvement programme has begun and at this stage the future viability of the crop in Tasmania appears assured.

1.3 Botanical description

Pyrethrum is a herbaceous perennial whose vegetative development is characterised by a rosette growth habit. The generative growth phase is expressed as the development of composite flowers borne on branched leafy stems, typically sixty to eighty centimetres in height. The leaves are deeply lobed and covered in fine glandular trichomes. The flowers are twenty to forty millimetres in diameter [although triploid clones have been reported with flower diameters significantly larger than diploid plants (Ottaro, 1977)] with an outer whorl of white ray or ligulate florets and the remainder of the receptacle covered with yellow disc florets. Pyrethrum displays a sporophytic self-incompatibility reaction, with proteins released by the pollen grains during the pollination process acting as recognition substances (Brewer, 1974. Brewer and Henstra, 1974). It is of interest to note that while pyrethrum is grown for its insecticidal constituents, the flowers are actually insect pollinated.

1.4 Pyrethrins

Six insecticidally active compounds, collectively called pyrethrins, have been isolated from pyrethrum. These are grouped into two fractions; the pyrethrin I fraction derived from chrysanthemic acid, containing pyrethrin I, cinerin I and jasmolin I, and the pyrethrin II fraction derived from pyrethric acid which contains pyrethrin II, cinerin II and jasmolin II (see Figure II.1.4). The ratio of the two pyrethrin fractions is important in determining the effectiveness of the insecticide.

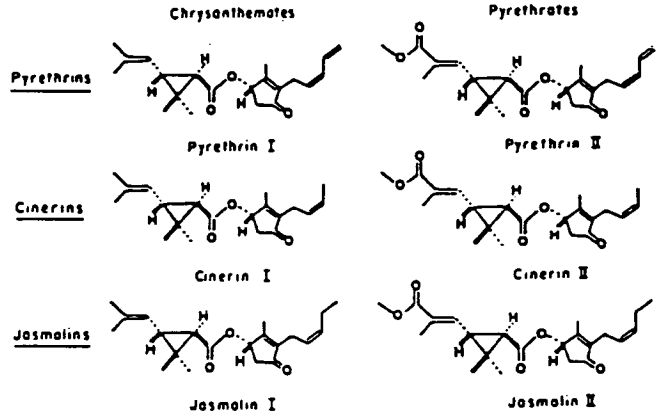


Figure II.1.4 Structures of the six insecticidal constituents of pyrethrum extract (from Bourne and Rosenthal, 1976).

The pyrethrins are concentrated in the flowers where they represent between one and two percent by weight on a dry matter basis at harvest (Ikahu and Ngugi, 1989), although workers are attempting to increase this yield through breeding programmes (eg. Bhat and Menary, 1984b). Approximately ninety four per cent of the pyrethrins are present in the secretory ducts and oil glands of the ovary or achene (Head, 1966). The oil glands are located externally on the ovary wall and the corolla while the secretory ducts are present within. Oil glands are also found on the surface of leaves, stems, leaf petioles and flower stalks, and secretory ducts are found in root, stem and leaf tissue where they are often associated with strands of vascular material (Zito, Zieg and Staba, 1983). During floral development, a change in the morphology of the oil glands on the achenes occurs, with the glands developing and filling from the bud stage, usually reaching a maximum when approximately three quarters of the disc florets are open before collapsing at the overblown stages (Bhat and Menary, 1984a). The increase in flower pyrethrins content is very closely associated with this development of the oil glands (Bhat and Menary, 1979) with each clone having a net accumulation pattern of its own. In some genotypes the pyrethrin content does not decline at the overblown stage and the oil glands remain full. It appears that the pyrethrins are produced in both the flowers and other plant organs by a number of different cell types; oil glands, resin ducts and mesophyll cells, and that the observed accumulation pattern during floral

development is a result of the translocation of pyrethrins within the plant coupled with the synthesis and the metabolic breakdown of the pyrethrins.

1.5 Insecticidal properties and uses

When the pyrethrin content of the flowers is at its maximum the flowers are harvested and dried before the crude product is isolated through solvent extraction. Refining of the crude extract yields the active insecticidal components which are mixed with solvents, emulsifiers, propellants and perfumes to make a multitude of different insecticide formulations (Anon, 1978). As a natural insecticide which is considered non-toxic to human beings, these pyrethrum formulations have become increasingly attractive to insecticide manufacturers in recent years. With many countries introducing legislation banning or restricting the use of an increasing number of synthetic insecticides, as well as the increasing environmental awareness being witnessed in industrialised countries, new market opportunities appear certain.

Apart from its lack of toxicity to human and mammalian life, the pyrethrins also possess many of the properties necessary for effective insect control. The pyrethrins are effective against a wide spectrum of insect pests, have a low level of insect immunity and a rapid knock down and killing action as well as a strong repellency action and a good flushing out effect (Anon., 1978). The insecticide is not persistent, rapidly decomposing in sunlight leaving no residual toxicity. Because of this, it is restricted in its uses to non-agricultural applications such as household, school, factory, hospital and other medical uses.

Synthetic substitutes have been developed which duplicate many of pyrethrins desirable properties. The closest synthetic substitutes are the pyrethroids which, like the pyrethrins, do not pose the environmental and health hazards that many other synthetic chemical insecticides do. None of the available pyrethroids combines the excellent knockdown, both early and late, and the adequate killing action of the pyrethrins (Winney, 1973). Synthetic insecticides are generally cheaper to produce and do not suffer from the production fluctuations of pyrethrins, caused primarily by climatic conditions.

2. Floral initiation and development in pyrethrum

Lack of adequate knowledge of the plant is proposed as one of the factors responsible for the failure of the early attemps to cultivate pyrethrum in Australia (Bhat and Menary, 1984a). Pyrethrum cultivation in Tasmania is confined to the arable regions lying close to or a few hundred metres above sea level and the prevalent growing conditions induce the plant to produce only one main flush of flowers in November - December. By comparison, in the major producing countries which are located close to the equator, pyrethrum is grown as a highland crop with the growing conditions allowing continuous flowering, with seasonal peaks, all the year round. A survey of the literature reveals few publications on the environmental requirements for floral initiation and development in pyrethrum, with the articles published generally being based on field observations in East African countries. Clearly a need exists to extend this volume of information to encompass the plants' responses to the environmental conditions encountered in Tasmania to ensure the continued viability of commercial pyrethrum production in the state.

While the volume of published information on floral initiation and development in pyrethrum is presently limited, a great wealth of information exists on the physiological and morphological aspects of the generative development of the related species *Dendranthema grandiflora*, formerly classified as *Chrysanthemum morifolium* (Anderson, 1987). *D. grandiflora*, the garden or glasshouse chrysanthemum, is a very important floriculture crop and as such has been extensively studied. Examination of the literature, supported by preliminary observations, indicated that similarities exist between the flowering behaviours of *T. cinerariaefolium* and *D. grandiflora*. For this reason the physiological and morphological changes during flower formation in *D. grandiflora* were used during this study as a flowering model for pyrethrum and are discussed accordingly in this review.

2.1 Morphological events

A scale of floral development stages has been proposed to describe certain macromorphological changes observed during pyrethrum bud and flower development (Head, 1966).

Stage	Description
I	Well developed closed bud
II	Ray florets vertical
III	Ray florets horizontal, first row of disc florets open.
IV	Approximately three rows of disc florets open.
V	An inflorescence with nearly all disc florets open.
VI	Early overblown condition, colour of disc florets diminishing but ray florets still intact.
VII	Late overblown condition, little colour remaining, disc florets still intact, ray florets dried out.
VIII	Disc florets fallen, stem dry 2cm below the flower head, suitable for collection of seed.

Figure II.2.1.a Scale of pyrethrum floral development stages (from Ikahu and Ngugi, 1989)

Parlevliet (1970) noted that vegetative shoots are distinguishable from those having initiated flowers as they are normally less than two centimetres long. The first macromorphological sign of floral initiation is stem elongation. By removing all initiated shoots from plants of eight different clones, Parlevliet was able to show that the number of days taken from initiation to flower bud opening (stage II of the above scale) was between 60 and 73 days. The number of days to picking (stage IV) varied between 68 and 82. These figures support the findings of Glover (1955) that plants do not begin yielding for 60 to 80 days after being cut back to within 10 to 15 centimetres of the ground. Bhat and Menary (1984a) found that the number of days taken by flowers to reach stage V from stage I (flower buds just beginning to open) was around thirty but varied slightly between clones. No information has been published on the histological changes associated with floral initiation in pyrethrum. While the above scale of floral development stages has proved satisfactory to define the physiological state of plants used in studies of flower development, the lack of detailed knowledge of the changes associated with floral initiation is an obvious area which must be addressed to ensure the validity of studies aimed at quantifying the early flowering responses of pyrethrum.

Detailed investigations of the morphology, organisation and growth of the apical meristem of *D. grandiflora* during flowering have been published. The morphological changes form the basis of an arbitrary scale of reproductive development which has been widely used to quantify the flowering responses of chrysanthemum (Cathey and Borthwick, 1957).

0 Stem terminal flat; typical of vegetative condition
1 Stem terminal slightly enlarged
2 Stem terminal forming receptacle; first bracts present
3 Receptacle spherical with twelve or more bracts around its rim
4 Receptacle becoming flattened; many bracts but no floret primordia present
5 Two or three rows of floret primordia on rim of receptacle
6 About six rows of floret primordia on receptacle
7 Receptacle covered with floret primordia except at tip
8 Entire receptacle covered with floret primordia
9 A few floret primordia not yet having beginnings of perianth
10 Perianth present on all florets
11 Reproductive organs present on all florets

Figure II.2.1.b Scale of generative apical development in *Dendranthema grandiflora* (from Cathey and Borthwick, 1957).

The initiation and further development of the reproductive meristems of cultivars 'Polaris' and 'Bittersweet' have been closely correlated with the size of the apex (Horridge and Cockshull, 1979. Cockshull and Horridge, 1980). Each stage of development is associated with a characteristic, narrow range of apical volumes, with different temperature and irradiance conditions having no influence on this relationship. Furthermore, there appears to be a critical size of apical dome, below which only leaf initiation occurs and above which bract and receptacle formation begins.

The critical stage during flower initiation at which the apex can first be defined as being reproductive is the moment that the changes it undergoes become irreversible (Schwabe, 1959). The irreversible committment to reproductive development in chrysanthemum is associated with apical stage II (Horridge and Cockshull, 1979). The cessation of leaf production by the apex takes place before the first bracts become visible and floral evocation ends (Van Ruiten and De Jong, 1984). The vegetative apical meristem can grow to 250 µm in diameter, is slightly domed and is surrounded by from nine to thirteen or more leaf primordia and young leaves which form the apical bud

(Cockshull, 1985). Floral initiation is marked by the enlargement of the apical dome to form a receptacle and by the formation of bract primordia, which are initiated more rapidly than leaf primordia and are distinguishable by retaining an entire margin rather than forming a dentate margin (Schwabe, 1959). These become the involucral bracts which enclose the receptacle and floret primordia. Floret initiation begins around the outer edge of the receptacle and proceeds acropetally until the entire receptacle is covered.

When grown under certain environmental conditions the apical meristem may form a flower bud which fails to grow rapidly. It may have enlarged involucral bracts and is normally surrounded by leafy lateral shoots arising from the axils of the upper leaves. These buds are frequently referred to in the literature as 'crown' or 'break' buds (eg. Kofranek, 1980. Cockshull and Horridge, 1980. Popham and Chan, 1952. Chan, 1950). The development of these buds has been retarded or arrested by the environmental conditions in which they are growing, but they retain the potential to develop normally (Popham and Chan, 1952). Cockshull and Horridge (1980) have shown that these 'crown' buds display the same correlation between apical size and stage of reproductive development as floral meristems which develop normally.

2.2 Temperature effects

Vernalisation is an essential requirement for flower initiation and development in pyrethrum. Field experiments conducted by Glover (1955) revealed that the yield of pyrethrum flowers in East Africa is inversely related to the mean maximum temperature and directly related to the number of hours at or below 16 °C some three months earlier. In equatorial regions, this vernalisation requirement explains why flowering is in general more prolific at higher altitudes and that the cultivation of pyrethrum below a certain altitude becomes uneconomic as many plants do not flower (often referred to in the literature as turning 'blind'). 'Blind' plants appear to be plants remaining vegetative simply because they have not received a sufficient inductive (vernalisation) treatment. Tuikong (1984) states that blindness is a recessive character, allowing the possibility, through breeding, of growing pyrethrum at lower altitudes in East African countries. Glover (1955), using blind plants in a controlled environment cabinet, showed that ten days continuous exposure to 16 °C is sufficient to stimulate the development of flower

buds. This cannot be regarded as the minimum vernalisation requirement as the plants were held in ambient conditions prior to and following the cold treatment and may have received a partial vernalisation stimulus from these conditions. The fact that one third of the untreated, control plants also flowered, albeit with a reduced flower number, supports this conclusion. No results have been published on the minimum duration or optimum temperature for vernalisation under controlled environment conditions even though vernalisation is accepted as the major environmental requirement for flowering in equatorial regions.

The yield of flowers and the pyrethrins content have been shown to increase with increasing altitude in East Africa. Muturi, Parlevliet and Brewer (1969) estimated that for every 360m in altitude there is an increase of 0.15% in total pyrethrins in the product. Using regression analysis on a series of mean temperature and percentage pyrethrins values, Kroll (1964) was able to predict a 0.03% increase in pyrethrins content for every 0.56 °C (1 °F) decrease in temperature over the range 12.5 to 17.2 °C. This correlation is due to the temperature effect on pyrethrin synthesis and metabolism during flower development. No conclusions can be drawn from this study on any possible effects during flower initiation as the stated correlation is between percentage pyrethrins measurements and mean temperature readings taken in the same month whereas Glover (1955) indicated that initiation occurs some three months before harvest.

Mean maximum temperature has similarly been shown to influence flower bud development. A multiple regression equation has been developed in which the monthly maximum and minimum temperatures account for 92 percent of the variation in flower yield (Mohandass, Sampath and Gupta, 1986). Flower number was influenced more by the accumulated degree days (calculated by subtracting a lower threshold from the monthly median temperature) in the month they were harvested than by the minimum temperature, with yield decreasing with higher values of accumulated degree days. As flower initiation occurs approximately three months before harvest, the variations in accumulated degree days or mean temperature would be expected to be exerting a greater influence on the monthly flower number through changes in the flower development rate whereas minimum temperature may be playing a greater role in flower initiation. An upper limit of mean maximum temperature of 24 °C which, if prolonged for a week or more, leads to inhibition of flower production (Glover, 1955) accounts for the observed yield decrease under high mean temperatures or high accumulated

degree days values. Again, this study appears to be examining the temperature effects on flower development, although a high temperature inhibition of flower initiation cannot be ruled out from the data presented. This lack of separation between the plants flower initiation responses and flower development responses to various environmental changes is a common problem when analysing the published data derived from field trials. The results of field trials are usually expressed in terms of final flower yield, thus encompassing both flower initiation and development, and so are of little use in establishing the environmental requirements for, and influences on, flower initiation.

While the vernalisation requirement for flowering in *D. grandiflora* has largely been eliminated from cultivars selected for year-round flower production, earlier reviews examined the requirement in some detail. Basal shoots must be exposed to low temperatures for at least three weeks to obtain the maximum acceleration of flowering (Schwabe, 1950) with a temperature of about 4 °C being more effective than one of 10 °C (Vince, 1955).

The vernalisation stimulus is perceived by the apical bud (Schwabe, 1954) and also by meristems in the leaf axils and at the base of the plant (Schwabe, 1955). Axillary meristems which are formed below a vernalised bud and begin to develop after the period of vernalisation, flower normally. The basal growing points, which normally remain dormant until after the plant has flowered, lose their vernalised state over time and have to be revernalised at the end of the growing season in order to flower (Schwabe, 1955). Schwabe (1957) also showed that vernalised shoots can be devernalised by exposure to high temperatures (>24 °C) in low-light conditions, but only if this treatment is given within three weeks of vernalisation. A devernalised shoot can be revernalised by exposure to a second chilling treatment. The unvernalised and devernalised states appear to be equivalent, with both resulting in vegetative growth and the production of leafy rosettes.

The influence of temperature on flowering is not restricted to vernalisation. Temperature affects flower initiation and flower development in both cold-requiring and photoperiodic *D. grandiflora* cultivars. The various chrysanthemum cultivars flower over a wide range of temperatures. Cathey (1969) classified all chrysanthemum cultivars into three response groups;

1. thermopositive, flowering inhibited by low temperature,
2. thermonegative, flowering inhibited by high temperaure
3. thermozero, flowering not sensitive to either high or low temperatures.

This arbitrary system of classification reflects the differences in the optimum temperature for flowering of the various cultivars as the temperature response curves are similar in shape. Most cultivars in use today would be classified as thermozero, for they are capable of flowering over a wide range of temperatures (De Jong, 1978).While flowering can be accelerated by growing plants under optimum temperature conditions, the leaf number on the flower stem may not differ from plants grown at higher or lower temperatures (Cockshull, 1979) indicating that temperature is influencing the rate of flower development rather than the initiation process. Analysis of clones selected for low temperature flowering shows that reduced sensitivity to temperature accounts for the ability to flower at low temperatures rather than a reduced optimum temperature requirement (De Jong, 1989a).

Karsson *et al* (1989a) examined the interaction of light intensity and day and night temperature on chrysanthemum flower initiation and development under short-day (SD) conditions, and developed a functional model to predict flowering. Under low photon flux density (PFD) conditions flowering was inhibited by day temperatures less than 10 °C or greater than 23 °C with a night temperature of 30 °C. Flowering was predicted to occur under all day and night temperature combinations in the range 10 °C to 30 °C at PFDs above 10.8 mole.m^{-2}.day^{-1}. The time to flower from the start of inductive conditions decreased non-linearly as PFD increased, with the greatest increases seen at lower PFDs (Karlsson *et al*, 1989b). Independent of PFD, the optimum day temperature for the cultivar studied was 17 °C and the optimum night temperature was 18 °C. Optimum temperature does, however, vary during flower development (Karlsson *et al*, 1989c., Whealy *et al*, 1987).

2.3 Photoperiod and irradiance effects

The photoperiodic response of pyrethrum has not been investigated. Glover (1955) points out that in one of the main growing regions in the highlands of Kenya, daylength varies by only around two minutes throughout the year. Given that daylength varies only slightly throughout the year in the main growing regions of East Africa, any photoperiodic response the plant possesses does not influence flower yield during the growing season and consequently photoperiodism has not been examined in these areas. The response of pyrethrum to different photoperiodic regimes is the area of flowering research which is most neglected and in need of clarification.

Very high rainfall accompanied by persistent cloud cover generally results in a reduction in pyrethrum flower yield. It has been shown experimentally that 90% shade can reduce yield by 50% (Muturi *et al*, 1969). It is not discussed as to whether this yield reduction is a result of an inhibitory effect on flower initiation or simply a consequence of reduced photosynthesis and therefore reduced growth rate. The effect of light intensity on flowering is obviously another area of flowering research in need of further study.

D. grandiflora is a quantitative short-day (SD) plant. The critical photoperiod varies from nine hours to sixteen hours depending on the cultivar and is influenced by both temperature and light intensity (Cathey, 1969). Flower bud development is also a SD response, but generally with a different critical photoperiod than initiation, and in certain cultivars is a qualitative SD response as plants are unable to develop their buds to anthesis in long days (LD). Flower initiation occurs eventually in all cultivars when grown in LD (Cockshull, 1985). Flower initiation under these conditions can be regarded as resulting from an autonomous induction process which proceeds independently of any external signal. Horridge and Cockshull (1979) suggest that the attainment of a critical size of apical meristem triggers this induction process (see Section II.2.1). This hypothesis has been incorporated into a mathematical model which can predict quantities such as the number of primordia initiated by the apex, plastochron duration and the apical dome mass before, during and after the transformation of the apical meristem from vegetative to reproductive development (Charles-Edwards *et al*, 1979).

Photoperiodic induction requires the presence of expanded leaves (Cathey, 1969), although one leaf can be sufficient (Tanaka, 1967). Seven or eight SD in succession are sufficient for rapid flower initiation, producing a similar final leaf number below the terminal flower bud as for plants held in continuous SD (Kofranek and Halevy, 1974). Four SD in succession are less effective, but still induce flower initiation before it occurs in LD and at a lower leaf number. When fewer than eight SD are given in succession it is generally only the terminal apical meristem that is committed to floral development, forming a 'crown' bud. As more SD are given, so the number of axillary meristems committed to flower initiation increases in a basipetal progression (Cockshull, 1972).

Flower initiation in SD occurs earlier the greater the daily integral of photosynthetically active radiation (400 to 700 nm) the plant receives in the range 0.31

to 2.50 $MJ.m^{-2}.day^{-1}$ (Cockshull, 1972). Higher PFDs also increase the rate of flower development (Hughes and Cockshull, 1971a). The flowering response is related to the daily total of radiant energy rather than to the maximum irradiance (Hughes and Cockshull, 1971b). In addition, plants are capable of integrating the radiant energy received over at least two days and then responding accordingly, as the effect of low light intensity in one day can be countered by the provision of high light in the following day (Cockshull and Hughes, 1972). Higher daily solar radiation integrals increase the rate of flower development and decrease the number of days to flowering and the variability of flowering (Noble and Ellis, 1990). Light quality (spectral energy distributions) also influence flower development, with blue light significantly reducing stem length and stimulating lateral shoot development (Mortensen and Stromme, 1987). The effect may be mediated by phytochrome as the light has a high red (R) content but it is suggested that the blue to red light ratio also influences plant morphogenesis.

The number of leaves produced in LD before autonomous induction is similarly irradiance dependent, and is also increased by high or low temperatures (Cockshull, 1979). This similarity to the SD responses suggests that the inductive processes of the two daylengths have features in common. The influences of irradiance and temperature on the flowering response indicate that photosynthates form an essential part of the floral stimulus.

Night break (NB) lighting is used commercially to inhibit flower initiation and to promote vegetative growth. The inhibition of flower initiation becomes more pronounced as either the duration (Sachs, Kofranek and Kubota, 1980) or the photon flux density (Kadman-Zahavi and Yahel, 1971) of the NB light increases when given near the middle of the night. Under SD (8 hr) maximal delay of flower initiation is achieved when the NB is given within a 4 hour period at the middle of the dark period and is diminished if it is preceded or followed by 10 hours or more of darkness (Horridge and Cockshull, 1989). The amount of photosynthetically active radiation received during the day can also influence the effectiveness of a NB treatment, with more energy being required during the NB when the daily integral is high (Sachs *et al*, 1980). Although there is no evidence that red light or dark reversion of phytochrome is changed by daytime irradiance, it is possible that the total phytochrome titre is affected and this in turn may determine the response to NB lighting.

Phytochrome interconversions form the basis of photoperiodic flower induction. NB treatments high in red light (R) are more effective than those with a high far red

(FR) content (Borthwick and Cathey, 1962). Kadman-Zahavi and Ephrat (1973) found that FR illuminations after an 8 hour photoperiod delayed flowering and completely inhibited flowering when combined with a threshold amount of NB illumination. On the other hand, 20 minutes of red light at the start of the dark period alleviated some of the inhibitory effects of the threshold NB lighting (illumination which delayed but did not prevent flowering). Low levels of the physiologically active far red form of phytochrome (P_{fr}) during the early part of the dark period appear to promote flowering, particularly during the four hour period near the middle of the dark treatment. Sensitivity to high P_{fr} decreases after this time, as demonstrated by the diminishing effects of NB lighting. Night break treatments altering this balance are inhibitory to flower initiation.

2.4 Meristem competence

Parlevliet (1970) noted an irregular variation in the flower yields of thirty five different clones in the first season after planting, but in the second and third seasons, a very regular periodicity in yields was observed. In the second and third seasons all clones showed a very similar periodicity, with yield troughs occurring simultaneously and yield peaks showing a maximum difference of fourteen days. He proposed that the irregular pattern in the first year is most likely due to differences in the quality of the splits and establishment of plantings. The possibility exists that the difference between the splits was in their competence to respond to inductive conditions. Although the implications of a period of juvenility or unresponsiveness to conditions normally conducive to floral initiation are significant in terms of yield reductions following planting, the conditions or parameters of meristem competence have not been elucidated for pyrethrum.

D. grandiflora seedlings exhibit a phase of juvenility during which they continue to produce leaves, even when growing in conditions that are normally conducive to rapid flower formation. This phase can last for many weeks in some seedlings (Cockshull, 1985). Similar periods of unresponsiveness have been observed in stem cuttings taken from plants which have flowered and so the material is no longer juvenile. Age has a profound influence in LD, for rooted cuttings form flower buds progressively earlier the older the shoot is from which the cutting is taken (Furuta and Kiplinger, 1955). It appears that flower bud initiation can occur only after the formation

of a minimum number of leaves, the number being a characteristic of each cultivar, through a physiological ageing of the apical meristem (Cockshull, 1976). The observation that plants must reach a minimum dry weight before becoming competent to flower (Hughes and Cockshull, 1971a) is consistent with the view that the apical meristem must reach a certain physiological age before it becomes responsive to inductive conditions.

The phase of juvenility appears to be a property of the shoot tip as the period of unresponsiveness is less evident in shoots arising from axillary buds on the main axis (De Jong, 1981a). A gradient of increasing readiness to flower exists from the base to the apex of seedlings. The apical meristem is, however, less competent to flower than the top axillary meristems (De Jong, 1981a). Axillary meristems released from apical dominance by decapitation of the main shoot are temporarily unresponsive to inductive conditions but flower more readily than the main apex of intact plants, with the length of the period of unresponsiveness varying with cultivar (De Jong, 1981b). Cuttings are competent to respond to SD throughout the year whereas seedlings must produce a minimum number of leaves before becoming competent to flower. The minimum leaf number is dependent on photon flux density and increases as radiation increases (De Jong, 1989b), but is not correlated with the rate of leaf initiation (Cockshull, 1976). This dependence on irradiance again implies a role for assimilates in the flowering process, but the lack of correlation with the rate of leaf initiation or growth rate indicates that the effect is not due to overall assimilate levels but may be due to changes in distribution patterns. Meristem competence may rely at least partially on the plant's ability to partition assimilates in a pattern which favours flower initiation and development. Hormonal factors may influence this and other physiological changes during this switch from juvenility to competence.

2.5 Defoliation effects

In Kenya, it is normal farming practice to cut away all the dead leaves and flower stems to within twelve to sixteen centimetres of the ground during the dormant season of each year, leaving a vegetative plant from which new flower stems arise. Attempts to cut back plants during the growing season result in yield losses due to the removal of developing flower buds. There is no compensating increase in yield at a later date (Glover, 1955).

Removing all mature leaves from plants for four weeks prior to vernalisation prevents floral initiation, while removing all mature leaves during the vernalising treatment has no effect on initiation (Brown, 1988). It is suggested that the former treatment may have maintained the plants in a juvenile-like state of unresponsiveness. Plants maintained in a state of unresponsiveness continue to grow vegetatively, a desired characteristic for nursery multiplication of plants under Tasmanian conditions.

Defoliation of *D. grandiflora* after three weeks of vernalisation does not prevent initiation (Schwabe, 1957) while low light intensity combined with high temperature applied three weeks after vernalisation results in devernalisation. It appears that the devernalisation response is not a direct result of a decrease in the plant's carbohydrate status.

2.6 Growth regulator effects

The cytokinin, 6-benzylaminopurine (BA), when applied as a foliar spray, increases the shoot to root ratio, leaf area and chlorophyll content of field planted pyrethrum splits. BA tends to increase shoot dry matter of older plants but has little effect on splits immediately after planting. Gibberellic acid A_3 (GA_3) increases leaf area and shoot dry matter at early stages of growth, and when applied with BA, can increase leaf area, shoot to root ratio, tiller number, rosette height, number of flower buds per plant, flower stem elongation and pyrethrins content, and slightly increase flower weight (Mnzava and Mpina, 1988a).

The same authors (Mnzava and Mpina, 1988b) have reported that silver nitrate, a potent anti-ethylene agent, delays flower senescence when applied at the stem elongation stage or the flower opening stage but not when applied at bud initiation. The treatment also tended to lower the pyrethrin content irrespective of the floral development stage at application. While flower fresh weight increased when treated at the opening stage, those treated in the bud stage weighed less than water treated controls. It is inferred that normal development of flowers and maturity of achenes, whose inhibition reduces pyrethrins content, might be regulated at least partially by ethylene.

The auxin transport inhibitor TIBA (2,3,5-Triiodobenzoic acid) and the growth retardant chlormequat chloride or CCC have both been shown to significantly increase flower yields (Mohandass, 1986). TIBA at 100 parts per million (ppm) or CCC at

250ppm applied monthly to three month old field planted seedlings increased the yield of dried flowers and also slightly increased the total pyrethrins content. The absence of data on the endogenous changes in hormone levels during the flowering of pyrethrum precludes any conclusions being drawn on the physiological significance of the above results.

Gibberellins are generally reported as promoting floral initiation and development in *D. grandiflora*. The concentration of endogenous gibberellins in plant extracts rises when plants are vernalised (Tompsett and Schwabe, 1974). Gibberellin A_3 can replace part or all of the vernalisation requirement in cold-requiring cultivars, although some negative results have been reported (Cockshull, 1985). While gibberellins and gibberellin-like substances have been reported both to promote (Pharis, 1972) or to have no effect on flower initiation (Cathey and Stuart, 1958), there is more evidence to suggest that they promote flower development and hasten anthesis (eg. Menhenett, 1979). In addition, extracts from apical buds of induced plants (Parups, 1980) and from young flower buds (Jeffcoat and Cockshull, 1972) contain high concentrations of gibberellin-like compounds. Flower stem extension can be reduced and flowering delayed by the application of a range of growth retardants. These growth restrictions can generally be reversed by subsequent applications of GA_1, GA_3, GA_5, or GA_{4+7}. GA_{13} is much less active while GA_5 has a relatively greater effect on the time of flowering than on shoot elongation (Menhennett, 1981). The response to GA_9 varies according to the retardant used as certain retardants not only block gibberellin biosynthesis but also wholly or partly restrict the metabolism or action of the endogenous gibberellins (Menhennett, 1982).

The most potent inhibitors of flower initiation are the unsaturated hydrocarbon gases, ethylene and propylene. An atmosphere containing between one and four volumes per million (vpm) of ethylene is inhibitory to floral initiation in SD when plants are continuously exposed (Tjia, Rogers and Hartley, 1969) and is inhibitory to flower development but not to flower initiation when plants are exposed intermittently (Rogers, Hartley and Tjia, 1969). Continuous exposure to atmospheres containing five hundred vpm propylene inhibits flower initiation both in SD and LD (Hand, 1979), indicating that the gas can block both the photoperiodic and autonomous flower-inducing processes. Ethephon, an ethylene generating compound, produces similar effect when sprayed onto plants at concentrations between 100 and 2000 parts per million (ppm) in SD (Cockshull and Horridge, 1978) and LD (Cockshull, Horridge and

Langton, 1979). Unlike ethylene and propylene, the effects of ethephon are persistent, perhaps because the plant tissues are stimulated to produce ethylene (Cockshull and Horridge, 1978). Ethephon delays flower initiation, reduces stem elongation and reduces apical dominance. The effect is generally greater when applied closer to the apical bud although ethephon is translocated out of the leaves within twelve hours (Stanley and Cockshull, 1989).

Auxins have also been reported to inhibit flower initiation in chrysanthemum. In the cold-requiring cultivar 'Sunbeam' auxin levels increase after vernalisation but decline rapidly, while a rise in auxin-oxidising activity is also observed (Tompsett and Schwabe, 1974). Extracts from vernalised plants held in LD contain higher auxin levels than those in SD and fail to flower. Low light intensity treatment or exogenous auxin applications reduce gibberellin-like substance levels and cause devernalisation. Jeffcoat and Cockshull (1972) also detected a rise in auxin activity in flower buds shortly after initiation, followed by a decline. Application of auxins alone (Graves, Adams and Winsor, 1977) or in combination with GA_3 (Cockshull, 1985) delay flower initiation in SD, and may also delay flower development (Lindstrom and Asen, 1967) although this is not always observed (eg. Menhenett, 1979). Graves *et al* (1977) concluded that auxin delays flower initiation but not development, with lateral shoots further down the stem showing the greatest delay. Plants treated with ethylene also contain higher levels of auxin than untreated plants (Tjia, Rogers and Hartley, 1969) suggesting that the two hormones may both be operating in the same inhibitory process.

Studies on the endogenous hormone levels of cold-requiring chrysanthemum cultivars reveal that vernalisation results in an increase in gibberellin levels, while auxin levels may initially rise but decline rapidly thereafter. The data from researchers applying hormones exogenously supports the theory that gibberellins form part of the flowering stimulus provided by vernalisation, and that a decrease in free auxin levels may be a requirement for initiation. The inhibitory effect of auxin may be at least partially mediated via the action of ethylene. This is, however, unlikely to be the full story on the hormonal regulation of flowering in chrysanthemum given the number of contradictory results published and the complex interactions observed between the hormones examined. The detailed nature of our knowledge on the environmental factors influencing flowering provides a sound background upon which further work on the endogenous controlling mechanisms must now be performed to increase our understanding of the physiological basis of flowering.

2.7 Other factors influencing flowering

The majority of studies on the flowering of pyrethrum have centred on the effects of cultivation practices such as plant spacing, irrigation, ridging and in particular fertilizer use. Conflicting reports on the effectiveness of nitrogen, potassium and phosphate applications in increasing flower yields have been published. Nitrogen has been reported to increase yields (eg. Alvord, 1976. Hussain and Ram, 1976) and to have no effect (eg. Rajeswara Rao, Singh and Prakasa Rao, 1983. Ngugi and Ikahu, 1989) and similarly both potassium and phosphate applications have been observed to cause yield responses ranging from no effect to significant increases (eg. Hussain and Ram, 1976. Rajeswara Rao *et al*, 1983. Sastry and Singh, 1990). It appears obvious that the nutrient status of the soil in which the plant is growing determines the effectiveness of nitrogen, phosphate and potassium applications and that the effect of fertilizers is on plant growth and not directly on flower initiation.

Sastry *et al* (1987) showed that increasing soil pH up to 6.5 - 7.5 significantly increased flower yield in acid soils, while an increase in soil reaction above 7.5 decreased yield. No differences were observed in pyrethrins content of flowers due to the treatments. Kroll (1962) also observed that raising the pH of acid soils can result in increased flower yields. An increase in the availability of certain mineral nutrients, particularly nitrogen and phosphorous, from the soil at neutral or slightly acid pH would result in more vigorous plant growth and hence increased flower yields. Similarly the yield increases associated with vesicular-arbuscular (VA) mycorrhizae innoculation of seedlings (Kandasamy *et al*, 1986) can be attributed to nutritional effects through increased phoshorous uptake.

Irrigation has been reported to increase flower yield (Kroll, 1963. Alvord, 1976) but cannot extend the flowering season in Kenya into the dry season (Kroll, 1963). Rand (1990) reported that water stress had no effect on flower initiation but resulted in a decrease in flower dry weight and flower number (possibly due to flower abortion), particularly when applied late in flower development, at flower stage II or later. Again it is likely that the effect of irrigation on flower yield is through a response in plant growth.

As these cultivation practices play no direct part in flower initiation under normal growing conditions they were not examined during this project. Where possible,

irrigation, pH and nutrient conditions both for field trials and glasshouse experiments were maintained near optimum levels for pyrethrum growth to remove any effect they may have on the flowering response.

2.8 Summary : flowering of pyrethrum

Serious deficiencies exist in our present understanding of the flowering process in pyrethrum, particularly under temperate, low altitude environmental conditions. Published research data are generally based on agronomic trials in tropical, high altitude regions which emphasise the effects of environmental and cultivation factors on the time from sowing to harvest and on yield components, but fail to differentiate between the effects on flower initiation and on flower development. It has been possible, through careful examination of these reports and the published material on the flowering of *D. grandiflora*, to ascertain the principal environmental influences on the flowering of pyrethrum.

A period of vernalisation is the principal requirement for the rapid initiation of flowers in pyrethrum. A night temperature below 16 °C for between two and six weeks has been cited as fulfilling the vernalisation requirement. Maximum temperature during the period of vernalisation can modify its effect, with mean maximum temperatures of 24 °C or higher for a week causing inhibition of flower bud initiation. Analysis of field data has shown that over ninety percent of yield variation is due to the combined effects of mean maximum and minimum temperature, indicating that, for equatorial, highland areas, a vernalisation stimulus under moderate maximum temperature conditions controls flower bud initiation and development in pyrethrum.

The observation that low light intensity may be inhibitory to flower initiation is supported by the published data on the flowering behaviour of *D. grandiflora*. Low light intensities combined with high temperatures have been shown to devernalise plants, while chrysanthemum flower development is hastened by increasing light intensities.

No photoperiodic requirement has been demonstrated for pyrethrum, principally because of the near constant daylength conditions in the major growing regions. If pyrethrum displays a similar flowering pattern to *D. grandiflora*, it can be expected to show a facultative SD response to photoperiod, although some chrysanthemum cultivars are day neutral.

The micromorphological changes at the apex during floral evocation and the physiological mechanisms which control these changes in pyrethrum are not known. The first priority for research in this area must be to identify the changes in apical morphology during evocation. Quantifying the plant's responses to environmental stimuli, and separating the effects on initiation and flower development, are then possible. The specific reactions involved in evocation can only be studied through an understanding of the morphological and physiological changes involved.

The few experiments that have been carried out with pyrethrum on the action of plant growth regulators indicate that they play a regulatory role in flower development and possibly initiation. However to determine the physiological significance, if any, of these observed responses to exogenous plant hormone applications the endogenous metabolism of plant hormones during flowering must be known. This area of research is needed to link environmental stimuli to the plant's morphological responses. Using the information available on the physiological and morphological aspects of floral initiation and development in chrysanthemum as a guide, this study examines the early changes during the flowering of pyrethrum and some of the physiological reactions controlling flowering.

The remainder of this review summarises our present understanding of the flowering process. The emphasis is placed on the early physiological and morphological events at the apex which are associated with floral initiation. Environmental stimuli and the correlative influences which affect these events are discussed along with the possible endogenous chemical substances which regulate the flowering process.

3. Floral initiation and development

The flowering process is generally divided into two phases; flower initiation and flower development. Despite the fact that the entire process is an orderly, integrated progression it is necessary for it to be divided as the two phases do not react similarly to environmental and internal variables and thus are not alike. Before addressing the process of flower initiation, the ability of the meristematic tissues to respond to inductive treatments must be reviewed.

3.1 Meristem competence

Not all shoot meristems can react to conditions that otherwise are known to promote flowering. In many species of higher plants, and particularly in woody plants, a period of juvenility exists between germination and the time when the plant becomes competent to respond to inductive treatments. A juvenile-like condition may also exist in plant material taken as cuttings from older, non-juvenile plants. The juvenile period in herbaceous plants is generally short and until recently was attributed to physiological limitations in other plant parts, especially the leaves, and not to meristem incompetence. Recent genetic and physiological studies have indicated that this is an oversimplistic view as juvenile meristematic cells may be intrinsically different from the meristematic cells of mature, competent shoots.

The juvenile phase commonly lasts until the plant has reached a minimum size or stage of development, after which reproductive development can be induced. The commitment to form flowers is correlated with the number of nodes produced by a meristem in tobacco (Singer and McDaniel, 1986) while in the raspberry, shoots of fewer than fifteen nodes do not respond to inductive treatments and blackcurrant cuttings with fewer than twenty nodes cannot be induced to initiate flowers (Schwabe and Al-Doori, 1973). In the latter case the juvenile-like condition is associated with the proximity of the shoot tips to the roots as aerial rooting on long, mature shoots prevents flower initiation. Gibberellin activity is detected in the roots and lower parts of the stems but not in the tops of long (mature) shoots, and it is suggested that this may be decisive in determining juvenility in the blackcurrant and possibly other species.

Juvenile meristems may be grafted as scions onto mature trees or vines bearing flowers and still not flower (Wareing, 1987). Some mechanism intrinsic to the apex must thus determine when the change over to the competent stage occurs. While studies such as those mentioned above indicate that factors such as node number, leaf area or distance from roots is critical for attaining meristematic competence, they offer little insight into the molecular mechanisms of meristem competence. Moreover, not all cases fit into the above generalisation, as grafting of some juvenile scions onto mature stocks can result in flowering (Wareing, 1987) and some active meristems in mature plants are not competent, for example rose axillary meristems under apical dominance (Cockshull and Horridge, 1977). Also mitotically active, non-zonated meristems may be competent in young plants and disappear in older meristems, for example the apical meristem in

Bidens (Poulhe, Arnaud and Miginiac, 1984) and the axillaries in *Pharbitis* (Owens and Paolillo, 1986).

In *Pisum*, the length of the juvenile phase is controlled by several genes (Murfet, 1985). Two of these genes have been shown to act in the leaves, and their activities decrease as the plant ages. A third gene acts at the apex and influences its ability to respond to the balance between floral promoter(s) and inhibitor(s). The *Veg* gene, when homozygous recessive, totally prevents flower initiation in the most favourable conditions or after grafting to the most promotory donors, but does not affect normal vegetative development. Studies of these and other genetic mutants offer the greatest opportunity to understand the molecular and cellular mechanisms of meristem competence.

Juvenility or meristem incompetence can be attributed to several physiological systems. Firstly, and most frequently observed in annual plants, floral expression by meristems may be restricted by insufficient photosynthetic capacity and/or other metabolic limitations in the leaves. Secondly, other correlative influences such as apical dominance and the activity of the root system may maintain meristems in a juvenile condition. These influences are discussed further in Section II.6. Thirdly, 'true' juvenility or the inability of a meristem to respond to floral stimuli produced in the plant can occur. This implies an intrinsic difference between cells in juvenile and competent meristems. The development of new techniques and protocols for the quantitative detection of minute quantities of proteins, genetic material and plant growth regulators offers hope that the molecular and cellular basis of juvenility may be unravelled in the near future.

3.2 Floral initiation

The morphological and physiological changes associated with the switch from vegetative to generative growth have been well reviewed (Bernier, 1988. Bernier *et al*, 1981b. Murfet, 1977. Zeevaart, 1976). Most of this work is based on a small number of species which are considered absolutely photoperiodic. These species have been considered ideal for flower initiation studies as a single inductive photoperiodic cycle results in an irreversible commitment to flowering, allowing experimental observations of physiological and morphological changes.

The events occurring in the apex that commit it to flower formation have been

termed 'evocation' (Evans, 1969), and are the least understood of the flowering processes. Using species requiring exposure to only one photoinductive cycle, the start of evocation is assumed to be the time of arrival at the apex of the leaf-generated floral stimulus. The situation becomes more complex for species requiring more than one photoinductive cycle or other inductive stimuli, and even for the model plants dramatic changes may be observed in the meristem prior to the arrival of the floral stimulus (Bernier, 1988). The start of evocation is thus difficult to determine accurately. Likewise the end of evocation or floral determination, the point at which the meristem is irreversibly committed to flower formation, has not been accurately characterised experimentally. Bernier (1981b) considers that floral determination of *Sinapis* occurs when inhibitors applied to the apex can no longer prevent flowering. This point occurs before any visible, macromorphological signs and at about the time most histological changes start, indicating that evocation is essentially a molecular and cellular process whose completion triggers the changes at the higher levels of organisation. Excision and tissue culturing of DN tobacco has revealed that internode tissue, not organised in meristems, becomes florally determined at about the same time as determination of the apical bud (Singer and McDaniel, 1987), supporting the observation that evocation is essentially a molecular and cellular process.

During the process of floral evocation and the subsequent formation of flowers, the apical cells begin to express a set of genes that are not expressed in the vegetative phase of growth, and these changes can later be detected by the presence of organ-specific proteins. Two views have been put forward concerning gene expression during evocation; the first states that the switch to flowering primarily requires a change in gene expression which sets in motion the complex sequence of all other changes while the second proposes an unspecific activation of the meristem, with changes in the expression of genetic information for the geometry of the generative growth pattern following the elimination of the vegetative growth pattern. To date, no changes in gene expression have been detected until after floral determination is morphologically evident (King, 1991). This suggests that an early but non-specific activation of the meristem occurs before floral-regulatory genes are expressed. Limitations in the resolution of the current techniques may, however, mean that early expression of floral genes has simply not been detected. More work in this field is necessary before either of the above views can be rejected.

New immunological and electrophoretic procedures for separation and analysis are being used to elucidate the molecular changes associated with evocation. Although

much of this work is preliminary, it appears that increased synthesis of all RNA fractions occurs early in the evocational sequence (Bernier *et al*, 1981b) and that the protein complement may be altered before flower primordia are initiated (Pierard *et al*, 1980). These changes are to be expected as flower development must be associated with a change in gene expression in the meristem, whether it be the primary action during evocation or a secondary result of other fundamental changes. In *Pharbitis*, the differences between evoked and non-evoked apical meristems is remarkably subtle with very few consistent quantitative differences in the pattern of polypeptides at the early stages of flower formation (Araki and Komeda, 1990).

A powerful combination of new gene isolation techniques is now yielding new information that will provide a conceptual understanding of the molecular basis of flower induction and development. Genes involved in the flowering process have been isolated and cloned from both *Sinapis* (Melzer, Majewski and Apel, 1990) and tobacco (Kelly *et al*, 1990). This work has lead to the division of the genes involved in flowering into two classes : the regulatory genes whose primary function is to modulate the activity of other genes, and the genes whose products form a functional part of the flower. Identifying and understanding the functions of these genes may provide some insight into the early events of flowering. A model based on the effects of three major regulatory genes in *Arabidopsis* has already been proposed for early floral organ development which accounts for the specification of all types of floral organs observed (King, 1991).

Other molecular changes reported during evocation include rises in sucrose and ATP levels, invertase activity, mitochondrion number and energy charge (Bernier *et al*, 1981b). Since only minute amounts of meristematic tissue are available for analysis, the need for sensitive preparatory and analytical techniques is obvious if meaningful measurements of molecular changes are to be made.

The cellular changes associated with floral evocation are more easily observed but, like the molecular changes, are not fully understood. While changes such as increased rates of cell division and synchronization of cell mitotic cycles are widely observed (Bernier, 1988), evocation can proceed under certain conditions without these changes occurring, making it impossible so far to define 'universal' evocational events. Evocation proceeds via a series or sequence of events which may proceed individually but conceivably the interaction of these sequences is necessary for evocation to proceed to the point of irreversible commitment by the meristem to flowering. As Bernier suggests, there are probably more ways than one for an apex to achieve the essential

changes associated with evocation.

3.3 Apical morphology and floral development

The changes in apical growth pattern observed during generative growth reflect the profound alteration from vegetative production of leaves to the formation of the various reproductive structures. Some changes are common to many, if not all, plants while others are specifically related to the kinds of reproductive structures that will be formed.The changes in apical morphology during the flowering of *D. grandiflora* have already been detailed (Section II.2.1) and it is my intention to review not just the changes observed for it and related species but also the many different and varied changes in apical growth patterns observed, with particular reference to those events which give an insight into the physiological processes involved.

Activation of the central zone of the apical meristem, resulting from the loss of the zonate pattern typical of many vegetative meristems, is one of the commonest and earliest changes during initiation. This change does not signify the end of evocation in *Dendranthema* as the resultant domed apices may persist in vegetative growth (Horridge and Cockshull, 1979). Nougarede (1967) describes meristems such as these as 'intermediate' (or prefloral) while Bernier (1988) suggests that evocation in the apex has started but cannot be completed as only some of the endogenous controlling factors are available.

While enlargement of the apical dome in *D. grandiflora* (Horridge and Cockshull, 1979) and other Asteraceae species (Sharman and Sedgley, 1988) is essential for flower initiation, as the apex must reach a critical minimum size for flowering to begin, an absolute size requirement is not a universal determinant of flower formation. The meristem of *Impatiens* does not change size during initiation (Lyndon and Battey, 1985) while the *Humulus* meristem becomes smaller (Thomas and Schwabe, 1970).

Plastochron, the time between initiation of successive primordia on the apical dome, is generally shortened during floral evocation and morphogenesis. This increase in the rate of primordia production has been noted in *D. grandiflora* as occurring after the first bract primordia are initiated (Charles-Edwards *et al*, 1979). The event can occur prior to the end of evocation , such as in *Sinapis* (Bernier, 1986) and in wheat (Kirby, 1974) but is generally most pronounced when the reproductive primordia are

initiated (Bernier *et al*, 1981b). Application of several plant growth regulators has been shown to shorten plastochron (Bernier, 1988) although endogenous changes during flowering have not been correlated with the observed changes in plastochron.

Primordial size often decreases during flowering (Bernier, 1988), particularly primordial reproductive structures. In *D. grandiflora*, this is seen in the production of leaves of reduced size and finally the loss of the dentate margin to produce the first involucral bract, of small size and simple shape (Cockshull, 1985). Leaves of small size and simple shape are commonly produced during evocation as a result of a strong inhibition of primordial growth (Bernier *et al*, 1981b). Again, the application of plant growth regulators has been demonstrated to affect leaf size and/or shape (Bernier, 1988).

As a result of meristem enlargement and the reduction in primordial size, whether relative to the meristem or absolute, changes in phyllotaxis occur during floral morphogenesis. More primordia can be initiated on the apical dome, changing the distribution pattern of the inflorescence structures compared with the vegetative leaf primordia distribution pattern.

Bolting, or rapid internode elongation, commonly accompanies floral initiation in rosette plants. Gibberellins are thought to play a major role in regulating internode elongation, with gibberellin A_1 (GA_1) demonstrated as being the active gibberellin in several species (Reid, 1990) while GA_4 is the active endogenous gibberellin in at least one species (Nakayama *et al*, 1991). Genetic and physiological studies have demonstrated that internode elongation and flower initiation are two separate processes in many, if not all, plants (Bernier, 1988).

These evocational events and the morphological changes associated with floral morphogenesis have in many cases been correlated with specific environmental stimuli. The problem for researchers is to understand the physiological, molecular and genetic processes which regulate these events. Before discussing the possible chemical and correlative controlling factors, the environmental stimuli which may form the basis of this regulation are discussed.

4. Environmental control

Plants display an enormous variety of flowering responses, representing a great diversity and subtlety of control mechanisms. These flowering responses are an adaptation to the various ecological niches plants occupy and are induced by external

environmental stimuli. The environmental stimuli act on the internal mechanism(s) regulating floral evocation. It is now becoming obvious that several endogenous factors are involved in the control of flower initiation, leading Bernier *et al* (1981b) to propose a model of multifactorial control of initiation. This model accounts for the diversity of responses seen in different species and the complexity of the evocation processes by assuming that flowering in all plants is under the control of several biochemical/physiological systems, all of which must be permissive if flowering is to occur. Different species may thus have different limiting factors, with the external stimuli which provides the appropriate balance or sequence of required factors triggering the whole process. With this in mind, the environmental factors covered in this review are discussed not as absolute controlling factors but as part of a harmonious integration of partial processes.

4.1 Photoperiod

The effect of photoperiod on flower initiation has been extensively reviewed by Bernier *et al* (1981a), Evans (1975), Vince-Prue, Thomas and Cockshull (1984) and Zeevaart (1976). While light has a promotory effect on flowering due to its influence on photosynthesis, the photoperiodic effects on initiation usually require light of energy below the photosynthetic compensation point.

In the past, species have been classified into the photoperiodic response groups short-day plant (SDP), long-day plant (LDP) or day-neutral plant (DNP) according to the critical daylengths the species needs to flower; less than a critical daylength for SDP, greater than a critical daylength for LDP, or with no critical daylength for DNP. These groups were further divided into absolute and facultative photoperiod responses as well as recognising the different daylength requirements for floral initiation and floral development in some species. These divisions have recently been shown to be somewhat arbitrary, with an ever-increasing list of plants, originally considered to be strictly photoperiodic, that can also be made to produce flower buds by a variety of other factors besides daylength (Zeevaart, 1976). For example, the SDP *Pharbitis* may be induced to form flowers under LD by low temperature, by high light intensity and by treatment with abscisic acid or a growth retardant (Zeevaart, 1976). Similar examples can be cited for a number of LDP.

Phytochrome appears to be the photoreceptor for the photoperiodic response.

Phytochrome occurs in two interconvertible forms, one with an absorption maximum in the red region at around 660nm (P_r) and the other absorbing predominantly far-red radiation at around 730nm (P_{fr}). The pool size of the physiologically active phytochrome, P_{fr}, is determined essentially by four processes :

1) phototransformation of P_r to P_{fr}
2) phototransformation of P_{fr} to P_r
3) destruction of P_{fr} to a spectrometrically undetectable form, and
4) reversion of P_{fr} to P_r in darkness.

These transformations are shown in the model below (Fig. II.4.1).

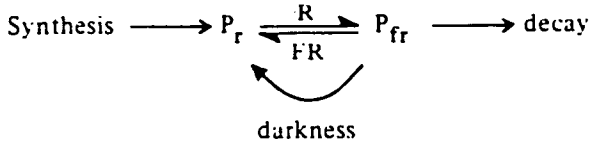


Figure II.4.1 Model of phytochrome interconversions (from Bernier *et al*, 1981a)

In sunlight, phytochrome continually cycles between the two forms, P_r and P_{fr}, and a photostationary equilibrium is maintained in the leaf tissue, with P_{fr} dominant at around 55 percent of the total phytochrome titre. This 'high' level of P_{fr} is necessary for floral induction. On transfer to darkness, the level of P_{fr} decreases through reversion to P_r and through thermochemical decay. It is thought that when the level of P_{fr} falls below a certain threshold the 'dark' processes of photoperiodic induction can proceed. For SDP, night break lighting or dawn after a short night re-establish the level of P_{fr} and so inhibit floral induction. By comparison, LDP require a period early in the night with high P_r followed by high P_{fr} during the second half of the night. The major difference between the requirements for high and low P_{fr} in SDP and LDP appear to be in the sequence in which the high and low P_{fr} processes must succeed each other in the long night (Zeevaart, 1976).

Complicating this rather simplistic scheme of phytochrome action is the circadian rhythm which underlies the measurement of the critical night-length. This involves the detection of the light/dark transition at dusk and the coupling of this information to the time-measuring system. Time-measurement in the SDP *Pharbitis* is initiated when, with no change in the spectral quality, the irradiance falls below a threshold value (Lumsden and Vince-Prue, 1984). The photoperiodic mechanism, at least in SDP, must

involve either two different 'pools' of phytochrome or two different modes of action. The two-pool concept suggests that part of the total P_{fr} pool is stable in darkness and is required for floral initiation, while part is rapidly lost following transfer to darkness and is involved in the perception of the light/dark transition and in the night-break effect. Alternatively, two P_{fr}-receptor complexes may exist, one of which is stable in darkness and the other not (Vince-Prue and Lumsden, 1987).

It is generally considered that phytochrome's major mode of action is through changes in membrane properties. Phytochrome seems to be present in a variety of membrane preparations and, in its P_{fr} form, is capable of binding *in vivo* to subcellular structures. This asssociation takes place within a few seconds, and is therefore fast enough to account for the fastest physiological effects related to membranes (Marme, 1977). Evidence is also increasing that circadian rhythms involve membrane changes and it has been suggested that oscillations in the properties of cell membranes are closely associated with the clock mechanism (Vince-Prue, 1978). Further, the restraints imposed on flowering by photoperiod often disappear under high or low temperatures. This may involve membrane changes since membrane fluidity is affected by temperature. Phytochrome-mediated membrane changes have also been proposed as a possible mechanism for the activation or release of compartmentalised gibberellins and possibly other endogenous plant growth regulators (Stoddart, 1976). *Ent*-kaurene biosynthesis has also been shown to follow an endogenous circadian rhythm in *Pisum*, implicating the circadian rhythm in the regulation of gibberellin biosynthesis (Moore, 1990). Phytochrome can act at the level of gene expression, altering the level of mRNAs in barley, and so could possibly direct activation or repression of genes expressing floral promoters or inhibitors (Dietzer, 1987). This observation may however be an indirect consequence of membrane changes.

The main site of perception of the photoperiodic stimulus in intact plants is the young expanded leaves (Vince-Prue, 1975). In chrysanthemum, exposure of the upper four leaves of one branch of a two-branched plant to SD results in flowering of both branches. This stimulus is graft transmissible (Weise and Seeley, 1964). In many species, photoinduction of a single leaf is sufficient to initiate flowering. The flower promoting substance(s), or floral stimulus, generated in the photoinduced leaf is exported to the shoot apex and is graft transmissible (Zeevaart, 1976). The flowering stimulus can be transferred from initiated donor leaves to receptor plants both of the same species and also between different species or even different families, although transfer between different response groups is not always observed and negative results

have been noted for certain species (Zeevaart, 1976).

In some species, non-induced leaves located between the shoot apex and induced leaves can be inhibitory to flower initiation (Zeevaart, 1976). Again, no flower-inhibiting substance has been identified as the photoperiodic inhibitor. Indeed, for the SDP *Xanthium*, instead of a particular inhibitory compound, it has been postulated that the rapidly expanding non-induced leaf might act as a sink for assimilates and the floral stimulus generated in the lower induced leaf (Zeevaart, Brede and Cetas, 1977). While substantial evidence exists for specific transmissible inhibitors of flowering originating in non-induced leaves of several species, in other species the inhibitory effect of non-induced leaves is via assimilate transport interference. In *Pharbitis*, Ogawa and King (1990) concluded that the inhibitory effect of non-induced leaves is due to both the presence of a specific photoperiodically sensitive inhibitor and to interference with the assimilate/floral stimulus co-transport in the phloem. The inhibitory effect may not be due to carbohydrate diversion, whereby the apex receives assimilates preferentially from non-induced leaves, but rather from prevention of the floral stimulus co-transport with assimilates in the vascular system (Papafotiou and Schwabe, 1990).

Despite this evidence for a translocatable floral stimulus, no ubiquitous flower initiating factor has been identified as yet. The same may be said for specific translocatable floral inhibitors. Given that only a small number of photoperiodic species have been studied, and that within this group are many plants whose photoperiodic requirements may be profoundly altered by other external stimuli, this failure to identify a ubiquitous floral stimulus supports the concept of multifactorial control of floral evocation.

4.2 Irradiance

Delayed flower development as a result of low light levels is seen in a number of plants, including chrysanthemum (Cockshull, 1972) (see Section II.2.3). The role of irradiance in flower initiation has been more widely studied in woody perennials than for herbaceous plants (Jackson and Sweet, 1972). In many fruit and forest trees there is evidence for a quantitative role for light in flower initiation in those species where photoperiod does not affect flowering.

Particularly in the case of LDP, investigation of photoperiodic induction may be confounded by an increase in assimilate supply, except in those species for which brief

light breaks effectively induce flowering. In some LDP, such as *Sinapis alba* and *Brassica campestris*, the rise in the supply of assimilates caused by greater irradiance may suffice for the induction of flowering even in SD. However, in *Lolium temulentum*, floral evocation is controlled by the photoperiodic processes, the response to which is amplified by high sugar supplies but not replaced by them (King and Evans, 1991).

Irradiance has a marked influence on both the temperature and the photoperiodic responses displayed by plants. Low photon flux densities can increase the number of SD required for flower initiation in many photoperiodic species. High irradiances have been shown to replace the photoperiodic requirement of the SDP *Pharbitis* var. Violet when grown in LD (Bernier, 1988). The devernalisation phenomenon observed in low temperature requiring species when grown under high temperatures and low light intensities is another example of irradiance modifying the plant's response to other external stimuli.

4.3 Vernalisation

Vernalisation, the low temperature requirement for flower initiation, is common in perennial rosette plants, grasses and several winter cereals. In most plants, the vernalisation stimulus is perceived by the apex and is not translocated within the plant. Transmission of the vernalised condition occurs through the process of division of the cells originally exposed to the cold, and thus only axillary meristems derived from a vernalised meristem can attain the vernalised state after this cold treatment (Napp-Zinn, 1987). Cultured plant material is only sensitive to cold if it contains actively dividing cells (Bernier *et al*, 1981a).

The physiological responses to vernalisation include an increase in the endogenous levels of gibberellins, possibly linked with bolting during flowering . The end-product of vernalisation is not gibberellin alone, as the chilling requirement in some species cannot be replaced by gibberellin applications (Napp-Zinn, 1987). Vernalisation in *Pisum* acts in the leaves to suppress inhibitor production and in the apex to alter the sensitivity to floral promoters and inhibitors (Murfet, 1985). Low temperature enhances *ent*-kaurene biosynthesis in dwarf *Pisum* cultivars but not tall cultivars ; however the lack of an increase in growth rate in dwarf cultivars indicates that endogenous GA biosynthesis remains blocked at some point beyond *ent*-kaurene in the biosynthetic pathway (Moore and Moore, 1991). Low temperature also alters membrane properties,

possibly contributing to or even mediating these responses.

It has recently been suggested that vernalisation may act at the molecular level to reduce DNA methylation in the genes for flower promoters, specifically at site(s) for the binding of regulatory proteins (King, 1991). This work is still preliminary, but merits further investigation as it neatly explains the non-graft transmissibility of the vernalisation response and the need for dividing cells for its expression.

In many cold-requiring species, high temperatures are known as devernalising since they oppose the effect of a low temperature treatment. To be effective, high temperatures must usually be applied immediately after vernalisation to suboptimally vernalised plants (Bernier *et al*, 1981a). This is not always the case as certain species cannot be heat devernalised while others, such as *Cheiranthus cheiri* and cold-requiring *D. grandiflora* cultivars may be devernalised after receiving supraoptimal vernalisation treatments (Bernier, 1988).

Consistent with the multifactorial approach, other external stimuli such as high temperature in *Scrophularia alata* (Larrieu and Bismuth, 1985) and exposure to SD at normal temperatures for certain cereals and grasses (Bernier *et al*, 1981a) can also substitute for the vernalisation requirement. Low temperatures may also counteract the inhibitory effects of low irradiance levels in various species, suggesting that assimilate supply, redistribution and utilization may also be essential factors in mediating temperature effects (Calvert, 1969).

4.4 Autonomous induction

The process by which many plants, even when grown continuously in 'non-inductive' conditions, eventually initiate flowers is termed autonomous induction. All they require is that environmental conditions are conducive for growth (Bernier, 1988). For self-inductive plants (plants having autonomous induction), light and other environmental factors may affect both flower initiation and development, including the prevention of flower bud abortion (Halevy, 1984).

Bernier (1988) asserts that the difference between autonomous, facultative and absolute species is only one of degree. The progression to flowering is part of a normal sequence, with photoperiod and vernalisation regulating the rate of progress towards flowering. This environmental regulation may vary from absolute, for example in strict photoperiodic species, or supplementary in species such as chrysanthemum where

flowering can be regarded as a result of autonomous induction in a non-inductive daylength and enhanced by photoperiodic induction in a flower-promoting daylength, to a minor role in autonomous or self-inductive species only requiring environmental conditions conducive to growth. Manipulation of external stimuli can alter many species' responses, changing the threshold values for the other environmental factors. Different environmental factors must be influencing different steps amongst the multiple and varied promotory and inhibitory processes occurring in the various plant parts and as such the importance of different organs changes according to the external conditions.

5. Chemical regulation

Environmental stimuli lead to the observed physiological, histological and morphological changes associated with floral evocation and flower development. The specific compounds which mediate these changes through particular biochemical reactions are as yet unknown although a large number of compounds exist which have been shown to promote or inhibit flower initiation and development.

5.1 Plant growth regulators

This chemical group includes the naturally occurring plant hormones and the synthetic plant growth regulators. For the purposes of this discussion, a plant hormone is defined as an organic substance, other than a nutrient (a substance which supplies either carbon and energy or essential mineral elements), active in very minute amounts, which is formed in certain parts of the plant and which usually is translocated to other sites where it evokes specific biochemical, physiological and/or morphological responses. Plant growth regulators fit the same description but need not be produced by the plant (Moore, 1989). The discussion centres on the five commonly recognised classes of plant hormones; abscisic acid, auxins, cytokinins, ethylene and gibberellins, and mention is made of other plant growth regulators only where they have been used to elucidate the physiological reactions controlled by the endogenous plant hormones.

Trewavas (1983, 1986) has recently questioned the validity of the concept of chemical control of flowering based on changes in the amount or concentration of the plant growth regulators. There is frequently a lack of correlation between hormone concentrations measured in tissues and the response of the tissues, and also the

response to exogenous applications of plant growth substances is seen over a much broader range of concentrations, in terms of orders of magnitude, than has been observed in endogenous changes. Firn (1986) points out that a change in sensitivity simply refers to an observation that the response to a given amount of a particular hormone has changed. This conceivably could be caused by a change in the concentration of the hormone receptors, a change in receptor affinity, or a change in a subsequent chain of events that may involve other hormones or other factors. Trewavas's assertion that sensitivity to growth substances and not their concentration may be the limiting factor to physiological responses is accommodated in the multifactorial model of flowering.

Recent work with plant hormone mutants in a number of species, coupled with new techniques for the extraction, purification and detection of endogenous plant growth regulator concentrations, have indicated that physiological responses to hormonal stimuli may be regulated both through changes in tissue sensitivity and changes in hormonal concentrations. Broadly speaking, plant hormone mutants may be split into two groups :

(1) those that influence hormone levels (synthesis mutants), and

(2) those that influence hormone response (sensitivity mutants).

Both mutant groups have been demonstrated to control shoot elongation in *Pisum*, influencing both GA synthesis and activity (Reid, 1990). Similar examples can be cited for auxin, ethylene and abscisic acid. It remains to be seen if evocational events are similarly controlled by changes in both hormone concentrations and tissue sensitivity.

Immunoassays, HPLC and GC-MS have increased the specificity and sensitivity of plant growth regulator detection making it possible to analyse smaller tissue samples such as apical meristems as well as partially alleviating the extensive purification procedures formerly required to remove interfering substances. Using monoclonal antibodies, immunoassay techniques have been reported with detection limits of sub-femtomole concentrations (Harris and Outlaw, 1990) allowing the quantitative analysis of certain plant growth regulators which previously was exceedingly difficult because of their low levels in plant tissues. These results must still be viewed cautiously; compartmentalization of hormones *in vivo*, the presence of inactive and conjugated forms and metabolism of hormones after sampling can all result in wrong assumptions being drawn from the measurement of endogenous hormone concentrations. Thus, the following results of endogenous hormone concentrations must be treated with caution

since the methods of determination in many instances suffer from various limitations and so the results may not be a true reflection of the endogenous levels of the active agents.

5.1.1 Abscisic acid

Exogenous applications of abscisic acid (ABA) may cause flowering in non-inductive conditions in the SD plants *Ribes nigrum* (El-Antably and Wareing, 1966) and, in high light and the presence of a growth retardant and sucrose, *Lemna paucicostata* 6746 (Kandeler, 1985). ABA may also promote flowering in several species of SDP in suboptimal inductive conditions. However it has also been reported to have no effect in others species and may even be inhibitory to a few SD species as well as the majority of LD species (Bernier *et al*, 1981b).

Determination of endogenous ABA levels has proved inconclusive, mainly due to the lack of sensitive detection techniques. Although ABA presumably acts in the shoot meristem (Kinet *et al*, 1975) a large bulk of leaf and bud material is generally extracted along with the apical meristem. Analysis of isolated shoot apices and leaves of the LDP *Lolium* reveal that the ABA level in the apex is significantly higher than in the leaves (King, Evans and Firn, 1977). Despite the fact that exogenous applications of ABA close to the apex can inhibit flowering, no consistent effect of photoperiod on the endogenous concentration of ABA was found in the two fractions analysed. In fact, results so far have yielded no consistent relationship between photoperiod and ABA content in plant tissues (Bernier *et al*, 1981b), leading Bernier to suggest that the critical factor is not the absolute level of ABA but short term changes in concentration. An equally valid hypothesis is that changes in tissue sensitivity mediate the observed responses. Further studies on endogenous ABA concentrations in apical tissue are needed to shed light on the role of ABA in flower initiation.

ABA is rapidly metabolised in plants and so far more than ten metabolites and conjugates have been identified. The main catabolic pathway involves two oxidation steps leading to the formation of phaseic and dihydrophaseic acid (Dorffling *et al*, 1986). ABA and its metabolites form conjugates, principally glucose esters and glucosides, which appear to be, at least in wheat seedlings, the final products of ABA metabolism (Lehmann, 1986). The ABA conjugates are neither translocation forms of ABA nor temporary storage forms. They are accumulated in cell vacuoles and do not

serve as a source of free ABA (Zeevaart and Creelman, 1988). Walton (1980) suggests that the glucose ester of ABA may be readily de-esterified by cell sap to release ABA, but as the conjugate studied was formed from the R-enantiomer of ABA and not the natural S-form its physiological significance is unknown. Evidence points to endogenous increases in ABA concentrations being a result of *de novo* synthesis and not metabolism of storage and transport conjugates.

5.1.2 Auxins

Studies on the role of auxins in flowering indicate that exogenous auxin can both inhibit and promote initiation in SDP and LDP, and that inhibition is far more widespread than promotion. Both excessive and very low concentrations of auxin may be inhibitory to flowering (Bernier *et al*, 1981b). Inhibition of flowering by high doses of auxin might not be specific to flowering since it may be related to a general auxin-induced inhibition of growth. *In vitro* studies on tobacco indicate that auxins do not oppose floral evocation but that their presence in a certain concentration range is an absolute requirement if flowers are to be formed (Barendse *et al*, 1985. Smulders *et al*, 1988).

Auxin action is also dependent on the prevailing light and temperature conditions. The total light flux influences the optimal auxin concentration for flowering in *Xanthium* and *Hyoscyamus* (Bernier *et al*, 1981b) while temperature may influence the effectiveness of auxin applications in chrysanthemum (Schwabe, 1970). The action of auxin is also time dependent; for example, in the SDP *Xanthium* the auxin NAA strongly inhibits flowering when given during the inductive long dark period while its activity decreases when applied after this period (Salisbury, 1955). Similar responses are observed in *in vitro* studies. This indicates that sensitivity to auxin may change during evocation both *in vivo* and *in vitro* or may reflect the requirements for auxin of the different processes in the evocational sequence.

Endogenous levels of auxin in *Xanthium* plants transferred from LD to inductive SD increase immediately after transfer, decrease a few days later before increasing once again when the inflorescence begins developing (Cooke, 1954). The initial increase may be associated simply with the sudden change in photoperiod and may play no part in the initiation of flowering. It is postulated that the subsequent decrease is related to flower initiation, and similar observations in other species are further evidence that a

decline in auxin concentration to a minimum level at the time of flower initiation is part of the evocational process.

The involvement of auxin in apical dominance is well documented (Martin, 1987). The temporary loss of apical dominance usually seen at the onset of flowering, leading to the precocious initiation of axillary buds, may be interpreted as a consequence of a reduction in the auxin supply from the young leaves of the apical bud. Another feature of evocation in which auxin is likely to be involved is the phyllotaxis alterations that commonly occur just before or at the onset of flower initiation.

It is generally agreed that indoleacetic acid (IAA) is the major, perhaps the only, native indole auxin of higher plants, with phenylacetic acid (PAA) also being a native auxin, but with much lower activity, in some species (Moore, 1989). IAA conjugates have been found in every species studied and probably all plants contain ester, glucosidic and amide conjugates which can act as storage forms or detoxification products. Conjugated auxins may be hydrolysed in plants to yield the active, free forms (Smulders *et al*, 1990), thus performing a regulatory role in maintaining a particular level of free auxin. Lilov and Zozikova (1986) found that the level of conjugated auxins in grape bud samples was higher in plants with intensified generative processes (flowering stimulated by the retardant chlorcholinechloride, CCC) than in control plants. Hydrolysis of conjugated auxins as well as biosynthesis of free auxin may be involved in maintaining the necessary concentration of active auxin during floral evocation and development.

5.1.3 Cytokinins

In a similar manner to the auxins, exogenous applications of cytokinins may cause both promotion or inhibition of flower initiation, although promotive responses are more common, and the response is dose dependent and influenced by the site and timing of the application. The range of cytokinin concentrations that promote or inhibit flowering also varies with the amount of other growth substances and carbohydrates present.

In *Chenopodium* (Krekule and Seidlova, 1977), *Anagallis* (Bismuth and Miginiac, 1984) and *Sinapis* (Bodson, 1985) inhibition or promotion of flowering has been observed depending on the amount of cytokinin applied and/or the timing of the

treatment. In several species, including Dendranthema (Pharis, 1972) and Chenopodium (Sawhney and Cumming, 1975) the promotory effect of cytokinin is greatly enhanced by the addition of gibberellin, while in Scrophularia the concentration of kinetin required to inhibit flowering is far lower in the absence of IAA than in its presence (Bernier et al, 1981b).

Cytokinins have been shown to be a requirement for flowering in many in vitro systems and from the point of view of development of virtually all plant tissue and cell cultures, the cytokinins and auxins are the most important classes of plant growth regulators (Horgan, 1987). Tran Thanh Van (1980), using thin cell layers of tobacco, found that cytokinin must be present for flowering, but vegetative buds are produced if the concentration is supraoptimal. The effect of cytokinins in vitro are very much dependent on the presence and concentration of other medium components, including sucrose, and so care must be taken in interpreting results from in vitro experiments.

Cytokinin applications have been shown to produce several responses which are typical components of evocation, including the release of axillary buds from apical dominance (Seidlova and Krekule, 1977) and an increased rate of leaf production (Miginiac, 1978). Vegetative meristems treated with cytokinin generally exhibit increased mitotic activity (Bernier et al, 1981b), and it is suggested that the early effect of an inductive LD on the mitotic activity of the meristem of Sinapis is mediated by a cytokinin.

Changes in the levels and/or metabolism of endogenous cytokinins have been observed with the onset of flowering. Cytokinin levels in the roots of Sinapis are lower in plants sixteen hours after induction than in vegetative plants while levels in the leaves are higher (Lejeune, Kinet and Bernier, 1988). The opposite is true in Xanthium, where a decline in cytokinin activity in leaves, buds and root exudate is observed after photoinduction (Wareing, Horgan, Hensa and Davis, 1977). An increase in zeatin riboside occurs in photoinduced leaves of a LD tobacco but not in those of a SD tobacco (Bernier, 1988).

5.1.4 Ethylene

Ethylene and its analogues propylene and acetylene induce rapid flower formation in pineapple and all other bromeliads so far studied and is widely use commercially to induce flowering in these species (Thomas, 1982). Outside of this group of plants, the

number of species examined is small and few conclusions can be drawn on the possible role of endogenous ethylene in regulating flower initiation.

Ethylene also induces flowering in mango (Chacko, Kohli and Randhawa, 1974) and apple trees (Williams, 1972) but inhibits flower initiation in the majority of other plants studied. In *Pharbitis*, ethylene can only inhibit flowering in the second half of an inductive long night and acts on the cotyledons, not the apex (Suge, 1974).

Synthesis of ethylene in higher plants proceeds along the sequence methionine to S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (Yang and Hoffman, 1984). Regulation of ethylene synthesis is thought to be primarily controlled at the SAM to ACC step, but formation and compartmentalisation of the conjugate N-malonyl-ACC may also play a regulatory role (Amrhein *et al*, 1986). In *Chenopodium rubrum*, where ethylene is postulated as playing an important role in the regulation of photoperiodic flower induction, induced plants produce significantly less ethylene than vegetative ones (Machackova *et al*, 1986). Interruption of the dark period by white light cancelled flowering and raised ethylene production almost to the level of vegetative plants. Study of the metabolism of exogenously applied ACC indicated that ACC conversion to ethylene is reduced by inductive short-day treatments.

Auxin induces ethylene synthesis in a variety of plant tissues and in many species ethylene and auxin have similar effects on flowering (Moore, 1989). Auxin inhibition of flower induction of *Pharbitis* has been shown not to be mediated by ethylene (Halevy, Spiegelstein and Goldschmidt, 1991). While some species have been shown to respond differently to auxin and ethylene, it is possible that at least some of the flowering responses observed after auxin applications are mediated through ethylene synthesis and that this mechanism plays a regulatory role in the physiological flowering processes.

5.1.5 Gibberellins

To date seventy nine structurally different gibberellins and more than ten gibberellin conjugates have been isolated and identified from higher plants and fungi. Individual species can contain several different gibberellins. The activity of the various gibberellins is related to their structure and can vary between species and possibly between different tissue types within plants (Pharis and King, 1985). Differences

between species in the observed responses to exogenous gibberellins may therefore be a reflection of differing biological activity or metabolism of the applied gibberellins. A knowledge of endogenous gibberellin levels and metabolism in any species is thus an advantage when analysing the physiological effects of exogenous gibberellins.

Exogenous gibberellins can elicit a flowering response in many LDP and cold-requiring rosette plants grown in non-inductive conditions (Pharis and King, 1985). Recent studies, however, have shown that there are a number of both cold-requiring species and LDP in which flowering is inhibited by GA_3 applications, and that some plants, such as *Pharbitis*, *Chenopodium* and *Dendranthema* may display flower promotion or inhibition depending on other environmental factors (Bernier *et al*, 1981b). The reaction in SDP is equally complex, ranging from promotive to markedly inhibitory or apparently unreactive. Response to GA is also a function of the stage of development reached by the plants at the time of treatment. In *Fuchsia*, the inhibitory action of GA_3 is at a maximum early during induction, declines rapidly as the time of application is delayed after induction and actually accelerates flower development if applied after perianth initials appear (Sachs, Kofranek and Shyr, 1967). Timing of application may account for some of the contradictory reports concerning the effects of exogenous gibberellins.

The reaction of Gymnosperms to exogenous gibberellins is one of enhanced flowering, with GA_3 being active in the Cupressaceae and the Taxodiaceae while the less polar gibberellins, especially the mixture of GA_4 and GA_7, show the greatest activity in the Pinaceae (Pharis and King, 1985).

The apparent complexity of the flowering response to exogenous gibberellins may be at least partially due to the complex nature of endogenous gibberellin metabolism. Studies of the endogenous levels of gibberellins have been hampered by the inability to isolate and detect the particular gibberellins which may be significant in any one species. Most studies are thus flawed by a limited methodology for separation, identification and quantification of the gibberellins. Also many early studies relating gibberellin levels to flowering did not specify the precise stage of reproductive development at the time of extraction and the observed changes may be related to flower development rather than initiation. These factors limit the conclusions which have so far been reached, with the one observation which is generally accepted being that levels of extractable gibberellins tend to be lower in SD than in LD or in SD with a light interruption of the long night (Bernier *et al*, 1981b).

In the *D. grandiflora* cultivar Shuokan, GA-like substances increase during low temperature treatment (Harada and Nitsch, 1959). After the third week of cold, GA levels are ten times higher than in unvernalised control plants. This increase precedes bolting by two weeks and corresponds to the minimum duration of cold treatment required for flower formation. An increase in gibberellins following low temperature treatments has also been demonstrated in winter wheat, winter rye, radish, olive and carrot (Bernier *et al*, 1981b). Although stem growth and flower initiation are usually associated processes, they can be more or less separated in many plants. Applications of GA_3 can cause stem elongation in a range of plants without resulting in flower formation, while growth retardant treatments which totally suppress stem growth do not effect flower initiation in a number of species, such as *Silene armeria* (Cleland and Zeevaart, 1970). The genetic analysis of Wellensiek on *Silene* points to localisation of genes associated with stem growth and flowering on the same chromosome, so that stem growth and flower initiation, although under different genetic control, are nevertheless often related (in Bernier *et al*, 1981b).

In the SDP *Pharbitis*, photoperiodic induction results in a substantial increase in the levels of free GA-like substances in the cotyledons and phloem exudate. The levels of $GA_{5/20}$-like and GA_{19}-like substances in the phloem exudate are significantly higher in photoinduced plants and thus could have some influence on the flowering of *Pharbitis* (Takeno and Cleland, 1990).

Conversely, a decrease in the level of extractable gibberellin-like substances has been noted during inductive treatments for the SD species tobacco, hemp, *Plumbago* and in the axillary buds of *Ribes nigrum*, but this may not indicate a dependence upon GA for flowering. In *Chenopodium*, GA levels drop after transfer to inductive SD while the growth retardant CCC also reduces GA levels but inhibits flowering. GA_3 can reverse the CCC-induced inhibition of flowering as can IAA and kinetin treatments. Also, GA levels are increased by IAA treatment and decreased by kinetin, and decreased even further by combined kinetin and CCC treatment which promotes flowering (Bernier *et al*, 1981b). This complexity makes it difficult to define a specific role for gibberellins in the flowering of SDP. Study of the LDP *Silene* indicates that changes in the diffusible gibberellins rather than the total gibberellin titre occurs during flower initiation and that an increase in gibberellin turnover or metabolism occurs under LD (Van den Ende and Zeevaart, 1971). In spinach, a LDP, photoperiod has a regulatory effect on gibberellin metabolism. SD and LD treatments increase the rates of

different metabolic interconversions, resulting in differing gibberellin complements between plants under the two photoperiods (Metzger and Zeevaart, 1982). Two enzymatic steps in the GA biosynthetic pathway are controlled by photoperiod, with LD conditions stimulating enzymatic activity (Gilmour *et al*, 1986).

Changes in the relative, but not the total, amounts of various gibberellins have been reported during induction for other LDP and it appears to be one of the key events in induction. Reversible conjugation of gibberellins, allowing rapid inactivation of biologically active GA's, and with the conjugates acting as a pool from which free GA's can be hydrolysed for subsequent biological function has been proposed as the physiological role of GA conjugates (Rood and Pharis, 1986), and may play a part in these observed changes in gibberellin metabolism. It is also possible that only certain gibberellins are involved in flower induction.

While gibberellins have been implicated as playing a role in the floral evocation of many, if not all plants, the extent to which they can influence flowering varies with species. The fact that in many species gibberellins can cause evocational changes such as precocious release of axillary meristems from apical dominance, changes in phyllotaxis and leaf shape, and a reduction in plastochron duration (Bernier *et al*, 1981b) without inducing flowering indicates that other factors are necessary for complete evocation. A fuller understanding of gibberellin metabolism will help determine their role(s) in evocation.

5.2 Assimilates

The 'nutrient diversion' hypothesis for the control of flowering postulates that induction involves the increased supply of photosynthetic assimilates to a critical part of the shoot apical meristem. This critical part of the meristem is relatively deprived of nutrients during vegetative development or must receive a higher level of assimilates for gene expression during evocation than for vegetative development (Sachs, 1977). Thus control of flowering by chemical or environmental factors may be an indirect result of their influence on assimilate supply and distribution and not upon specific morphogenetic processes in the shoot apex.

Photon flux is one of the key parameters in many plant processes, influencing a range of biological and chemical systems including photosynthesis, transpiration, membrane transport properties, phloem loading, nitrate and sulphate reduction,

translocation and numerous other processes. Changes in photon flux will affect a large number of plant processes, making it difficult to link photosynthesis to the control of flower induction. The most compelling evidence that photosynthesis has great importance for floral initiation is that high photon flux densities, in the photosynthetically active region, override and replace the photoperiodic signal in many LDP, for example *Anagallis* and *Brassica* (Friend, Deputy and Quedado, 1978) and *Sinapis* (Bodson, King, Evans and Bernier, 1977). In *Dendranthema*, the hypothesis that apical dome size is critical for the transition from vegetative to reproductive development gives photosynthesis a major role. Charles-Edwards *et al* (1979) developed a mathematical model for this transition in which flowering is almost entirely governed by photosynthesis, which promotes apical dome growth, and an inhibitor, which controls primordial initiation and, indirectly, flowering. Further evidence for the role of assimilate partitioning in the flowering of chrysanthemum is that the night interruption irradiance required to prevent floral initiation is a direct function of the daytime irradiance (Sachs, Kofranek and Kubota, 1980), suggesting that night break lighting inhibits flowering by diverting assimilates away from the apical dome and so higher night break photon flux densities are required to overcome high daytime irradiance. Cockshull and Hughes (1967) found that the proportion of total dry matter that was diverted to flowers as a whole was highly correlated with their stage of development, demonstrating an assimilate dependence during flower development.

While it is evident that high photon flux densities will elevate carbohydrate levels via photosynthesis, the actual rate of photosynthesis does not appear to play a part in floral induction. In some species, plants will flower when the photosynthetic rate is the same as in vegetative plants (Bernier *et al*, 1981b). Partitioning of assimilates within the plant and particularly within the apical bud is proposed as the mechanism controlling the observed photosynthetic effects. Increased carbohydrate levels in the apical bud during initiation have been reported in both photoperiodic and cold-requiring plants. In *Sinapis*, significant increases in the contents of soluble sugars and starch in the apical bud are observed in the early hours of an inductive LD or after displaced SD induction (Bodson, 1977). As displaced SD induction and normal SD conditions have the same period of photosynthesis, the increase in the level of soluble sugars in the buds of induced plants indicates that redistribution of photosynthetic assimilates is required for flowering in *Sinapis*. Remobilization of carbohydrate reserves may be important in this process as a significantly lower percentage of respiratory carbon

dioxide released at night from plants under inductive conditions is from carbon dioxide fixed during the previous light period than for non-induced plants (Bernier, 1988).

High levels of soluble sugars in the apical bud do not always result in induction, however, as one SD at high photon flux increases soluble sugar levels in *Sinapis* but results in only partial evocation. In *Pharbitis*, the assimilate supply to the apex remains unchanged during evocation (Ogawa and King, 1991), while in *Bougainvillea*, gibberellin treatments inhibit flowering without significantly lowering the level of soluble solids in the bud (Ramina, Hackett and Sachs, 1979). In *Lolium temulentum* floral evocation is controlled by the photoperiodic processes, the response to which is amplified by high sugar supplies but not replaced by them (King and Evans, 1991). Distribution of assimilates within the apical bud may explain this seemingly contradictory result, as a particular carbohydrate level is one of several necessary but not individually sufficient events in the transition to flowering.

Sucrose or glucose applications promote flowering in several SDP and LDP, even in non-inductive conditions. *In vitro*, the optimal sugar concentration for flower production by non-photosynthetic explants is generally above that for vegetative bud formation (Sachs, 1987). Time of application, however, often determines the effect on flowering of carbohydrate applications. Exposure to high intensity light during the first half of an inductive LD promotes flowering in *Sinapis* whereas it is inhibitory during the second half (Bodson, 1985). Removal of carbon dioxide from the air reverses both effects, indicating that the effect is related to photosynthate input. Also, plants treated with sucrose before a displaced SD flower more readily than if the treatment is applied after the SD. Both are given during what would have been normal light periods, but the early application comes at a time when it could be conveniently added to light of a displaced SD to give the effect of an inductive LD (Bodson *et al*, 1978). In wheat and sunflower, CO_2 enrichment promotes flowering but again only when applied during a critical, relatively short period (Marc and Gifford, 1984).

The observed effects of chemical and environmental factors on flowering are an indirect result of influences on assimilate supply and distribution according to the nutrient diversion hypothesis. Treatments which promote activity in sinks competing with the apical meristem will appear as floral inhibitors while factors favouring assimilate diversion to the apical meristem, including removal or restriction of competing sinks, will be seen as floral promotors. Differentiating between the effects of assimilates and other endogenous chemical substances during evocation is complicated

by the fact that photosynthates and any other leaf induced floral stimuli are simultaneously translocated in the phloem. While the effects of photosynthesis and assimilate transport are difficult to separate from the proposed physiological reactions associated with plant growth regulator action, the diversity and sophistication in control of flowering indicates that assimilate partitioning cannot fully explain the control of floral evocation. The nutrient diversion hypothesis does not account for the timing of events required in the leaves and the apex, including photosynthetic inputs. For the majority of plants, assimilate supply does not appear to be the sole signal for flower initiation and should be regarded, along with the other chemical factors discussed, as controlling one or more of the evocational processes which, in the appropriate balance or sequence, result in flower initiation.

5.3 Other endogenous metabolites

A range of endogenous metabolites not associated with the five classical groups of plant growth regulators or assimilates have been shown to influence flowering in several species. Nucleic acid constituents, amino acids, amides, phenolics, phenolamines, steroids, vitamins and others have all been shown to affect the initiation of flowers. The great variety of chemicals influencing floral evocation is a reflection of the complexity of the process. All substances analysed change in amount or distribution at the time of flower initiation but it is generally impossible to decide whether or not these changes are causally related to flower formation or a consequence of the process.

Proline has been implicated in the flowering process, particularly in cold-requiring species. In *Cheiranthus* and cabbage, proline accumulates in the buds of vernalised plants at the outset of flower initiation and decreases afterwards. Proline content is, however, associated with stress conditions and the observed increases during vernalisation may be due to cold stress rather than evocation as the proline content of the biennials beet and carrot is low at the time of initiation but peaks during the cold treatment before initiation occurs (Bernier *et al*, 1981b). Proline, as well as phenylalanine and tyrosine, have been shown to promote flower formation in root explants of *Cichorium* (Paulet, 1985). Several steps in the flowering of *Lemna* are influenced by exogenous applications of amino acids which can be either promotive or inhibitory (Maeng and Khudairi, 1973. Khurana, Tamot and Maheshwari, 1988). Also, a polypeptide has recently been isolated which induces flowering in *Lemna*

paucicostata at a very low concentration (Kozaki, Takeba and Tanaka, 1991).

The phenolic compound salicylic acid has been demonstrated as promoting flowering in *Lemna* (Cleland and Tanaka, 1979) but it is apparently not important in *Xanthium* where endogenous levels do not change in response to photoinduction (Cleland and Ajami, 1974). Chlorogenic acid has also been implicated in the flowering of *Lemna* (Umemoto, 1971) and *Nicotiana* (Zucker, Nitsch and Nitsch, 1965). Other changes in the apical and overall levels of phenolic compounds during evocation have been reported but their role in the process is still questioned. Endogenous phenolics are largely compartmentalised or stored in bound forms in plant cells and so the extracted levels reported may not indicate the levels of physiologically active compounds within the plant (Umemoto, 1971).

As with phenolic compounds, exogenous applications of steroids or vitamin E may promote, inhibit or have no effect on flowering depending on species and on experimental conditions. Steroids, studied because members of the chemical group are active animal sex hormones, are usually reported as floral stimulating hormones, although the mode of action is not known. Likewise, vitamin E applications can promote flowering, but changes in endogenous levels generally do not correlate with flower initiation (Bernier et al, 1981b).

The experimental evidence points to a combination of the plant growth regulators and assimilates, necessary in specific sequences and balances, as controlling floral evocation. New regulatory factors may yet be discovered although it is unlikely that any new factor will be a ubiquitous 'florigen', controlling flowering. The possible role of other substances such as phenolics or steroids, which have usually been studied in an attempt to identify a unique florigenic compound, cannot be ruled out. Modification of extraction methods and quantification procedures are necessary to determine the role of these substances in flower initiation.

6. Correlative Influences

The apical and axillary meristems are the sites of floral morphogenesis but represent only a small part of the whole plant. Other plant organs, both adjacent and distant, influence flower initiation at the developing meristematic points in various ways. Organ correlations provide an independent mechanism of regulating flowering and as such are more obvious in plants displaying a neutral or quantitative

photoperiodic response rather than strict photoperiodic species where they are less apparent and/or masked (Krekule, 1979). Self-inductive plants (those having autonomous induction), such as chrysanthemum, display strong organ correlations during the flowering process.

Examination of the correlative influences of plant organs has generally been performed by removing or destroying the given organ and observing the plant's subsequent behaviour. Replacement of the removed part by another one of similar or different location in the plant, or by various exogenous chemical applications, can also be used to determine the organ's role in the complex network of interactions between the various plant parts. *In vitro* studies of excised meristematic material, and more recently explants devoid of meristems, have been used to remove many of the correlative influences acting in whole plant systems. Explants from excised apical meristems still possess leaf primordia and in many cases roots regenerate so that the *in vitro* systems are not devoid of correlative influences. Explants composed of thin cell layers of epidermal and subepidermal cells are the simplest flowering systems so far examined, but interactions between the tissue layers still influence flowering.

6.1 Apical dominance

Of the many shoot apical meristems formed during plant growth, only a small percentage are allowed to actively develop and flower at any one time. The apical bud meristem is commonly dominant to some degree over the lateral, often axillary, bud meristems. The reservoir of dormant and undifferentiated meristems provides additional growing points should the apical bud be damaged or removed. Release of the lateral, axillary meristems from the regulating effects of the dominant apical bud is frequently observed during evocation.

Apical dominance ranges from inhibition of lateral buds at a very early stage of development, so that the bud consists of only a group of organised apical meristematic cells, such as in *Tradescantia* through to incomplete inhibition, such as in *Coleus*, where lateral buds continue to grow as leafy shoots, although still subject to an inhibitory influence from the apical bud (Phillips, 1975). Since auxin synthesised by the apical bud appears to be the principle correlative signal for both complete and incomplete apical dominance, it is possible that in both cases cell division activity at the lateral apex is suppressed (Thimann, Sachs and Mathur, 1971). Complete apical

dominance would then involve blocking of apical cell division activity whereas developmental processes proceed in lateral buds under incomplete apical dominance but at less than maximum rates.

The nutrient diversion hypothesis has been used to explain the mode of action of correlative inhibition of axillary buds by asserting that the apical bud is a strong sink for assimilates and so axillary bud growth is limited by a lack of assimilates. Phillips (1975) argues that this view is oversimplistic and that plant hormones, particularly auxins and cytokinins, are part of the mechanism of action of the correlative signal which also involves nutrients. In *Pharbitis*, the continued outgrowth of lateral buds, and possibly also the initiation of bud outgrowth, is dependent on a nutritive signal from the root system. IAA has a significant role in this process, modifying the effects of the nutritive signal, but the specific function of the hormone is not fully understood (Prasad, Hosokawa and Cline, 1989). Basipetal transport of auxin is a critical factor in the control of axillary bud growth in *Phaseolus* (Tamas *et al*, 1989) but the inhibitory effect is not due to accumulation of auxin in the bud (Lim and Tamas, 1989). Auxins, produced in the apical bud, are transported basipetally and are thought to control cytokinin activity in the lateral buds either by directly controlling synthesis in the buds or by influencing the distribution of root synthesised cytokinins.

Direct cytokinin applications to buds has been demonstrated to produce lateral bud outgrowth in *Chenopodium* (Seidlova and Krekule, 1977), soybeans (Ali and Fletcher, 1970) and other species. Subsequent applications of IAA or GA_3 to shoots in soybean allows the shoot to develop considerably further than only cytokinin applications, indicating a sequential role for the plant growth regulators in lateral bud outgrowth (Ali and Fletcher, 1971). Levels of ABA have been recorded to decrease significantly in buds released from apical dominance (Tucker and Mansfield, 1973) suggesting that it may play a role in the regulatory process. Also, IAA has been shown to markedly stimulate acropetal transport of ABA in pea epicotyl segments (Borkovec and Prochazka, 1989), and it is possible that the control of apical dominance involves IAA-stimulated changes in ABA distribution.

Ethylene is reported to be involved in at least two discrete mechanisms in the control of apical dominance : the release of lateral buds from inhibition and their subsequent growth and development. High ethylene concentrations in the region of the apical meristem promote lateral bud growth, possibly by inhibiting apical growth and so allowing assimilates to flow to other sinks, or by influencing auxin transport. While

high ethylene levels in the region of lateral buds prevents their outgrowth, ethylene is necessary for the further development of shoots once they are released from apical dominance, again indicating a sequential role for plant growth regulators in lateral shoot growth (Yeang and Hillman, 1984). Most of the evidence indicates that correlative inhibition of lateral bud growth involves a deficiency in the lateral buds of some essential factor or factors required for their further development. It seems most likely that the lateral buds lack both an adequate supply of cytokinins and assimilates, and that auxin produced by the apical bud and transported basipetally influences assimilate distribution and plant growth regulator translocation to maintain this correlative inhibition of lateral bud growth.

The inhibitory influence of the apical bud on the flowering behaviour of lateral buds is expressed in several ways. A basipetally decreasing potential to flower at axillary positions, or flowering gradient, is commonly observed, for example in day-neutral tobacco (McDaniel and Hsu, 1976). Each axillary bud produces a number of nodes before flowering, the number being dependent on the bud's position on the main axis. It is the physiological status of the plant and not the number of nodes on the bud at the time of release from apical dominance that influences this node-counting process of the bud (McDaniel, Sangrey and Singer, 1989). Excised buds, however, flower at approximately the same time and same leaf number no matter what the position they were taken from, indicating that some correlative influence from the apical bud rather than physiological age or state of the bud determines flowering. The basipetal gradient of flowering potential can be reversed by girdling the stem. In plants with a basipetal gradient of flowering, gibberellins, auxins and cytokinins also follow a basipetal gradient while abscisic acid follows an acropetal gradient. Reversing the flowering gradient by stem girdling also reverses the gradients of all four of these plant growth regulators (Lozhnikova *et al*, 1989).

In several species, for example *Fuchsia* (Sachs, Kofranek and Shyr, 1967), terminal inflorescences are apparently never produced, even with strong floral induction, resulting in indeterminate flowering on axillary shoots.

Other plants initiate axillary flowers immediately after release from apical dominance, examples being wild cherries (Webster and Shepherd, 1984) and roses (Cockshull and Horridge, 1977). Axillary meristems in cold-requiring perennials flower automatically when released from apical dominance. The effect of chilling is seen primarily as a means of reducing the growth of the terminal meristem, releasing

the axillary meristems from its dominance. Observations with cold requiring chrysanthemum cultivars support this theory. Decapitation of non-vernalised plants results in accelerated inflorescence initiation on distal lateral shoots (Vince and Mason, 1954). Also devernalisation of axillary meristems results from growing plants, after a chilling treatment, under conditions that result in strong apical dominance (Cockshull, 1985). The correlative influence of the terminal bud has a regulatory function in the autonomous flowering process in chrysanthemum.

6.2 Root inhibition

Excised apices of the SDP *Chenopodium* and the LDP *Scrophularia* initiate flowers *in vitro* irrespective of the daylength conditions, indicating that an inhibitory influence is acting *in vivo*. This inhibitory influence has been shown to be the roots (Miginiac, 1978). As mentioned in Section II.3.1, proximity to the root system totally prevents *Ribes* meristems from flowering (Schwabe and Al-Doori, 1973), with similar inhibition also observed in tobacco (McDaniel, 1980). The inhibitory effect of roots in *Chenopodium* and *Scrophularia* can be replaced by a cytokinin application while the effect in *Ribes* is attributed to root generated gibberellins.

Flowering may be promoted in several species by restricting root growth through root pruning or water stress (Miginiac and Sotta, 1985). High temperature and root removal both promote flowering in SD in the LDP *Silene* (Wellensiek, 1968). Apparently roots have a detrimental effect on flower initiation in *Silene* and this effect is inactivated at high temperatures.

The role of roots is not always inhibitory. The rosette LDP *Rudbeckia* is unable to bolt and flower when deprived of roots, even when its leaves are fully induced. Bernier (1988) notes that the effect of roots is on flower initiation as flowering can occur without bolting. Generally, however, the initiation of flowers appears to be inhibited by excessive root growth.

6.3 Role of mature leaves

The role of mature leaves in photoinduction of photoperiodic plants has been well documented (eg. Bernier, 1988). Detached leaves of *Perilla* can be photoinduced in the absence of buds and roots, and these leaves can be grafted onto derooted receptor

shoots where they cause flower formation (Zeevaart and Boyer, 1987). The sole organ interaction apparently required is thus the one way transport of a signal from leaves to the apices. The various correlative influences discussed above and the inability of other species to respond in a similar way to *Perilla* indicate that the situation is not this simple in other non-perennial photoperiodic plants.

The inhibitory effects of non-induced leaves in photoperiodically sensitive species may be due to specific inhibitors or to interference in assimilate transport (see Section II.4.1). The possibility that inhibitors may be produced in non-inductive daylengths and/or in inductive conditions makes it difficult to accept the one-way transport of the floral stimulus from induced leaves to the apex as the sole organ correlation in photoperiodically sensitive species. Also, a decline over time in the activity of the gene(s) expressing the inhibitor may be responsible for the autonomous induction process, as proposed for *Pisum* (Murfet, 1985).

The involvement of leaves in the mediation of the effects of environmental factors tends to mask the correlative influences in other plant organs. *In vitro* studies show that relatively high sugar concentrations are required for flowering of excised apices (Krekule, 1979). Similarly in intact photoinsensitive plants sugar feeding can often promote flowering while defoliation is inhibitory (Zeevaart, 1976). The evidence points to a largely nutritive role for mature leaves in correlative, non-photoperiodic effects in intact plants but the possibility exists that inhibitors from the leaves may influence the autonomous flowering process.

6.4 Organ correlations within the apical bud

Short-range correlations at the shoot apex are difficult to study owing to the small quantity of material available for analysis. The transition of the apical bud from vegetative to reproductive development is usually associated at the anatomical level with a general activation of the meristem, resulting in increased mass and structural complexity. Thus, shifts can be envisaged in correlations of young organs at the apical meristems, due to changing relative growth rates (Krekule, 1979). In *Arabidopsis*, evidence exists that the communication between adjacent developing regions in reproductive meristems which results in the type and position of the floral organs formed, does not involve the plant hormones (Bowman, Smyth and Meyerowitz, 1989).

Flowering of excised vegetative apices depends on factors from young leaves, scales or cotyledons. Young leaves or scales can replace the low temperature requirement for flowering in apices excised from *Iris* bulbs (Rodrigues Pereira, 1962). The promotive effects of scales can be demonstrated even when the pieces of these organs are implanted in the same medium but separated from the apices, suggesting the production of one or more diffusible floral promoters. Charles-Edwards and co-workers (1978) in their flowering model for chrysanthemum, assume that each newly formed primordium acts as a point source of a substance which can inhibit further primordia initiation. A new primordium can only be initiated when the concentration of the inhibitor falls to a critical level, also providing positional information for the next primordium to be initiated. This model can predict certain features of chrysanthemum flowering and indicates that short-range correlations within the apical bud play a large part in flowering.

6.5 Flower development

When plants enter the reproductive phase, other complex organ interactions develop. Several studies suggest that the apical bud controls the development of axillary flowers or inflorescences in the same fashion that it controls development of vegetative shoots (Kinet *et al*, 1985). Competition for assimilates between reproductive structures and axillary shoots is an indirect result of apical dominance and the greater the number of axillary shoots competing with the reproductive structures the greater the impact on development.

Besides apical dominance and the influences of the vegetative organs of the plant (root system, leaves, axillary shoots), strong correlations between and within inflorescences and flowers have been observed. In barley, cessation of spikelet occurs concurrently with stamen initiation in the most advanced spikelet, possibly due to the production of an inhibitor, thought to be a gibberellin, in the stamens (Cottrell, Dale and Jeffcoat, 1981). Flowers and fruits developing in an inflorescence markedly alter the fate of younger flowers in many species through both inhibitory and stimulatory influences. Alternate bearing in apple is thought to be related to the developing seeds exerting a strong inhibitory influence on flower initiation (Buban and Faust, 1982). Similarly, development of the various floral organs shows strong correlations. These correlative influences may be interpreted in terms of either competition between various

organs for metabolites or production of inhibitors or promoters by some organs.

6.6 Plant growth regulator transport

The possible involvement of plant growth regulators in both the mediation of the effects of environmental factors and in correlative influences, particularly long distance organ interactions, requires some understanding of their translocation within the plant. A major gap in our knowledge of plant growth regulators relates to the absence of information on their translocation between organelles, including from source leaves into sink tissues (Lenton, 1984).

Auxins are synthesised primarily by the young leaves in the developing buds and by root tips, but enlarging leaves, flowers, fruits and seeds are also rich sources, and appear to be translocated in cells associated with the phloem (Matthysse and Scott, 1984). The polar, basipetal transport of auxin is well documented. It is believed that auxin is transported by two elements in the plasma membrane; a symport (an electrogenic plasma membrane carrier) and a polarly distributed exit carrier. Auxin uptake into the cytoplasm is driven by a pH difference and an electric gradient, while the efflux is via the exit carriers, and the process may be inhibited by metabolic inhibitors or low oxygen, indicating that it is an active process (Hertel, 1986). Polar basipetal movement diminishes in older parts of stems, coleoptiles and petioles.

Cytokinins, originally thought to be synthesised only in the roots, have now been reported in areas of actively dividing tissue in the plant (Chen *et al*, 1985). Cytokinins produced in the roots are transported in the xylem, probably as conjugated forms (Letham and Palin, 1983) as conjugated glucoside forms occur naturally in the phloem (Moore, 1989). Metabolism of conjugated forms may play a large part in cytokinin transport and activity as glycosylation to inactivate free cytokinins produces a potential reservoir of bound cytokinins (Saha, Nagar and Sircar, 1985).

Ethylene appears to be produced in all plant tissues. Translocation of its precursors, SAM and ACC, and their conjugates has been reported (Yeang and Hillman, 1984). Gibberellins, like auxins and cytokinins, may be removed from the growth regulating system by temporary storage as a glucoside or ester, allowing translocation and regeneration. Translocation of the gibberellins is without polarity and takes place readily in the phloem or xylem (Moore, 1989). Recent results have cast doubt on the proposed function of conjugated GAs as transport forms, suggesting

rather that certain free GAs are translocated (Takeno and Cleland, 1990). Likewise, the transport of ABA does not appear to be polar, but may be regulated by auxin (Phillips, 1975). Movement of ABA from the roots to the shoots occurs in the xylem, while it is transported out of senescing leaves in the phloem. The lipophilic, protonated form permeates freely across membranes, whereas the dissociated anion is impermeable (Zeevaart and Creelman, 1988). Consequently the distribution between different compartments is determined by the difference in pH between the compartments. Accumulation of ABA in leaves under stress may be at least partly due to an induced reduction in internal phloem pH causing decreased loading of ABA (Baier and Hartung, 1991).

A novel approach to the investigation of the transport of the leaf-induced floral stimulus is the use of electric currents applied to the leaves of photoinduced plants. Direct current (DC) applied to photoinduced leaves of *Chenopodium* during the period when the floral stimulus is apparently transported from the leaves, prevents flowering (Adamec *et al*, 1989). The DC treatment is not permanent and may be reversed by a second inductive treatment (Machackova, Pospiskova and Krekule, 1990). It is proposed that this inhibition of flowering might result from a disruption of the normal transport of the floral stimulus from the leaves, and so the approach to be followed is now to examine the distribution of specific nutrients and plant growth regulators during the DC application.

Hormone binding at the site of action is another area of research where our understanding is sadly lacking. The objective of all those engaged in receptor research is to understand how the growth regulator stimulus is transduced into the observed physiological responses. As this research is still in its infancy and the results are still preliminary, plant growth regulator binding is not discussed in this review. The topic is discussed in reviews by Napier and Venis (1990), Moore (1989) and Venis (1985).

6.7 Hormone-directed transport of metabolites

Assimilate partitioning and growth substance distribution are generally viewed as essential factors governing the establishment of the complex interactions existing within plants. It is not easy to separate the respective roles of these two processes partly because plant growth regulators can be translocated in the phloem alongside assimilates. Evidence exists that the plant hormones may be able to influence metabolite

transport and distribution.

Foliar sprays of GA_3 or BA stimulate ^{14}C-photosynthate transport to the shoot tips of *Vitis* (Shindy and Weaver, 1967) while BA treatment of the roots enhances transport to these organs (Shindy, Kliewer and Weaver, 1973). Metabolite transport to the cut sections of decapitated seedlings is stimulated by hormone applications in a wide range of plant material (Patrick, 1976), indicating a causal relationship between the plant hormones and metabolite transport rather than a direct effect of hormone induced changes in sink strength.

The effectiveness of particular exogenous hormone applications depends, at least to some extent, on the physiological state of the treated plant or plant part. The stimulatory effects of both natural and synthetic auxins on the movement of assimilates to decapitated stem stumps has been recorded by Bowen and Wareing (1971). While they failed to demonstrate any influence of cytokinins and gibberellins, their stimulatory effect has been recorded by other workers (eg. Hayes and Patrick, 1985. Mullins, 1970). Mullins also found that ethylene promotes and abscisic acid inhibits transport.

The mechanism by which hormones exert their influence on metabolite transport remains unclear, and may involve either direct and/or indirect effects on transport processes. Hormones may influence both source strength, by affecting the net photosynthetic rate of leaves, and sink strength. The transfer of shoot-produced hormones to mature leaves would seem minor compared to that of root-produced hormones and thus the mobilising ability of root sinks is more likely to act via hormonal regulation of photosynthesis than shoot sinks (Patrick, 1982). It is considered that sink activity depends on the rates at which endogenous metabolite pools are depleted and/or on the activities of cellular nutrient-uptake mechanisms, both of which are susceptible to hormone action. As Patrick and Wareing (1973) discuss, it is possible that the nature of decapitation experiments results in inherent artifacts in the experimental systems which gives indirect hormonal effects.

Direct hormonal effects on metabolite transport are concerned with the phloem transport process. This may involve sieve-tube loading mechanisms, sieve-tube unloading mechanisms or the longitudinal transfer of assimilates in the sieve tubes. In the case of GA_3 the stimulation of assimilate transport appears to be due to increased activity of the phloem unloading process (Patrick and Mulligan, 1989). Most work has been done on IAA-induced metabolite transport and several studies have indicated a direct effect on the phloem transport process (Patrick, 1982). Available results do not

allow definitive conclusions to be drawn on the mechanism of hormonal effects on metabolite transport, but sufficient evidence exists, particularly for IAA and GA_3, to warrant the continued pursuit of studies on the possible direct effects on phloem transport processes.

7. Conclusion

The bewildering array of flowering responses described in the previous pages belies the fact that the number of species examined represents only a small percentage of the total number of flowering plants. Who knows what complex and varied control mechanisms will be revealed as more species are studied. The challenge for geneticists, morphologists and physiologists has been to develop a unifying model for floral evocation and morphogenesis which incorporates the myriad of regulatory factors observed and can explain the specific events of evocation and morphogenesis common to all plants.

The concept of multifactorial control of flowering, as proposed by Bernier *et al* (1981b), provides the best model for flowering research. According to the model, floral evocation is controlled by a complex system of interacting events, with an appropriate balance or sequence of these events triggering evocation. Flower development is also controlled by a similar sequence of specific determination steps (Kinet *et al*, 1985). Different species may require different sequences of evocational events, and not all specific steps need occur for the apex to become florally committed.

Past research has so far given us an insight into the enviromental factors which control these specific evocational events. Attention has now focused on the molecular and genetic nature of the evocational sequences. The participation of the known endogenous plant growth regulators is widely acknowledged, and it now remains to relate changes in overall concentrations, proportions, metabolisms and fluxes of these substances to the successive steps of the flowering process. Changes in tissue sensitivity to the plant growth regulators and the role of assimilates and their distribution during flowering are also receiving more attention. Finally, the genetics of flowering is being investigated in some detail. Gene expression is ultimately responsible for the development of reproductive structures, and so an understanding of the regulatory gene products associated with each evocational event may provide an insight into the endogenous signal molecules controlling these processes.

To date, flowering research has concentrated mainly on a small number of sensitive photoperiodic species that offer an ideal system to examine photoinduction. Not all the observed evocational events are present in these systems, and indeed these species cannot be considered representative of the flowering response in all plants. For this reason the scope of flowering research needs to be extended to include defined experimental systems centred on other evocational events and regulatory processes. A full understanding of the physiological and genetic basis of flowering will only be possible when each of the partial processes regulating floral evocation and morphogenesis is analysed.

While it is outside the scope of this study to analyse in detail the physiological processes controlling flowering in pyrethrum, it is hoped that the results will not only give us sufficient understanding of the flowering process to allow successful commercial exploitation of the crop but also provide a basis from which future studies correlating endogenous chemical changes with floral evocation and morphogenesis can follow. As mentioned previously, the morphological events associated with flowering, and the environmental parameters affecting flower initiation and development must be covered before further research is undertaken. It then seems logical to examine a broad range of endogenous changes during flowering to determine which events are well correlated with the evocational sequence. Detailed investigation of these events can then proceed.

Photosynthetic and assimilate partitioning studies appear to be an essential area of research in the flowering of pyrethrum. In the literature, assimilates are given a major role in the flowering of chrysanthemum, and studies on the environmental control of flowering in pyrethrum suggest a similar regulatory action. The role of the plant growth regulators in the flowering of many species, including chrysanthemum, necessitates the inclusion of a quantitative examination of endogenous levels in this study. Exogenous application of plant growth substances will supplement this work. This study encompasses these areas of research in flower initiation and morphogenesis in pyrethrum, *Tanacetum cinerariaefolium.*

III General Materials and Methods

1. Plant Material

1.1 Controlled Environment Studies

At the commencement of this research project, two pyrethrum clones, labelled CIG 3 and CIG 11, were grown commercially in Tasmania. Unless otherwise specified, one or both of these clones were used in all controlled environment experiments; where only one clone was used, CIG 3 was chosen. New material was generated through tissue culture, transferred from the media to 'Speedling' trays for adjustment to ambient environmental conditions before being planted in 15 cm pots. The potting medium was a 50:50 (v/v) mixture of sand and peat moss, with 40 g ground limestone, 40 g 'Limil' (a liming agent) and 80 g 'Osmocote' (encapsulated slow release fertilizer) added per forty litres of sand/peat moss mixture. Plants were maintained in a glasshouse at the Horticultural Research Centre, University of Tasmania, where the minimum and maximum temperatures were 30 °C and 20 °C respectively. Watering was carried out by an automatic overhead watering system once a day, or twice a day when necessary during summer. Nutrient solution (N. Hoaglands) was applied weekly after the 'Osmocote' capsules in the potting mix were exhausted. 'Calibre' and 'Kelthane' were applied where necessary to control red spider mite infestations.

Preliminary experiments indicated that a juvenile-like condition exists in plant material generated through tissue culture. To ensure that experimental plants were competent to flower, all plant material was maintained under glasshouse conditions for at least two months before being selected for experimental use. Selection of experimental plant material was based on physiological uniformity.

1.2 Field trials

Field trials were located at two sites, Ouse and Bushy Park. Both sites are located in the Derwent Valley in Southern Tasmania, and are 76 and 120 metres above sea level respectively. The yearly mean minimum and maximum temperatures (average of the last

twenty years at least) were : Ouse 5.9 and 18.1 °C, and Bushy Park 6.1 and 17.4 °C respectively. Bushy Park soil is dark-coloured with an upper profile of uniform loam to clay loam field texture and friable crumb structure. The soil at Ouse is similar to that at Bushy Park but has a slightly lighter field texture.

Two separate trial plots were maintained at the Ouse location. The first plot was a five year old planting of clonal material formerly used in a multilocation trial for the plant improvement programme. Ten plants each of eight clones, including CIG 3 and CIG 11, were arranged in rows with an inter-row spacing of 70cm, and 50cm between plants within each row. This arrangement was replicated four times with the clonal rows distributed randomly within each block. The Bushy Park plot was arranged identically, also being a part of the former multilocation trial. The second plot at Ouse consisted of clones CIG 3 and CIG 11 planted in June 1988 in a 80m x 20m area at the recommended commercial spacings : 45cm between plants and 50cm between rows, with each group of three rows separated by 80cm to allow machinery to move within the plot.

The sites were irrigated during the active growing period at a rate dependent on the prevalent conditions. Weeds were controlled by a combination of manual and chemical control measures. Plots were regularly sprayed with 'Ronilan' and 'Sumisclex' to control crown rot caused by *Sclerotinia sclerotiorum* and *S minor*. Fertilizer (NPK 5:6:9 at 50 $Kg.ha^{-1}$) was applied in early Spring each year.

2. Environmental Control

2.1 Glasshouses

Plants were grown in the main glasshouse (an air conditioned glasshouse) and an evaporatively cooled glasshouse at the Horticultural Research Centre (HRC), University of Tasmania. Maximum/minimum temperatures were 30/20 °C and 33/12 °C respectively. No artificial lighting was used in the glasshouses. Natural photon flux densities varied from 600 $\mu mol.m^{-2}.s^{-1}$ to 1200 $\mu mol.m^{-2}.s^{-1}$. Daylength in Hobart varies from 15.30 monthly mean hours of daylight (sunrise to sunset) in December to 9.04 in June.

2.2 Light Tunnels

Three light tunnels at the HRC were utilised. The light tunnels were each 1.5m x 4m in area, light proof and lined with polystyrene blocks 50mm thick. Constant night temperatures were maintained using thermostatically controlled, refrigerated cooling elements. Temperature was monitored continuously using a Foxbro electronic chart recorder. The temperature was found to vary by approximately +/- 2 °C ; the fluctuations were cyclic and each full cycle was completed within ten minutes.

Daylength control was provided by programming the light tunnel trolleys to emerge from the tunnels into the main glasshouse at the HRC for the required period of time. Day temperature and photon flux density thus varied according to the prevalent natural conditions in the main glasshouse (see Section III.2.1). Artificial lighting within the tunnels was used where necessary to extend the natural daylength for long-day treatments. Lighting was provided by two mercury vapour lamps suspended 1.5m above the plants and 10 white fluorescent tubes 2.0m above the plants, providing a combined PFD of 150 $\mu mol.m^{-2}.s^{-1}$ at the plant apex level.

2.3 Growth Cabinets

Four custom-constructed controlled environment cabinets were used during the course of this study. Temperature was controlled by a thermostatic sensing device connected to a heating unit and a combined condensing unit and cooling element. Temperature was found to vary by +/- 1 °C in the range 5 °C to 40 °C. Photoperiod was maintained by 16 Sylvania 40W cool white fluorescent tubes and 4 Sylvania 100W incandescent globes, giving a PFD of 280 $\mu mol.m^{-2}.s^{-1}$ at the plant apex level. PFD was found to vary with the age of the fluorescent tubes, which had to be replaced regularly. A switching mechanism to reduce the PFD by fifty percent was also used.

3. Observation Methods

Pyrethrum displays a rosette growth habit during vegetative growth. Lateral shoots develop from the main shoot axis as axillary meristems are released from apical dominance. For the purposes of this study, shoots with two or more mature, expanded leaves were considered as developing lateral shoots. Each lateral shoot was numbered

according to its subtending leaf on the main stem, with the lowest leaf (first leaf initiated) numbered one and subsequent leaves numbered in an acropetal progression. Lateral shoots developing from the main shoot axis are denoted as secondary shoots while axillary shoots developing from secondary shoots are denoted as tertiary shoots, and further shoots quaternary and so on. Numbering of these shoots followed the same scheme as for secondary shoots, using the leaf numbers on the appropriate lateral.

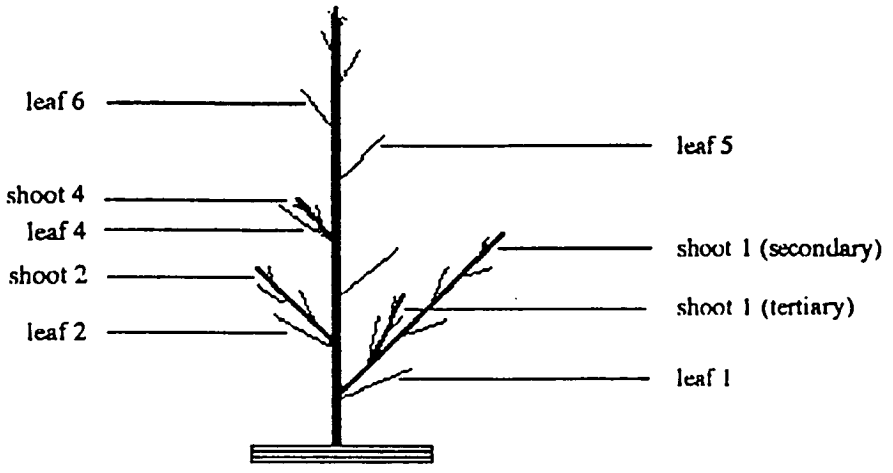


Figure III.3 - Schematic diagram showing leaf and lateral shoot numbering scheme.

For the investigation of the environmental control of flower initiation, plants were defined as florally committed when stem elongation was first observed, this being the earliest macromorphological sign of flower initiation. These results were verified through further observation of floral development. The following measurements were then recorded :

a) the elapsed time between the imposition of the treatment and the observation of floral commitment, and

b) the position of the committed meristem(s).

Destructive observation of plants, in the day temperature and PFD experiments, enabled measurement of all florally committed meristems through histological examination. In these cases the number of actively dividing vegetative and reproductive meristems was counted and expressed as a percentage of the total number of meristems at the time of harvest.

The scale of flower development proposed by Head (1966) (see Section II.2.1) was used for the investigation of the environmental control of flower development. Plants were harvested when the majority of flowers had reached stage II, ray florets vertical. The following measurements were then recorded :

a) the elapsed time between the imposition of the treatment and harvest,
b) the elapsed time between the observation of floral commitment and harvest,
c) the number of flower stems,
d) the number of flowers and flower buds,
e) the average height of the flower stems.

The field trials were harvested when the majority of flowers in each treatment block had reached flower development stage IV, approximately three rows of the disc florets open. The date of harvest was recorded for each experimental treatment. The following measurements were also taken :

a) the average height of the flower stems,
b) the number of flowers per plant,
c) the degree of lodging in each plant (rated on a scale of 1-6, one being no lodging and six being lodging where all flower stems were close to perpendicular).

Where appropriate, measurements were taken of small, representative subsamples from individual treatments. Also estimates of flower number or yield were used in the defoliation and timing of 'Cultar' (Paclobutrazol) application experiments by rating on a scale of 1-6 and validated by comparing with representative subsamples in which flowers were individually counted.

The pyrethrins content of harvested flowers was determined through spectrophotometric analysis following the procedure of Beckley (1950). Flower samples at stages IV and VI were collected from treatment plots and dried at 50 °C for 48 hours. The dry weights were recorded and the samples ground into a powder. 0.5g samples of the pyrethrum powder were then extracted with petroleum ether (40-60 °C boiling point) diluted with ethanol and the absorbance measured spectrophotometrically at 227nm. Pyrethrin content was calculated by multiplying the absorbance by 2.144 and expressed as a percentage of the sample dry matter content.

4. Scanning Electron Microscopy

Vegetative and floral buds were dissected in water, using a stereo microscope to provide a clear view and so aid in the removal of material covering the apical meristem. Apical material selected for scanning electron microscopy (SEM) was fixed in 4% gluteraldehyde in sodium phosphate buffer (0.1M, pH 7.2) for 24 hours at 4 °C. Following fixation the specimens were washed with chilled buffer and distilled water before being dehydrated in a graded ethanol series, culminating in immersion in 100% ethanol. The specimens were then washed in acetone and subsequently critical point dried using a Polaron E-3000 Critical Point Drier.

The critical point dried specimens were mounted on electron microscope stubs using electroconductive silver paint. Orientation of the material was arranged to best facilitate observations in the SEM. Specimens were gold coated using a Blazers Sputter Coater. Observations were carried out with a Phillips 505 SEM at approximately 15kV. Electron micrographs were recorded on a Rolex 120 camera, using Ilford FP4 film and developed in Ilford ID-11.

5. Radioimmunoassay

5.1 Tissue sampling

Leaf samples selected in each experiment consisted of mature, fully expanded leaves of approximately the same physiological age. Bud samples taken in the field trial consisted of vegetative and floral buds at the early developmental stages with all leaves above one centimetre in length removed, and flower buds at the later developmental stages with all leaf material removed. Care was taken to collect samples at approximately the same time of the day in all trials to limit diurnal variation of plant growth regulator (PGR) levels. All samples were immediately frozen in liquid nitrogen to prevent degradation. They were then freeze-dried, ground to a powder and stored at -18 °C.

5.2 Extraction and purification

The ground plant material (0.5g dry mass) was extracted with 10ml 80%

methanol (MeOH) (containing 20 $mg.l^{-1}$ butylated hydroxytoluene) by shaking overnight at 4 °C. The extract was centrifuged in teflon centrifuge tubes at 20,000 rpm for 12 minutes and the pellet rinsed with a further 10ml 80% MeOH and recentrifuged. The supernatants were combined and the organic phase removed in a Speed-Vac concentrator (Savant, USA). Ammonium acetate (0.01M, 10ml) was added to the remaining aqueous phase and the extract purified using the method described by Roberts, Menary and Hofman (1991).

Briefly, the extract was purified through a series of connected columns consisting of polyvinylpolypyrrolidone (PVPP, 5ml bed volume, with fines removed by decantation), DEAE Sephadex (3ml bed volume) and a SepPak C_{18} cartridge. The two former columns were conditioned with 20ml 1.0M ammonium acetate followed by 25ml 0.01M ammonium acetate, and the latter with 5ml 100% MeOH and 5ml 0.001M ammonium acetate. The extract was passed through the columns, followed by an additional 30ml 0.01M ammonium acetate.

The SepPak cartridge containing the cytokinins was removed and rinsed with 5ml distilled water. The cytokinins were then eluted with 5ml 60% MeOH. The PVPP column was detached from the Sephadex column and a fresh SepPak C_{18} cartridge (rinsed with 5ml 100% MeOH and 5ml 1% acetic acid) placed beneath the Sephadex column. The acidic PGRs were eluted from the Sephadex with 15ml 1.0M acetic acid. The SepPak cartridge containing the acidic PGRs was removed and rinsed with 5ml distilled water, and the acidic PGRs eluted from the SepPak with 5ml 50% MeOH. The SepPak was flushed with a further 5ml 80% MeOH to remove the remaining indolylacetic acid (IAA) fraction. The 50% MeOH wash was found to elute all the gibberellin (GA) and abscisic acid (ABA) but not all the IAA, while the 80% wash eluted all the IAA but also eluted a non-specific inhibitor to the ABA radioimmunoassay (see Section III.5.4).

The extraction and purification procedure separated the free from the bound and conjugated forms of the acidic PRGs, but did not separate the cytokinin bases from their respective ribosides (Hofman, unpublished data).

The cytokinin and acidic PGR fractions were evaporated to dryness, redissolved in MeOH and an aliquot equivalent to 0.01g dry mass (GA and ABA), 0.025g dry mass (IAA, zeatin (Z) and dihydrozeatin (DHZ)) or 0.05g dry mass (isopentenyladenosine (IPA)) dispensed in triplicate into polypropylene test tubes (12x55mm). All acidic hormones were methylated with ethereal diazomethane and the

organic solvents removed under vacuum. The samples were stored at -18 °C until analysed.

The percentage recovery of each PGR was calculated by adding known amounts of tritiated PGR (in duplicate) to the plant material prior to overnight extraction. Following the normal extraction and purification procedure subsamples were taken in triplicate for counting. Percentage recoveries were calculated in this way for each PGR after extraction and purification of all samples from each experiment. The percentage recoveries were generally above 90%, the lowest value being 70% for IAA in the defoliation trial.

5.3 Radioimmunoassays

The PGRs were quantified with previously developed and characterised radioimmunoassays (Cutting *et al*, 1983. Cutting *et al*, 1986. Hofman *et al*, 1985. Hofman *et al*, 1986. and Hofman, 1990). Standards were made in MeOH, and the required amounts dispensed into polypropylene test tubes. The MeOH was removed and the standards and samples dissolved in 0.1ml of radiolabelled tracer (containing approximately 30,000 dpm) in phosphate buffered saline (pH 7.4) containing 0.1% gelatine. [1,2(n)-^{3}H] gibberellin A_1, 3-[5(n)-^{3}H] indolylacetic acid and DL-*cis,trans*-[G-^{3}H] abscisic acid were purchased from Amersham International while tritiated Z, DHZ and IPA were produced by N.J.Roberts. Antibody (0.1ml, diluted to provide 30-50% binding) and 0.25ml of 0.1% bovine serum (or phosphate buffered saline for the ABA assay) were then added, the mixture vortexed, and incubated at 37 °C for 30 minutes. Ammonium sulphate (0.75ml, 90% saturated) was added to precipitate the bound fraction and the suspension was centrifuged at 7,000 rpm for 16 minutes. The pellet was rinsed with 50% saturated ammonium sulphate, re-centrifuged and the supernatant decanted. The pellet was redissolved in 0.25ml distilled water, 2.5 ml scintillant (Packard Optifluor) added, the test tubes capped and shaken, and the radioactivity determined using a Beckman LS 5801 scintillation counter.

Polyclonal antibodies were used in this study and as such each assay was not specific to the PGR being examined. Each of the cytokinin antibodies displayed significant cross-reactions with the corresponding ribosides. Thus the Z assays determined the combined concentrations of zeatin and ribosylzeatin, the DHZ assay detected dihydrozeatin and dihydroribosylzeatin and the IPA assay detected

isopentenyladenine and isopentenyladenosine. Both the IAA and ABA antibodies showed no significant cross-reactions with other naturally occurring PGRs while the GA antibody was used to assay the combined concentrations of GA_1 and GA_3 with a lesser cross-reaction to GA_{20}.

5.4 Validation of Immunoassays

The need to validate all PGR immunoassays for each type of plant material sampled is described by Pengelly (1985). Two procedures for checking on the presence or absence of specific and non-specific inhibitors were used in this study. Flower bud and mature leaf samples were analysed for each hormone to be assayed, using internal standards and extract dilution techniques of validating the immunoassays. The internal standard technique involves adding a constant amount of plant material extract to each point of the standard curve, and counting this standard curve as an unknown using the previously described RIA procedure. The amount of PGR detected is then plotted against the amount of PGR added (ie. the standard curve), with slopes differing from additivity indicating the presence of an inhibitor(s) of PGR binding. Similarly, the extract dilution technique involves assaying a range of extract volumes or weights, and plotting extract volume or weight against the amount of PGR found. If the plot is linear and shows additivity, the extract is free of specific inhibitors (except for the possibility of inhibitors with the same affinity characteristics to the antibody as the desired PGR displays). Direct comparison of samples with analytical methods, such as GC-MS, would provide unequivocal evidence of chemical purity, but such methods were not avaliable during this study.

The results obtained through the two validation steps discussed above indicated the absence of inhibitors in the extracts, but the possibility exists that specific inhibitors with the same affinity constant as the desired PGR may be present. A non-specific inhibitor to the ABA assay was found to be eluted from the acidic hormone SepPak with 80% MeOH but was not eluted with 50% MeOH, and therefore two separate washes were used, as described in Section III.5.2.

6. Statistics

An analysis of variance (ANOVA) table was calculated for each growth and flowering measurement in all experiments. Where values were significant, the Least Significant Difference (LSD) was calculated at the five percent probability level.

For any experiments which utilised time as a treatment, and where the values were significant, a test of homogeneity of the variances was calculated using Duncans multiple range test, determining the least significant range (LSR).

Statistical analysis of the changes in plant growth regulator concentrations determined by radioimmunoassay was not performed unless sample replicates were analysed. While extractions and assays were replicated for each sample, it was considered that statistical analysis of these results would reflect the variation due to the extraction and assay procedures and so could not be used to detect any differences between samples unless a number of sample replicates was analysed.

The following Apple Macintosh applications were used for the analysis of data and in the compilation of this thesis :

WriteNow 2.0

Cricket Graph 1.0

Delta Graph 1.5

Excel 1.0

Statview SE + Graphics 1.03.

IV Results and Discussions

IV.A Morphological Observations

1.1 Introduction

The general macroscopic descriptions used in the scale of floral development stages put forward by Head (1966) for pyrethrum have been widely used to define the physiological state of plants used in studies of flower development. The early morphological changes have not been described. Detailed descriptions of inflorescences and their surface topography at the various stages of differentiation are required for quantitative studies on the effects of environmental conditions and other treatments on floral initiation and development.

Detailed investigations of the morphology, organisation and growth of the apical meristems of several members of the Asteraceae have been published. Floral development of the apical meristem is best documented in the genus *Dendranthema* where a scale of generative apical development is widely used to quantify the early flowering responses of *D. grandiflora* (Cockshull, 1985). Other members of the family Asteraceae have similar patterns of apical development during flowering (Sharman and Sedgley, 1988).

This study investigates the morphological development of the apical meristem of *T. cinerariaefolium* prior to and after floral initiation by scanning electron microscopy. These changes are then correlated with the previously published macroscopic descriptions.

1.2 Materials and Methods

Scanning electron microscopy (SEM) preparation and procedure was as described in Section III.4. Meristem samples were taken from plants grown under as wide a range of environmental conditions as possible ; short days (10hr, 20-30 °C day temperature, 20 °C or 6 °C night temperature), long days (16hr, 20-30 °C day temperature, 6 °C night temperature), low photon flux density conditions under short days, natural conditions (field grown plants) and plants treated with growth retardants.

Apical and basal buds were examined as well as secondary and tertiary buds on developing flower stems.

The stage of apical development of each sample was recorded after viewing with the scanning electron microscope and the diameter of each apical meristem was measured from the micrographs taken. Measurements of the apical dome diameter were taken from the widest point on or above the axils of the two most recently formed leaf or involucral bract primordia. During the later stages of inflorescence development, the diameter of the dome was measured immediately above the whorl of bract primordia, irrespective of the presence of floret primordia.

1.3 Results

1.3.1 Scanning Electron Microscopy (Histological descriptions)

Apical morphology during floral development in pyrethrum undergoes similar changes to those described by Cathey and Borthwick (1957) for *D. grandiflora*. The vegetative apical meristem is flat or slightly domed and surrounded by from 10 to 15 leaf primordia and young leaves to form an apical bud. Short internodes, causal to the plants rosette growth habit, result in a large degree of overlapping of the leaf primordia in the vegetative bud. Lengthening of the internodes accompanies flowering, while the apical meristem enlarges and the apical height increases to give a pronounced dome structure. The first involucral bracts are initiated on the apical dome and are distinguishable from leaf primordia as they have an entire rather than a dentate margin. The apex may revert to a vegetative growth pattern after doming of the meristem, resulting in a return to the rosette habit after several elongated internodes have formed. Reversion was never observed after the first involucral bract had been initiated.

Further floral development is characterised by the rapid enlargement of the apical dome, forming a receptacle. The involucral bracts are initiated around the rim of the receptacle, which then begins to flatten. Floret primordia are initiated around the rim of the receptacle after between 26 and 38 bracts have been initiated. Floret initiation proceeds centripetally until the entire receptacle is covered. Floret differentiation, which may begin before the receptacle is completely covered, proceeds until the perianth is present on all florets, and is followed by the differentiation of reproductive organs.

The development of the reproductive apical meristem may be divided into stages

based on a slightly modified scale of development from that proposed by Cathey and Borthwick (1957) for *D. grandiflora.*

Stage	Description
0	Shoot meristem flat, typical of vegetative condition
1	Meristem slightly enlarged
2	Doming of the meristem to form a receptacle, and associated internode elongation ; first involucral bracts initiated
2.2	Four involucral bracts initiated
2.4	Six involucral bracts initiated
2.6	Eight involucral bracts initiated
2.8	Ten involucral bracts initiated
3	Receptacle spherical with twelve or more bracts initiated
4	Receptacle becoming flattened ; many bracts but no floret primordia
5	Two or three rows of floret primordia on the rim of the receptacle
6	About six rows of floret primordia on the receptacle
7	Receptacle covered with floret primordia except at the tip
8	Entire receptacle covered with floret primordia
9	A few floret primordia not yet with perianth initials
10	Perianth primordia present on all florets
11	Primordia of reproductive organs present on all ray florets

Figure IV.A.1.3.1.a Scale of apical development stages for pyrethrum. Modified from Cathey and Borthwick (1957).

Each stage in the development of the apical meristem is associated with a narrow range of apical diameters, indicating that inflorescence initiation and development are closely related to apex size. Plates 1-4 show typical vegetative meristems, which invariably have apical diameters less than 220µm, and may be flat or slightly domed. The diameter of the apical dome at the transition between vegetative and floral development is approximately 220µm, and the bract primordia are initiated at apical diameters between 220µm and 340µm. Plate 5 shows the doming of the apex when the first bract primordium is initiated. Apical doming is more pronounced during apical stage 2 as more bract primordia are initiated, as shown in plate 6.

Plates 7 and 8 show the significantly broadened receptacle at apical stage 2.8. The receptacle continues to broaden during stages 3 and 4 which are associated with apical diameters of between about 340µm and 500µm. The first florets are initiated on

Plate 1

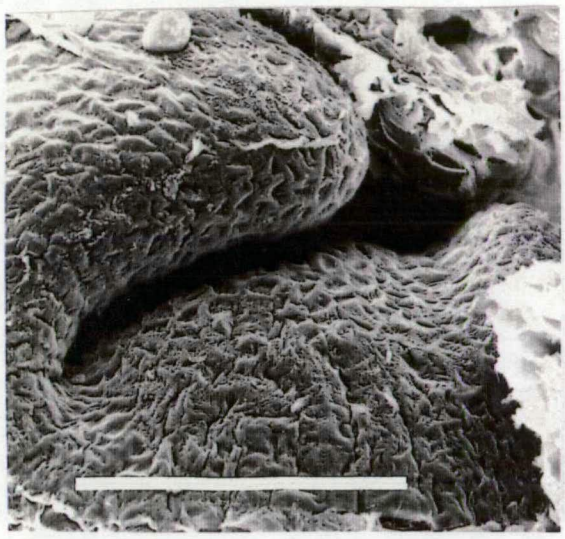


Plate 2

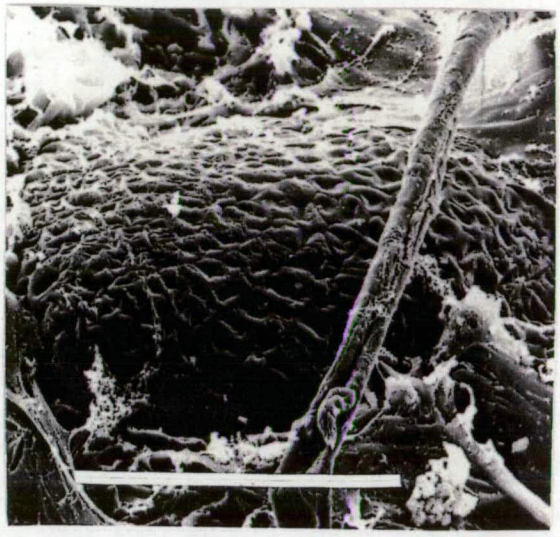


Plate 3

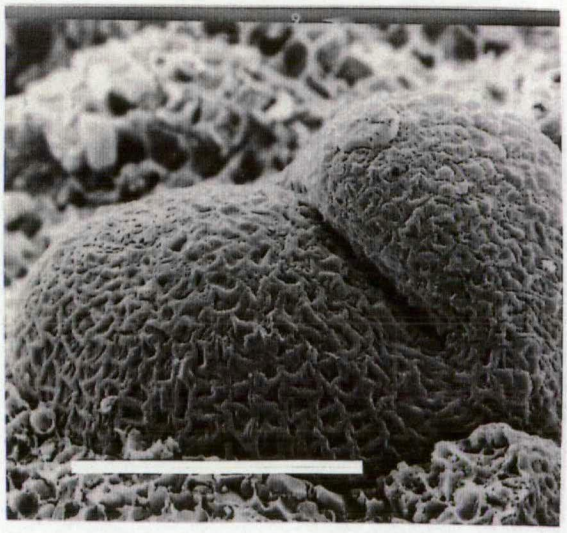


Plate 4

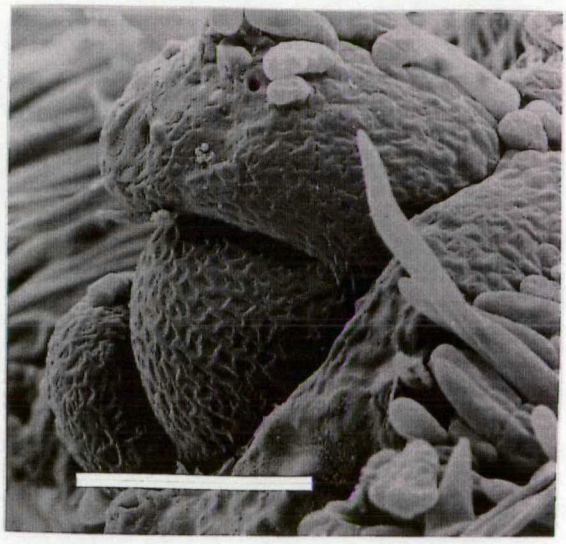


Plate 1 - SEM of vegetative bud, stage 0 (shoot meristem flat). Note the leaf primordia overlapping the meristem. Bar = 0.1mm

Plate 2 - SEM of vegetative bud, stage 0. All the leaf primordia have been removed. Bar = 0.1mm

Plate 3 - SEM of vegetative bud, stage 1 (meristem slightly enlarged). Note the slight doming of the apical meristem. Bar = 0.1mm

Plate 4 - SEM of vegetative bud, stage 1. The meristem was dissected from a leaf axil near the base of a flower stem. Bar = 0.1mm

Plate 5

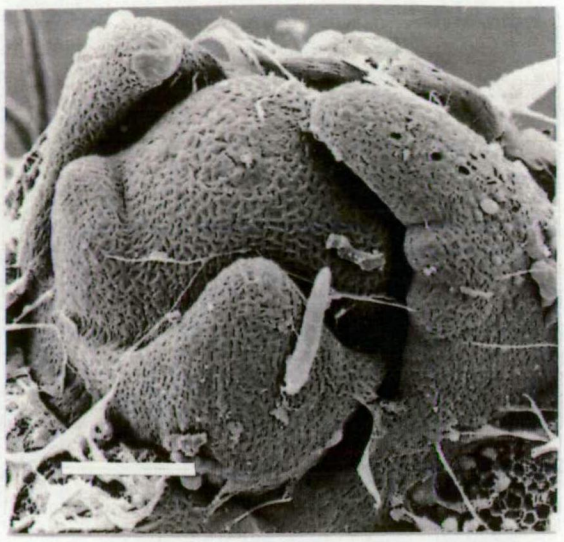


Plate 6

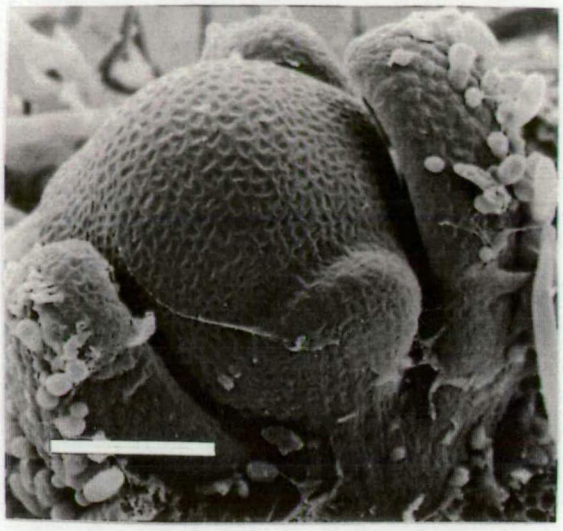


Plate 7

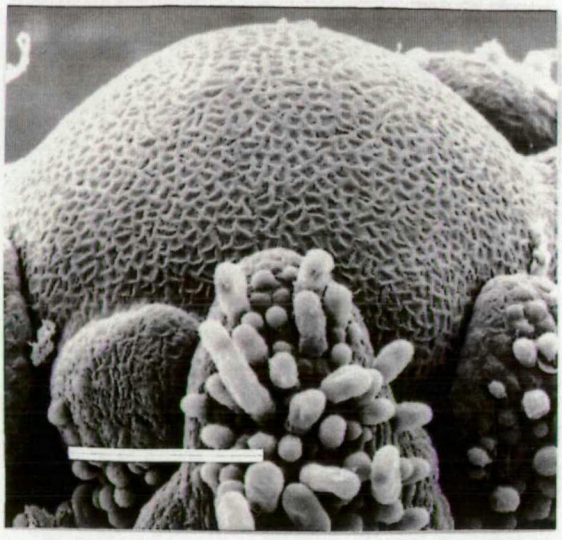


Plate 8

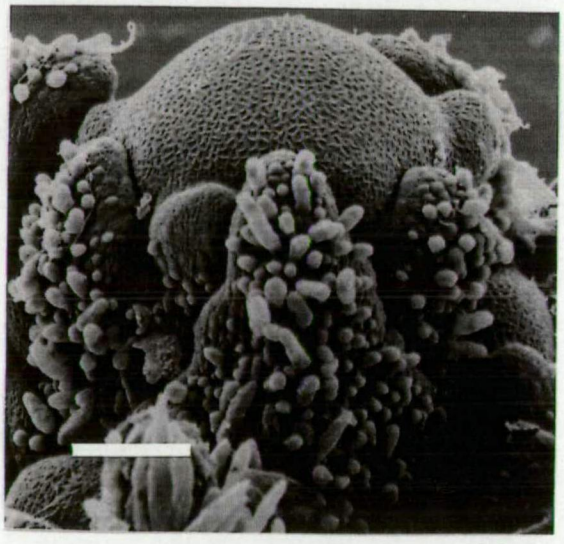


Plate 5 - SEM of meristem at apical stage 2.2. The last leaf primordium and three of the first four bract primordia are visible. Bar = 0.1mm

Plate 6 - SEM of meristem at apical stage 2.2. Note the pronounced doming of the apical meristem. Bar = 0.1mm

Plate 7 - SEM of meristem at apical stage 2.8. The doming is still pronounced and the receptacle is becoming broader. Bar = 0.1mm

Plate 8 - SEM of meristem at apical stage 2.8. View of the same apex as shown in Plate 7, showing the axillary meristems in the leaf axils. Bar = 0.1mm

the rim of the receptacle when the meristem diameter is approximately 500μm, as shown in Plate 9. Apical stages 6,7 and 8 describe the continuing initiation of floret primordia until the entire receptacle is covered, and are associated with discrete ranges of apical diameters in the range 500μm to 2500μm. Plates 10-12 represent typical apical meristems at stages 5, 6 and 7.

Perianth development has begun on almost all the florets on the receptacle in Plate 13. Plate 14 shows florets at the various stages of perianth development as the five perianth initials form on the rim of the floret primordia and extend toward the centre of the primordia, before completely covering the centre of the floret as shown in Plate 15. The abundance of glandular trichomes surrounding the floret can be seen clearly in this micrograph. Plate 16 shows the development of a ray floret near the rim of the receptacle.

Over the period of this study, electron micrographs of meristems dissected from plants grown under the range of conditions described above were examined and the apical stages and diameters recorded. A plot of apical stage against diameter shows the relationship between shoot apex size and inflorescence initiation and development (Figure IV.A.1.3.1.b). For each of the 90 apices measured, no matter the conditions under which the plants were grown, the stage of apical development was associated with a narrow range of apical diameters.

Low night temperature conditions were most conducive to floral initiation and development. Significant doming of apices could be observed approximately 20 days after transfer of plants to these conditions, and the earliest observation of an apex which had initiated an involucral bract was 28 days after transfer. The initiation of the first involucral bract was predicted to occur 25 to 30 days after the imposition of inductive conditions, as the bract primordia take several days to reach a size where they are recognisable as bracts and this point was seen at between 28 and 35 days under optimal inductive conditions. The timing of this stage, apical stage 2, and all the subsequent stages varied according to the environmental conditions under which the plants were grown. Under the most favourable conditions, stage 3 was reached after approximately 38 days, stage 5 after 44 days and stage 10 after 70 days.

Plates 17-20 show the phyllotaxis alterations during floral initiation and development as seen during primordial initiation. Vegetative growth is characterised by leaf arrangement in a spiral phyllotaxis, with the outer margins of the older leaf

Plate 9

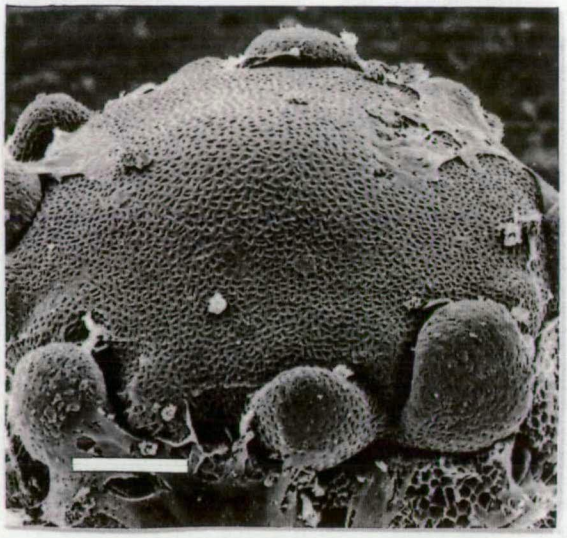


Plate 10

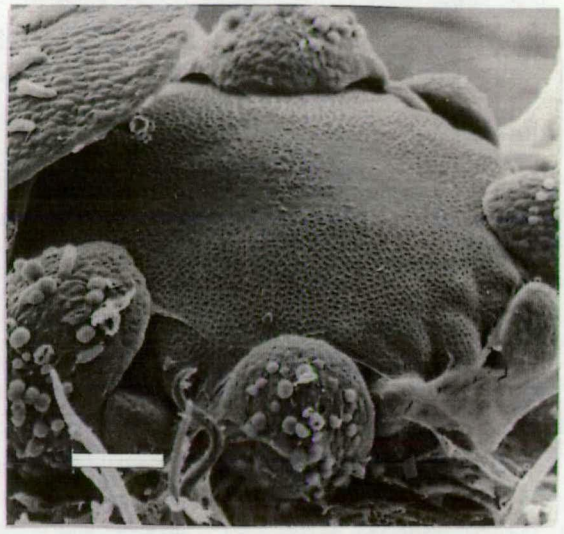


Plate 11

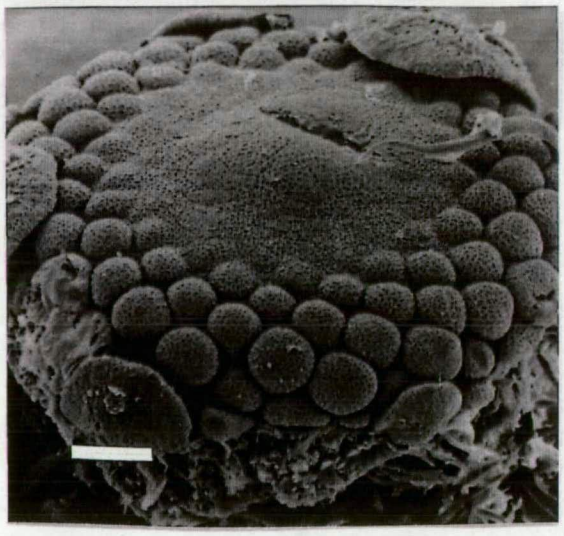


Plate 12

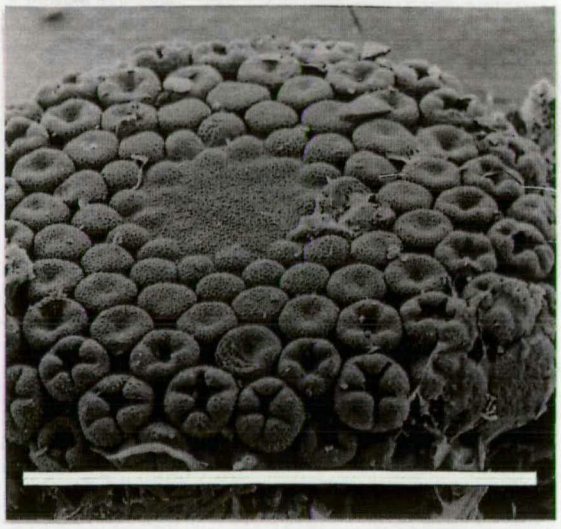


Plate 9 - SEM of meristem at apical stage 5. Note the row of floret primordia forming on the rim of the receptacle, which is beginning to flatten. Bar = 0.1mm

Plate 10 - SEM of meristem at apical stage 5. The second row of floret primordia is visible on the rim of receptacle. Bar = 0.1mm

Plate 11 - SEM of meristem at apical stage 6. Initiation of floret primordia continues towards the centre of the receptacle in a spiral arrangement. Bar = 0.1mm

Plate 12 - SEM of meristem at apical stage 7. Note that the receptacle is covered with floret primordia except at the tip, and that the outer rows of floret primordia have perianth initials. Bar = 1mm

Plate 13

Plate 14

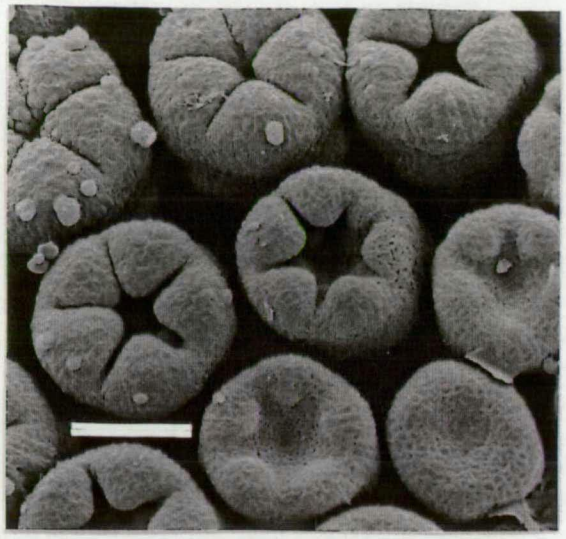


Plate 15

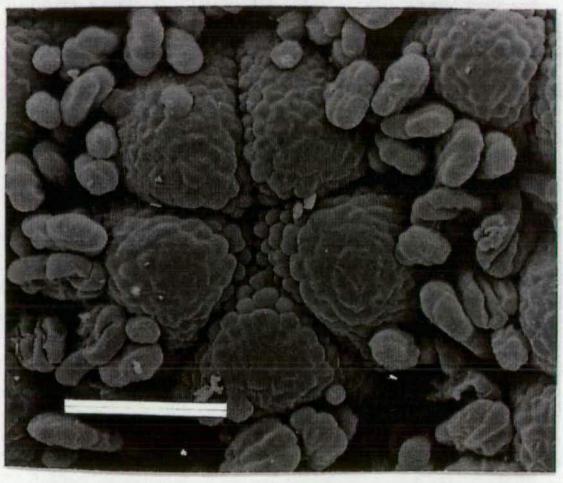


Plate 16

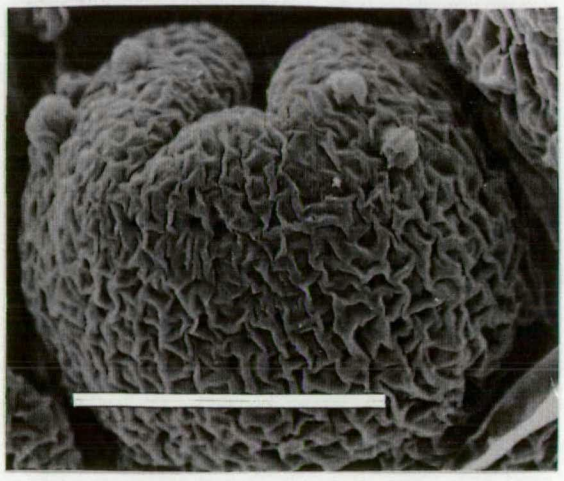


Plate 13 - SEM of meristem at apical stage 9. Several ray floret primordia are visible on the edge of the receptacle, surrounding the disc floret primordia which are at various stages of perianth development. Bar = 1mm

Plate 14 - SEM of disc floret primordia, showing the development of the five perianth initials. Bar = 0.1mm

Plate 15 - SEM of disc floret primordium, showing the five perianth initials which have converged to cover the centre of the primordium. Note the large number of glandular trichomes surrounding the primordium. Bar = 0.1mm

Plate 16 - SEM of a ray floret primordium. Note that only three of the perianth initials develop and merge to form a single ray or stipulate structure. Bar = 0.1mm

Plate 17

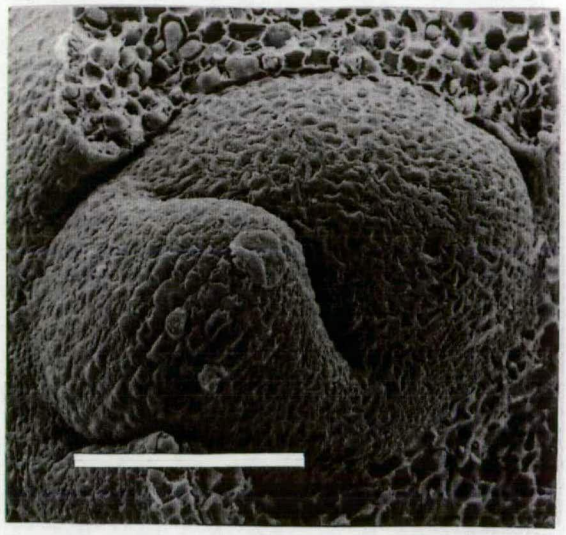


Plate 18

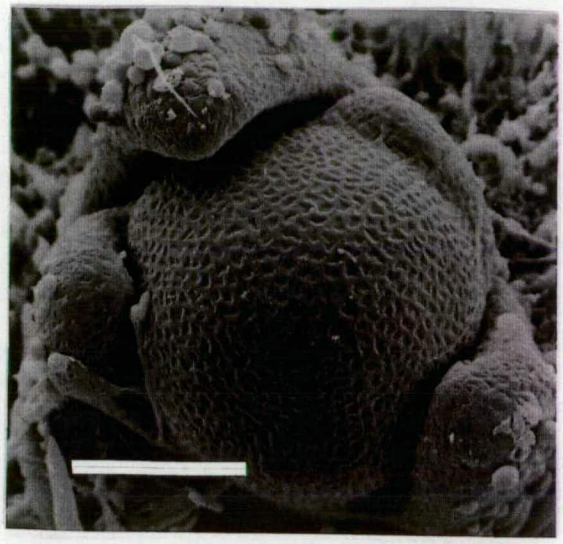


Plate 19

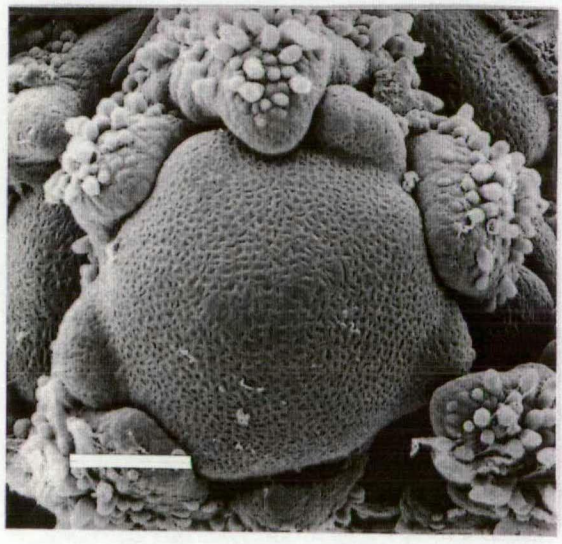


Plate 20

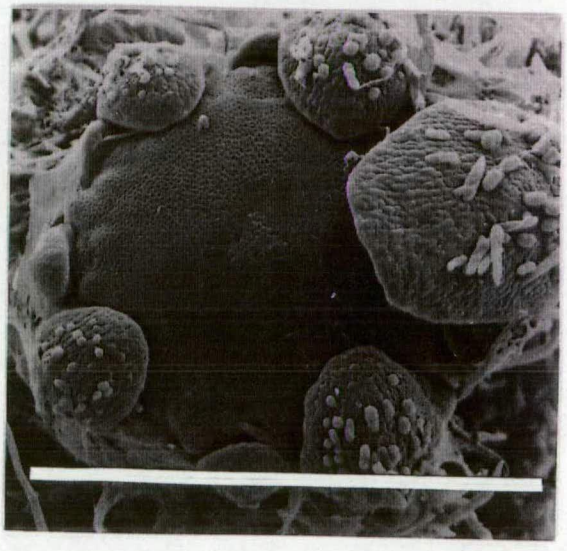


Plate 17 - SEM of vegetative bud, apical stage 1. The second youngest leaf primordia has been removed, but its remains can be seen enclosing the meristem. Bar = 0.1mm

Plate 18 - SEM of meristem at apical stage 2.2. As the meristem enlarges, the angle between successive primordia is reduced and the relative primordia size decreases. Bar = 0.1mm

Plate 19 - SEM of meristem at apical stage 2.8. The angle between primordia has been further reduced. Bar = 0.1mm

Plate 20 - SEM of meristem at apical stage 5. Floret primordia are initiated in rows around the rim of the receptacle. Floret initiation continues towards the centre of the receptacle in a spiral pattern. **Bar = 1mm**

Figure IV.A.1.3.1.b

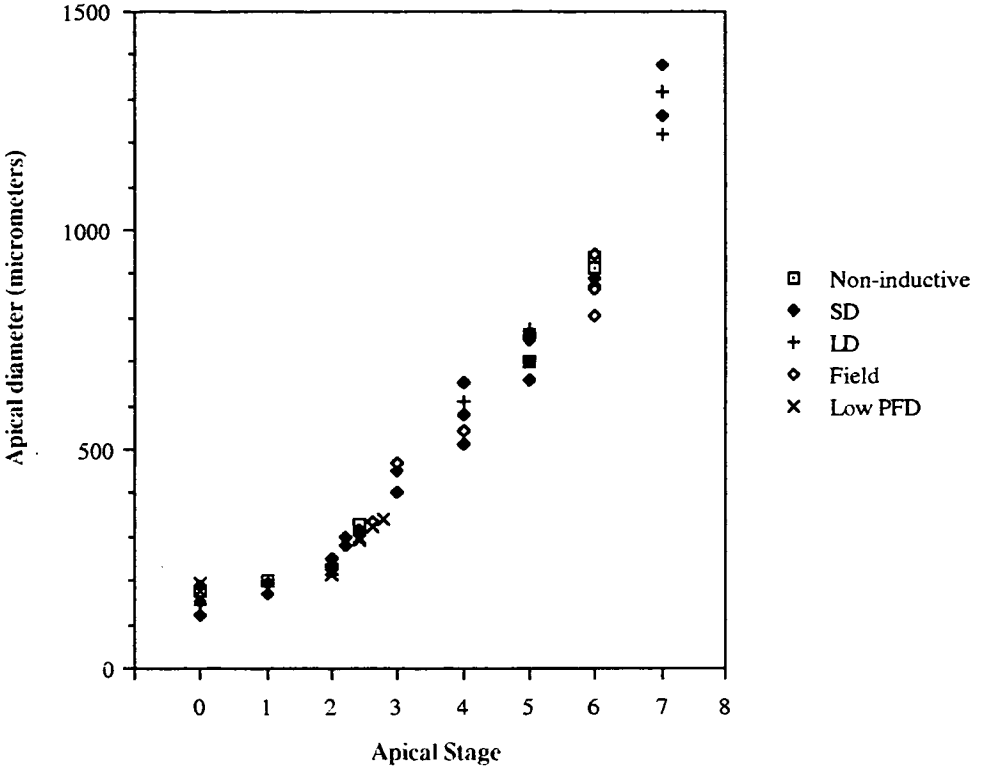


Figure IV.A.1.3.1.b Plot of apical diameter against apical stage

Apical diameter was measured from electron micrographs taken of apices dissected from plants held under the range of conditions listed above. Stage of apical development was established using the scale described in Figure IV.A.1.3.1.a

primordia extending around the stem to almost completely enclose the apical meristem. The spiral phyllotaxis is continued during floral initiation and development but as apical diameter increases the relative size of the primordia decreases, allowing a greater number of primordia to be present on the apical dome. This alteration in phyllotaxis is explained in Charles-Edwards' (1979) model of flowering in chrysanthemum; each primordia is postulated to be the source of production of an inhibitor to primordial initiation, and it is assumed that the initiation of new primordia can only take place when the concentration of the inhibitor drops to below a critical value. The inhibitor is postulated to move by diffusion, and to be degraded at a rate proportional to its concentration, so that the spatial arrangement of primordia is altered as the apical diameter increases. As flower development continues the pastochron duration decreases and so a larger number of primordia are seen around the enlarged receptacle. These primordia may compete with the apical dome for substances moving acropetally to the meristem and so the growth rate of the dome declines and is eventually entirely consumed in the production of floral primordia.

1.3.2 Macromorphological Descriptions

The timing of the macroscopic floral development stages (Head, 1966. see Figure II.2.1.a) was found to vary under different environmental conditions, and under optimal conditions to closely follow those reported by Bhat and Menary (1984). Flower buds first began to open around 65 to 70 days after being transferred to inductive conditions and reached stage II, ray florets vertical and almost perpendicular to the disc florets, around twelve days later.

The first macromorphological sign of flower initiation is the elongation of the internodes below the meristem. This event can precede the end of evocation, the point at which the meristem is irreversibly committed to generative growth, as stem elongation can occur without evocation being completed. Meristems examined at the first sign of stem elongation were at apical stages 1 or 2, indicating that apices may revert to vegetative growth up until the time that the first bract primordium is initiated.

The earliest observation of stem elongation was 25 days after plants were transferred to inductive conditions, corresponding to the earliest predicted time of initiation of the first bract primordia. These events may not be related as apices at stages

2 and 2.2 were dissected from plants in low PFD or high temperature conditions before any signs of stem elongation were observed. Similarly, axillary buds on flower stems may contain meristems which have reached apical stage 2 or above before internode elongation begins.

After approximately 50 to 55 days the flower bud, consisting of a large number of involucral bracts covering the receptacle, becomes visible as all the leaf primordia surrounding the meristem at the time of initiation expand and develop. The apex at this point is generally at stage 7 or 8 and the flower stem is typically around 10-15cm in height. By the time the flower bud begins to open, the meristem has reached apical stage 11, although under certain situations such as water stress or low PFD, the florets at the centre of the receptacle may have aborted.

1.3.3 Abnormal Flower Development

Under optimal inductive conditions, the flower stem will reach a height of between 60 and 80cm. However, under marginally inductive conditions, flowers may be borne on flower stems significantly shorter than this, typically around 30 to 40cm. Flower stem heights are not restricted to these two discrete ranges but vary between 30 and 80cm. However the percentage of stems between 40 and 60cm in height is small. It appears that the normal flower development sequence includes a period of rapid flower stem elongation, but that this event is not an essential part of the sequence. Under conditions not favourable to this event, flower development can proceed with reduced stem elongation.

As has already been mentioned, the elongation of internodes below the meristem may precede the end of evocation. Vegetative, rosette growth was observed after a short period of stem extension in plants which were transferred from inductive to 'non-inductive' conditions before floral evocation was completed. This return to vegetative growth was never observed after the first bract primordia was initiated by the meristem, as no bracts or leaves with entire margins were observed as part of one of these vegetative shoots. This indicates that the initiation of the first bract primordia marks the irreversible commitment to floral development by the apex.

When plants are transferred to non-inductive conditions after the completion of floral evocation, the further development of the floral meristem may be arrested. The apex may remain at this arrested stage of development or may abort. If the flower bud

Plate 21

Plate 22

Plate 21 - Flowering behaviour of plants in inductive (left) and 'non-inductive' (right) conditions.

Plate 22 - Flower stem initiated under 'non-inductive' conditions through an autonomous induction process. The developing flower stem has arisen from the vegetative, rosette shoot developing in one of the leaf axils on the original flower stem whose flower bud has aborted along with the flower buds developing from the uppermost two leaf axils, as can be seen to the left of the developing flower stem.

does not abort, it is possible to continue its generative development by transferring the plant back to inductive conditions. When the plant is kept in non-inductive conditions, the meristems in the leaf axils on the flower stem may begin to actively divide in the vegetative growth pattern producing rosette, vegetative shoots on the flower stem (see Plate 22). Further flower stems may initiate from these shoots if the plant is subsequently transferred to inductive conditions, as shown by Plates 21 and 22. Flowers initiated under non-inductive conditions through an autonomous induction process generally do not fully develop, and follow this growth pattern.

1.3.4 Autonomous Induction

The process by which many plants, even when grown continuously in 'non-inductive' conditions, eventually initiate flowers is termed autonomous induction. All they require is that environmental conditions be conducive for growth (Bernier, 1988).

A period of vernalisation, or low night temperature, was found to be the major environmental requirement for rapid flower initiation in pyrethrum. Night temperatures of 18 °C or greater were considered non-inductive, and rapid flower initiation was never observed with this treatment irrespective of the prevalent daylength, photon flux density and day temperature (in the range 15 °C - 30 °C) conditions. However, in the population held in the evaporatively cooled glasshouse at the HRC under 'non-inductive' conditions, a small percentage of plants was observed to initiate flower buds each year. The number of plants flowering varied throughout the year, with the greatest frequency in late spring and autumn when the temperatures were moderate and the daylength extended. An autonomous induction process was thought to control flowering under these conditions, with the frequency of flowering being greatest when growth rate was at its maximum.

1.4 Discussion

During vegetative development, the apical meristems of the shoots of higher plants initiate successive primordial units composed of a leaf and its associated stem internode and axillary meristem. The leaf primordia produced by the vegetative meristem of pyrethrum are initiated in a spiral arrangement. The stem internodes produced during vegetative development are particularly short, resulting in a rosette

growth habit. The phase change produced by floral initiation terminates this vegetative development pattern and is followed by initiation of the various primordial units associated with generative development. Doming of the apical meristem and the subsequent formation of an enlarged, flattened receptacle causes a spatial rearrangement of primordial organisation and so the phyllotactic pattern is altered.

The pattern of apical development during the flowering of pyrethrum is very similar to that described for *D. grandiflora* (Horridge and Cockshull, 1979), and a modified scale of apical development has been proposed (Figure IV.A.1.3.1.a). As for *D. grandiflora*, the size of the apical dome at the point of floral initiation falls into a narrow range of apical diameters, and each subsequent stage of inflorescence development is likewise associated with a characteristic range of apical diameters. This relationship was observed under a range of environmental conditions, including flower buds formed in 'non-inductive' conditions through an autonomous induction process. This correlation between apical size and development allows treatment effects on flowering in pyrethrum to be assessed by using the diameter of the apical dome as a quantifiable criterion.

The first macromorphological sign of flower initiation, the elongation of internodes below the evoked meristem, occurs at around the time of floral determination and as such is a valuable, non-destructive observation for assessing treatment effects on flower initiation. Stem elongation may, however, precede the end of floral evocation, necessitating the further observation of plants to ensure that flower initiation has occurred. Stem elongation cannot be used to gauge the further progress of floral development as examination of abnormal, shortened flowering stems indicates that there is little correlation between the later stages of floral development and stem height.

The lack of correlation between stem elongation and flower development suggests that the two processes are separate. Stem elongation was observed to proceed without flower initiation, but no examples of flower initiation and development were observed without stem elongation. Thus there appears to be some link between stem elongation and floral evocation, with internode elongation either being one of the necessary partial processes of evocation or an indirect consequence of one of these processes. The development of shortened flower stems under marginally inductive conditions suggests that the physiological processes mediating internode elongation, either causal to flower initiation or a consequence of evocational processes, are sensitive to environmental conditions and may be modified by environmental stimuli.

The arrested development of flower buds under 'non-inductive' conditions is similar to the 'crown' or 'break' bud condition described for *D. grandiflora*. However, while the arrested bud may be surrounded by vegetative lateral shoots, the buds in pyrethrum were never observed to have enlarged involucral bracts. The buds were more likely to abort than to remain in an arrested state of development, suggesting that they had lost the ability to compete successfully for photosynthetic assimilates. The arrested state may simply represent a meristem at a normal development stage which cannot develop further as the assimilate supply has been partitioned to other plant parts. Thus returning the plant to inductive conditions, or conditions which promote sufficient assimilate partitioning to the developing floral apices, may result in the recommencement of normal development provided that the bud has not aborted.

The role of assimilates in the flowering of pyrethrum is further implicated by the relationship between apical size and stage of development. As each stage of development is characterised by a narrow range of apical sizes, the timing of the stages must be related to the assimilate supply to the developing apices. The question which must be answered is whether the supply of assimilates to the apex is causal to the evocational and developmental processes or a consequence of these processes.

IV.B. Environmental Effects

IV.B.1 Juvenility and Meristem Competence

1.1 Introduction

Many species display a period of juvenile growth where they continue to develop vegetatively even when held in normally inductive conditions. Similar periods of unresponsiveness have also been noted for cuttings taken from mature plants. Preliminary experiments indicated that a juvenile-like condition exists in pyrethrum material regenerated through tissue culture. Observation of field planted tissue cultured material and vegetatively propagated plant segments or splits also revealed a difference in the timing of floral initiation between different sized plants, possibly due to a lack of meristematic competence to flower in smaller plants. These observations point to a minimum size or physiological age requirement for plant material to respond to inductive conditions.

1.2 Materials and Methods

Separate meristem competence experiments were completed using tissue culture material, splits and seedlings. CIG 3 plants regenerated through tissue culture were transferred from the 'Speedling' trays in which they were propagated into 15cm pots when they had on average 3 to 4 expanded leaves, and moved onto one of the light tunnel trolleys at the HRC under 12 hr daylength and 18 °C night temperature.

Mature CIG 3 plants were vegetatively divided to provide splits with between 4 and 6 mature, expanded leaves and no developing lateral shoots. These splits were planted in 15cm pots and moved to the light tunnel trolley set as described above.

Seeds collected from a CIG 3 x CIG 11 cross were germinated in standard potting mix on a heated mistbed. The seedlings were transferred to individual 5cm pots when the first leaf was fully expanded, and moved to the light tunnel as described above.

Groups of plants were transferred, weekly in the case of tissue culture material and splits or monthly for seedlings, from the 18 °C / 12 hr light tunnel to a light tunnel

set at 6 °C night temperature and 12 hr daylength. Each date of transfer was considered a separate treatment as the plants were of different chronological as well as physiological ages when placed under inductive conditions. Five tissue culture explants, eight or more seedlings and four splits were transferred at the appropriate times. Initially larger numbers of seedlings and splits were transferred to allow for the loss of some plants, with the remainder being reduced to the desired number by selection based on physiological uniformity. Likewise the number of plants held in the 12 hr / 18 °C light tunnel was reduced by discarding plants which failed to establish or grew poorly. This procedure ensured some degree of uniformity within the somewhat variable split and seedling populations.

Upon transfer to the inductive 12 hr / 6 °C conditions the number of mature leaves and the number of developing shoots, including the terminal shoot bud, were recorded for each plant. At the first macromorphological sign of flower initiation, flower stem elongation, the number of mature leaves and the number of developing shoots were again recorded along with the position of the flowering shoot (from Figure III.3) and the number of days to initiation. If the flowering shoot was found to be tertiary, the total shoot number and the number of secondary shoots were recorded as well as the position(s) of the secondary shoot(s) from which the tertiary shoot(s) developed. The results are tabulated in Appendices IV.B.1.a (tissue culture explants), IV.B.1.b (splits) and IV.B.1.c (seedlings).

Mature leaf samples were taken monthly during the course of the seedling experiment for radioimmunoassay analysis of plant hormone levels. The RIA procedure is described in Section III.5. A representative plant was selected from each treatment (each date of transfer from non-inductive 12 hr / 18 °C conditions to inductive 12 hr / 6 °C conditions was considered a separate treatment) and the mature, expanded leaves were removed for analysis. Sampling did not commence until month three (March) as the plants were too small to yield an assayable sample before this time. To accommodate the loss of plants for RIA sampling each month, the number of plants transferred to inductive conditions was large enough so that eight or more plants from each treatment remained from which to record the flowering data. The endogenous hormone levels measured through radioimmunoassay during the seedling experiment are tabulated in appendices IV.B.1.d-i.

1.3 Results

1.3.1 Tissue Cultured Material

The aim of this experiment was to establish if plant material generated through tissue culture displays a period of juvenile-like behaviour and, if so, to determine the physical limitations which may be regulating this behaviour. The experimental design was such that the point at which the plants reached meristem competence could be determined and the leaf and shoot number at this point counted. Assuming that the plant's rate of development towards competence was similar in the inductive and the non-inductive conditions, it was expected that all plants would flower at approximately the same time, irrespective of the date of transfer, up until the time that the plants held in the non-inductive conditions became competent to flower. From then on, all plants were predicted to flower at a constant time after transfer to the inductive conditions, and the total age of the plants from the commencement of the experiment to the point of flower initiation should increase in weekly increments.

The results are shown graphically in Figures IV.B.1.3.1.a, IV.B.1.3.1.b and IV.B.1.3.1.c. As expected, the trend in Figure IV.B.1.3.1.a is for a short period where the time of initiation (total days to initiation) does not change irrespective of the time of transfer from non-inductive to inductive conditions, followed by an increase in the time of initiation as the week of transfer was increased. The plants appear to have lost their juvenile characteristics after two weeks in the non-inductive conditions. Thus all plants transferred to inductive conditions in weeks 0, 1 and 2 initiated flowers at approximately the same time as each of the plants reached meristem competence when in the inductive conditions. After two weeks, plants were no longer juvenile when transferred to the inductive conditions and therefore initiated flowers after a given period of time in the inductive conditions, as shown in Figure IV.B.1.3.1.a by the fixed number of days under vernalising conditions before initiating flowers. This fixed number of days to initiation for mature, tissue cultured pyrethrum plants is between 30 and 35 days.

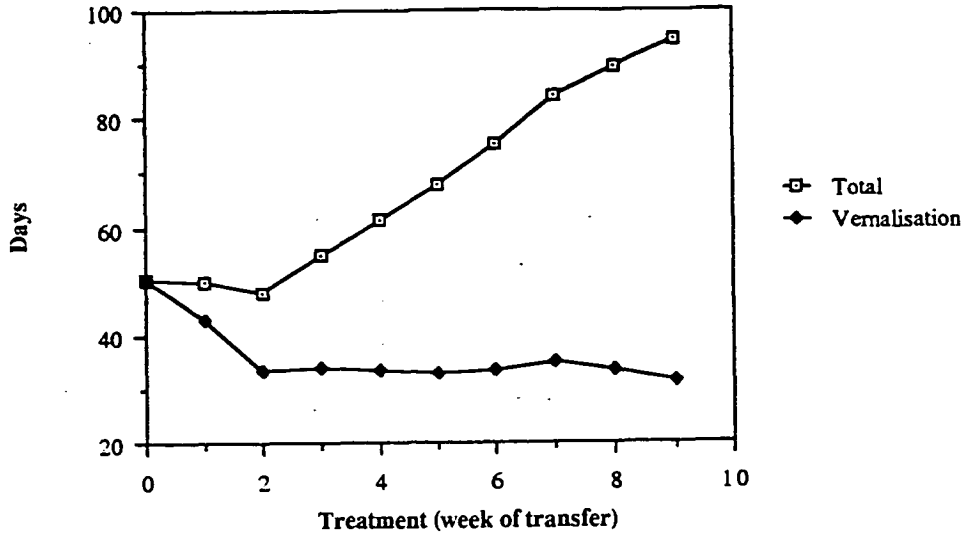
Two weeks after the experiment commenced, the plants had an average of 9.8 mature leaves and 2.8 developing shoots. At the time of flower initiation, when stem elongation was first observed, all the plants transferred to the inductive conditions in the juvenile state had on average 31 mature leaves and a total of around 6 developing

Figure IV.B.1.3.1.a - Total number of days between the commencement of the experiment and flower initiation (Total), and the number of days spent under vernalising conditions (Vernalisation). Treatment refers to the time that the plants were held under non-inductive conditions before being transferred to the inductive (vernalising) conditions.

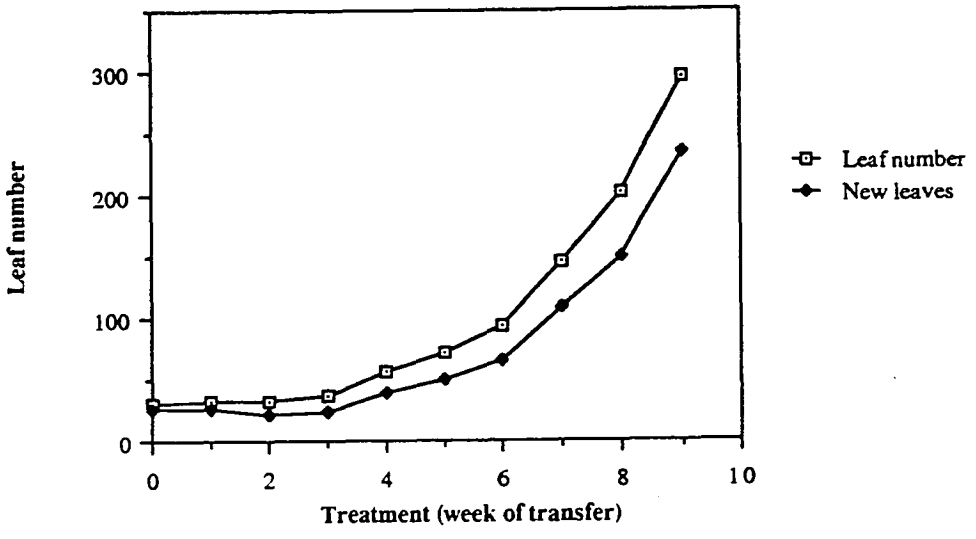
Figure IV.B.1.3.1.b - Number of mature leaves at the time of flower initiation (Leaf number) and the number of leaves which had fully expanded between the time that the plants were moved to the inductive conditions and flower initiation (New leaves).

Figure IV.B.1.3.1.c - Number of developing shoots at the time of flower initiation (Total shoots), and the number of secondary shoots (Secondary) and the position of the secondary shoot which first initiated flower stem elongation (Position).

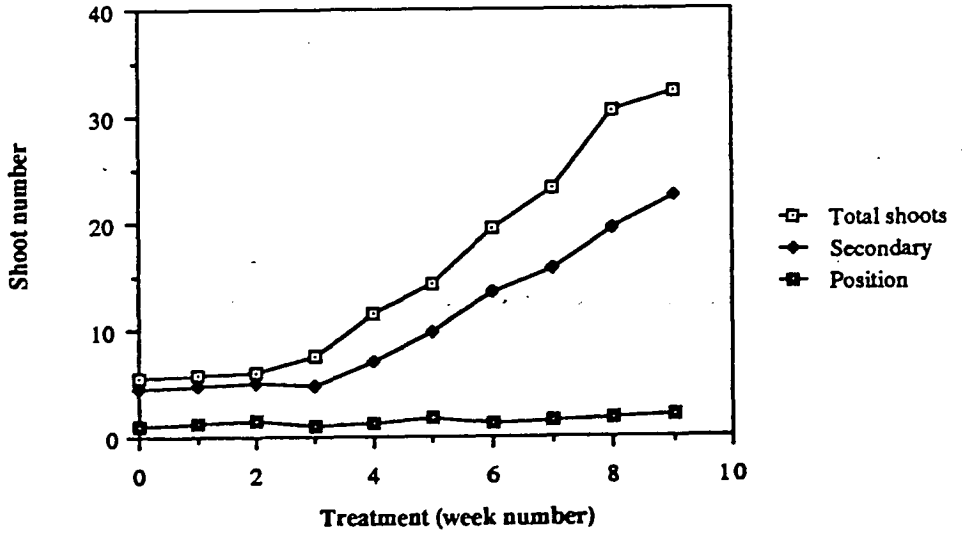
IV.B.1.3.1.a



IV.B.1.3.1.b



IV.B.1.3.1.c



shoots, this number consisting of five developing lateral shoots and the main shoot apex.

At the time of flower initiation, the number of leaves on plants which were transferred when juvenile (weeks 0, 1 and 2) was the same irrespective of the date of transfer, as shown in Figure IV.B.1.3.1.b. The number of leaves increased with increasing weeks of transfer after two weeks. The plants became competent to respond to the inductive conditions after two weeks and so the longer the plants were kept in the non-inductive conditions after this time the greater the number of leaves they initiated before flowering. The number of leaves initiated by the plants when held in the inductive conditions displayed a similar trend to that of the total leaf number. However, rather than a constant leaf number for the first two weeks the number of new leaves decreased. This decrease corresponds to the decreased period of time spent in the inductive conditions by the plants. The number of leaves produced by the plant under inductive conditions does not directly influence the flowering process, indicating that the end of the juvenile phase is associated with the attainment of a certain size or physiological age rather than a result of a change in growth rate.

The observations of the number of developing shoots at the point of flower initiation supports this conclusion. Figure IV.B.1.3.1.c reveals the regular shoot number at flower initiation of the plants transferred to inductive conditions while still in the juvenile state. The number of shoots increases with increasing time spent by the mature plants in the non-inductive conditions before transfer to the inductive conditions. Tertiary shoots also begin to develop during this period of vegetative growth, as seen by the increasing difference between total shoot number and secondary shoot number. The plants become competent to respond to inductive conditions before any tertiary shoots develop as all the juvenile plants transferred to inductive conditions lacked tertiary shoots at the time of flower initiation.

The most interesting measurement from this experiment was the position on the main shoot axis from which the flower stems were initiated. The secondary shoots which initiated floral development (including those shoots where the actual flower stem was tertiary) were always in the most basal or second most basal positions. This was true of plants transferred to the inductive conditions in both the juvenile and the mature states. The terminal shoot apex was never observed to initiate flowers, and always continued to grow vegetatively. Likewise, the terminal apex of the secondary lateral shoots on which tertiary shoots were developing did not flower. The meristem in the

basal leaf axil, position 1 of the numbering scheme outlined in Section III.3, is the first to be released from apical dominance as the plant develops, and it appears that the attainment of the mature state is linked to the development of the first lateral released from apical dominance. The meristem may not necessarily be competent to flower as soon as it is released from apical dominance, as plants which were transferred to inductive conditions while still juvenile often had one or two developing secondary shoots but still displayed a delay in flower initiation compared with mature plants. Meristematic competence is thus obtained after a certain period of development upon release from apical dominance, but is lost again by the shoot apex at the point where tertiary shoots begin to develop.

1.3.2 Splits

The results obtained using the vegetatively divided pyrethrum splits corroborate the findings of the tissue cultured material experiment. The variability within the results is, however, greater due to the lack of uniformity within the plant population as a result of the poor establishment and early growth of many of the splits, and the leaf abscision associated with this problem.

The juvenile-like phase for the splits lasted for between four and five weeks from the beginning of the experiment. Figure IV.B.1.3.2.a shows that the first five treatments, plants transferred to the inductive conditions on weeks 0 to 4, all initiated flowers at the same time, while plants transferred after four weeks displayed an increase in the time of initiation as the time spent in the non-inductive conditions was increased (later weeks of transfer). All plants transferred after four weeks spent similar periods of time, around 35 days, in the inductive conditions before flowering.

Figure IV.B.1.3.2.b reveals a similar trend to that of the tissue cultured plants in the number of leaves at flower initiation. Similar leaf numbers were observed for the first five treatments followed by steadily increasing leaf numbers for plants which were transferred to the inductive conditions from five weeks after the commencement of the experiment and onwards. The number of mature leaves formed while the plants were in the inductive conditions showed a decrease over the first five treatments (weeks 0 to 4) before rising rapidly with increasing week of transfer after four weeks.

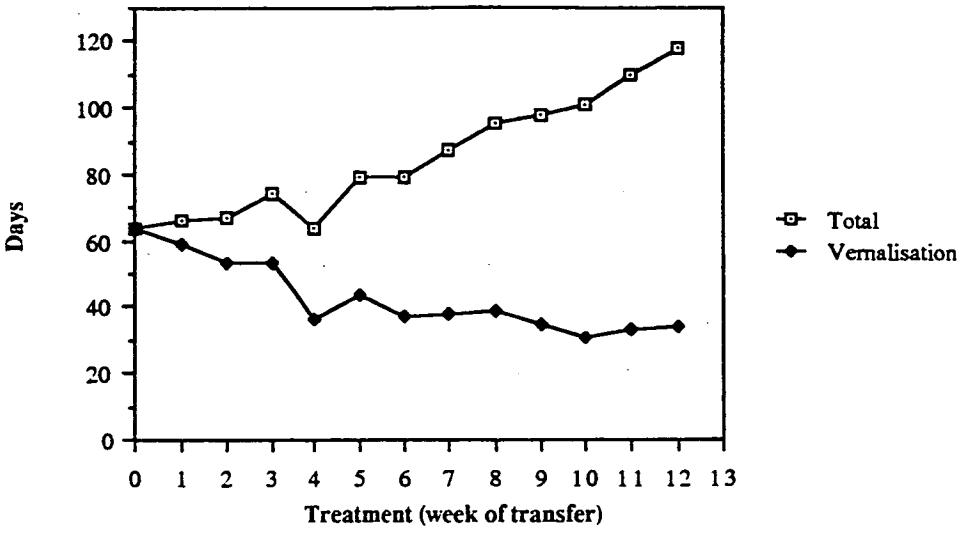
The position of the flowering meristem was again found to be the most basal developing shoot, either in the first or the second leaf axil. The average number of

Figure IV.B.1.3.2.a - Total number of days between the commencement of the experiment and flower initiation (Total), and the number of days spent under vernalising conditions (Vernalisation). Treatment refers to the time that the plants were held under non-inductive conditions before being transferred to the inductive (vernalising) conditions.

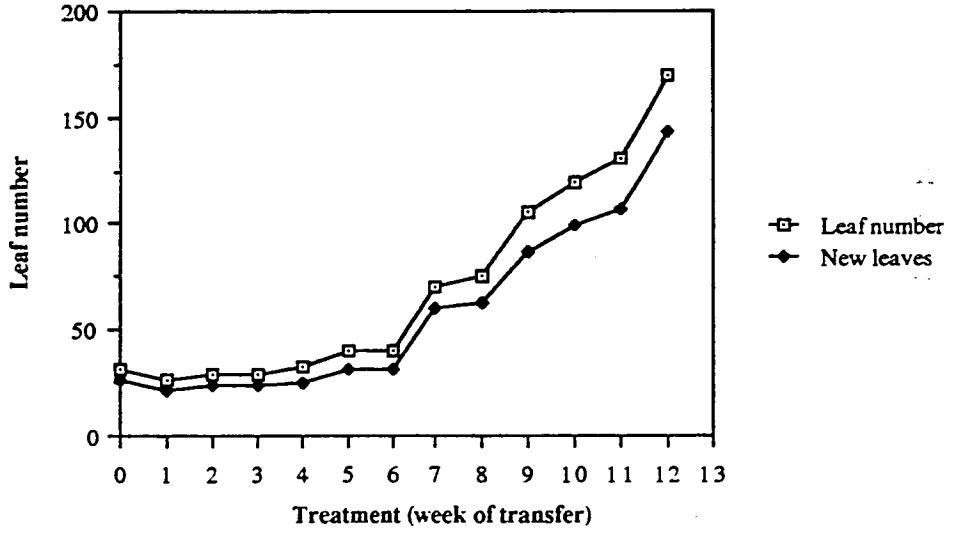
Figure IV.B.1.3.2.b - Number of mature leaves at the time of flower initiation (Leaf number) and the number of leaves which had fully expanded between the time that the plants were moved to the inductive conditions and flower initiation (New leaves).

Figure IV.B.1.3.2.c - Number of developing shoots at the time of flower initiation (Total shoots), and the number of secondary shoots (Secondary) and the position of the secondary shoot which first initiated flower stem elongation (Position).

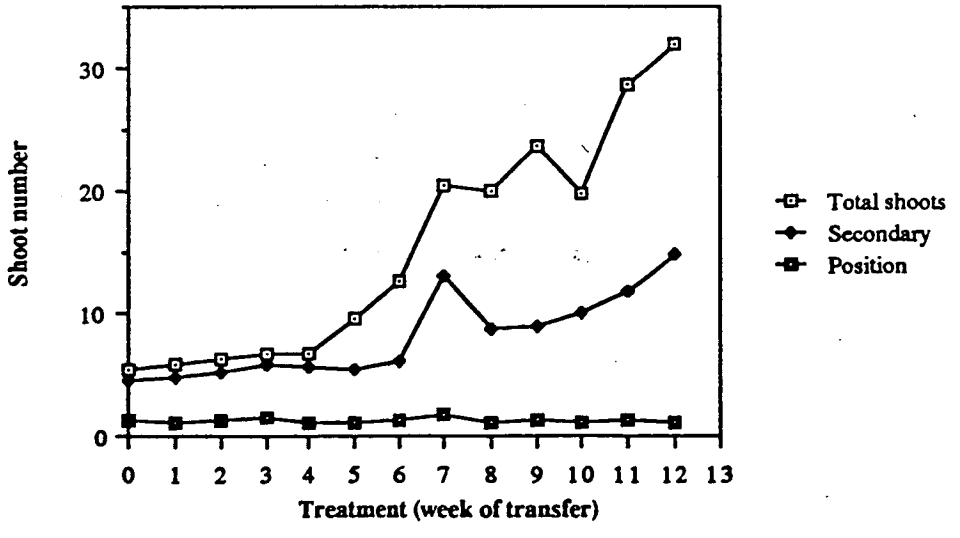
IV.B.1.3.2.a



IV.B.1.3.2.b



IV.B.1.3.2.c



shoots developing at the time of flower initiation, as shown in Figure IV.B.1.3.2.c, varied between 5.5 and 6.8 for plants which were transferred to the inductive conditions while still in the juvenile-like state but increased for older, mature plants. Mature plants also tended to have developing tertiary shoots at flower initiation while only secondary shoots were present on plants which were in the juvenile-like state when transferred.

At the phase change between the juvenile-like and mature conditions, after being held for four weeks in the inductive conditions, the plants had an average of 7.5 mature leaves and 2.8 developing shoots. The number of leaves was less than observed for the tissue cultured plants, possibly because of abscission of the mature leaves during the establishment of the split material in the pots. Meristem competence, however, again appeared to be related to the release of the lateral shoots from apical dominance.

1.3.3 Seedlings

The seedlings exhibited a much longer chronological period of juvenile growth than either the tissue cultured or split plants. This was at least partially due to the extremely slow growth rate of the seedlings, as the number of leaves and developing shoots at the point of initiation were similar to those of the earlier experiments. The small size of the seedlings when they were potted may have contributed to their slow early growth rate.

The trends shown in Figure IV.B.1.3.3.a for flowering time and time in the inductive conditions are the same as those described earlier for the tissue cultured plants and for splits, but the timing differs as discussed above. The seedlings appear to have attained the mature state between six and seven months after the experiment commenced. At this point the number of days required in inductive conditions before flower initiation becomes steady at around 40 days while the total time from the commencement of the experiment to flower initiation begins to increase.

Until the plants reached maturity, the number of mature leaves at flower initiation varied only slightly between 18 and 25, and the corresponding number of leaves formed during the time spent in the inductive conditions decreased with the plant's age at transfer, as seen in Figure IV.B.1.3.3.b.

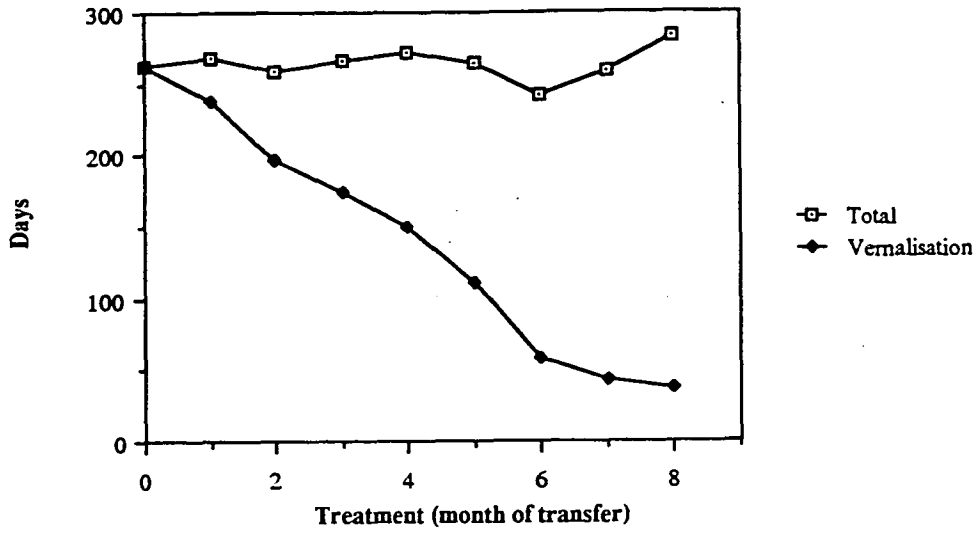
Figure IV.B.1.3.3.c indicates that meristem competence is associated with the

Figure IV.B.1.3.3.a - Total number of days between the commencement of the experiment and flower initiation (Total), and the number of days spent under vernalising conditions (Vernalisation). Treatment refers to the time that the plants were held under non-inductive conditions before being transferred to the inductive (vernalising) conditions.

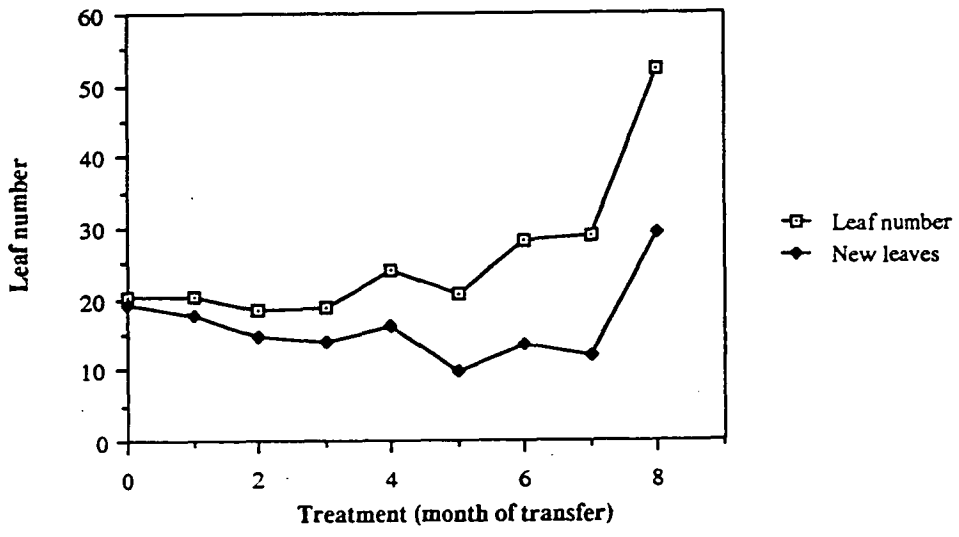
Figure IV.B.1.3.3.b - Number of mature leaves at the time of flower initiation (Leaf number) and the number of leaves which had fully expanded between the time that the plants were moved to the inductive conditions and flower initiation (New leaves).

Figure IV.B.1.3.3.c - Number of developing shoots at the time of flower initiation (Total shoots), and the number of secondary shoots (Secondary) and the position of the secondary shoot which first initiated flower stem elongation (Position).

IV.B.1.3.3.a



IV.B.1.3.3.b



IV.B.1.3.3.c

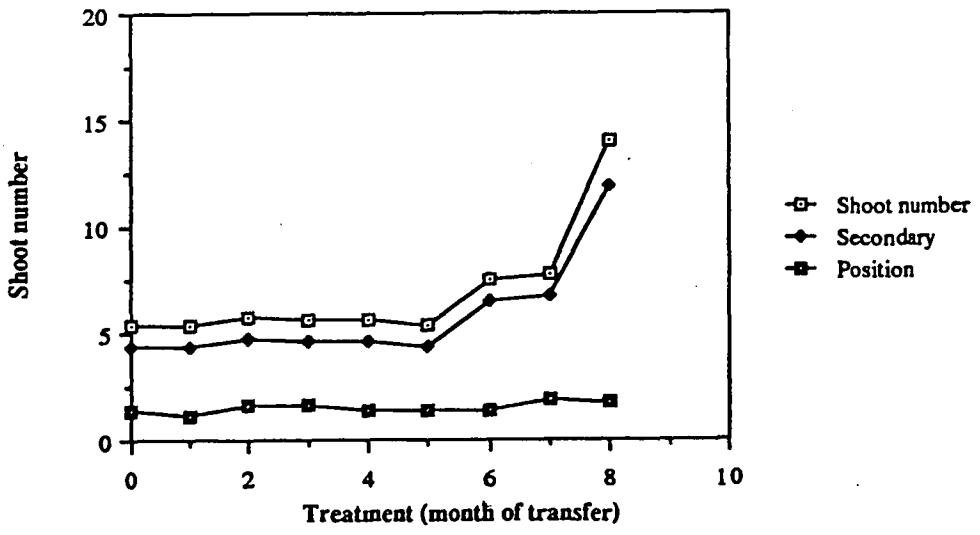


Figure IV.B.1.3.3.d

	Tissue culture		Splits		Seedlings	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Flowering time	50.89	5.46	71.61	21.43	261.81	20.85
Leaf number	31.17	3.40	30.25	5.46	21.33	6.86
Shoot number	5.81	0.54	6.33	1.05	6.53	1.23
Flowering position	1.22	0.43	1.21	0.42	1.38	0.49

Figure IV.B.1.3.3.d - Table of means and standard deviations of time from the commencement of the experiment to flower initiation (Flowering time), the number of mature leaves (Leaf number) and shoots (Shoot number) at the time of flower initiation, and the position of the first secondary shoot to initiate flower stem elongation (Flowering position) for all plants which were juvenile when transferred to inductive conditions in each of the three experiments.

release of laterals from apical dominance as the position of the flowering stem is always near the base of the plant, in the axil of one of the lowest three leaves. When the plants reached maturity in inductive conditions, the number of developing shoots at flower initiation is around 5.5, and increases for plants which have reached maturity in non-inductive conditions before being transferred to inductive conditions. Tertiary shoots also begin developing soon after the mature state is reached.

Figure IV.B.1.3.3.d shows the means and standard deviations of the measurements taken at flower initiation from each of the three experiments for all plants which were juvenile when transferred to the inductive conditions. The mean time from the beginning of the experiment to flower initiation differs markedly between the three experiments, and also between plants within each experiment, as indicated by the large values for the standard deviation, a measure of variance. The difference between experiments may be due to an inherent difference in the juvenile characteristics of the three plant types, or simply due to experimental differences such as the slow growth rate of the seedlings after transplantation. The variation within each experimental population indicates that chronological age is not a critical determinant in plants attaining the mature state, but that other factors associated with the plant reaching a certain size or physiological age control the process. The number of developing shoots at flower initiation and the position of the first flowering stem are similar for all three plant types, supporting the hypothesis that meristem competence is linked to the release of lateral shoots from apical dominance. The low leaf number of the seedlings at this point may be explained by leaf abscission due to overwatering of the plants, but the possibility exists that plant development differs between seedlings and splits and tissue cultured plants, enabling the seedlings to lose apical dominance after initiating fewer leaves than the other plants.

1.3.4 Endogenous Hormonal Changes

The gibberellin content of the mature leaf tissue shows a decline with ageing of the plant material in the non-inductive conditions (control treatment), and this may be related to the phase change between the juvenile and the mature states. The transfer of plants to the inductive conditions always resulted in an increase in the GA concentration although the increase was less for plants transferred at later dates. The GA concentration decreased with time after the plants were transferred but generally

Figure IV.B.1.3.4.a - Endogenous gibberellin concentrations in mature leaf samples from seedlings. Control plants were maintained in non-inductive conditions while plants under inductive conditions were moved on the dates indicated. Complete results are tabulated in appendix IV.B.1.d.

Figure IV.B.1.3.4.b - Endogenous indolylacetic acid concentrations in mature leaf samples from seedlings. Control plants were maintained in non-inductive conditions while plants under inductive conditions were moved on the dates indicated. Complete results are tabulated in appendix IV.B.1.e.

Figure IV.B.1.3.4.c - Endogenous abscisic acid concentrations in mature leaf samples from seedlings. Control plants were maintained in non-inductive conditions while plants under inductive conditions were moved on the dates indicated. Complete results are tabulated in appendix IV.B.1.f.

Figure IV.B.1.3.4.a

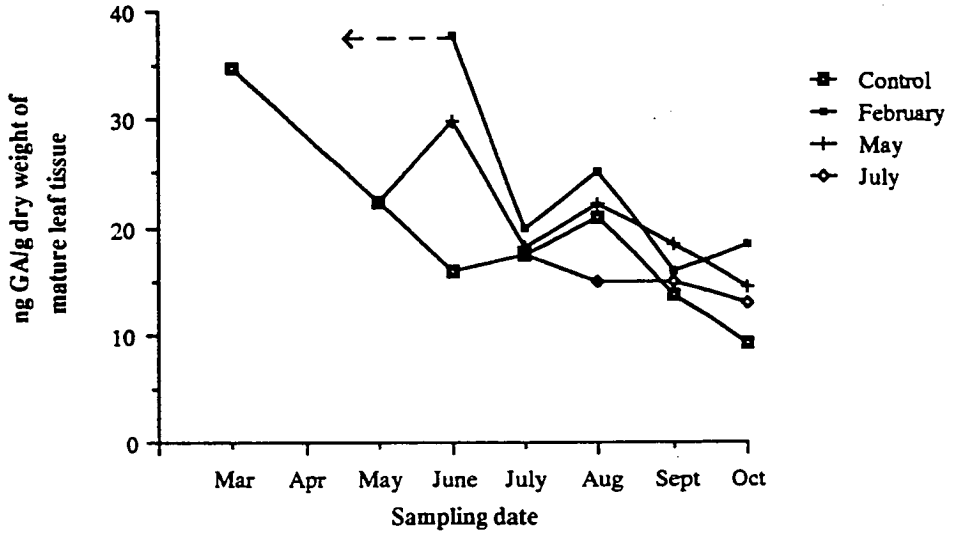


Figure IV.B.1.3.4.b

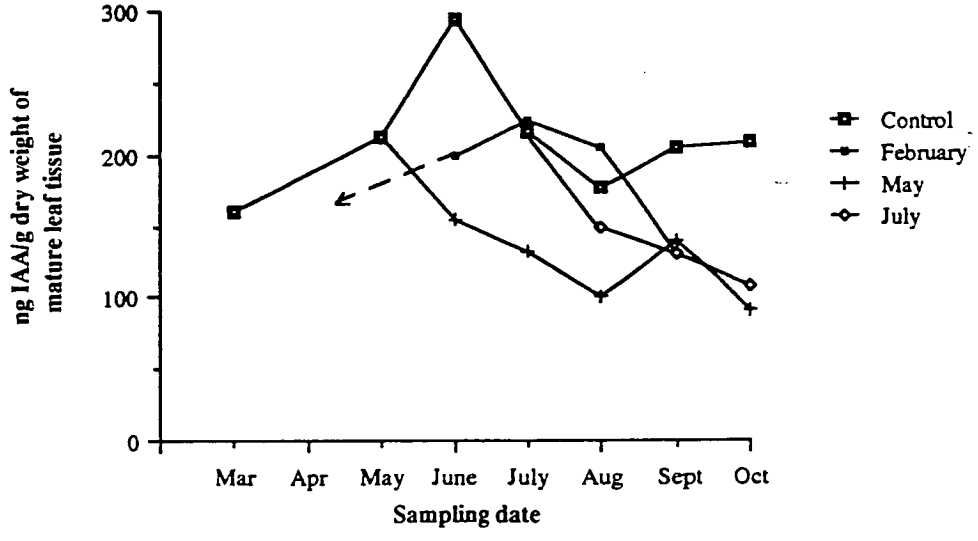


Figure IV.B.1.3.4.c

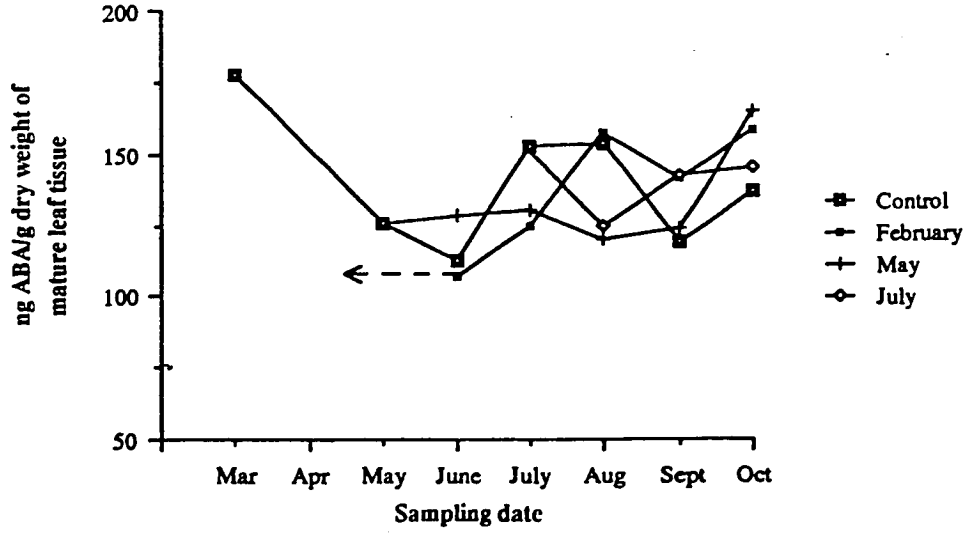


Figure IV.B.1.3.4.d - Endogenous zeatin concentrations in mature leaf samples from seedlings. Control plants were maintained in non-inductive conditions while plants under inductive conditions were moved on the dates indicated. Complete results are tabulated in appendix IV.B.1.g.

Figure IV.B.1.3.4.e - Endogenous dihydrozeatin concentrations in mature leaf samples from seedlings. Control plants were maintained in non-inductive conditions while plants under inductive conditions were moved on the dates indicated. Complete results are tabulated in appendix IV.B.1.h.

Figure IV.B.1.3.4.f - Endogenous isopentyladenosine concentrations in mature leaf samples from seedlings. Control plants were maintained in non-inductive conditions while plants under inductive conditions were moved on the dates indicated. Complete results are tabulated in appendix IV.B.1.i.

Figure IV.B.1.3.4.d

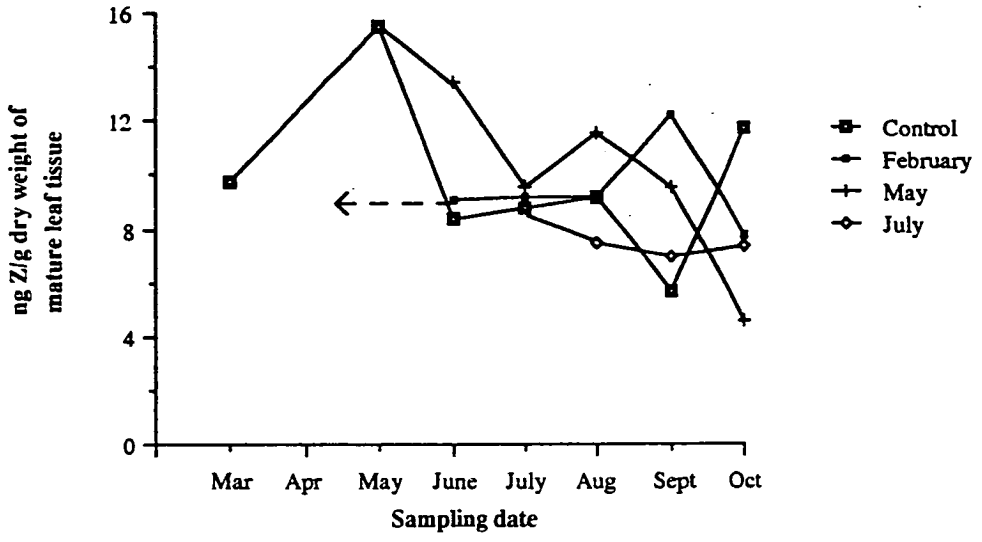


Figure IV.B.1.3.4.e

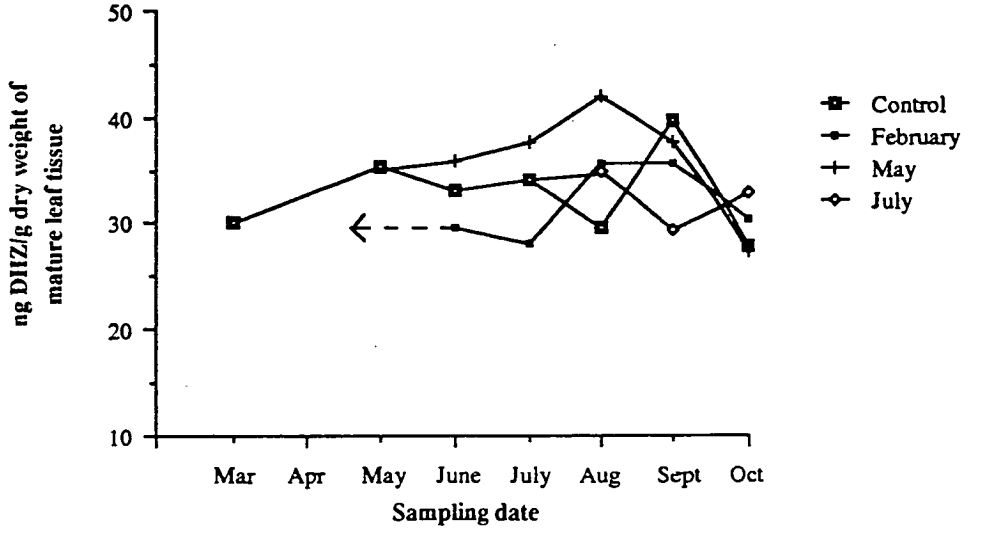
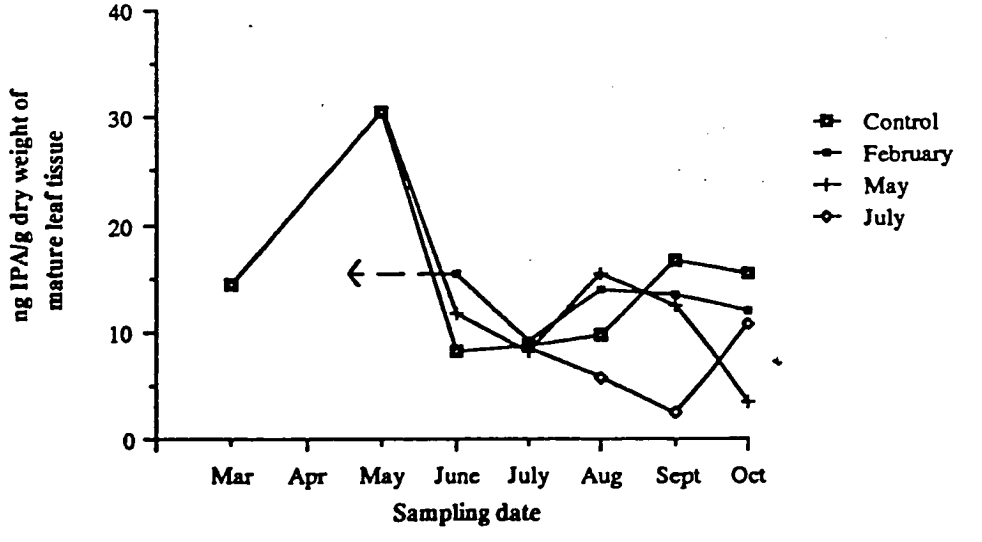


Figure IV.B.1.3.4.f



remained above the concentrations of control plants. Figure IV.B.1.3.4.a shows these trends for four of the treatments; the control GA concentration decreases with time, while the concentrations in February, May and July increased above the control after transfer but to a decreasing degree for the later dates.

The indolylacetic acid concentration increased with time as the seedlings were held in the control, non-inductive conditions, reaching a peak at the June sampling date, before declining to values of around 200 ng/g dry weight of mature leaf tissue. The period of decline corresponded to the time, between June and August, where the plants passed from the juvenile to the mature state. This trend is shown in Figure IV.B.1.3.4.b. Inductive conditions promoted a decline in IAA concentration prior to and after flower initiation was first observed. The decline in IAA concentration was most marked after the plants had reached the mature state.

Abscisic acid concentration varied between 177 and 112 ng/g dry weight of mature leaf tissue when held in non-inductive conditions, but displayed no correlation with the attainment of the mature state. ABA concentration tended to increase after flower initiation for most treatments, as shown in Figure IV.B.1.3.4.c, but showed no consistent deviations from the control concentration before this point.

The concentrations of cytokinins, namely zeatin, dihydrozeatin and isopentyladenosine did not appear to be correlated with the juvenile condition in the seedlings. The zeatin concentration (Figure IV.B.1.3.4.d) may decrease after flower initiation, but the very low concentrations present in the plant tissue suggest that this observed change is not an important regulatory mechanism and may in fact simply be due to experimental variation. The dihydrozeatin concentration (Figure IV.B.1.3.4.e) did not vary significantly from the control, indicating that it plays no part in the regulation of flower initiation or juvenility. IPA, while being present in low concentrations, tended to decrease in the leaf tissue after flower initiation when compared with the control concentrations (Figure IV.B.1.3.4.f). Thus IPA appears to be the cytokinin most likely to be involved in the regulation of the flower development process.

1.4 Discussion

Seedlings, tissue cultured pyrethrum plants and vegetatively divided splits from mature plants exhibit a period of juvenile-like behaviour in which they are not

competent to respond to normally inductive treatments. The juvenile phase lasts until the plant has reached a minimum size or stage of development, but does not depend on chronological age. Flower bud initiation in *D. grandiflora* can occur only after a minimum number of leaves have formed (Cockshull, 1976), and a similar link between leaf number and the phase change between the juvenile and the mature states is seen in pyrethrum. Plants are competent to respond to inductive conditions after six to ten leaves have fully expanded.

The attainment of meristem competence is linked to the release of axillary shoots from apical dominance. The number of nodes produced by the apical meristem is correlated with this release, and the number of leaves produced is a measure of the number of primordial units (leaf, internode and axillary meristem) initiated. Thus the abscission of leaves in the seedling and split experiments resulted in lower leaf numbers at flower initiation than for tissue cultured plants, even though similar numbers of nodes were probably present in all three plant types.

Unlike *D. grandiflora*, the apical meristem of pyrethrum remains vegetative during flower initiation, and only the axillary meristems produce flowers; a similar situation occurs in *Anagallis* (Bernier, 1971). The first axillary meristem released from apical dominance, usually in either of the two most basal leaf axils, is the earliest meristem to become competent to respond to inductive conditions, but reverts to a juvenile-like condition as the shoot ages. Similarly, the axillaries of *Pharbitis* lose their meristematic competence as the shoot ages (Owens and Paolillo, 1986). Also the axillary meristems may not be competent to respond to inductive treatments immediately upon release from apical dominance, in a similar way to axillary meristems of *D. grandiflora* released from apical dominance by decapitation of the terminal shoot (De Jong, 1981b).

The correlative influence of apical dominance appears to mediate the juvenile condition in pyrethrum. Meristems become competent to flower a short time after release from apical dominance, while older axillary meristems lose their competence. The short lag period between release from apical dominance and becoming competent to flower may correspond to the time required for the apex to reach the critical size required for flower initiation. The factors mediating the juvenile-like condition of older meristems may be related to the reduction in the sink strength of the shoot apex when axillary shoots begin developing, or due to other correlative factors and possibly even an intrinsic difference between cells in these and competent meristems.

Auxins and gibberellins appear to be the major plant growth regulators controlling the juvenile condition in pyrethrum seedlings. The gibberellin content of the mature leaves, which represents the whole plant, above ground status as the leaves make up the bulk of the plants mass, decreases as the seedling ages. This agrees with the findings of Schwabe and Al-Doori (1973) that juvenility in black currants is determined by gibberellins produced by the roots. After the gibberellin levels decrease below a maximum threshold, meristems may be florally evoked. The effect of gibberellins cannot be entirely inhibitory as inductive conditions elevate gibberellin concentrations, particularly after flower initiation.

The observed increase in auxin concentration during the early growth of the seedlings corresponds to the increase in growth rate of the terminal shoot apex following transplanting of the seedlings. The release of lateral shoots from apical dominance is likely to be related to a decrease in auxin content in their vicinity, due to the growth dilution effect as the plant's size increases as well as a localised effect as the terminal apical meristem extends further from the basal nodes. Basipetal transport of IAA from the shoot apex is generally thought to control apical dominance by regulating assimilate distribution and plant growth regulator translocation, limiting the supply of assimilates and cytokinins to the lateral meristems. The further development of the lateral shoots may be stimulated by GA or IAA applications (Ali and Fletcher, 1971) indicating a sequential role for the plant growth regulators. The small increase in GA concentration in July and August in the non-inductive conditions may be part of this sequential series, and likewise the increase in IAA in September and October. Assimilate supply is also thought to be linked to lateral shoot outgrowth, and it is possible that photosynthetic limitations may be involved in the early apical dominance of seedlings.

Neither the ABA nor the cytokinin concentrations show any link with juvenility. As apical dominance is thought to involve auxin-mediated changes in cytokinin distribution and/or metabolism at the lateral shoots, the expected cytokinin fluxes associated with the release of these shoots from apical dominance may be localised and not detectable on a whole plant level. The trend for cytokinin concentrations to decrease in the mature leaves after flower initiation while the ABA concentration increases may reflect the altered assimilate distribution pattern as vegetative growth is suppressed and the flower bud develops rapidly.

IV.B.2 Night Temperature and Duration of Vernalisation

2.1 Introduction

The literature review revealed vernalisation as the major environmental requirement for the rapid initiation of flowering in pyrethrum. Glover (1955) reported that the yield of pyrethrum flowers in East Africa is directly related to the number of hours at or below 16 °C at the time the flowers were initiated, three months before harvest. No results have been published on the minimum duration or optimum temperature for vernalisation under controlled environment conditions despite the fact that it is accepted as the major environmental requirement for flowering in equatorial regions. The influence of vernalisation on flowering in cool temperate regions has not been examined.

The vernalisation stimulus is thought to be perceived by the shoot apex and is not translocated within the plant. Transmission of the vernalised condition occurs through the process of division of the cells originally exposed to the cold, and thus only axillary meristems derived from a vernalised meristem can attain the vernalised state after this cold treatment (Napp-Zinn, 1987). Vernalisation is usually accompanied by an increase in gibberellin concentration, although gibberellins alone are not the end-product of the treatment.

2.2 Materials and Methods

CIG 3 and CIG 11 plants were selected from the population held in the evaporatively cooled glasshouse at the HRC under non-inductive conditions and placed in low night temperature conditions in the HRC light tunnels. Three tunnels were each programmed to receive ten hours of natural daylength at ambient day temperatures. Night temperatures of 6 °C, 12 °C and 18 °C were set in the respective tunnels, with 18 °C considered as non-vernalising. Four plants of each clone were placed in the 18 °C tunnel as control treatments. Four plants of each clone were moved at weekly intervals over the next five weeks from both the 6 °C and 12 °C tunnels to the 18 °C tunnel, resulting in a range of plants which had received from one to five weeks vernalisation at 6 °C or 12 °C. The plants were examined regularly, and the dates of

flower initiation and flower stage II (first flowers with ray florets vertical) recorded. After three months, the plants were harvested and the number of flower stems, flowers and flower buds recorded. No significant differences were observed between the behaviours of the two clones, and so the results were pooled for statistical analysis.

The fluctuations in plant growth regulator concentrations during vernalisation were analysed using radioimmunoassay. Two groups of ten CIG 3 plants, both under ten hour daylength and 20-30 °C day temperature but with 6 °C (inductive) and 18 °C (non-inductive) night temperatures respectively, were held in the light tunnels for plant hormone analysis. Every fifth day for a period of forty days mature leaf samples were taken from all plants in both tunnels to give representative samples from both inductive (vernalising) and non-inductive (non-vernalising) conditions. The RIA procedure was followed as per Section III.5. The samples collected on day 25 could not be analysed due to a fault in the freeze-drier which resulted in degradation of the plant material over a period of several days during the freeze-drying procedure.

For each sampling date, three extractions were analysed in triplicate by RIA. At each date the difference in PGR concentration between unvernalised and vernalised material was compared by the Students 't' test. Least significant differences (LSD) were calculated at P=0.05. Individual analysis of each tissue type over time was achieved using Duncans multiple range test, and the least significant ranges (LSD) were calculated at P=0.05. Prior to this, a test of homogeneity established which sampling dates could not be compared statistically.

2.3 Results

2.3.1 Optimum Temperature and Duration of Vernalisation

The minimum vernalisation treatment at which flower initiation was observed was 1 week at 6 °C or 2 weeks at 12 °C. No plants were seen to initiate flowers at any time under 18 °C night temperature or with 1 week of 12 °C night temperature conditions. However these conditions represent only marginally inductive treatments, and it was found that for rapid flower initiation and development in a high percentage of plants, the minimum vernalisation requirement was 2 weeks at 6 °C or 3 weeks at 12 °C.

Figure IV.B.2.3.1.a shows that night temperatures of 6 °C for 2 or more weeks

Figure IV.B.2.3.1.a

Vernalistion	6 °C	12 °C
Control	-	-
1 week	1	-
2 weeks	7	2
3 weeks	6	6
4 weeks	7	6
5 weeks	8	8

Figure IV.B.2.3.1.a - Number of plants flowering.
Plants were harvested 90 days after the start of the experiment and the number of plants, out of a total of eight, with developing flowers and flower buds counted.

Figure IV.B.2.3.1.b

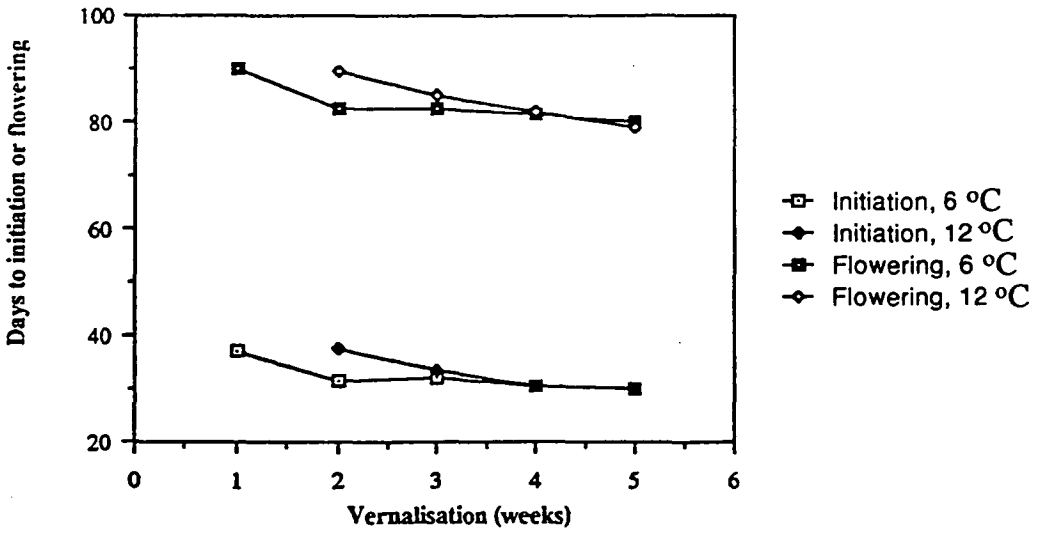


Figure IV.B.2.3.1.b - Flowering time.
Mean number of days to flower initiation (stem elongation) and to flower stage II (ray florets vertical). Plants were held under 6 °C or 12 °C night temperature for the lengths indicated. The calculated LSD values (0.05) for differences between the means for time of initiation at 6 °C and 12 °C are 2.9 and 3.8 respectively, and for flowering time 3.9 and 4.4 respectively.

Figure IV.B.2.3.1.c

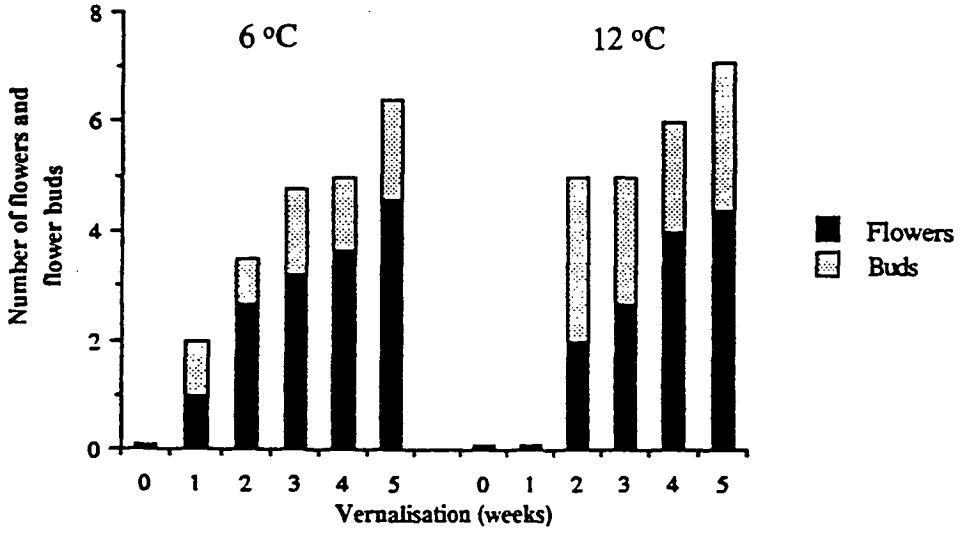


Figure IV.B.2.3.1.c - Average number of flowers (stage II and above) and visible flower buds per flowering plant at harvest, 90 days after vernalising treatments had begun.

Figure IV.B.2.3.1.d

Vernalisation	6 °C	12 °C
Control	-	-
1 week	0	-
2 weeks	50	100
3 weeks	40	50
4 weeks	35	50
5 weeks	29	38

Figure IV.B.2.3.1.d - Number of abnormal, shortened flower stems formed under each vernalisation treatment expressed as a percentage of the total number of flower stems formed.

and 12 °C for 3 or more weeks are required for flower initiation in the majority of plants. Similarly, 2 weeks at 6 °C or 3 weeks at 12 °C are required for the rapid initiation and development of flower buds, as shown in Figure IV.B.2.3.1.b. Flower initiation may occur at less than this requirement, but is significantly delayed in comparison with the longer vernalisation treatments.

Further observations of the plants when harvested supports the conclusion that periods of vernalisation less than the stated minimums can be considered marginal. The number of flowers and flower buds at the time of harvest increased with increasing lengths of vernalisation (Figure IV.B.2.3.1.c). The greater number of flowers on plants given the 6 °C treatment is balanced by a larger number of flower buds after the 12 °C treatment, reflecting the faster rate of flower initiation after the 6 °C treatment and indicating that the two treatments are otherwise similar. Figure IV.B.2.3.1.d reveals the decreasing probability of shortened flower stems (see Section IV.A.1.3.3) developing as the duration of the vernalising treatments is increased. Again this trend is indicative of the marginally inductive treatments represented by the shortest periods of vernalisation.

2.3.2 Endogenous Hormonal Changes

The gibberellin and auxin concentrations were found to change significantly soon after the imposition of the vernalisation treatment, as was seen in the meristem competence experiment. The differences between the mean gibberellin concentrations of the vernalised and the non-vernalised plants were statistically significant on day 15 and from days 30 to 40, as shown in Figure IV.B.2.3.2.a. No statistically significant changes in gibberellin concentration were observed in the non-vernalised plants during the experiment. Under vernalising conditions, the gibberellin concentration in the plants increased within five days of the imposition of the cold treatment but the changes did not become statistically significant until thirty days of cold treatment had been received. Flower initiation was noted in the majority of plants after 30 days in the vernalising conditions, and so the rise in gibberellin levels at this time may explain the stem elongation observed thereafter. The increased gibberellin concentrations prior to this time may be associated with earlier evocational events, but it is not the sole evocational stimulus as gibberellin levels were seen to rise well before initiation during the meristem competence experiment (Section IV.B.1.3.4) and in several instances

Figure IV.B.2.3.2.a - Endogenous gibberellin concentrations in mature leaf samples of plants held in vernalising and non-vernalising conditions. Mature leaf tissue was sampled every five days from the time that the plants were placed in the vernalising conditions. Each point represents the mean of three replicates of each of three sample extractions.

Figure IV.B.2.3.2.b - Endogenous indolylacetic acid concentrations in mature leaf samples of plants held in vernalising and non-vernalising conditions. Mature leaf tissue was sampled every five days from the time that the plants were placed in the vernalising conditions. Each point represents the mean of three replicates of each of three separate sample extractions.

Figure IV.B.2.3.2.c - Endogenous abscisic acid concentrations in mature leaf samples of plants held in vernalising and non-vernalising conditions. Mature leaf tissue was sampled every five days from the time that the plants were placed in the vernalising conditions. Each point represents the mean of three replicates of each of three separate sample extractions.

Figure IV.B.2.3.2.a

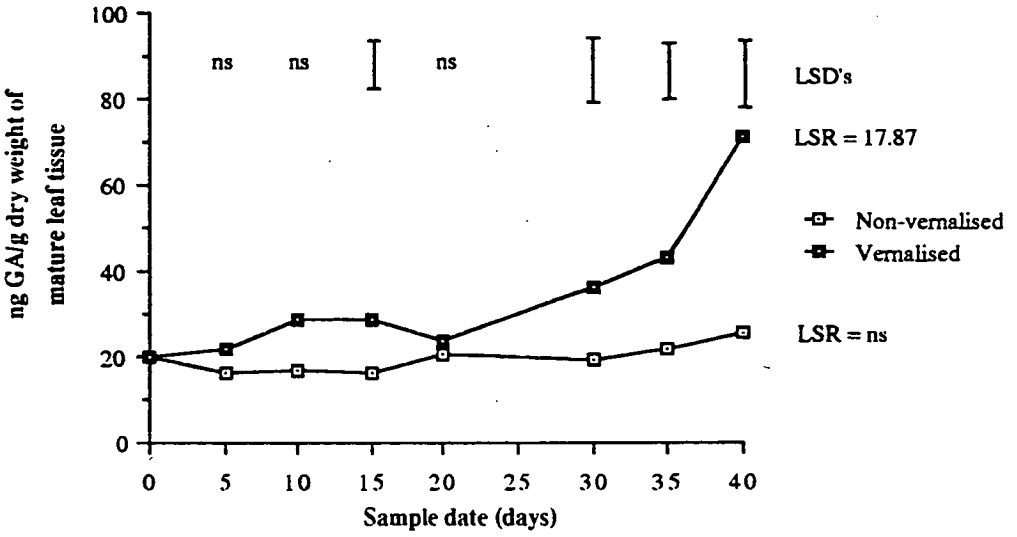


Figure IV.B.2.3.2.b

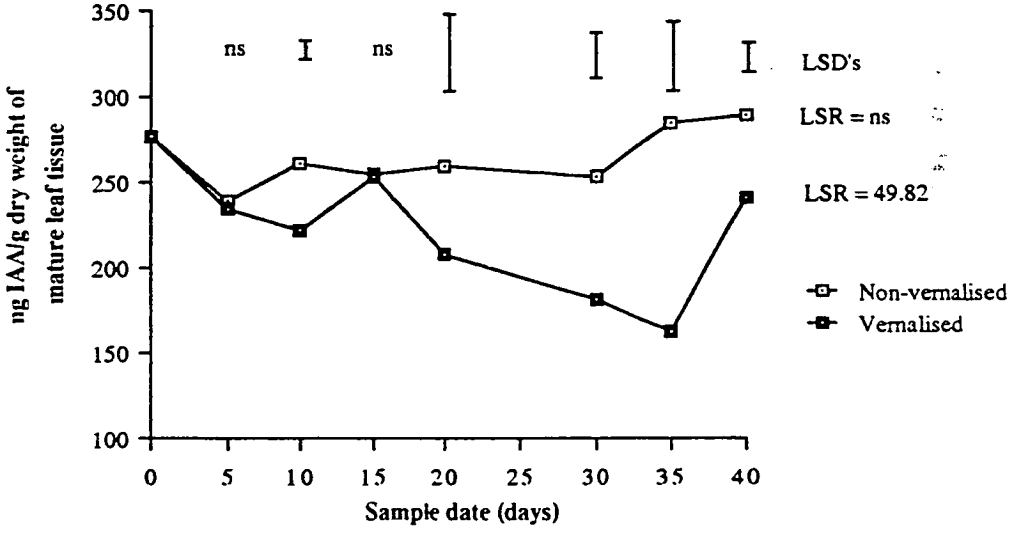


Figure IV.B.2.3.2.c

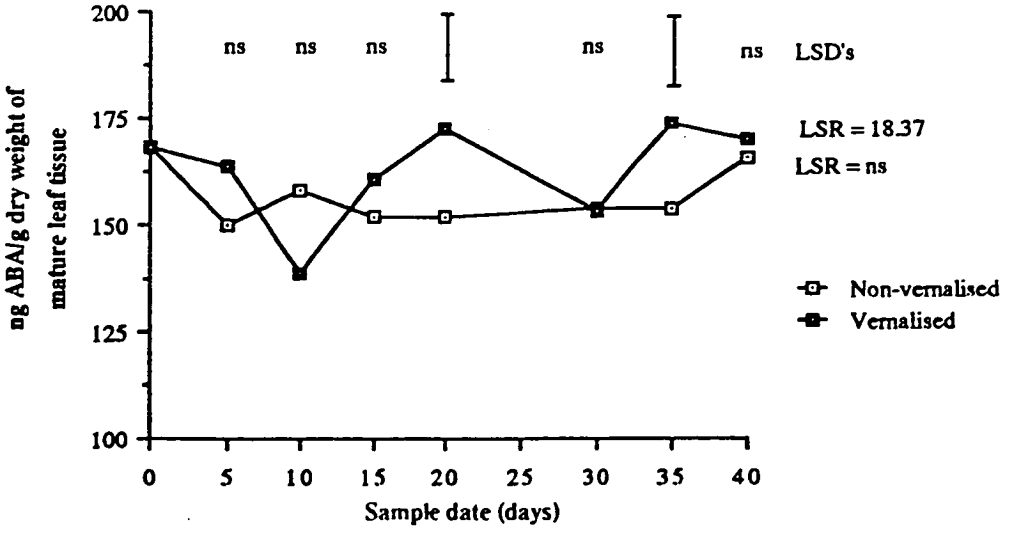


Figure IV.B.2.3.2.d - Endogenous zeatin concentrations in mature leaf samples of plants held in vernalising and non-vernalising conditions. Mature leaf tissue was sampled every five days from the time that the plants were placed in the vernalising conditions. Each point represents the mean of three replicates of each of three sample extractions.

Figure IV.B.2.3.2.e - Endogenous dihydrozeatin concentrations in mature leaf samples of plants held in vernalising and non-vernalising conditions. Mature leaf tissue was sampled every five days from the time that the plants were placed in the vernalising conditions. Each point represents the mean of three replicates of each of three separate sample extractions.

Figure IV.B.2.3.2.f - Endogenous isopentyladenosine concentrations in mature leaf samples of plants held in vernalising and non-vernalising conditions. Mature leaf tissue was sampled every five days from the time that the plants were placed in the vernalising conditions. Each point represents the mean of three replicates of each of three separate sample extractions.

Figure IV.B.2.3.2.d

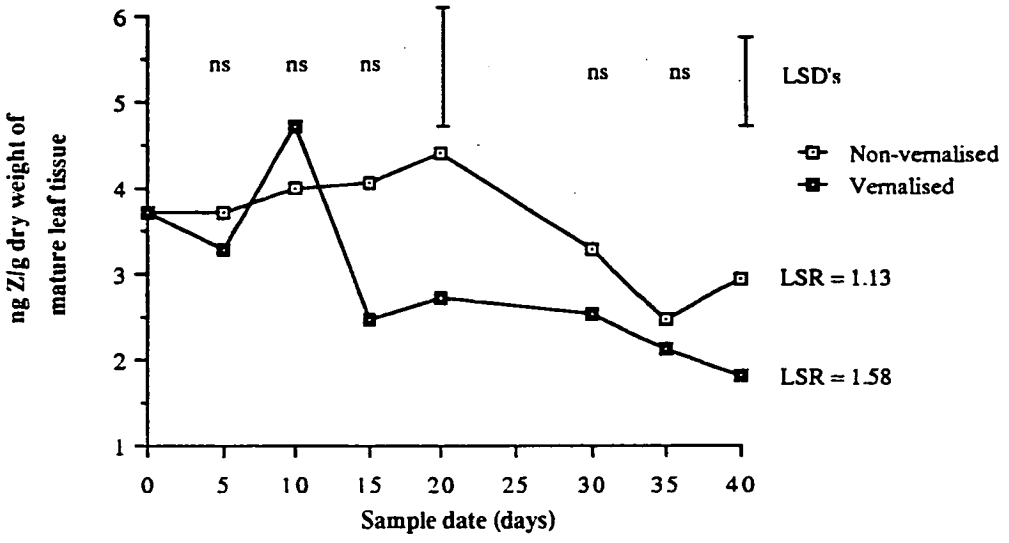


Figure IV.B.2.3.2.e

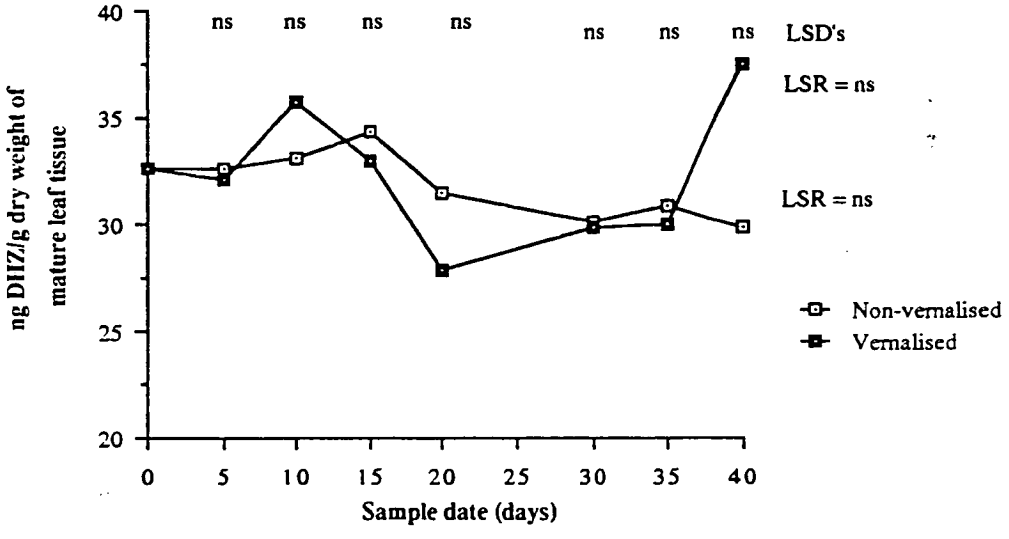
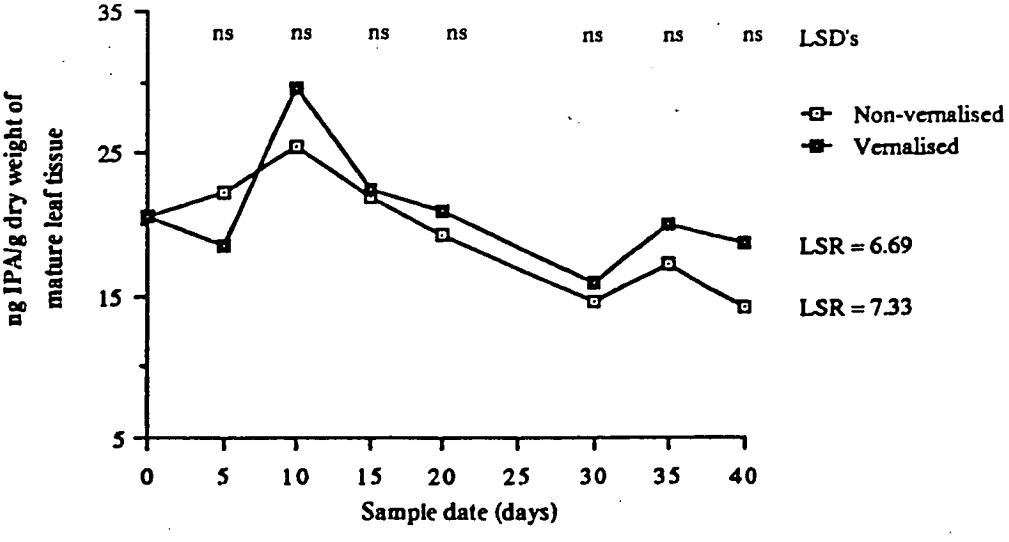


Figure IV.B.2.3.2.f



were only slightly above that of non-vernalised plants at the time of initiation.

Figure IV.B.2.3.2.b shows the decrease in IAA concentration due to the vernalisation treatment. Again the largest differences between the hormone levels in the non-vernalised and vernalised plants are around the time that flower stem elongation was first observed. These results are consistent with the observations made in Section IV.B.1.3.4, where the attainment of meristem competence and subsequent flower initiation were thought to be associated with the loss of apical dominance due at least partially to a decrease in auxin concentration. The significant decline in IAA concentration observed by day 10 indicates that auxin mediates some of the early evocational events. Two weeks vernalisation at 6 °C has been shown to be sufficient to promote rapid flower initiation, and the decline in IAA concentration during this time and before the commitment to flowering becomes irreversible, points to auxin as a possible inhibitor to processes necessary for floral evocation. The proposed influence of auxin on assimilate translocation and possibly also on the distribution of other plant growth regulators may be the physiological action of auxin in mediating some of the early evocational processes as well as in apical dominance and the release of lateral shoots.

ABA concentration does not vary significantly between plants held in the non-vernalising and vernalising conditions. As shown if Figure IV.B.2.3.2.c, there was a tendency for the ABA levels to be higher in vernalised plants but the scale of this variation and the overall pattern of concentration fluctuations in both vernalising and non-vernalising conditions indicates that vernalisation does not directly influence ABA levels.

Similarly the cytokinin concentrations, on a whole plant basis, do not seem to be directly affected by vernalisation and do not vary significantly between vegetative and florally induced plants. The concentration of zeatin (Figure IV.B.2.3.2.d) did show a decline in vernalised compared with non-vernalised plants from day 15 onwards, but is probably not linked to flower initiation as the concentration in non-vernalised plants decreased to similar levels on days 35 and 40 without being florally evoked. The low concentrations of zeatin in the vernalised plants at this stage may be linked to flower development and the rapid stem elongation seen at this time. The concentrations of dihydrozeatin (Figure IV.B.2.3.2.e) and isopentyladenosine (Figure IV.B.2.3.2.f), however, do not vary between the vernalised and non-vernalised plants at this or any other stage during the period examined.

As stated in Section IV.B.1.3.4, the analysis of mature leaf samples reflects the whole plant hormonal status and may not detect the localised fluctuations which mediate certain correlative influences such as apical dominance, and may have an important role in flower initiation.

2.4 Discussion

Under short days and with day temperature in the range 20-30 °C, the minimum vernalisation requirement in pyrethrum is 2 weeks at 6 °C night temperature. A night temperature of 12 °C will result in vernalisation if plants are exposed for 3 or more weeks, while an 18 °C night temperature will not stimulate flower initiation through the vernalisation process. These results agree with Glover's (1955) findings that temperatures of 16 °C or below are required for vernalisation.

Flower initiation may occur in a small percentage of plants after shorter periods of vernalisation but the end of evocation, as well as the further development of the flower bud, is significantly delayed. The flower stems which develop under these conditions are likely to be significantly shortened, and the number of flowers and flower buds is reduced. The number of flowers formed is increased and the percentage of shortened flower stems decreased as the duration of vernalisation is increased. The stated minimum periods of vernalisation must thus be considered somewhat arbitrary, and are used in this study to indicate the conditions which promote rapid flower initiation and development in the majority of plants.

Marginally inductive periods, where vernalisation is observed to occur at less than the stated minimum durations, provide a sufficient stimulus to promote flower initiation but the delayed timing of initiation as well as the altered development pattern suggests that some or all of the physiological processes involved in initiation and development are proceeding at a reduced rate. Pyrethrum displays a quantitative response to vernalisation, with longer periods in vernalising conditions providing the plant with a greater inductive signal leading to larger numbers of flowers, longer flower stems and more rapid flower initiation and development.

One of the effects of vernalisation is to increase the concentration of free gibberellins in the plant. This increase becomes more pronounced as the duration of vernalisation increases, and may mediate some of the physiological processes which result in the plants quantitative response to vernalisation. As stated in Section

IV.A.1.3.1, the irreversible commitment to flowering occurs after around 25 days in inductive conditions, and so the increase in gibberellin concentration during this period indicates that some of the processes mediated by gibberellins may be involved in floral evocation. Gibberellins have been reported to be involved in the flowering of many rosette species, and increased gibberellin concentrations have been correlated to several evocational events but are not considered the sole inductive stimulus. While the role of gibberellins in flower initiation is not fully understood, it is generally accepted that increased gibberellin concentrations play a major role in mediating flower stem elongation during flowering, and the high gibberellin concentrations observed during stem elongation indicate that this is the case in pyrethrum. This may also explain the high percentage of shortened stems observed under marginally inductive conditions.

The role of auxin in the flower initiation process of pyrethrum appears to be inhibitory, through suppression of lateral shoot growth by apical dominance. IAA levels decline during vernalisation, and this decrease may initiate the outgrowth of lateral shoots whose meristems are competent to respond to the other evocational processes stimulated by vernalisation. The role of auxin during flower initiation may not be limited to the control of apical dominance, as a decline in auxin concentration is commonly observed during flower initiation in a number of different species where the release of lateral shoots from apical dominance is not necessary for flower initiation.

The cytokinin and abscisic acid concentrations are not directly affected by vernalisation, but a possible role for these hormones during flower initiation cannot be ruled out. Localised fluxes of hormones, particularly in the region of the lateral buds during release from apical dominance, have not been examined and may reveal the physiological processes involved during flower initiation. The results of this experiment and the meristem competence experiments, which examined the hormone concentrations on a whole plant basis in order to gain an insight into the possible chemical regulation of initiation, indicate that gibberellins and auxins are the plant growth regulators whose concentrations are affected by inductive environmental conditions. While these changes can be used to explain the process of flower initiation through the release of lateral buds from apical dominance, the possibility exists that localised fluxes of other plant growth regulators or changes in tissue sensitivity to the plant growth regulators may mediate some or all of the evocational processes.

Inductive, vernalising conditions appear to cause apical dominance to be

weakened by decreasing the IAA concentration, and the evocational processes may then direct the axillary meristem to floral development. Gibberellins and auxins may mediate some of these evocational processes, and gibberellins are strongly implicated in the stimulation of stem elongation during flower development. The promotive effect of vernalisation is thus dependent on the release of lateral buds from apical dominance, and so the longer the duration of vernalisation the greater the number of axillary buds released and the larger the number of flower buds initiated. If, as has been observed in other species including *Dendranthema*, the vernalisation stimulus is not translocated within plants, then the response to vernalisation depends on the physiological state of the plant and in particular the physiological age of its axillary shots. The vernalised condition can be transmitted through cell division, allowing lateral shoots on each flower stem to form flowers even under non-inductive conditions. However, as reported in Section IV.A.1.3.3, vegetative shoots may develop as laterals on flower stems in certain conditions, indicating that the vernalised condition is not the sole inductive requirement.

IV.B.3 Temperature and Light Effects on Photosynthesis

3.1 Introduction

According to the multifactorial theory for the control of flowering, the supply of photosynthetic assimilates may control one or more of the evocational processes which, in the appropriate balance or sequence, result in flower initiation. As indicated in the literature review (Section II.5.2) assimilates appear to be essential for both flower initiation and development in many species. The control of flowering in Charles-Edwards (1979) mathematical model for *D. grandiflora* is almost entirely governed by photosynthesis, which promotes apical dome growth, and an inhibitor which controls primordial initiation and, indirectly, flowering. This model fits the observed relationship between apical dome size and the stages in apical development, including the critical apical dome size at the transition to flowering. The relationship between apical diameter and the stages in apical development during flowering described for pyrethrum (Section IV.A.1.3.1) indicates that flower initiation and development are also dependent on assimilate supply in this species .

The rate of photosynthesis is modified by leaf maturity and various physical environmental variables including temperature and photon flux density (PFD). Clearly a knowledge of the effects of these factors on photosynthesis would be beneficial both in terms of experimental design and in the interpretation of experimental results. The aim of this experiment was to investigate the effects of temperature and PFD on the 'apparent' rate of photosynthesis in mature and immature leaves of pyrethrum. This measurement of the 'apparent' rate of photosynthesis cannot be used to accurately predict the 'true' rate of photosynthesis, which takes into account the effects of photorespiration, dark respiration and oxygen inhibition of photosynthesis, but it provides sufficient information to aid in the interpretation of experimental results on the effects of environmental factors on flower initiation.

3.2 Materials and Methods

3.2.1 Infra Red Gas Analyser, Leaf Chamber and Gas Supply

Photosynthetic measurements were recorded using an LCA-3 portable Infra Red Gas Analyser (IRGA) from the Analytical Development Company Limited. The LCA-3 is a battery powered, 'open' system IRGA for the measurement of transpiration and photosynthesis, incorporating a controlled air supply for a plant leaf chamber, a differential (in time), auto-zeroing, solid state carbon dioxide analyser together with a data processing and storage facility.

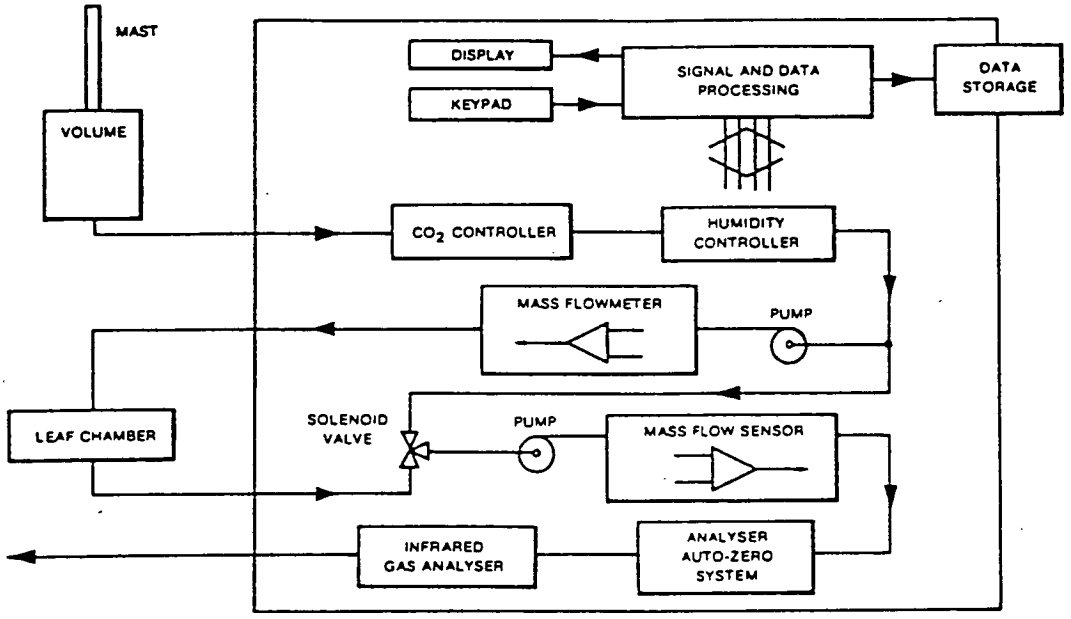


Figure IV.B.3.2.1 - Diagrammatic representation of the LCA-3 system

The LCA-3 system is shown in Figure IV.B.3.2.1. Air may be drawn from the atmosphere via a mast and volume to buffer changes in ambient carbon dioxide concentration or, as used in this experiment, released directly from a gas cylinder into the system. The air is passed through a carbon dioxide controller that can adjust the proportion of carbon dioxide from ambient continuously through to zero. The humidity of the air can be adjusted in a similar way by the humidity controller. The air is then pumped at a controlled rate determined by the mass flowmeter to the leaf chamber. On its return, either the chamber air or reference air is selected by the solenoid valve for analysis as dictated by the analyser operating mode. The air to be analysed is then

drawn by a second pump at the rate determined by the mass flow sensor and delivered to the analysis section. Ahead of the analyser itself is an auto-zeroing system which operates on a five second cycle time alternately presenting sample air and zero carbon dioxide air to the analyser. After passing through the analyser the air was vented to the atmosphere. The absorption by the sample of the infrared radiation in the 4.26 μm band, emitted by the modulated source, is measured by a pyroelectric detector. The operation of the whole instrument is under microprocessor control. Measurements and results were shown on the analyser display panel and also recorded on the data storage card located in the instrument base.

A Parkinsons Leaf Chamber, model PLC (B), from the Analytical Development Company Limited, was used in this study. The leaf chamber was designed to allow maximum air mixing, and included a quantum flux sensor which recorded photon flux density and a copper-constantan thermocouple, which was placed inside the leaf chamber on the undersurface of the leaves to measure temperature.

Air from a pressurised gas cylinder was passed though a flowmeter and fed into the IRGA system inlet at a constant flow rate. The flow rate was varied according to the tissue type in the leaf chamber and the experimental temperature and PFD conditions so that carbon dioxide differential readings were always less than ten percent of the carbon dioxide concentration in the air (35 - 37 parts per million, ppm). The air was passed through the humidifier in the LCA-3 before entering the leaf chamber.

The IRGA was calibrated with two gas mixtures of known carbon dioxide concentration, 300 and 365 ppm and the remainder nitrogen, before and after measurements were recorded for each leaf used. Air cylinders were checked by direct measurement in the IRGA, and only gas mixtures with a carbon dioxide concentration between 355 and 370 ppm were used.

3.2.2 Temperature and Photon Flux Density Conditions

A copper water jacket was fitted to the base of the leaf chamber to control the temperature in the chamber. The air line from the gas cylinder to the LCA-3 was passed through a copper coil in the water bath feeding the water jacket to aid in temperature control. Also, as far as possible, the room temperature was maintained at the temperature of the leaf chamber to prevent condensation in and around the leaf chamber.

Light was supplied by a 400 W mercury vapour lamp suspended above the leaf chamber. A glass water table separated the light source from the leaf chamber to prevent excessive heating of the chamber by the lamp. Photon flux density was controlled by inserting varying thicknesses of 'Sarlon' shade cloth between the light source and the leaf chamber, and was measured using a Lambda LI-185 meter fitted with a quantum flux sensor (the sensor in the leaf chamber was found to be inaccurate at high PFD's). The quantum flux sensor measured photosynthetically active radiation (400-700nm) and results are expressed in $\mu mol.m^{-2}.s^{-1}$. Measurements of PFD were made at leaf height and were corrected for the chamber and water jacket.

3.2.3 Measurement of Leaf Area

Descriptions of the photosynthetic rate are generally expressed as $\mu mol.m^{-2}.s^{-1}$. Hence it is necessary to accurately calculate the leaf area used in each measurement. As the leaves used in the experiment did not fill the area of the leaf chamber, it was necessary after each series of measurements on individual leaves to cut out the leaf section held in the leaf chamber and pass it though a planimeter to record its area.

Before the rate of net CO_2 exchange was measured on individual leaves, their stomatal conductance was recorded and only leaves with high values of stomatal conductance were selected for photosynthetic measurements. Each leaf was allowed to equilibrate in the leaf chamber for five minutes before measurements were taken. The CO_2 differential between air before entering and after leaving the leaf chamber was measured after the equilibration time. Ten separate recordings were stored in the LCA-3 memory card for each leaf at each set of experimental conditions. Measurements were recorded in this way on the effects of temperature and PFD on CO_2 exchange for three plants each for both mature leaves and immature leaves.

3.2.4 Calculation of Net Assimilation Rates

The net assimilation rate of CO_2 into the plant tissue is a measure of the plants apparent rate of photosynthesis; CO_2 is assimilated into the plant by the 'true' photosynthesis process, but is also released through the respiratory processes. The assimilation rate, or rate of CO_2 exchange, is calculated by the following formula :

$$A = (f/s) \times \Delta C$$

where A = assimilation rate ($\mu mol.m^{-2}.s^{-1}$)

f = mole flow of air ($mol.s^{-1}$)

$= f_v \times (1/22.4) \times [273.15/(273.15+T)] \times (p/101.3)$

f_v = volumetric flow of air ($l.s^{-1}$)

T = temperature recorded during measurement (°C)

p = atmospheric pressure during measurement (kPa)

s = photosynthetic surface area of the leaf (m^2)

$\Delta C = CO_2$ differential (ppm)

Net CO_2 exchange was calculated for mature and immature leaves over a range of PFD's between 0 and 940 $\mu mol.m^{-2}.s^{-1}$ at temperatures between 5 and 35 °C (at 5 °C intervals). Full results are shown in appendices IV.B.3.3.a and IV.B.3.3.b.

3.3 Results

The effects of temperature and PFD on the net rate of CO_2 assimilation in mature leaves and immature leaves are shown in Figures IV.B.3.3.a and IV.B.3.3.b respectively. At 940 $\mu mol.m^{-2}.s^{-1}$, net CO_2 assimilation reached a maximum in both immature and mature leaves at 25 °C and decreased with increasing temperatures above this. At this and all other points the net rate of CO_2 assimilation was lower in immature than in mature leaves. The temperature response curve changes shape as PFD decreases, and the maximum rate of CO_2 exchange occurs at lower temperatures. At 0 $\mu mol.m^{-2}.s^{-1}$ PFD, the measured rate of CO_2 exchange is due to dark respiration, which increases with increasing temperature. The rate of dark respiration is greater in

Figure IV.B.3.3.a

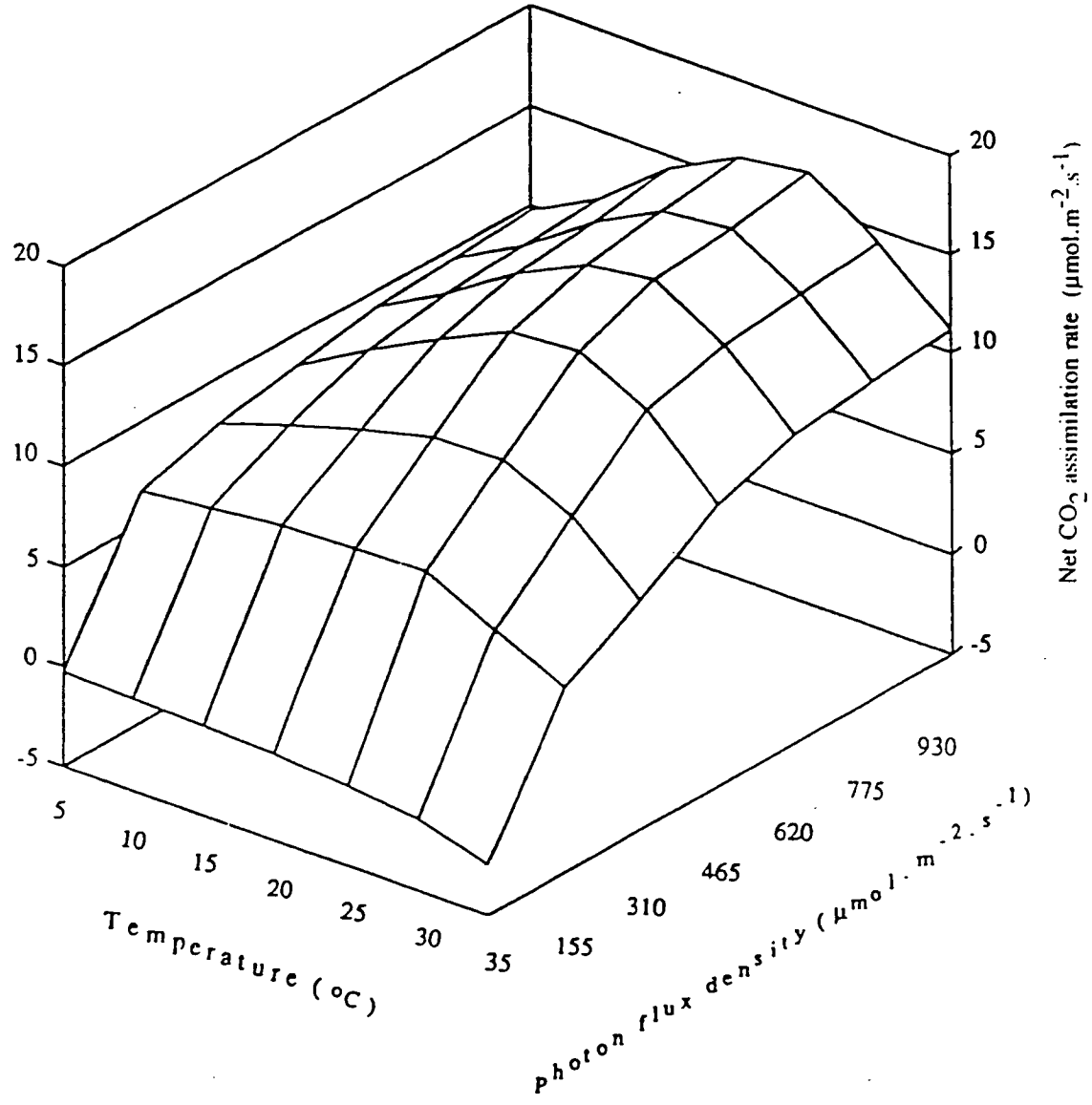


Figure IV.B.3.3.a - Net assimilation rate in mature leaves.
PFD was varied between 0 and 940 $\mu mol.m^{-2}.s^{-1}$. Net CO_2 exchange was measured at temperatures of 5 °C to 35 °C in 5 °C intervals.

Figure IV.B.3.3.b

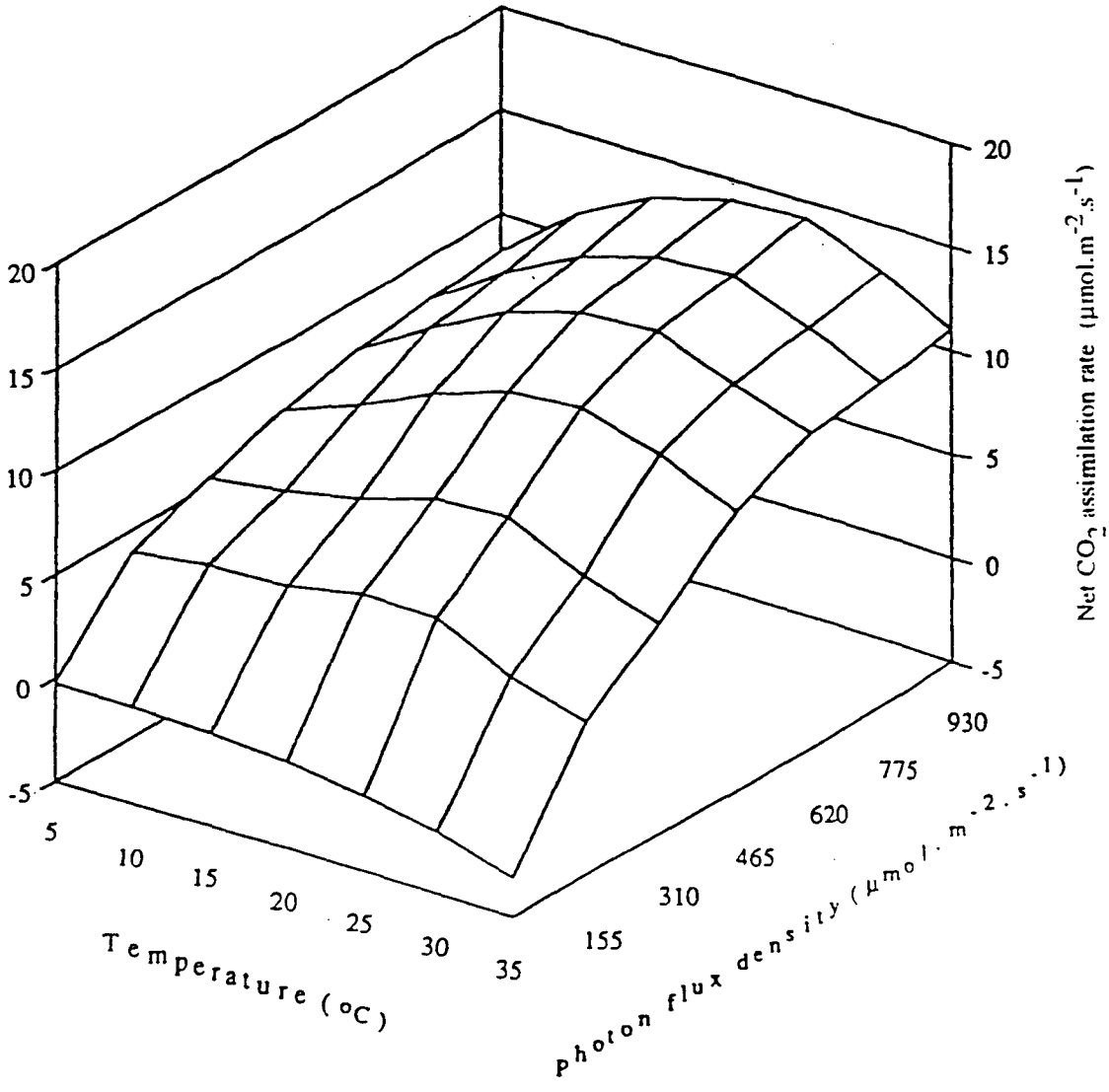


Figure IV.B.3.3.b - Net assimilation rate in immature leaves.
PFD was varied between 0 and 940 $\mu mol.m^{-2}.s^{-1}$. Net CO_2 exchange was measured at temperatures of 5 °C to 35 °C in 5 °C intervals.

immature leaves and results in the compensation point, where photosynthetic CO_2 assimilation is balanced by respiratory CO_2 release, occurring at higher PFD's in immature than in mature leaves, particularly at high temperatures.

Light saturation occurred at PFD's of between 350 and 500 $\mu mol.m^{-2}.s^{-1}$ at 20 °C and above. Below 20 °C, net CO_2 assimilation increased with increasing PFD up to 300 - 350 $\mu mol.m^{-2}.s^{-1}$ and then remained constant with increasing PFD. Light saturation occurs at lower PFD as temperature is limiting the rate of CO_2 exchange.

3.4 Discussion

'Apparent' photosynthesis can be considered to have four components ; 'true' photosynthesis, photorespiration, dark respiration and oxygen inhibition of photosynthesis. The rate of dark respiration increases with temperature, particularly above 20 °C, and is one of the factors contributing to the observed decrease in the rate of 'apparent' photosynthesis above 25 °C. While not examined in this experiment, photorespiration characteristically increases with temperature and the rate of 'true' photosynthesis reaches a maximum at a slightly higher temperature than 'apparent' photosynthesis before declining (Hall and Rao, 1989). 'Apparent' photosynthesis increases with temperature as a result of the increasing rate of 'true' photosynthesis, and declines after reaching its maximum rate at 25 °C as a result of the rapid increase in the respiratory processes at higher temperatures combined with falling rates of 'true' photosynthesis. The net rate of CO_2 fixation ('apparent' photosynthesis) is a measurement of the balance between production and utilization of photosynthate, and indicates the level of photosynthate produced by the leaf which is available to the plant.

The net photosynthetic capacity of the immature leaves is considerably lower than the mature leaves. Leaf primordia are net importers of photosynthates, and continue to act as sinks for assimilates until they reach a sufficient size to fulfil their own energy demand. The lower rate of net CO_2 exchange can mainly be attributed to the higher dark respiration rate in the immature leaves. Dark respiration is made up of two main components, growth respiration and maintenance respiration. The growth respiration component is greater in immature leaves due to the need to expend energy in the expansion of tissue ; building new structural materials and new cell organelles. The source-sink relationship between mature leaves and immature leaves is examined further in Section IV.B.6 by ^{14}C feeding experiments and autoradiography.

Flowering of pyrethrum in Tasmania occurs during spring and early summer when the light integral is increased, compared with winter, as a result of less cloud cover and longer daylengths . Temperature also increases, leading to an increased rate of net CO_2 exchange, provided the temperature does not exceed 25 °C, and also increasing the rates of photorespiration and dark respiration. Clearly the plants photosynthetic activity will be enhanced during this period provided the temperature does not increase above 25 °C.

Vernalisation has been demonstrated to be the principal requirement for rapid flower initiation and development in pyrethrum. Night temperatures in late autumn and winter in Tasmania fulfil the plants vernalisation requirement and yet rapid flower bud development is not observed until spring. Examination of apices dissected from field grown plants reveals that floral evocation proceeds through the winter months but that apical development does not proceed past apical stages 2 or 3. It is speculated that floral development is arrested during this period because of assimilate source limitations. Low temperatures, low average PFD's and short daylengths all serve to decrease the plants photosynthetic capacity, and may prevent the necessary assimilate requirement for floral development from being met. The virtual cessation of apical activity and the slow rate of expansion of immature leaves under these conditions is a clear indication that photosynthate is limiting growth. However environmental conditions in winter may also act in other ways to reduce the sink strength of the developing apices or by influencing the supply of other essential metabolites for floral development.

This hypothesis to explain the observed flowering behaviour of pyrethrum in Tasmanian conditions is based on the assumption that vernalisation and an adequate assimilate supply represent inductive conditions. This assumption is justified on the basis of the previously published data on the flowering of pyrethrum and of *D. grandiflora.* To test this hypothesis, the effects of PFD and temperature on the distribution of assimilates during flowering must be studied, and the possible role of photoperiod examined.

IV.B.4 Daylength and Photoperiod Effects

4.1 Introduction

Photoperiod is the most widely studied environmental flowering response. While light has a promotory effect on flowering due to its influence on photosynthesis, the photoperiodic effects on initiation usually require light of energy below the photosynthetic compensation point. The photoperiodic response may vary from absolute to facultative and can describe daylength requirements above and below a critical value. The classification of species into various photoperiodic response groups has recently been shown to be somewhat arbitrary, with an ever increasing list of plants, originally considered to be strictly photoperiodic, that can be made to produce flower buds by a variety of other factors besides daylength.

Phytochrome appears to be the photoreceptor for the photoperiodic response. New evidence indicates that phytochrome-mediated changes in the metabolism of endogenous plant growth regulators may not be the sole plant photoperiodic response. In *Pharbitis*, Ogawa and King (1990) concluded that the inhibitory effect of non-induced leaves is due to both the presence of a specific photoperiodically sensitive inhibitor and to interference with the assimilate/floral stimulus co-transport in the phloem. Failure to identify a ubiquitous photoperiodically produced floral stimulus also suggests that the photoperiodic response is not simply production of a chemical stimulus to flowering but rather a promotion of one or more of the many evocational processes.

The photoperiodic response of pyrethrum has not been investigated. As discussed in the literature review (Section II.2.3) daylength varies only slightly throughout the year in the main growing regions of East Africa and therefore is unlikely to influence flower yield. However in Tasmania, daylength varies from 15.30 monthly mean hours of daylight (sunrise to sunset) in December to 9.04 in June, and the plant produces only one main flush of flowers in November-December and a smaller flush in March-April. While vernalisation has been shown to be the major environmental stimulus for flower initiation, it is possible that photoperiod may modify the flowering response, resulting in the observed differences in flowering behaviour of pyrethrum under Tasmanian conditions and East African conditions.

4.2 Materials and Methods

Mature CIG 3 and CIG 11 plants were selected from the population held in the non-inductive conditions in the evaporatively cooled glasshouse at the HRC on the basis of physiological uniformity. Ten plants of each clone were placed in light tunnels under 8 hour, 16 hour and 24 hour daylengths respectively for the daylength experiment. Natural daylight was sufficient to fill the requirements of the 8 hour treatment, while artificial lighting was used to extend the daylength for the 16 hour and 24 hour treatments. These two treatments received 11 hours natural daylight, with artificial lighting extending the daylength to the required length as described in Section III.2.2. The PFD of the artificial lighting at plant height was 150 $\mu mol.m^{-2}.s^{-1}$ while PFD during natural daylight hours varied from 600 to 900 $\mu mol.m^{-2}.s^{-1}$. Each tunnel received 8 hours of 6 °C temperature, corresponding to the dark period in the 16 hour treatment. This ensured that an identical vernalisation stimulus was received by each plant. The temperature during the natural daylight hours varied between 23 and 27 °C, and except for the 8 hour vernalisation period, was set at 20 °C when the plants were inside the light tunnels. Plants were examined regularly, and the time of flower initiation noted. Individual plants were harvested when they first reached flower stage II and the date recorded. No differences were observed between the flowering times of the two clones and so the results were combined for statistical analysis.

The photoperiod experiment involved three treatments and ten CIG 3 plants per treatment. Two light tunnels were set as described above for 8 hours daylength and 16 hours daylength (12 hours natural light and 4 hours artificial light). A third tunnel was set with 8 hours natural daylength and a 1 hour night break after 7 hours darkness. Night break lighting was provided by five white fluorescent tubes and one mercury vapour lamp, giving a combined PFD of 75 $\mu mol.m^{-2}.s^{-1}$ at plant height. Temperature was again set at 6 °C for 8 hours for all treatments, and 20 °C for the remainder of the period inside the light tunnels. Temperature during the natural daylight hours varied from 20 to 28 °C and PFD from 600 to 1200 $\mu mol.m^{-2}.s^{-1}$. The time of flower initiation and flower stage II were recorded as described above.

4.3 Results

4.3.1 Daylength

Figure IV.B.4.3.1 shows the effect of daylength on flower initiation and development in pyrethrum. The time to initiation was significantly increased by the 8 hour daylength treatment and similarly the time taken to reach flowering stage II was significantly longer in the 8 hour than the 16 or 24 hour daylength treatments. The rate of flower development was reduced by the 8 hour daylength as seen by the increased time between initiation and flower stage II when compared with either the 16 hour or 24 hour daylength treatments.

Flowering appears to be a quantitative response to long days. Both flower initiation and development are promoted by 16 and 24 hour daylengths. Two possible explanations for the reduced rate of flower initiation and development under an 8 hour daylength exist. Firstly, the reduced daily light integral associated with the 8 hour daylength restricts the total level of photosynthate produced by the plant, and results in decreased rates of all growth processes. Secondly, the difference in the lengths of the light periods between the three treatments may result in endogenous hormonal changes mediated through the photoreceptor, phytochrome. The significant increase in the timing of flower stage II under the 24 hour daylength points to endogenous chemical changes partially controlling the flower development process as this treatment received the longest light integral. Examination of the photoperiodic response will aid in the interpretation of this data, and in explaining which of the two processes, individually or in combination, mediate the observed reduction in the rates of flower initiation and development under short days.

4.3.2 Photoperiod

Delays in the time of flower initiation and flower stage II were observed under both 8 hours daylength and 8 hours daylength with night break lighting. Figure IV.B.4.3.2 shows the delays in initiation and flowering under these two treatments compared with 16 hours daylength. The period of night break lighting did not affect the flowering of plants under 8 hours daylength.

The rates of initiation and flower development under 8 hours daylength and 16

Figure IV.B.4.3.1

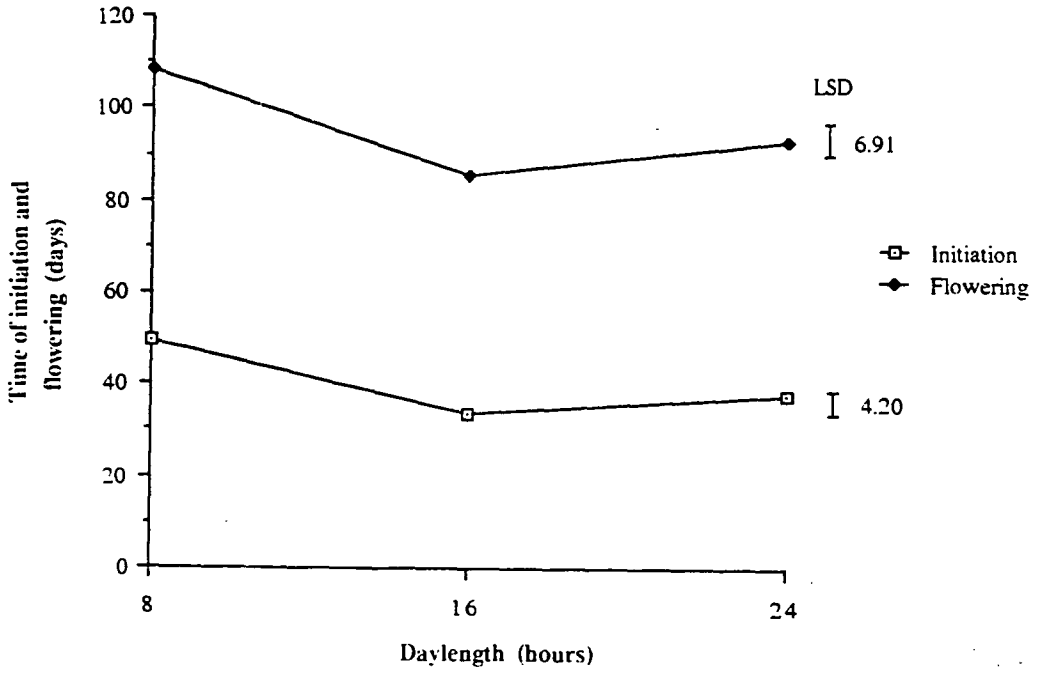


Figure IV.B.4.3.1 - Effect of daylength on flower initiation and development. Time of flower initiation was recorded when stem elongation was first observed. Time of flowering was recorded when the first flower reached flower stage II, ray florets vertical.

Figure IV.B.4.3.2

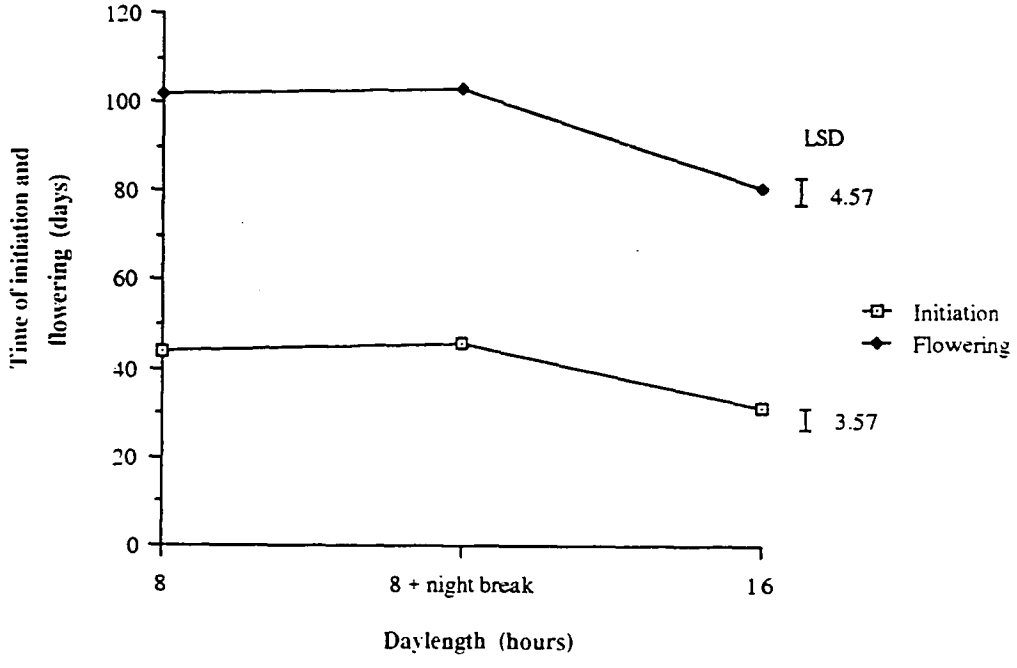


Figure IV.B.4.3.2 - Effect of photoperiod on flower initiation and development. Time of flower initiation was recorded when stem elongation was first observed. Time of flowering was recorded when the first flower reached flower stage II, ray florets vertical.

hours daylength were increased in this experiment compared with the corresponding rates in the daylength experiment. The daylength experiment was carried out in late autumn / early winter and the photoperiod experiment in spring when the average photon flux density and temperature were higher. Either of these parameters may have influenced the growth rate of plants through their effect on the rate of photosynthesis (Section IV.B.3.4) and the higher temperature may also have increased sink activity.

4.4 Discussion

Daylength has a quantitative effect on both flower initiation and development in pyrethrum, with both processes promoted by long days. The flowering behaviour of pyrethrum thus diverges from the proposed model based on the flowering of *D. grandiflora*. *D. grandiflora* is a quantitative short day plant, although some day neutral cultivars exist (Cathey, 1969). The inhibitory effect of short days on the flowering process in pyrethrum appears to be mediated by restricting the photosynthate production and not via the phytochrome reactions. In this regard, pyrethrum must be considered a day neutral species as its daylength reaction is to the daily light integral and not to photoperiod.

Night break lighting in the middle of the dark period failed to stimulate flower initiation or development in short day conditions. Night breaks of shorter duration and lower PFD have been demonstrated to be effective in *D. grandiflora* (Kadman-Zahavi and Yahel, 1971). This indicates that the night break used in this experiment was sufficient to influence any phytochrome reactions involved in the possible photoperiodic processes. As the treatment had no effect, the conclusion that must be drawn is that phytochrome mediated processes do not control flower initiation and development.

Daily light integral appears to be the critical element in the effect of daylength treatments. The reduced photosynthetic capacity associated with shorter days may influence the assimilate distribution pattern within the plant as well as the overall level of photosynthate. As apical size and stage of apical development are related during flowering (Section IV.B.1.3.1), the supply of assimilates to the developing meristem will influence the rate of apical development. The decreased rate of flower development observed under 24 hours daylength cannot be explained in this way, but is probably caused by the lack of a dark period in the treatment preventing certain reactions

involved in photosynthesis and other plant processes from occurring. If the daily light integral influences flowering, then changes in photon flux under constant daylength will alter the rates of flower initiation and development.

IV.B.5 Day Temperature and Photon Flux Effects

5.1 Introduction

Both day temperature and photon flux have been demonstrated to influence the flowering of pyrethrum. Glover (1955) proposed an upper limit of mean maximum temperature of 24 °C which, if prolonged for a week or more, leads to the inhibition of flower production. Unfortunately, it is not evident from Glover's work if the inhibitory effect of high temperature is on flower initiation or on flower development. Similarly, the effects of low and moderate day temperatures on initiation and flower development have not been adequately separated in previous publications.

Flower yield was reduced by 50 percent when plants were grown in 90 percent shade (Muturi *et al*, 1969). Whether this yield reduction is a result of an inhibitory effect on flower initiation or simply a consequence of reduced photosynthesis and therefore reduced growth rate is not discussed. Daylength appears to affect the rates of flower initiation and flower development through changes in the daily total light integral and its influence on photosynthesis (Section IV.B.4.3.1). Changes in PFD also affect photosynthesis (Section IV.B.3.4) and may reasonably be expected to influence flower initiation and development because of this.

Higher PFD's increase the rate of flower development in *D. grandiflora* (Hughes and Cockshull, 1971a). The flowering response is related to the daily total of radiant energy rather than the maximum irradiance (Hughes and Cockshull, 1971b). Low PFD's combined with high temperatures (>24 °C) may result in devernalisation of vernalised shoots (Schwabe, 1957). If PFD and temperature influence the flowering of pyrethrum in a similar way, the effects of vernalisation will be greatly modified throughout the year under Tasmanian conditions by these environmental factors. The effects of temperature and photon flux were examined in this series of experiments.

5.2 Materials and Methods

5.2.1 Day Temperature

The four controlled environment cabinets described in Section III.2.3 were used

in this experiment. Each cabinet was set with a 12 hour daylength and 6 °C night temperature. Day temperatures were maintained at 10 °C, 15 °C, 20 °C and 25 °C. Twelve CIG 3 plants were placed in each cabinet and examined every second day when they were hand watered.

After seventy days, only 12 plants had shown any signs of stem elongation. It was considered that the low PFD in the cabinets, which varied from 280 $\mu mol.m^{-2}.s^{-1}$ to 190 $\mu mol.m^{-2}.s^{-1}$ may have been inhibitory to floral initiation and development. The experiment was terminated at this point and the number of plants with elongating stems recorded.

To establish if the low PFD was inhibiting flower initiation and development the plants were transferred to two light tunnels with a PFD of 600 - 900 $\mu mol.m^{-2}.s^{-1}$ (natural daylight) or kept in two of the growth cabinets. Daylength in the tunnels and cabinets was kept at 12 hours, and day temperature at 20 °C in the cabinets or 20 - 25 °C in the tunnels. Night temperatures of 6 °C and 20 °C were set in the two tunnels and the two cabinets. Three plants from each of the original treatments were labelled and randomly selected for transfer into each of the tunnels and the cabinets. After thirty days all plants were dissected and the number of developing floral and vegetative shoots recorded as well as the average stage of apical development of the flowering shoots.

5.2.2 Photon Flux / Vernalisation Interaction

Two lengths of vernalisation at three night temperatures and three photon flux densities were combined in all possible combinations. The 15 treatments consisted of three or six weeks at night temperatures of 6 °C or 12 °C and PFD's of 200, 350 or 800 $\mu mol.m^{-2}.s^{-1}$, with control treatments of 18 °C night temperature at each PFD. Daylength for each treatment was 12 hours. A PFD of 200 $\mu mol.m^{-2}.s^{-1}$ was maintained in three growth cabinets with 20 °C day temperature and 6 °C, 12 °C or 18 °C night temperature. 'Sarlon' shade cloth covered part of each light tunnel to reduce the PFD to 350 $\mu mol.m^{-2}.s^{-1}$ from the ambient 800 $\mu mol.m^{-2}.s^{-1}$ (average of values recorded over ten days, with three readings taken each day). Day temperature ranged from 20 to 25 °C and the respective tunnels were set at 6 °C, 12 °C or 18 °C night temperature.

Each treatment was given to 5 CIG 3 plants. After receiving 3 or 6 weeks

vernalisation the plants were transferred to the 18 °C night temperature light tunnel under 800 $\mu mol.m^{-2}.s^{-1}$ PFD. One plant per treatment was selected for dissection at the time of transfer to determine the stage of apical development. Mature leaf samples of these plants were taken for RIA analysis of plant growth regulator concentrations. Statistical analysis of the RIA results was not performed as only single replicates were taken. Control plants under PFD's of 200 $\mu mol.m^{-2}.s^{-1}$ and 350 $\mu mol.m^{-2}.s^{-1}$ were transferred to 800 $\mu mol.m^{-2}.s^{-1}$ after six weeks. RIA samples were only taken from control plants under 800 $\mu mol.m^{-2}.s^{-1}$ PFD conditions.

Plants were examined regularly and scored when they reached flowering stage II. After 108 days the experiment was terminated and each plant dissected. Each developing shoot was examined and classed as vegetative (normal), vegetative (short stem), floral (short stem) or floral (normal). Vegetative short stem plants were those in which vegetative rosette development occurred after a short period of stem elongation. Floral short stem shoots were those in which abnormally short flower stems developed (Section IV.A.1.3.3).

5.3 Results

5.3.1 Day Temperature

After seventy days in the growth cabinets only twelve plants had shown any sign of stem elongation ; three plants under 10 °C day temperature, four plants each under 15 °C and 20 °C and one plant under 25 °C. It was concluded that low PFD was inhibitory to flower initiation and development as flower initiation and development were observed to occur normally under 20 °C day temperature at higher PFD in the vernalisation experiment (Section IV.B.2.3.1). The aim of the redesigned experiment was to determine if this conclusion was valid.

High PFD conditions accelerated the rate of flower initiation and development. Figure IV.B.5.3.1.a shows that the stage of apical development was significantly higher in both high PFD treatments (600 - 900 $\mu mol.m^{-2}.s^{-1}$) compared with the low PFD, growth cabinet treatments (280 $\mu mol.m^{-2}.s^{-1}$) for each of the original day temperature conditions except 25 °C. The 25 °C plants in the light tunnels under high PFD were at a similar stage of apical development as the 25 °C plants in the growth

Figure IV.B.5.3.1.a

Treatment	10 °C	15 °C	20 °C	25 °C
LT 20/20	8.00	8.00	8.00	2.00
LT 20/6	8.00	7.67	7.33	2.67
GC 20/20	2.75	2.75	3.00	3.00
GC 20/6	3.33	2.50	2.50	2.33

Figure IV.B.5.3.1.a - Average stage of apical development (from Figure IV.A.1). Plants were transferred from the day temperatures listed at the top of the table to the treatments listed on the left after seventy days and harvested thirty days later. Treatments were light tunnels (LT) at 600 - 900 $\mu mol.m^{-2}.s^{-1}$ PFD or growth cabinets (GC) at 280 $\mu mol.m^{-2}.s^{-1}$ and 20 °C / 20 °C (20/20) day and night temperature respectively, or 20 °C / 6 °C (20/6) day and night temperature respectively.

Figure IV.B.5.3.1.b

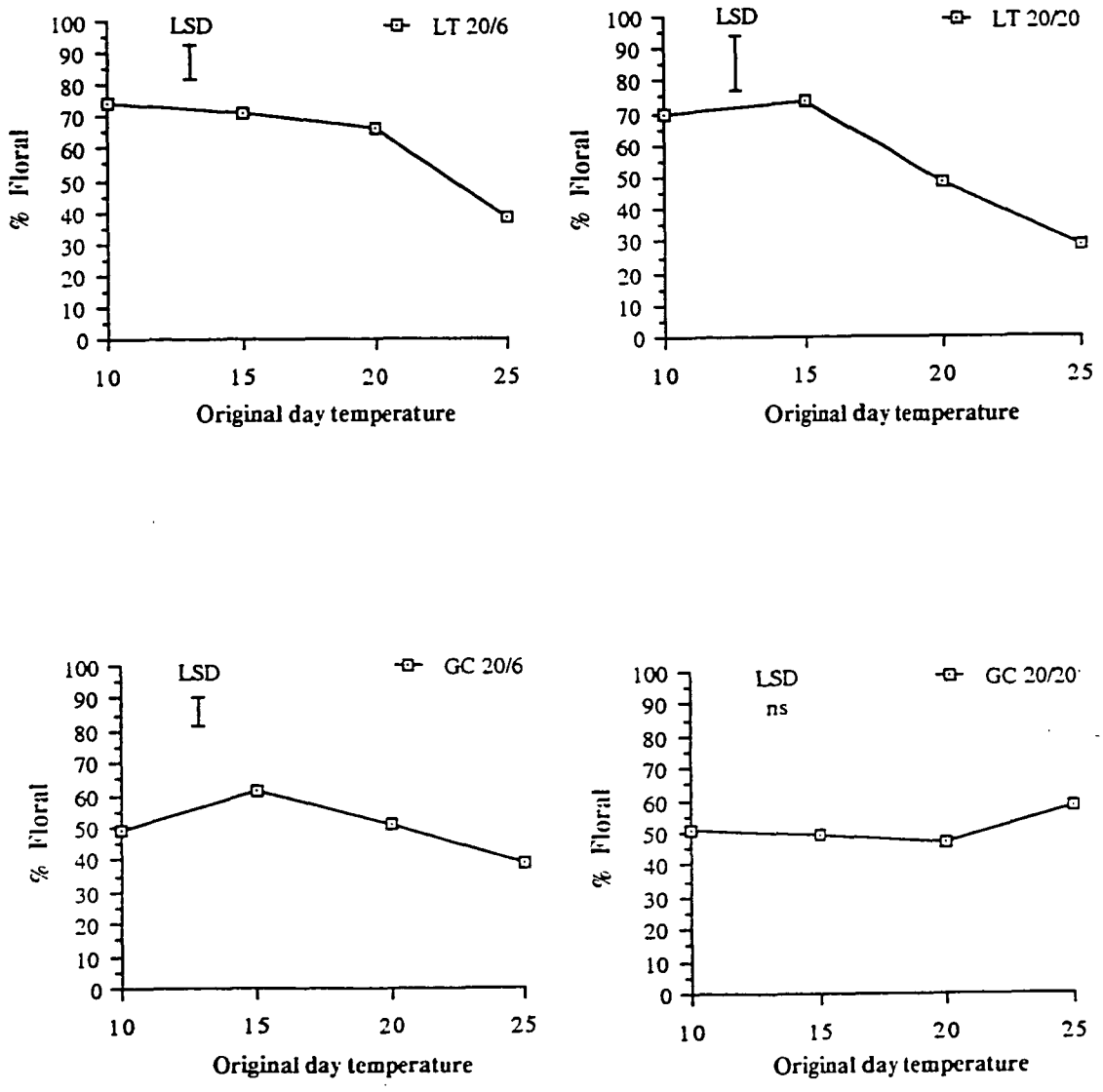


Figure IV.B.5.3.1.b - Number of floral shoots expressed as a percentage of the total shoot number. Treatments were light tunnels (LT) at 600 - 900 $\mu mol.m^{-2}.s^{-1}$ PFD or growth cabinets (GC) at 280 $\mu mol.m^{-2}.s^{-1}$ and 20 °C / 20 °C (20/20) day and night temperature respectively, or 20 °C / 6 °C (20/6) day and night temperature respectively.

cabinets, suggesting that flower initiation was inhibited or significantly delayed by the original treatment.

The number of floral shoots (expressed as a percentage of the total number of shoots) also increased under high PFD conditions. Figure IV.B.5.3.1.b shows that seventy percent or greater of shoots were floral under the high PFD conditions for the 10 °C and 15 °C plants. The floral percentage dropped significantly for the 20 °C and 25 °C plants in the light tunnel under 20 °C night temperature, and similarly for the 25 °C plants in the 6 °C tunnel. Again this indicates that the original 25 °C day temperature conditions were inhibitory to floral initiation. The percentage of floral shoots in the 6 °C light tunnel was higher than in the 20 °C tunnel for both 20 °C and 25 °C plants as the former night temperature was sufficient for vernalisation.

The floral percentages of the plants in the growth cabinets were lower than the corresponding plants in the light tunnels. The 25 °C treatments were the only exception as the 6 °C treatments had similar percentages and the growth cabinet 20 °C treatment had a higher floral percentage than the corresponding light tunnel treatment. One plant in the growth cabinet 20 °C treatment had a very high floral percentage, resulting in the observed discrepancy. Each of the growth cabinet treatments resulted in a floral percentage of around fifty, indicating that the low PFD conditions were preventing floral development rather than initiation.

5.3.2 Photon Flux / Vernalisation

Figure IV.B.5.3.2.a shows that maximum flowering was only seen after six weeks at 6 °C under each of the PFD conditions or six weeks at 12 °C under 800 $\mu mol.m^{-2}.s^{-1}$ PFD. A smaller number of plants flowered after three weeks at 6 °C under each of the PFD conditions and after three weeks at 12 °C under 800 $\mu mol.m^{-2}.s^{-1}$ PFD. No plants were observed to initiate flowers under any of the other treatments, including the controls. Rapid flower development was promoted by high PFD combined with a 6 °C vernalising temperature, while flower development was delayed by lower PFD's and the 12 °C vernalising temperature conditions.

The effect of PFD in modifying the vernalisation response can clearly be seen in Figures IV.B.5.3.2.b, IV.B.5.3.2.c and IV.B.5.3.2.d. At high PFD (Figure IV.B.5.3.2.b) six weeks vernalisation stimulates the largest flowering response. Three weeks at 6 °C or six weeks at 12 °C also elicit a strong flowering response while three

Figure IV.B.5.3.2.a

Vernalisation temperature	Vernalisation length (weeks)	Photon flux density	No. of plants flowering	Flowering time (days)
18	-	800	0	-
18	-	350	0	-
18	-	200	0	-
6	3	800	3	84.7
6	3	350	2	101.5
6	3	200	2	94.5
6	6	800	4	83.5
6	6	350	4	90.0
6	6	200	4	97.0
12	3	800	2	103.5
12	3	350	0	-
12	3	200	0	-
12	6	800	4	89.0
12	6	350	0	-
12	6	200	0	-

Figure IV.B.5.3.2.a - Effect of photon flux density and vernalisation on the number of plants flowering and flowering time (flower stage II, ray florets vertical). Plants were transferred after 3 or 6 weeks from the vernalising conditions (6 °C or 12 °C) to 18 °C night temperature conditions. Control plants were maintained in the non-vernalising 18 °C conditions.

Figure IV.B.5.3.2.b

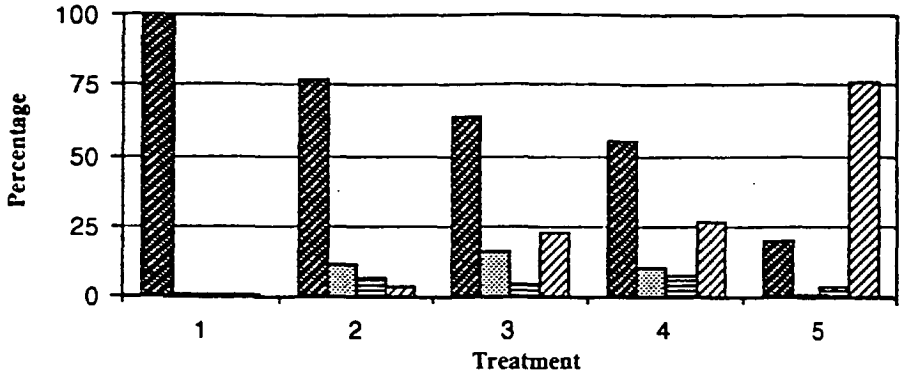


Figure IV.B.5.3.2.c

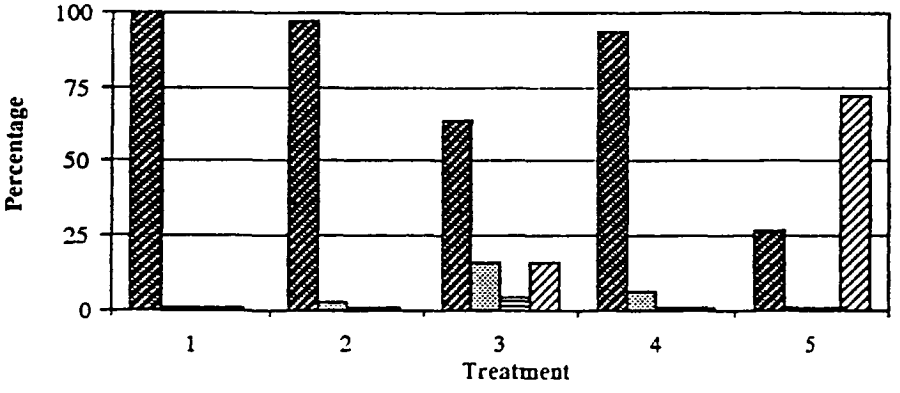
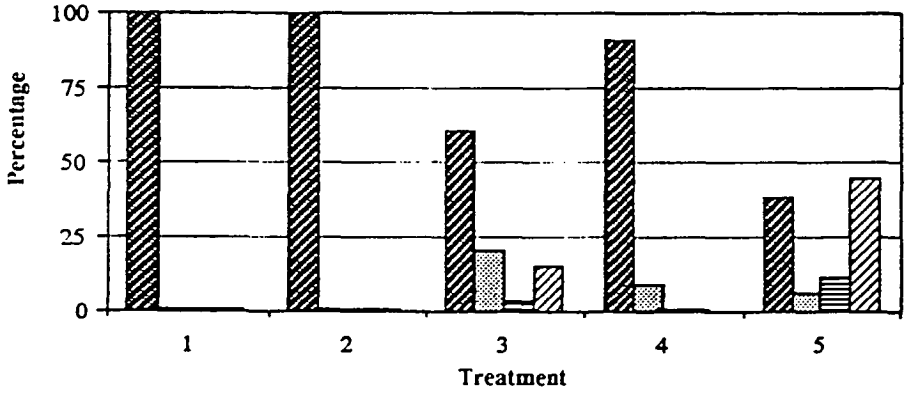


Figure IV.B.5.3.2.d



Treatments

1 - Control, 18 °C
2 - 12 °C, 3 weeks
3 - 6 °C, 3 weeks
4 - 12 °C, 6 weeks
5 - 6 °C, 6 weeks

Shoot Type

- Vegetative (normal)
- Vegetative (short)
- Floral (short)
- Floral (normal)

Figure IV.B.5.3.2.b - Shoot types under 800 $\mu mol.m^{-2}.s^{-1}$ PFD

Figure IV.B.5.3.2.c - Shoot types under 350 $\mu mol.m^{-2}.s^{-1}$ PFD

Figure IV.B.5.3.2.d - Shoot types under 200 $\mu mol.m^{-2}.s^{-1}$ PFD

weeks at 12 °C results in a small percentage of flowering stems. The latter three treatments also resulted in a number of shortened stems, both vegetative and floral, which was an indication that the conditions are only marginally inductive (Section IV.B.2.4). This conclusion is supported by the delays observed in the timing of flower stage II under these treatments (Figure IV.B.5.3.2.a).

At a PFD of 350 $\mu mol.m^{-2}.s^{-1}$ the percentage of floral shoots is reduced from those noted above. Figure IV.B.5.3.2.c shows that six weeks at 6 °C again promotes flowering and three weeks at 6 °C also results in a significant flowering response. No flower stems were seen after 12 °C vernalising treatments although a small number of short, vegetative stems were noted.

Figure IV.B.5.3.2.d shows that the 200 $\mu mol.m^{-2}.s^{-1}$ PFD treatment reduced the percentage of normal flower stems after six weeks at 6 °C and resulted in a number of shortened vegetative and floral stems forming. It appears that the six weeks at 6 °C vernalising treatment tends to become marginally inductive as PFD decreases. The conditions were also marginally inductive after three weeks at 6 °C, producing a small number of both normal and shortened flower stems as well as a large percentage of short vegetative shoots. Decreasing the PFD tended to shift the balance of shoot development from floral to vegetative, and caused a large number of intermediate, shortened stems when conditions became marginally inductive.

5.3.3 Endogenous Hormonal Changes

Changes in photon flux resulted in marked changes in gibberellin metabolism within the plant. Figure IV.B.5.3.3.a shows that the gibberellin concentration of mature leaf tissue was significantly reduced by the 200 $\mu mol.m^{-2}.s^{-1}$ PFD treatment regardless of the prevalent vernalising conditions. Vernalising conditions increased the gibberellin concentration under 800 $\mu mol.m^{-2}.s^{-1}$ PFD, particularly the six weeks, 6 °C treatment. Vernalisation also resulted in a smaller increase at 350 $\mu mol.m^{-2}.s^{-1}$ PFD, the exception being the three week , 6 °C treatment where the gibberellin concentration was less than in the control plants. As stated earlier, no statistical analysis of these results was carried out as the sampling was not replicated. While the samples were taken from plants chosen as representative of their treatment, it is possible that anomalies may exist in this data due to plant differences rather than treatment difference

The photon flux treatments appeared to have little effect on the endogenous

Figure IV.b.5.3.3.a Endogenous gibberellin concentration in mature leaf samples. Plants were vernalised for 3 or 6 weeks at the temperatures indicated. Photon flux densities were maintained at 200, 350 or 800 $\mu mol.m^{-2}.s^{-1}$. The 18 °C treatment was a non-vernalising control.

Figure IV.b.5.3.3.b Endogenous indolylacetic acid concentration in mature leaf samples. Plants were vernalised for 3 or 6 weeks at the temperatures indicated. Photon flux densities were maintained at 200, 350 or 800 $\mu mol.m^{-2}.s^{-1}$. The 18 °C treatment was a non-vernalising control.

Figure IV.b.5.3.3.c Endogenous abscisic acid concentration in mature leaf samples. Plants were vernalised for 3 or 6 weeks at the temperatures indicated. Photon flux densities were maintained at 200, 350 or 800 $\mu mol.m^{-2}.s^{-1}$. The 18 °C treatment was a non-vernalising control.

Figure IV.B.5.3.3.a

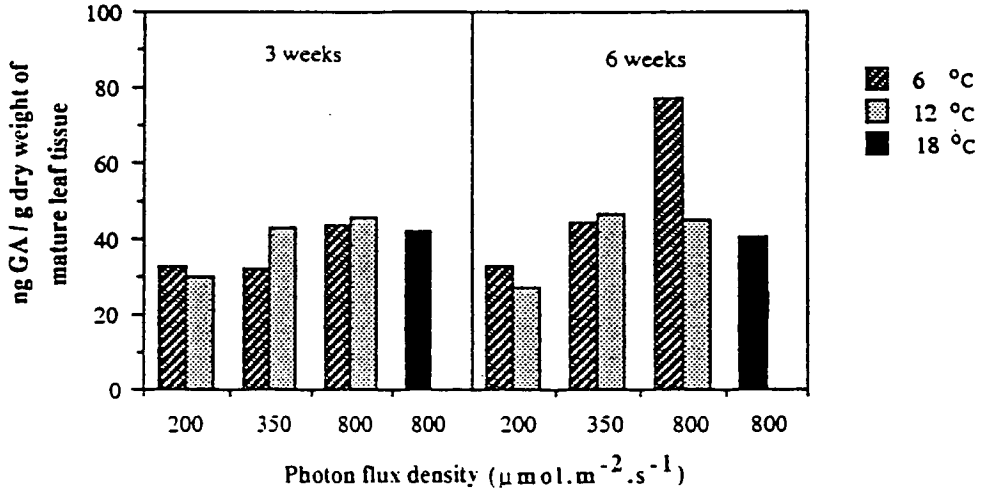


Figure IV.B.5.3.3.b

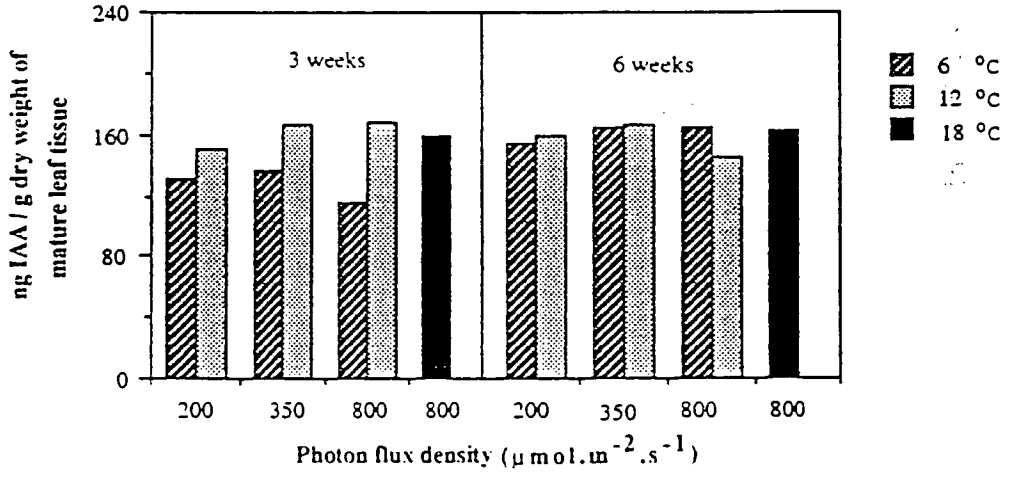


Figure IV.B.5.3.3.c

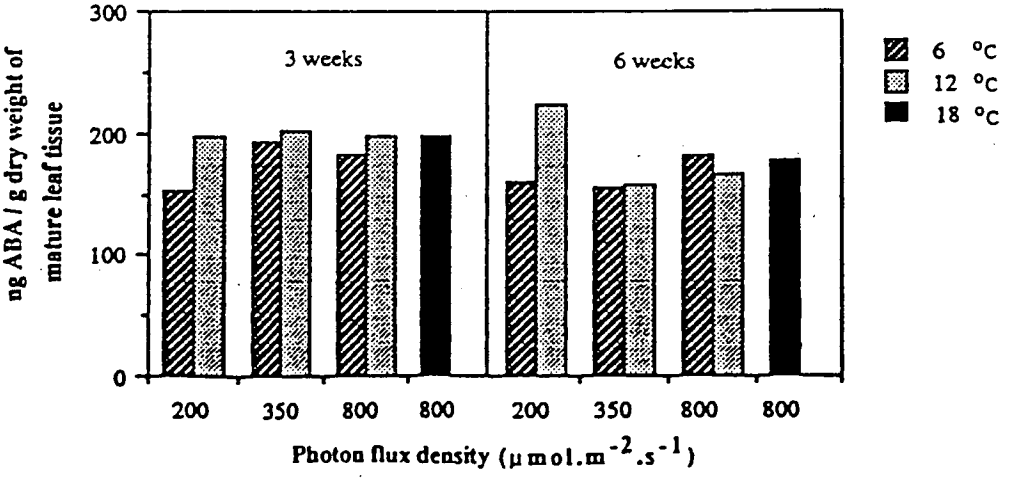


Figure IV.b.5.3.3.d Endogenous dihyrozeatin concentration in mature leaf samples. Plants were vernalised for 3 or 6 weeks at the temperatures indicated. Photon flux densities were maintained at 200, 350 or 800 $\mu mol.m^{-2}.s^{-1}$. The 18 °C treatment was a non-vernalising control.

Figure IV.b.5.3.3.e Endogenous isopentyladenosine concentration in mature leaf samples. Plants were vernalised for 3 or 6 weeks at the temperatures indicated. Photon flux densities were maintained at 200, 350 or 800 $\mu mol.m^{-2}.s^{-1}$. The 18 °C treatment was a non-vernalising control.

Figure IV.B.5.3.3.d

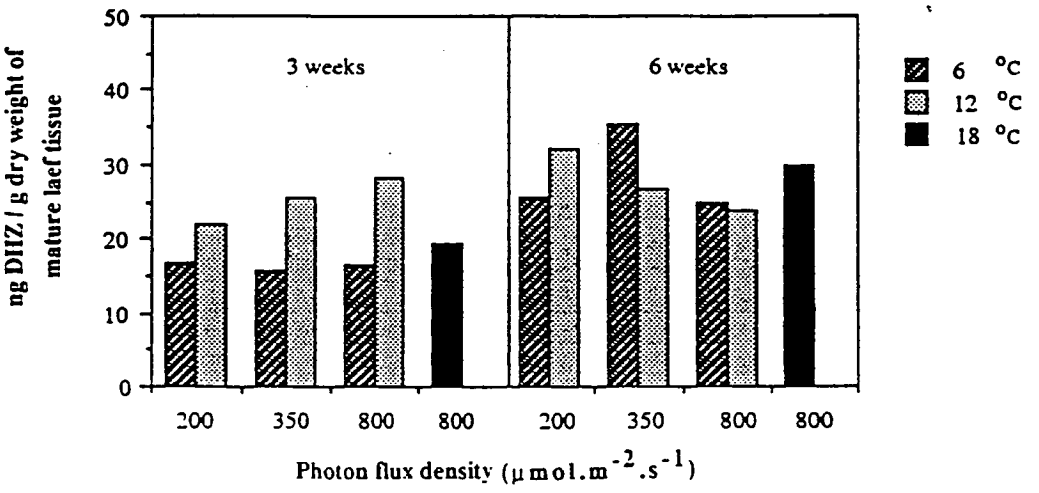
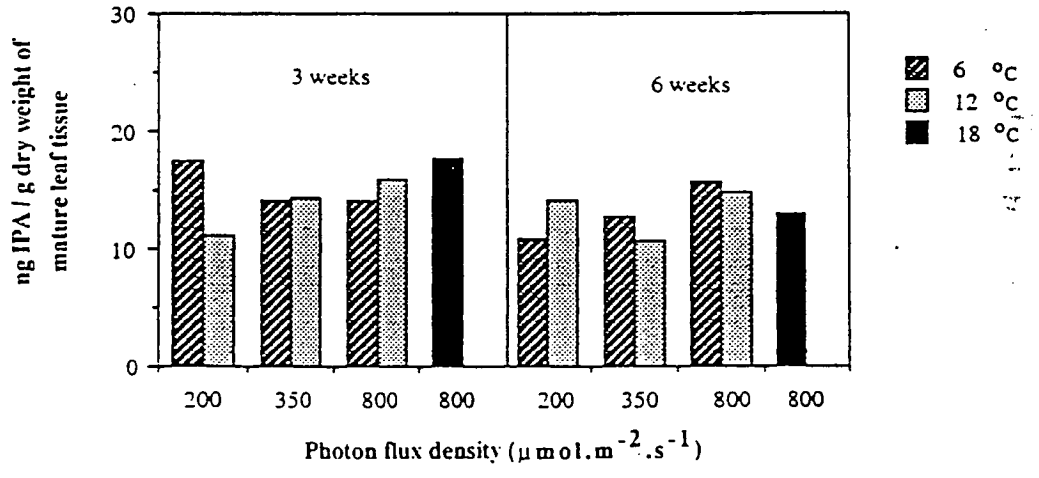


Figure IV.B.5.3.3.e



concentrations of the other plant growth regulators examined. Figure IV.B.5.3.3.b shows the variations in IAA concentration. Three weeks vernalisation at 6 °C reduced the IAA concentration at each PFD, but six weeks had no effect. However, stem elongation was observed in both the 800 $\mu mol.m^{-2}.s^{-1}$ and 350 $\mu mol.m^{-2}.s^{-1}$ PFD treatments at this sampling date, and increased IAA concentrations may accompany this process (Section IV.B.2.3.2).

The ABA concentration (Figure IV.B.5.3.3.c) remains reasonably steady between the treatments and does not appear to be influenced by either PFD changes or vernalisation. A similar conclusion may be drawn for both dihydrozeatin (Figure IV.B.5.3.3.d) and isopentyladenosine (Figure IV.B.5.3.3.e). Unfortunately the zeatin content of the tissue samples could not be assayed as the percentage recovery of the hormone during the extraction procedure was low (70 %) and insufficient quantities were recovered for RIA analysis.

5.4 Discussion

Flower development is retarded by low photon flux density conditions regardless of day temperature. Only twelve plants out of a total of forty eight displayed macromorphological signs of flower initiation after seventy days in otherwise inductive, vernalising conditions. Flower development was promoted by transfer to high photon flux density conditions under both vernalising and non-vernalising night temperatures. The percentage of floral shoots was also greater in plants under high photon flux density than under low photon flux density conditions. As the percentage of flowering shoots in non-vernalising conditions was higher under high photon flux density than low photon flux density, flower initiation must be limited by the latter conditions. Either the rate of flower initiation is decreased under low photon flux conditions or the evocational sequence is completed except for one or more processes requiring higher photon fluxes.

High day temperature combined with low photon flux resulted in a devernalisation-like effect. Plants maintained under low PFD and a day temperature of 25 °C were not able to respond to the otherwise inductive 6 °C vernalising night temperature. When transferred to high PFD conditions, a greater percentage of flower stems was initiated under vernalising than non-vernalising conditions, indicating that

the plants had not responded to the original vernalisation treatment. The average stage of apical development was also reduced, supporting this conclusion. This is not a true devernalisation response as the plants appear to be prevented from responding to the vernalisation treatment rather than the vernalisation stimulus being reversed by a later treatment.

The effect of low photon flux appears to be in reducing the ability of the plants to respond to otherwise inductive conditions. The effect is not totally inhibitory as initiation can occur under low PFD conditions. Six weeks at 6 °C under a PFD of 200 $\mu mol.m^{-2}.s^{-1}$ stimulated rapid flower initiation and development, although the percentage of floral shoots was less than in plants under higher PFD's. The rate of flower initiation was reduced by decreasing photon flux, and conditions which were marginally inductive under higher PFD's became non-inductive when lower PFD's were used.

The occurrence of shortened flower stems and vegetative rosette shoots on elongated internodes indicates that many of the evocational requirements are satisfied but that environmental conditions are not fully conducive to flower initiation. One or more factors must be limiting the evocational sequence so that normal development cannot occur. If returned to non-inductive conditions before the meristem is committed to flowering the evocational requirements for limited internode elongation may be satisfied but further limitations prevent evocation from proceeding. Thus vegetative rosette growth continues after a short period of internode elongation. If the meristem is florally committed, then shortened flower stems may result if the factors required for normal flower stem elongation are limited.

Assimilate supply may limit the rate of floral initiation as the meristem must reach a critical size before floral commitment can occur. A reduction in assimilate supply to the meristem, either through a decrease in the rate of photosynthetic CO_2 assimilation or a change in the pattern of assimilate distribution, may conceivably reduce the rate of floral initiation. In this way, low photon flux conditions may reduce the rate of flower initiation. The inhibition of flower initiation observed at 25 °C cannot be attributed simply to a reduction in photosynthesis as the rate of net CO_2 assimilation (Section IV.B.3.3) is greater at this temperature than at 10 °C and 15 °C, conditions which were not inhibitory to flower initiation. The inhibitory effect must be due to changes in the pattern of assimilate distribution , or other endogenous chemical changes which prevent the evocational sequence from being completed.

Low photon flux conditions affected gibberellin concentration. A reduction in gibberellin concentration was observed under each of the low PFD treatments irrespective of the temperature or duration of vernalisation. Gibberellin concentration has previously been shown to increase during vernalisation (Section IV.B.2.3.2). Gibberellins may mediate one or more of the evocational processes, and the low photon flux effect may be due to this reduction in gibberellin levels. The short flower stems observed under marginally inductive conditions are probably a result of the reduced gibberellin concentration. The fact that, in the case of the short vegetative shoots, some stem elongation was observed under these conditions, and before evocation was completed, indicates that at least one of the evocational events is not mediated by overall gibberellin levels.

Of the other endogenous plant growth regulators examined, only auxin concentration appeared to be affected in any way by low PFD conditions. The auxin concentration may have been slightly reduced by the 6 °C vernalisation treatment but the decline was not as marked as noted earlier (Section IV.B.2.3.2). Low PFD conditions may maintain auxin concentrations at a high level, thus restricting axillary bud outgrowth and so reducing the flowering response.

As mentioned previously, using mature leaf samples for RIA analysis gives an indication of the overall plant status but does not identify localised plant growth regulator fluxes which may mediate certain plant responses.

IV.B.6 Autoradiography

6.1 Introduction

The relationship between apical diameter and the stages of apical development during flowering described for pyrethrum (Section IV.A.1.3.1) indicates that flower initiation and development are dependent on assimilate supply. Low light intensity can result in a devernalisation effect (Section IV.B.5.4), where plants held in otherwise inductive, vernalising conditions continue developing vegetatively. While defoliation experiments with chrysanthemum have indicated that the devernalisation response is not due entirely to assimilate limitations (Schwabe, 1957), the relationship between apical size and stage of development indicates that reduced assimilate supply may be one of several factors limiting floral evocation in devernalising conditions. In many plants, the evocational assimilate requirement is met by a redistribution of assimilates under inductive conditions rather than an increase in the net rate of CO_2 assimilation. The sink strength of potential floral meristems is thus increased under inductive conditions and assimilate distribution is altered during floral initiation and development to favour these meristems.

The aim of this experiment was to use autoradiography to investigate the distribution of photosynthate during floral initiation and development. The assimilate distribution pattern was also examined under devernalising conditions to ascertain the role of assimilates in this process.

6.2 Materials and Methods

6.2.1 Source of Isotope and Generation of $^{14}CO_2$

Sodium bicarbonate ($NaH^{14}CO_3$) was purchased from Amersham International in vials containing 1ml of $NaH^{14}CO_3$, specific activity 58 mCi/mmole, radioactive concentration 2 mCi/ml. This was diluted with 3.0ml of distilled water and adjusted to pH 9.4 with 0.01N NaOH to make a diluted solution of 0.5 mCi/ml.

$^{14}CO_2$ was generated with the aid of a Shimshi apparatus (Figure IV.B.6.2.1). In this the $NaH^{14}CO_3$ was placed in a boiling tube and sealed in the generating cylinder

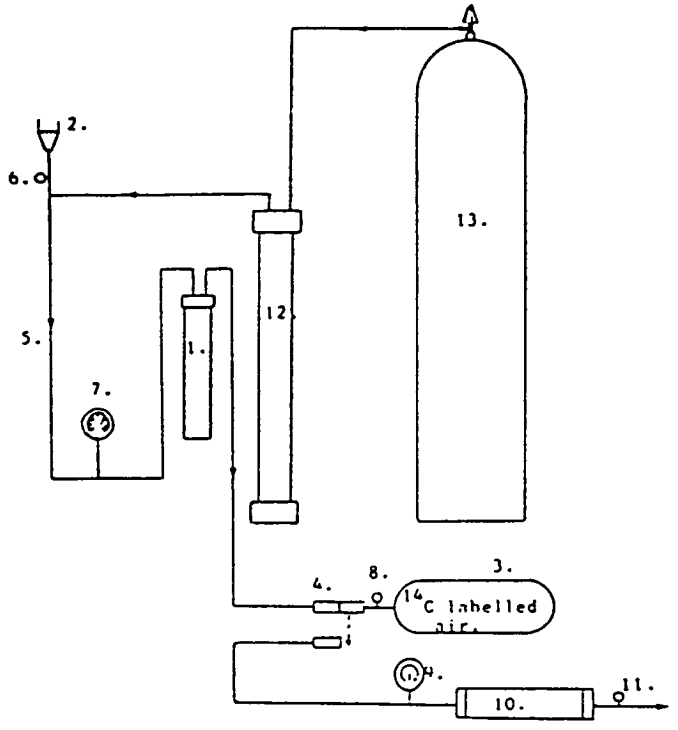


Figure IV.B.6.2.1 - Diagramatic representation of the Shimshi apparatus

1. ^{14}C generating cylinder
2. Separator funnel containing 1N H_2SO_4
3. ^{14}C storage cylinder
4. Snap connectors
5. Air and acid connecting line
6. Acid line stop tap
7. Generating pressure gauge
8. Storage cylinder stop tap
9. Out-flow pressure gauge
10. Releasing cylinder
11. Control release tap
12. Acid trap (NaOH pellets)
13. 'G' size air cylinder

(1); the storage cylinder (3) was connected to the top of the generating cylinder (1) using snap connectors (4). Approximately 15ml of 1N H_2SO_4 was pipetted into the separator funnel (2). After releasing the acid into the line (5), this was sealed by closing the tap (6) and air was used to push the acid into the generating cylinder (1). Reading the pressure gauge (7), the total pressure of air and generated $^{14}CO_2$ was brought up to five atmospheres over approximately five minutes. The storage cylinder was then sealed by turning tap (8) and was connected to the outflow pressure gauge (9) and releasing cylinder (10). $^{14}CO_2$ was released from the cylinder (10) by reading the gauge (9) and controlling the output tap (11) on the releasing cylinder (10).

Pilot experiments (not shown) were set up to optimize the level of ^{14}C for autoradiography studies. It was found that 5 μCi per plant of $^{14}CO_2$ was sufficient. The number of plants to be fed determined the amount of ^{14}C placed in the Shimshi machine, as described above. Normally four plants were fed at a time, and hence 20 μCi of $^{14}CO_2$ was placed in the Shimshi apparatus.

6.2.2 Application of $^{14}CO_2$

Two techniques for introducing $^{14}CO_2$ into plants were tested ; the first involved feeding $^{14}CO_2$ to whole plants. In this case the pots were sealed in plastic bags and plants were placed in an airtight perspex box 1.0m long, 0.5m high and 0.3m wide. The second involved feeding single leaves (Plate 23) which were exposed by placing inside a glass column 0.3m long and 3cm internal diameter. The column was clamped is such a way that the natural position of the leaf relative to the plant was not disturbed. A split rubber bung was placed over the base of the lateral and forced into the column, this was sealed first with plasticine and then with a layer of vaseline.

Both methods required an estimation of the total leaf area to be fed. A large number of mature and newly expanded leaves were passed through a planimeter and the average areas for both leaf types calculated. Leaf area of whole plants was estimated by counting the leaf numbers and multiplying by the average areas calculated. The calculated average areas were used as an estimate for individual leaf areas, and leaves at either end of the size range were not chosen for feeding. Feeding took place at 20 °C (inside the perspex box and inside the glass columns) with a photon flux density of 200 $\mu mol.m^{-2}.s^{-1}$. Hence, from Figure IV.B.3.3.a, it was possible to approximate the rate of CO_2 uptake and so calculate the required flow rate of air into the feeding

Plate 23

Plate 23 - Apparatus for feeding up to four leaves simultaneously. The Shimshi apparatus and gas cylinder were connected to a flowmeter and the line split to feed four individual feeding tubes surrounding selected leaves. Expired $^{14}CO_2$ and unfixed $^{14}CO_2$ was collected in an NaOH trap. The perspex chamber on the right hand side of the photograph was used for feeding whole plants.

chamber.

Plants were allowed to equilibrate in unlabelled air being supplied at the calculated flow rate at 20 °C and 200 $\mu mol.m^{-2}.s^{-1}$ for approximately 30 minutes. Unlabelled air was then switched off and air containing the isotope fed at the calculated flow rate. The air was bubbled through an NaOH solution after passing through the chamber to trap any CO_2 remaining in the air. Once the Shimshi storage cylinder was empty, the supply of unlabelled air was resumed. The storage cylinder was then flushed with unlabelled air and this was then passed over the plants. Subsequently the feeding chambers were flushed with unlabelled air until a total of four times the chamber volume had been replaced.

A sub-sample of the NaOH trap was taken for calculation of $^{14}CO_2$ content by liquid scintillation counting which showed that approximately 95 percent of the $^{14}CO_2$ was incorporated as photosynthate by the plant.

6.2.3 Plant Treatment

Feeding of whole plants was found to result in too much material to be effectively handled during the dissection and autoradiography procedures. For this reason the main experiments all involved feeding single leaves and only the shoot from which the leaf originated was subsequently dissected and examined by autoradiography. Selection of leaves to be fed was based on physiological uniformity, with leaves on secondary shoots with six to eight expanded leaves and one or two developing tertiary shoots being chosen.

An initial trial experiment was conducted to determine the rate of translocation of ^{14}C-labelled assimilates. Basal leaves of secondary shoots on plants held in 'inductive' and 'non-inductive' conditions were fed and the plants returned to their respective conditions before dissection. Plants were dissected at the following times after feeding : 12 hours, 24 hours, 3 days, 7 days and 14 days. A sample of root tissue as well as the main shoot tip, selected secondary shoot tips and leaves, and all of the leaves and developing shoots on the fed secondary shoot were examined by autoradiography.

Two further experiments were performed to determine the source/sink relationships within individual secondary shoots during floral initiation and development. The first experiment involved feeding plants at different stages of floral development while held in 'inductive', vernalising conditions. Two types of plant

tissue, the most basal mature leaf or the newest expanded leaf on secondary shoots selected as described above, were fed and the entire shoot examined by autoradiography. The selected leaves were fed after plants had been held in inductive conditions for the following periods : 0 days, 14 days, 28 days, 42 days and 56 days. Two plants, with either the most basal mature leaf or the newest, apical expanded leaf fed, were maintained in 'non-inductive' conditions while each of the other fed plants were transferred to 'inductive' conditions after feeding. Plants were dissected five days after feeding.

The second experiment examined the effects of devernalising conditions (25 °C day and night temperature, 100 $\mu mol.m^{-2}.s^{-1}$ PFD during the 10 hour day) on assimilate distribution during flower initiation. The most basal mature leaf or the newest expanded leaf on secondary shoots selected as described above, of plants held in 'non-inductive' conditions or in 'inductive' conditions for three weeks, were fed and the plants transferred to either 'inductive', vernalising conditions or devernalising conditions. The plants were dissected five days after feeding and the fed secondary shoots examined by autoradiography.

6.2.4 Dissection and Drying

Plant tissues selected for autoradiography were dissected, then pressed and dried at 60 °C for three days. Tissue selection depended on the experiment. Where whole plants were exposed, a sample of root material was excised as well as a selection of developing shoots and leaves, and the apical section of the main shoot axis. A small sample of root material was also examined from each of the plants in the translocation time trial along with the entire fed secondary shoot and several leaves and shoot tips from unfed shoots.

Only the secondary shoots which included fed leaves were dissected in the two main experiments. The expanded leaves were individually dissected and arranged in order from basal to apical for drying, with the fed leaf being tagged for identification. The larger expanding leaves were also removed to allow dissection of the apical bud. The apical stem section containing the bud was dissected longitudinally to allow the apical tissue to be placed flat on X-ray paper. Developing tertiary shoots were similarly dissected to reveal the apical portion, although it was usually not necessary to remove

the leaves.

6.2.5 Autoradiography

Under a Kodak safelight filter (type GBX-2), dried plant material was placed on Kodak X-ray paper (type X-omat AR film) in such a way that the tissues to be investigated (i.e. the buds) were closest to the paper. The plant material was placed on the paper in order from basal to apical, with each tertiary shoot placed immediately above its subtending leaf. The paper was laid on a piece of blotting paper on top of a sheet of stainless steel. Several sheets of absorbent paper were then placed over the top of the tissue followed by another sheet of stainless steel. Up to ten individual sheets of X-ray paper were stacked in this way, then wrapped in cellotape to prevent slipping, covered with two light-proof plastic bags and finally placed in a plant press at room temperature.

Ideal exposure time was found to be eight days. Development of the X-ray paper involved removal of all the plant material, placing the paper in Kodak liquid X-ray GBX developer for five minutes at 20 °C, followed by a 30 second rinse in still water and then fixing for two to four minutes in Kodak liquid X-ray GBX fixer and replenisher. Finally the X-ray paper was rinsed for five minutes in running water and air dried at 20 °C.

6.3 Results

6.3.1 Whole Plant Feeding and Translocation Time

Areas containing radioactive label were easily seen as shadowing on the X-ray film. As the darkness of the shadowing was related to the radioactivity of the photosynthate in the plant tissue, a crude quantitative estimation of ^{14}C levels was possible. A grey scale ranging from zero to five (Figure IV.B.6.3.1), white being zero and black five, is used to describe the level of shading seen on the X-ray paper.

A quantitative comparison of activities between plants using X-ray sheets was not made statistically since only one replication was taken. Also, at the time of exposure some leaf areas were considerably greater than others, and photosynthetic rates may have varied between plants. Hence quantitative comparisons between and within

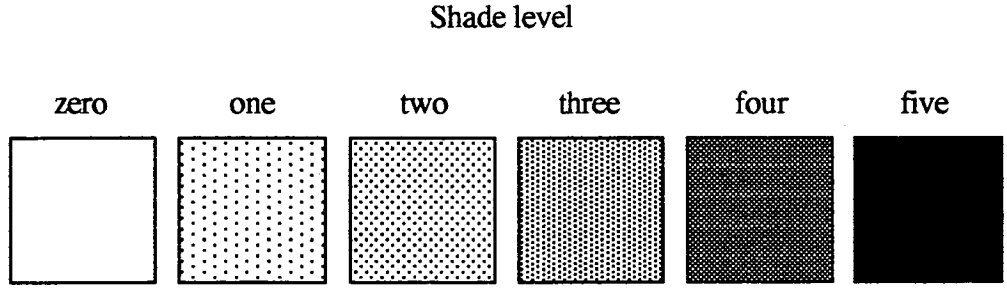


Figure IV.B.6.3.1 - Shade intensities used for comparison of ^{14}C distribution within the plant as revealed by autoradiography.
Shade zero indicates no ^{14}C activity (no change to the X-ray paper after development), whereas shade five shows high isotope levels (the X-ray paper was black after developing).

experiments have only been made tentatively. The grey scale is most useful for making comparisons between ^{14}C distribution on the one X-ray paper. This gives an estimation of the relative amounts being distributed between various tissue types, such as leaves and buds, and also between the various developing shoots and the root system.

Where whole plants were fed, ^{14}C was found to be distributed throughout the plant but was most concentrated (shade five) in the expanding leaf tissue and the bud tissue. Within each shoot there was a basipetal decrease in ^{14}C concentration in the mature leaves of each shoot, from shade three to four in the newest expanded leaves to shade one in the most basal leaves. The ^{14}C concentration in the root samples was also very low with shade three the highest reading, seen in the root tips and in the vascular system connecting adjacent splits. No difference was observed between the ^{14}C distribution patterns in plants under inductive and non-inductive conditions.

Translocation of ^{14}C from a fed basal leaf to other tissue types within a secondary shoot was not observed until three days after feeding. Plants dissected 12 hours or 24 hours after feeding had ^{14}C activity only in the fed leaf (shade five). After three days, the ^{14}C activity in the fed leaf was rated at shade four while the apical bud was shade two. Shade two spots were seen where both immature leaves and the apical meristem were exposed to the X-ray paper. The apical and axillary buds were rated according to the darkest spots observed in the relevant bud section.

Seven days after feeding, the ^{14}C concentrations in the fed leaf and the apical bud were still rated as shades four and two respectively, and the developing tertiary bud in the fed leaf was shade four. After fourteen days the ^{14}C concentration in the shoot decreased markedly, with the fed leaf rated as shade two and the apical bud as shade one. Respiratory loss of ^{14}C as $^{14}CO_2$ could explain this decrease over time, although it is possible that a smaller amount of ^{14}C was initially incorporated into the plant when fed.

In each of the plants used in the translocation time experiment, very little ^{14}C activity was found in the roots examined and none was seen in the adjacent leaf and shoot material from outside the fed secondary shoot. For this reason the root system was not dissected in further experiments and only the fed secondary shoots were examined. As significant amounts of ^{14}C were observed to be translocated from the fed leaf to other shoot tissue between three and seven days after feeding, dissection of plants was carried out five days after feeding in the two main experiments.

6.3.2 Flower Initiation and Development

Figure IV.B.6.3.2 shows the changes in assimilate distribution patterns within secondary shoots during floral development. During vegetative development and floral evocation the terminal bud and its expanding leaves are strong sinks for photosynthate while axillary buds are very weak importers of photosynthetic assimilates, even those produced by subtending leaves. The fed apical leaves proved to be poor exporters of photosynthates to the remainder of the plant during this period, exporting minor concentrations of labelled assimilate to the apical bud or being the only tissue with ^{14}C at the time of dissection. In contrast, basal leaves were all observed to export significant concentrations of ^{14}C-labelled assimilates to the apical portions of the plant, and must be considered to be the plant's major assimilate source.

The first treatment in which ^{14}C was observed in an axillary bud at the same concentration as the apical bud was when the basal leaf of a plant given two weeks vernalisation was fed, and resulted in a ^{14}C concentration measured as shade two. The plant had been held in vernalising conditions for a total of nineteen days, as the plant was dissected five days after feeding, which is slightly less than the minimum vernalisation requirement described in Section IV.B.2.3.1 and indicates that assimilate diversion to the axillary meristem is part of the evocational sequence. The axillary bud remains a weak sink for assimilates at this point, failing to attract any assimilate from the fed apical leaf and having the same ^{14}C concentration as the apical bud when the basal leaf was fed.

At each successive stage of floral development, the ability of the lateral, axillary bud to attract photosynthetic assimilates from within the secondary shoot is enhanced. Flower stem elongation was evident after four weeks vernalisation, and after eight weeks the flower stems were approaching leaf canopy height and the flower buds were visible. The axillary bud became the major sink for assimilates during this period of development as the proportion of ^{14}C label from fed basal leaves was greater in the axillary buds than in either the fed tissue or the apical bud. Labelled photosynthate from the apical leaf was also partitioned to the developing flower buds, and by week eight had attracted a greater proportion of the assimilate than the apical bud. Plates 24 and 25 show the X-ray papers after autoradiography of plants fed after 0 weeks and 8 weeks in vernalising conditions, revealing the trend of assimilate partitioning away from the fed leaf into the developing flower buds. While it is not possible to compare assimilate

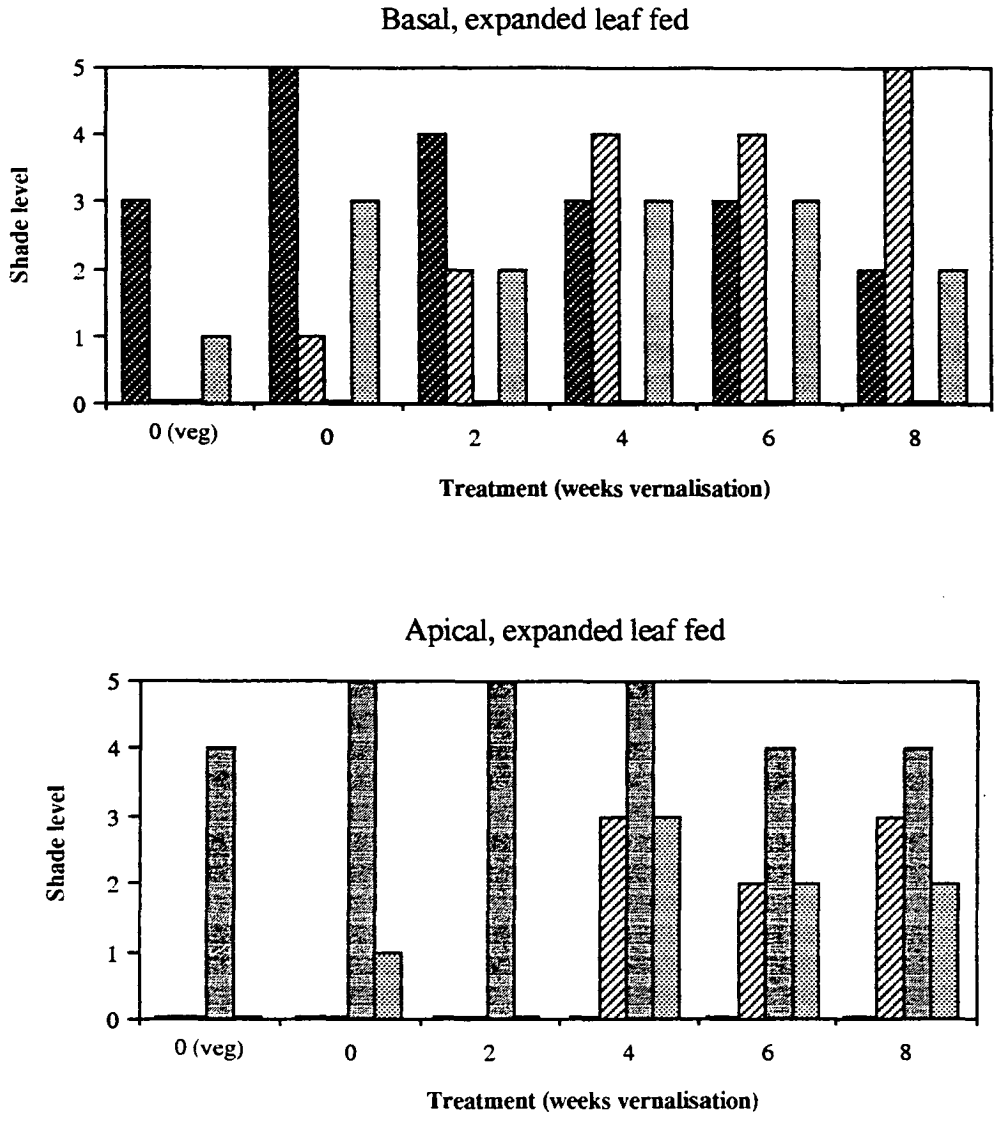


Figure IV.B.6.3.2 - ^{14}C distribution during flower initiation and development.
Shade intensities (from Figure IV.B.6.3.1) estimate the ^{14}C concentration in lateral shoots with the most basal or the most apical expanded leaf fed. Plants were fed after 0,2,4,6 or 8 weeks vernalisation. The veg plants were held in non-vernalising conditions and all other plants in vernalising conditions for five days after feeding before dissection.

Plate 24

Plate 25

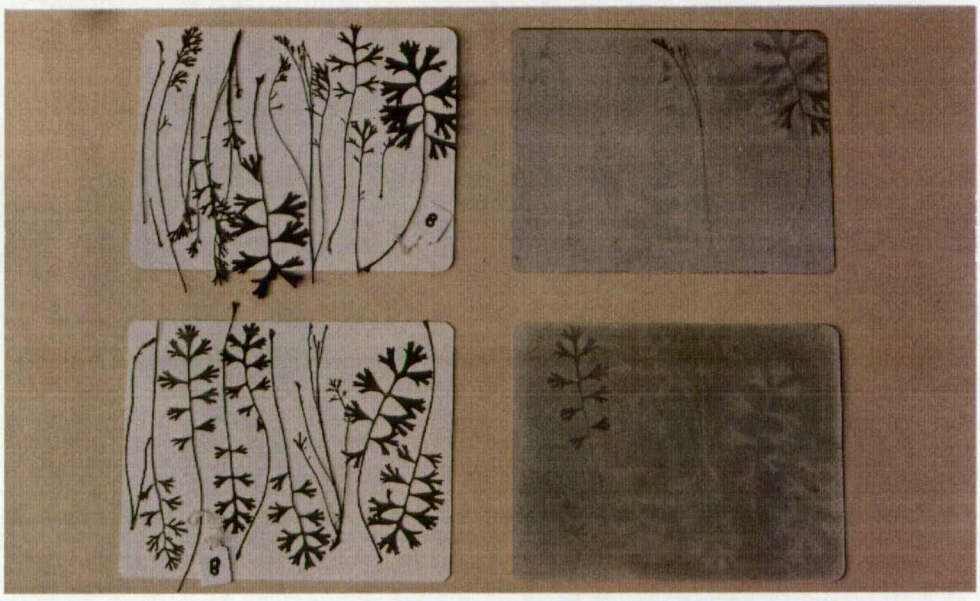


Plate 24 - ^{14}C distribution within lateral shoots with the most basal (top autoradiograph in photo) and most apical (bottom) expanded leaf fed. The plants received no vernalisation before feeding and five days vernalisation before dissection. The dissected shoots are laid out from basal (right) to apical (left) with axillary shoots placed next to their subtending leaf. Bar = 25cm

Plate 25 - ^{14}C distribution within lateral shoots with the most basal (top autoradiograph in photo) and most apical (bottom) expanded leaf fed. The plants received 8 weeks vernalisation before feeding and five days vernalisation before dissection. The dissected shoots are laid out from basal (right) to apical (left) with axillary shoots placed next to their subtending leaf. Bar = 25cm

levels in the various tissue types at the different developmental stages using the quantitative shade measurements, it is clear from the results that the proportion of assimilates being partitioned into the axillary buds increases during floral initiation and development.

6.3.3 Devernalisation

Devernalising conditions reduced the relative sink strength of the axillary meristems. Figure IV.B.6.3.3 shows that no ^{14}C-labelled assimilates were detected in the axillary buds of plants held in devernalising conditions after feeding. This was true for both basal and apical leaves fed on vernalised (3 weeks) and unvernalised (0 weeks) plants. The corresponding plants transferred to vernalising conditions after feeding had very different assimilate distribution patterns. Unvernalised plants (0 weeks) partitioned a major proportion of the ^{14}C fed to the basal leaf to the apical bud but some of the label was detected in the axillary bud. The majority of the ^{14}C fed to the apical leaf remained within the fed leaf, with the apical bud receiving a low concentration rated as shade one. Vernalised plants, fed after three weeks in vernalising conditions, partitioned a major proportion of the ^{14}C fed to the basal leaf to the developing axillary buds. Two axillary buds were developing on the fed secondary shoot, and both had a ^{14}C concentration scored as shade four. In contrast the apical bud was scored as shade three and the fed leaf as shade one. When the apical leaf was fed the photosynthate again remained largely within the fed tissue with only a low concentration detected in the apical bud. Plates 26 and 27 show the X-ray plates of the vernalised shoots after feeding and being placed in vernalising or devernalising conditions respectively. These clearly show the greater proportion of photosynthate partitioned into the axillary buds from the basal leaf under vernalising conditions.

6.4 Discussion

Feeding whole plants resulted in detectable amounts of ^{14}C in all tissue types, including the roots. ^{14}C concentration was greatest in the regions of active cell division and expansion, the immature leaves and shoot and root tips. While this indicates that assimilates are partitioned to these regions, the amounts exported or imported within the individual shoots could not be estimated. For this reason, the feeding of individual

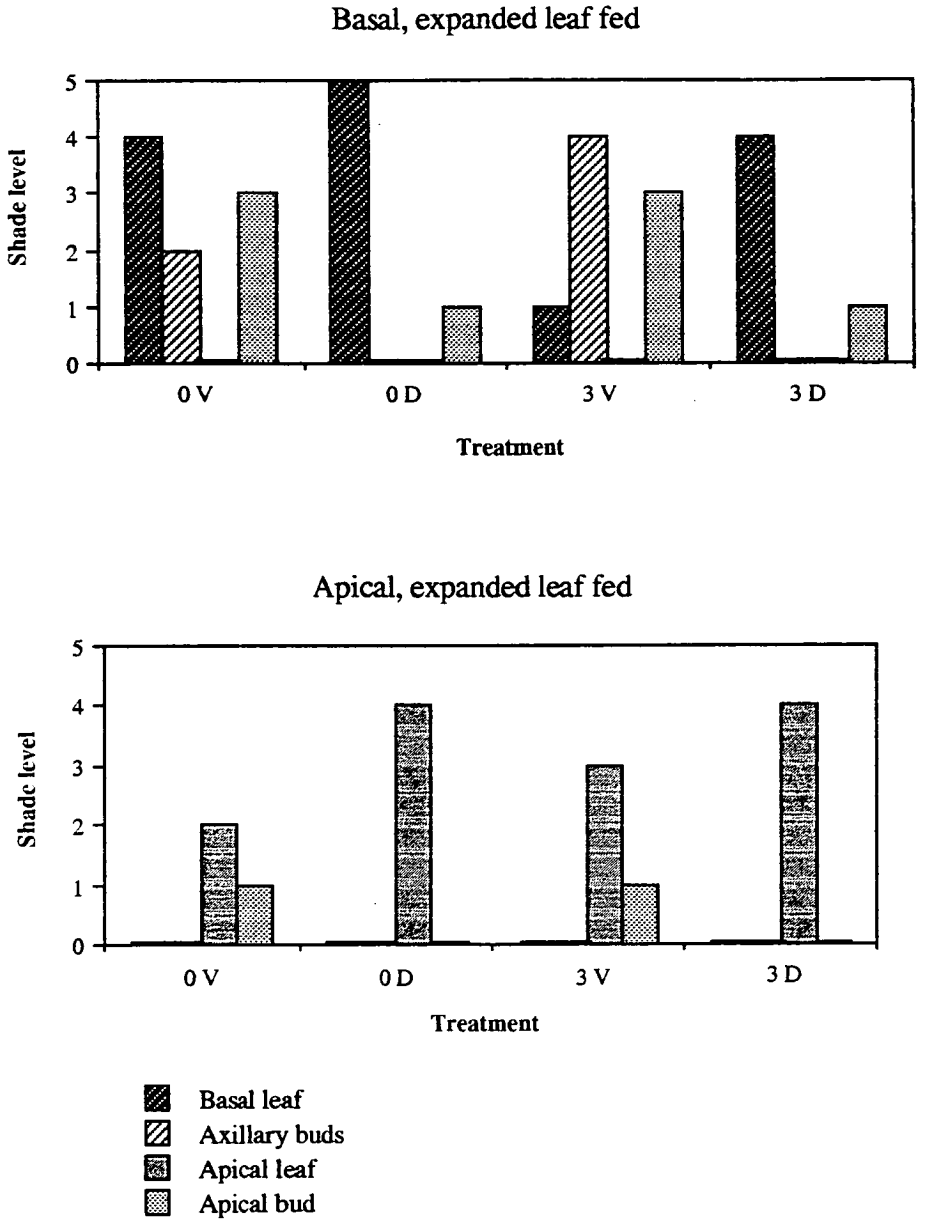


Figure IV.B.6.3.3 - ^{14}C distribution under vernalising and devernalising conditions. Shade intensities (from Figure IV.B.6.3.1) estimate the ^{14}C concentration in lateral shoots with the most basal or the most apical expanded leaf fed. Plants were fed after no vernalisation (0) or after three weeks vernalisation (3) and transferred to vernalising (V) or devernalising (D) conditions for five days before dissection.

Plate 26

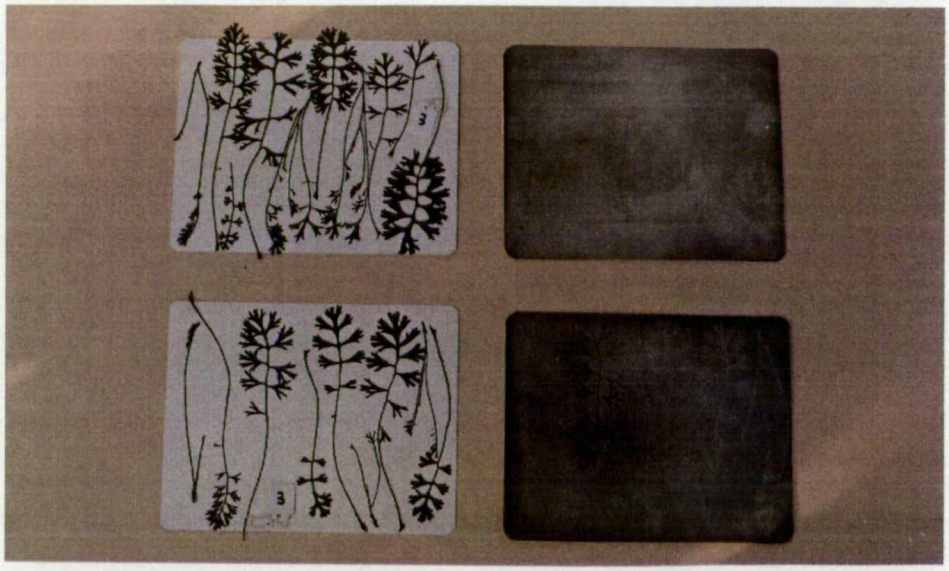


Plate 27

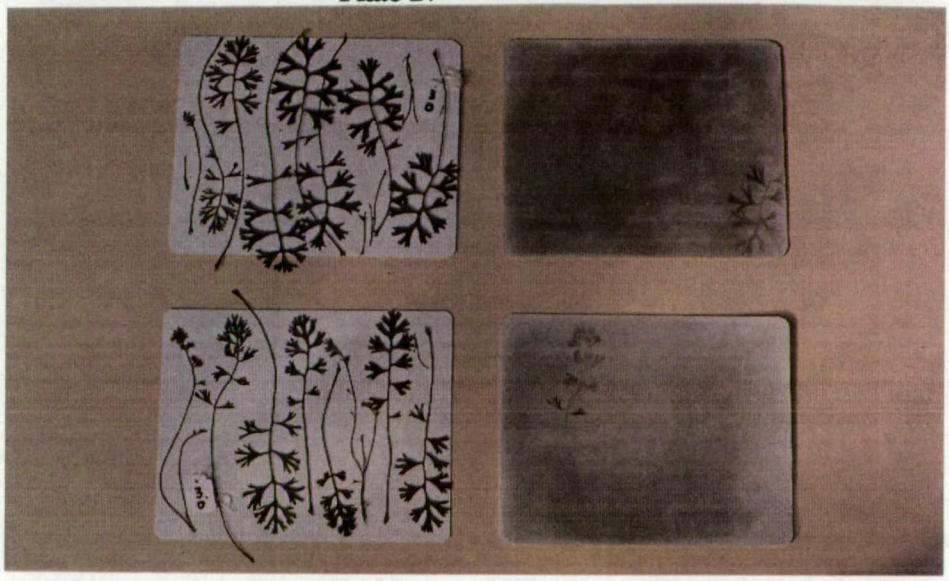


Plate 26 - ^{14}C distribution within lateral shoots with the most basal (top autoradiograph in photo) and most apical (bottom) expanded leaf fed.The plants received 3 weeks vernalisation before feeding and five days vernalisation before dissection.The dissected shoots are laid out from basal (right) to apical (left) with axillary shoots placed next to their subtending leaf. Bar = 25cm

Plate 27 - ^{14}C distribution within lateral shoots with the most basal (top autoradiograph in photo) and most apical (bottom) expanded leaf fed. The plants received 3 weeks vernalisation before feeding and five days devernalisation (high temperature and low photon flux density) before dissection. The dissected shoots are laid out from basal (right) to apical (left) with axillary shoots placed next to their subtending leaf. Bar = 25cm

leaves proved the most adequate means of investigating the assimilate distribution relationships during flowering.

Twenty four hours after feeding, no ^{14}C was detectable in the areas of high meristematic activity while detectable levels were high in the fed leaves. The meristematic regions were found to accumulate a significant proportion of the ^{14}C after three days and after seven days. There appears to be a decrease in the ^{14}C detectable after fourteen days, probably due to respiratory turnover of fixed ^{14}C. To ensure that mobilisation and translocation of labelled assimilates within the fed shoot occurred, five days translocation before dissection was chosen for the main experiments. This extended translocation time did, however, increase the treatment period and the extra five days in vernalising, devernalising and non-inductive conditions must be taken into account when analysing the results.

Exposure to vernalising conditions resulted in increased meristematic activity in the axillary buds, leading to flower initiation and development. This increase in axillary bud growth occurs at the same time that the sink strength of the axillary buds increases. The concentration of ^{14}C in the axillary buds began to increase after plants were held for 19 days in vernalising, 'inductive' conditions (2 weeks in vernalising conditions plus five days after feeding). Labelled photosynthate produced by the basal leaf accumulates in the axillary bud and the apical bud at similar concentrations at this time. Labelled photosynthate from the most apical expanded leaf was not partitioned to the axillary bud until after 33 days vernalisation. Basal leaves are the primary exporters of photosynthates in the shoot, while apical expanded leaves retain a large proportion of the ^{14}C fed to them. The increase in sink activity of the axillary buds occurred before the end of initiation, supporting the hypothesis that the partitioning of assimilates to the axillary meristems is an essential part of the evocational sequence in pyrethrum.

The sink strength of the axillary buds continues to increase as flower development proceeds. After 61 days in vernalising conditions, the flower buds were the dominant sink for assimilates in the shoot, attracting ^{14}C-labelled photosynthate from both basal and apical leaves. This observation is in accordance with the relationship between apical diameter and stage of development describe in Section IV.A.1.3.1 which indicated a strong reliance on assimilates for apical development. The competition between vegetative growth and floral development within the plant is altered by vernalising conditions to favor the floral development of the axillary buds.

Devernalising conditions alter the assimilate distribution pattern within the plant by reducing the sink strength of axillary buds or preventing the translocation of assimilates to them. Low PFD conditions have been shown to reduce the net rate of CO_2 exchange (Section IV.B.3.4) in mature and immature leaves. The amount of photosynthate produced by the leaves is thus reduced, and they become less effective exporters of assimilates. Devernalisation appears to be at least partially due to the fact that the assimilate supply to the axillary meristems is not sufficient for certain evocational processes to occur. Cessation of flower bud development, and flower abortion, in devernalising conditions may also be explained by assuming that assimilate supply is insufficient for development. While it is possible that other endogenous chemical changes produced by devernalising conditions mediate the process, the evidence suggests that reduced assimilate supply to the axillary meristems forms part of the inhibitory effect of devernalisation.

IV.C Manipulation of Flowering

IV.C.1 Trimming

1.1 Introduction

One of the aims of this study was to gain a sufficient understanding of the flowering process to aid in the commercial exploitation of the crop. Trimming plants in the field is one method of manipulating the flowering behaviour of pyrethrum which offers potential for commercial application. The current production system in Tasmania is based on clonal material which is multiplied through vegetative division of nursery plants. Trimming may be used to reduce floral growth, stimulate vegetative production and therefore potentially increase the number of vegetatively divided splits available from each nursery plant. This observed pattern of growth is a result of the changes produced by trimming influencing the physiological processes which have been shown to stimulate the flowering response. The morphological effects caused by trimming of nursery plants were examined to gain a better understanding of these processes.

The effect of timing of trimming treatments on the flowering of pyrethrum under field conditions in Tasmania was also examined in this study. In both Tasmania and East Africa, it is standard practice to cut away all dead leaves and flower stems during the dormant season to stimulate new vegetative growth. Yield reductions occur if the plants are trimmed during the growing season in East Africa (Glover, 1955). As the dormant season is very much longer in Tasmania than in East Africa, it is desirable to establish the optimum time for trimming during the dormant season to increase flower yields.

The physiological changes resulting from trimming were examined in a series of glasshouse trials. It has previously been shown that the removal of mature leaves prior to vernalisation prevents floral initiation but has no effect during vernalisation (Brown, 1988). It was not shown if this effect was due to nutritional factors or other chemical changes. This area was investigated by holding trimmed plants under different environmental conditions and by removing flower stems without defoliating plants.

1.2 Materials and Methods

1.2.1 Field Trials

Two field trials were conducted at Ouse. The field plot planted in 1988 at Ouse was used for both trials and plant cultivation conditions at the site were as described in Section III.1.2. The first trial was conducted in 1988 immediately after the tissue cultured CIG 3 and CIG 11 plants were established at the site, and was aimed at identifying the effects of trimming on the number of flowers and splits when the nursery area was harvested. The second trial was carried out in 1990 and investigated the effect of the timing of trimming treatments on the flowering behaviour of three-year-old plants.

Three treatments were replicated ten times in a completely randomised design for the first trial. Each replicate consisted of thirty plants. The treatments were no trimming (control), trimming once (on 7/10), and trimming twice (on 20/9 and 27/10). Plants were trimmed to a height of approximately three centimetres with a brush cutter, removing most immature leaves and flower stems but leaving several mature leaves on each plant.

The plants were harvested on 22/12 and the number of flowers per plant recorded. Two plants per replicate, representing a total of twenty plants per treatment, were selected randomly for splitting. The plants were vegetatively divided and the number of splits recorded. The ease of splitting was noted for each treatment.

The second field trial investigated the effect of the timing of trimming treatments on the flowering of three year old CIG 3 plants. Twelve treatments were replicated twice in completely randomised design. Each replicate consisted of twenty plants. The treatments consisted of trimming on the following dates : 1/2, 1/2 and 1/11, 1/4, 1/4 and 1/11, 1/6, 1/6 and 1/11, 1/8, 1/8 and 1/11, 1/10, 1/10 and 1/11, no trimming, and trimmed once on 1/11.The plants were trimmed with a brush cutter approximately two centimetres above ground level.

The trial was harvested on 22/12. Three plants from each replicate were chosen randomly and the following measurements recorded : height of the flower stems, degree of lodging, total flower number and the average stage of flower development.

1.2.2 Endogenous Hormone Analysis

Mature leaf samples were taken every two months, beginning on 1/2, during the course of the timing experiment. Endogenous hormone concentrations were determined by RIA, as described in Section III.5.

In conjunction with this study, the endogenous hormonal changes throughout the year in mature leaf tissue and bud samples were examined. Mature leaf tissue and vegetative or floral buds of CIG 3 plants were sampled approximately every fifteen days from 15/8/1989 to 1/10/1990 for analysis by RIA. Samples could not be taken in mid May 1990. Insufficient leaf material was collected for isopentyladenosine analysis. Due to the large number of samples taken, extractions were not replicated and statistical analysis of the results was not performed.

1.3.3 Glasshouse Trials

Two glasshouse trials were conducted involving trimming of flowering CIG 3 plants. The first involved trimming plants 1cm, 3cm and 5cm above ground level at three different times : flower stage 0 (when flower stems were emerging from the leaf canopy), flower stage IV (approximately three rows of the disc florets open), and flower stage VII (late overblown stage, disc florets still intact and ray florets dried out). A further treatment, trimming twice (at flower stage 0 and at the same time as the flower stage VII trimming treatment), as well as an untreated control were also conducted. Each treatment was replicated five times. Plants were maintained in non-vernalising, 12 hour daylength (20-25 °C, 600-900 $\mu mol.m^{-2}.s^{-1}$ PFD) conditions before harvesting. Plants were harvested when they reached flower stage IV, and the number of flowers per plant recorded.

The second glasshouse trial involved trimming developing flower stems but not leaves. Flower stems were trimmed at three different heights when the flower buds were at flower development stage I (flower buds just beginning to open). The treatments were : cutting flower stems above the first leaf on the stem (leaving one elongated internode, axillary apex and subtending leaf), cutting flower stems above the second leaf on the stem, and cutting flower stems above the third leaf on the stem. Untreated control plants were also kept.

The experiment was divided into two blocks, with each treatment replicated five

times in each block. One block was maintained in long day conditions; 12 hours natural daylight (20-25 °C, 600-1200 $\mu mol.m^{-2}.s^{-1}$ PFD, 18 °C night temperature) and 4 hours artificial light (20 °C, 150 $\mu mol.m^{-2}.s^{-1}$ PFD). The second block was maintained in short day conditions; 8 hours natural daylength (20-25 °C, 600-1200 $\mu mol.m^{-2}.s^{-1}$ PFD, 18 °C night temperature). Treatments were harvested when the first flower reached stage IV and the following measurements recorded : number of flower stems, height of flower stems and the number of flowers (stage II and above) and flower buds. The number of axillary shoots on the trimmed flower stems developing vegetatively and the number of axillary buds which had aborted or were not developing were also recorded.

1.3 Results

1.3.1 Field Trial - Trimming of Nursery Plants

Figures IV.C.1.3.1.a and IV.C.1.3.1.b show the effects of trimming on the number of flowers per plant and the number of splits per plant at harvest. Both trimming treatments significantly reduced the number of flowers per plant. The flower number was significantly lower in twice-trimmed plants than in plants trimmed once. The number of splits was significantly increased by trimming, and the number of splits was significantly higher when plants were trimmed twice compared with the single trimming treatment. Plates 29 and 30 show the effects at harvest time of the single trimming and dual trimming treatments respectively. Plate 28 shows the untrimmed control plants at the same time. It was noted that the untrimmed plants were woody and very difficult to split whereas dividing the trimmed plants was much quicker and easier.

1.3.2 Field Trial - Timing of Trimming

Trimming three-year-old plants in February, April, June and August significantly increased the flower yield over untreated plants at harvest. Figure IV.C.1.3.2.a shows these yield increases as well as the significant yield reduction associated with trimming in October. All treatments involving trimming on two occassions caused significant reductions in flower yield irrespective of the time that the first trimming was carried out. The yield recorded for these treatments may be slightly low as the average stage of

Figure IV.C.1.3.1.a

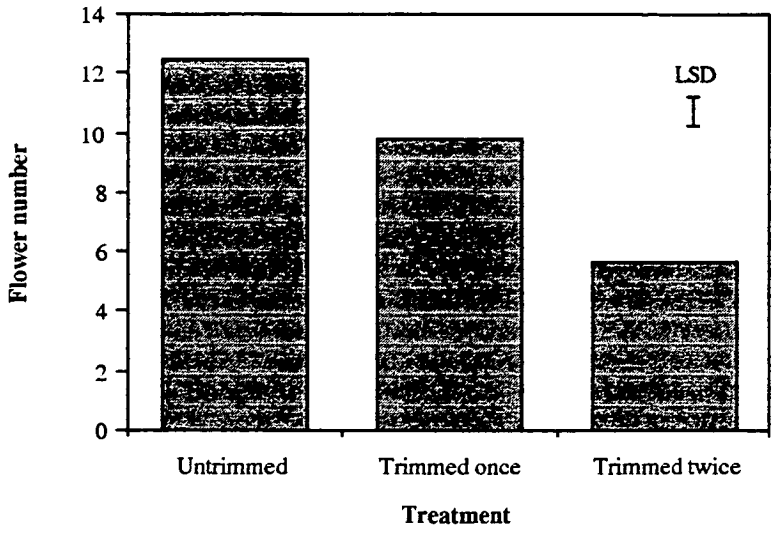


Figure IV.C.1.3.1.b

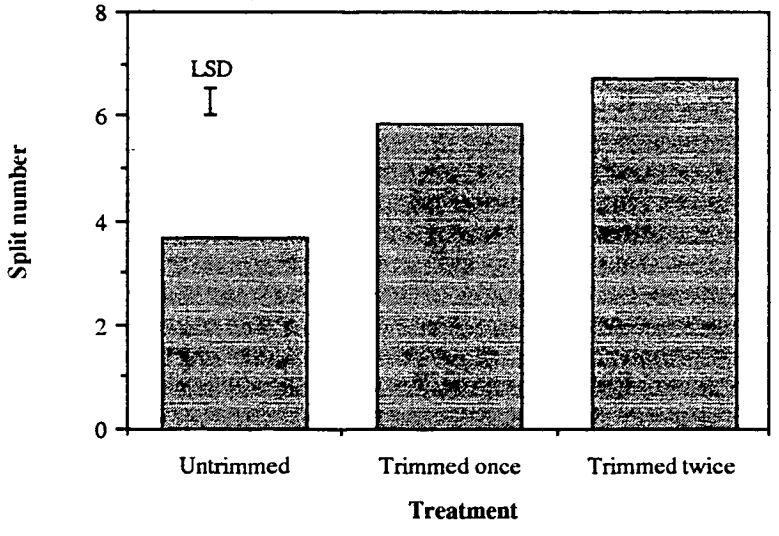


Figure IV.C.1.3.1.a - Effect of trimming on the flower yield of nursery plants. The treatments were no trimming, trimmed once (on 7/10/1988) and trimmed twice (on 20/9/1988 and on 27/10/1988).

Figure IV.C.1.3.1.b - Effect of trimming on the split yield of nursery plants. Plants were vegetatively divided and the number of viable splits recorded. Viable splits were those considered suitable for commercial planting.

Plate 28

Plate 29

Plate 30

Plate 28 - Untrimmed, control plants at harvest.

Plate 29 - Plants trimmed once, on 7/10, at harvest.

Plate 30 - Plants trimmed twice, on 20/9 and 27/10, at harvest.

Figure IV.C.1.3.2.a - Effect of trimming treatments on flower number at harvest. The plants were trimmed once on the dates indicated, or left untrimmed (none). For each of the treatments involving two trimmings, the second trimming was carried out on 2/11. Flower numbers for the plants trimmed twice may be slightly low due to the early stage of flower development of these plants at harvest.

Figure IV.C.1.3.2.b - Effect of trimming treatments on stage of flower development at harvest. The stage of flower development was estimated using Figure II.2.1.a.

Figure IV.C.1.3.2.c - Effect of trimming treatments on flower stem height at harvest. Flower stem heights for the plants trimmed twice are not given due to the early stage of flower development of these plants at harvest.

Figure IV.C.1.3.2.d - Effect of trimming treatments on the degree of lodging of flower stems at harvest. Lodging was estimated on a scale of 1 to 6, with 6 representing the highest degree of lodging. The degree of lodging of twice trimmed plants was not estimated due to the early stage of flower development of these plants at harvest.

Figure IV.C.1.3.2.a

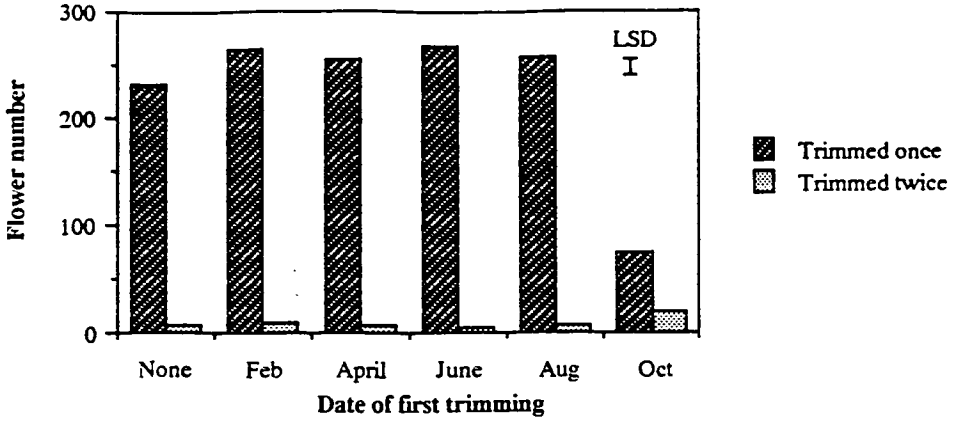


Figure IV.C.1.3.2.b

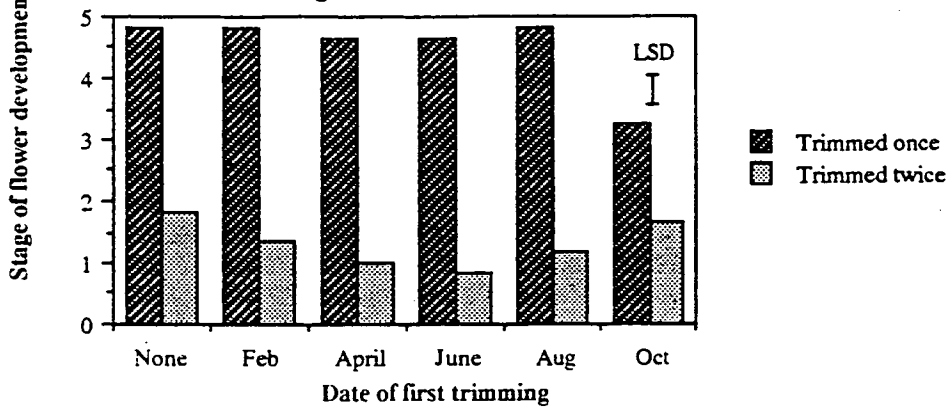


Figure IV.C.1.3.2.c

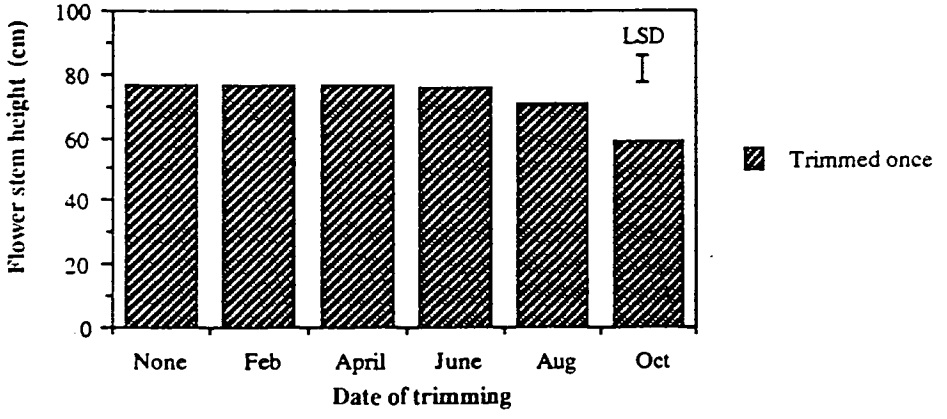
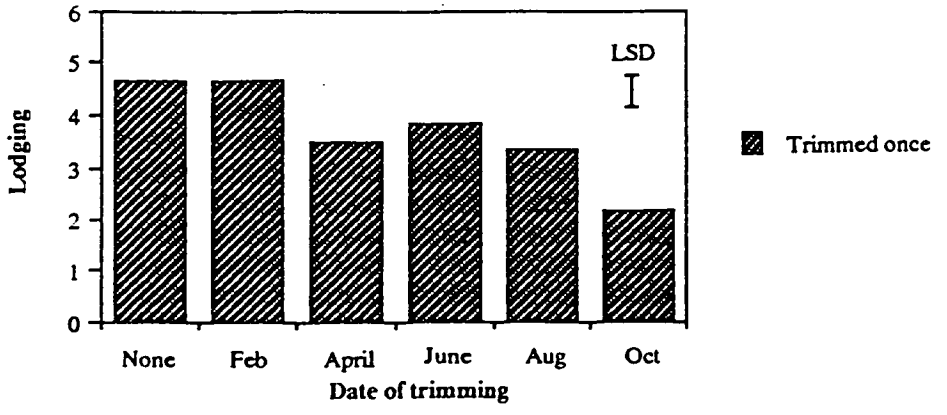


Figure IV.C.1.3.2.d



flower development of these plants was low at harvest. If the plants had been allowed to develop to flower stage IV, it is likely that lateral shoots on the flower stems may have developed as flower buds, increasing the total flower number. Figure IV.C.1.3.2.b shows that the average stage of flower development of the plants trimmed on two occasions was significantly lower than that of the control plants and the plants trimmed once. The plants trimmed once in October were also at a significantly earlier stage of flower development at harvest compared with the untrimmed plants and the plants trimmed once in February, April, June or August. However the measured flower yield for this treatment included all flowers and flower buds, and it is unlikely that any more flower buds could have developed to maturity by the time that the plants would have been ready to harvest commercially.

As the flower stems of the twice trimmed plants were at an early stage of development, stem height and lodging were not recorded for these plants. The stem height and degree of lodging recorded for the plants trimmed once in October may also have been low due to the early stage of development of these plants. Figure IV.C.1.3.2.c shows that flower stem height was significantly reduced by trimming in August compared with each of the other treatments (the exception being the October treatment , as discussed above). While not reducing flower stem height, trimming once in April, June or August significantly reduced lodging when compared with the untrimmed plants. No difference was observed between the untrimmed plants and the plants trimmed in February.

1.3.3 Endogenous Hormonal Changes

The trimming treatments resulted in significant changes in endogenous hormone concentrations. The concentration of gibberellin in the mature leaves decreased after trimming as shown in Figure IV.C.1.3.3.a. On 1/12, the gibberellin concentration in plants which were trimmed on two occasions was significantly lower than in the corresponding plants which had been trimmed once. The October treatments were the exception, where trimming once in October resulted in similar gibberellin concentrations to trimming in October and again in November. It should be noted that the concentration in the single October trimming treatment was significantly lower on 1/12 than in each of the other single trimming treatments. The gibberellin concentrations

Figure IV.C.1.3.3.a - Effect of trimming on endogenous gibberellin concentration in mature leaf tissue. The graph on the left shows the effect of each of the treatments on the GA concentration on 1/12. The x-axis shows the date of treatment of the plants trimmed once. When plants were trimmed twice, the second trimming was performed in November. The graph on the right shows the changes in GA concentration through the year in plants trimmed once on the dates indicated.
ns = differences between means not statistically significant

Figure IV.C.1.3.3.b - Effect of trimming on endogenous indolylacetic acid concentration in mature leaf tissue. The graph on the left shows the effect of each of the treatments on the IAA concentration on 1/12. The x-axis shows the date of treatment of the plants trimmed once. When plants were trimmed twice, the second trimming was performed in November. The graph on the right shows the changes in IAA concentration through the year in plants trimmed once on the dates indicated.
ns = differences between means not statistically significant

Figure IV.C.1.3.3.c - Effect of trimming on endogenous abscisic acid concentration in mature leaf tissue. The graph on the left shows the effect of each of the treatments on the ABA concentration on 1/12. The x-axis shows the date of treatment of the plants trimmed once. When plants were trimmed twice, the second trimming was performed in November. The graph on the right shows the changes in ABA concentration through the year in plants trimmed once on the dates indicated.

Figure IV.C.1.3.3.a

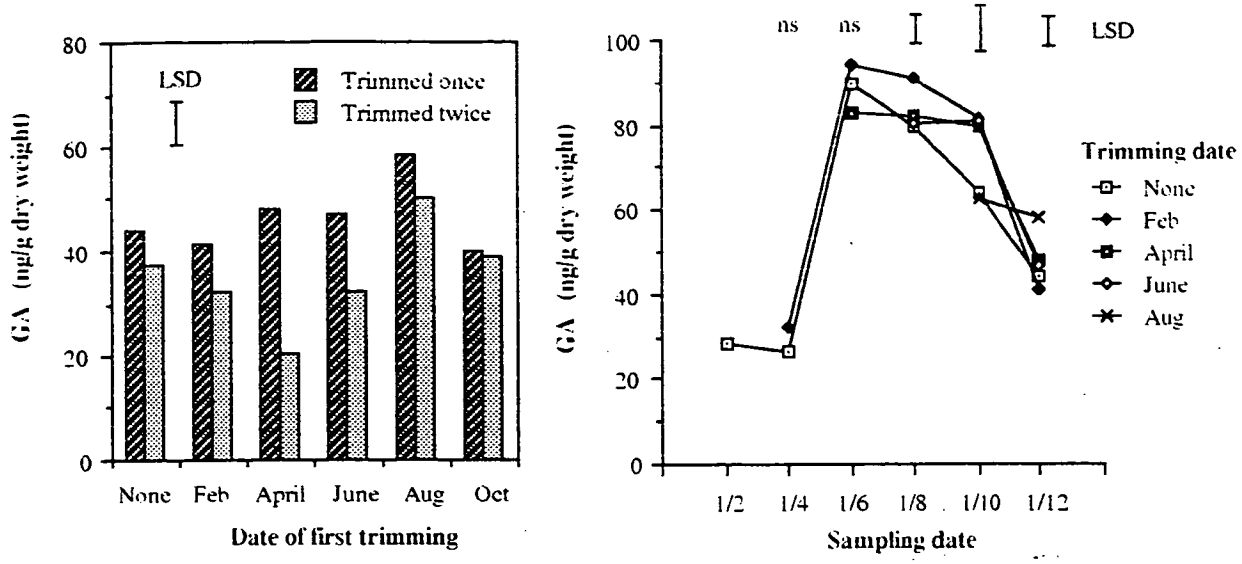


Figure IV.C.1.3.3.b

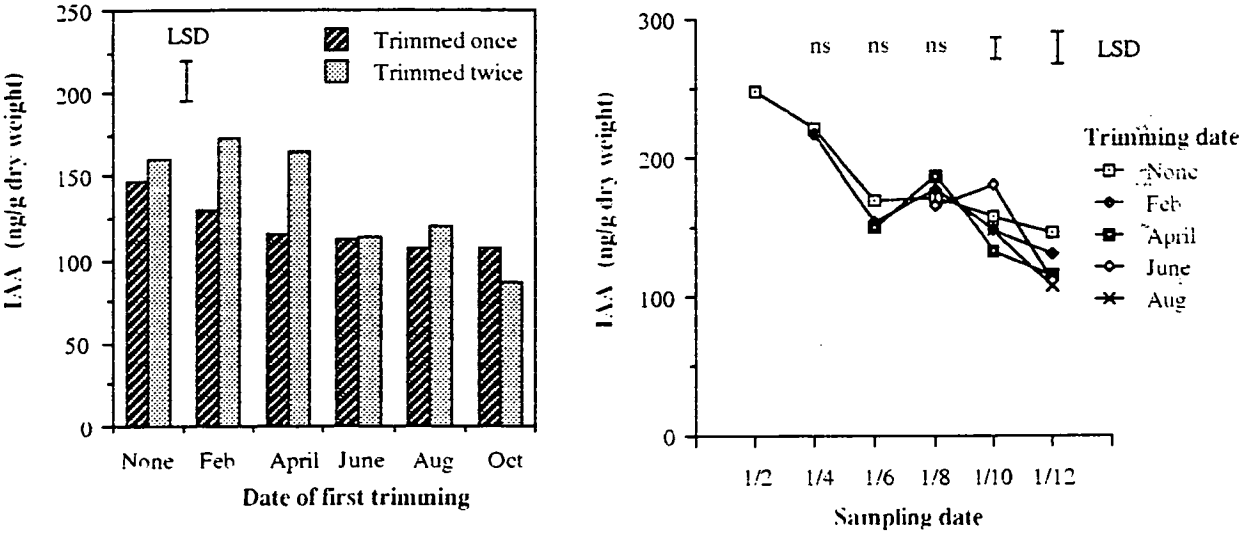


Figure IV.C.1.3.3.c

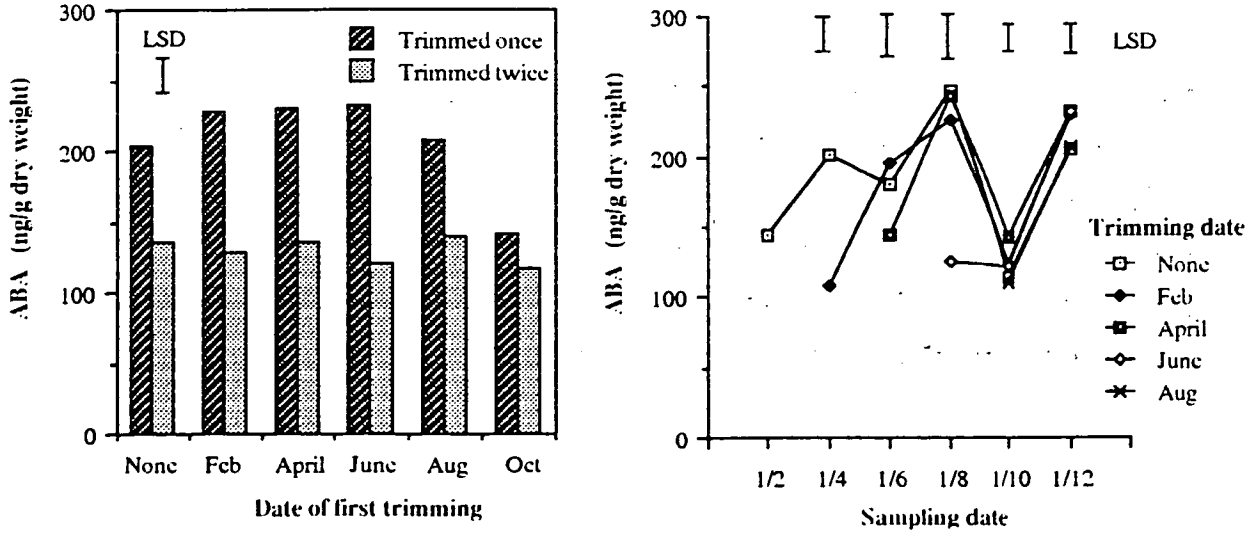


Figure IV.C.1.3.3.d - Effect of trimming on endogenous zeatin concentration in mature leaf tissue. The graph on the left shows the effect of each of the treatments on the Z concentration on 1/12. The x-axis shows the date of treatment of the plants trimmed once. When plants were trimmed twice, the second trimming was performed in November. The graph on the right shows the changes in Z concentration through the year in plants trimmed once on the dates indicated.
ns = differences between means not statistically significant

Figure IV.C.1.3.3.e - Effect of trimming on endogenous dihydrozeatin concentration in mature leaf tissue. The graph on the left shows the effect of each of the treatments on the DHZ concentration on 1/12. The x-axis shows the date of treatment of the plants trimmed once. When plants were trimmed twice, the second trimming was performed in November. The graph on the right shows the changes in DHZ concentration through the year in plants trimmed once on the dates indicated.
ns = differences between means not statistically significant

Figure IV.C.1.3.3.f - Effect of trimming on endogenous isopentyladenosine concentration in mature leaf tissue. The graph on the left shows the effect of each of the treatments on the IPA concentration on 1/12. The x-axis shows the date of treatment of the plants trimmed once. When plants were trimmed twice, the second trimming was performed in November. The graph on the right shows the changes in IPA concentration through the year in plants trimmed once on the dates indicated.
ns = differences between means not statistically significant

Figure IV.C.1.3.3.d

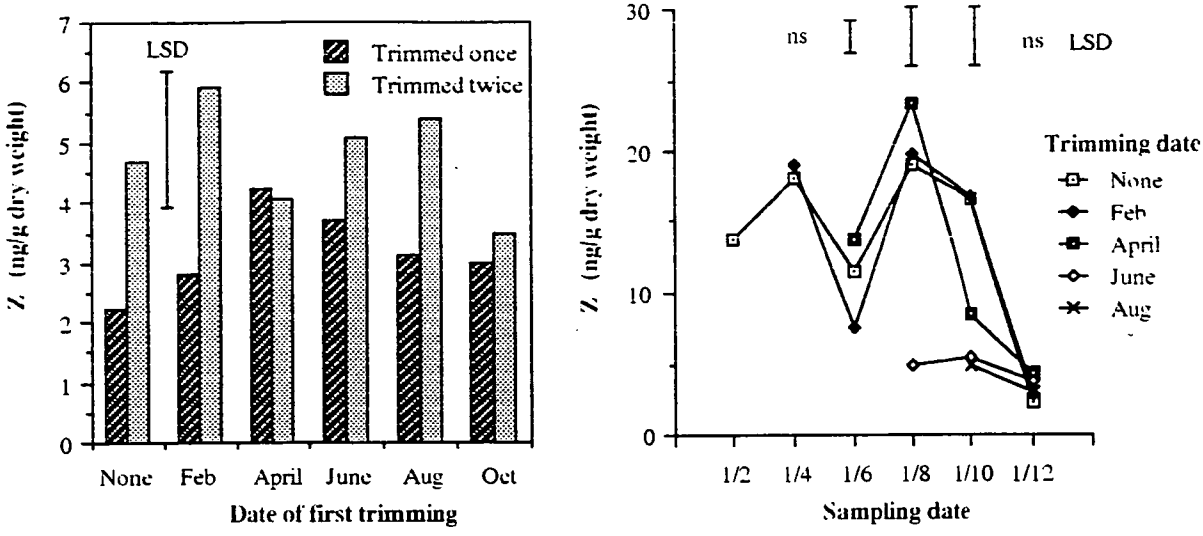


Figure IV.C.1.3.3.e

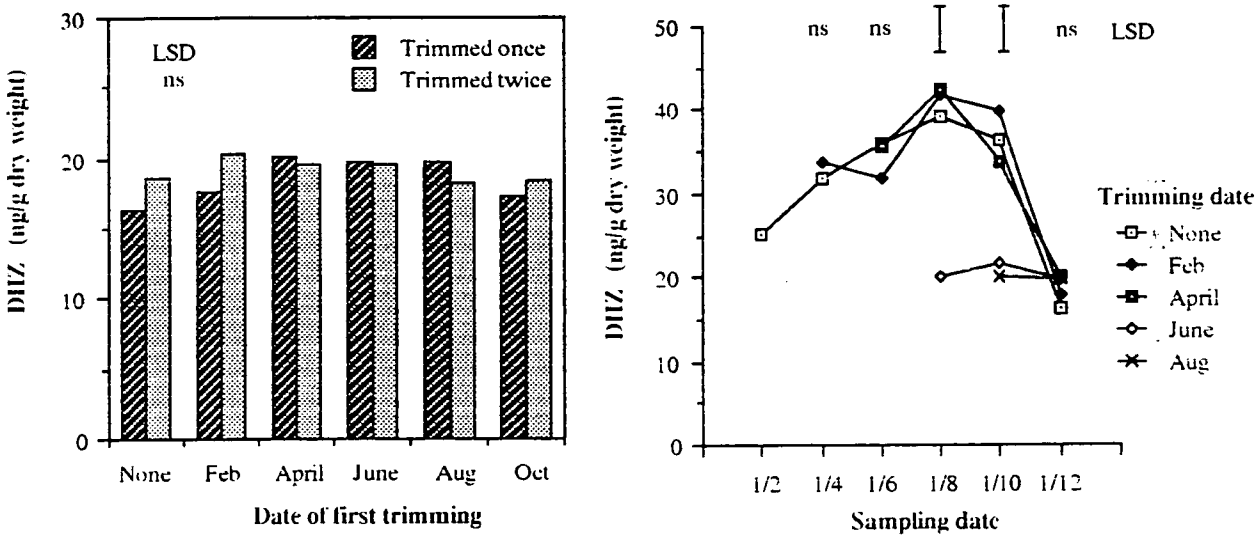
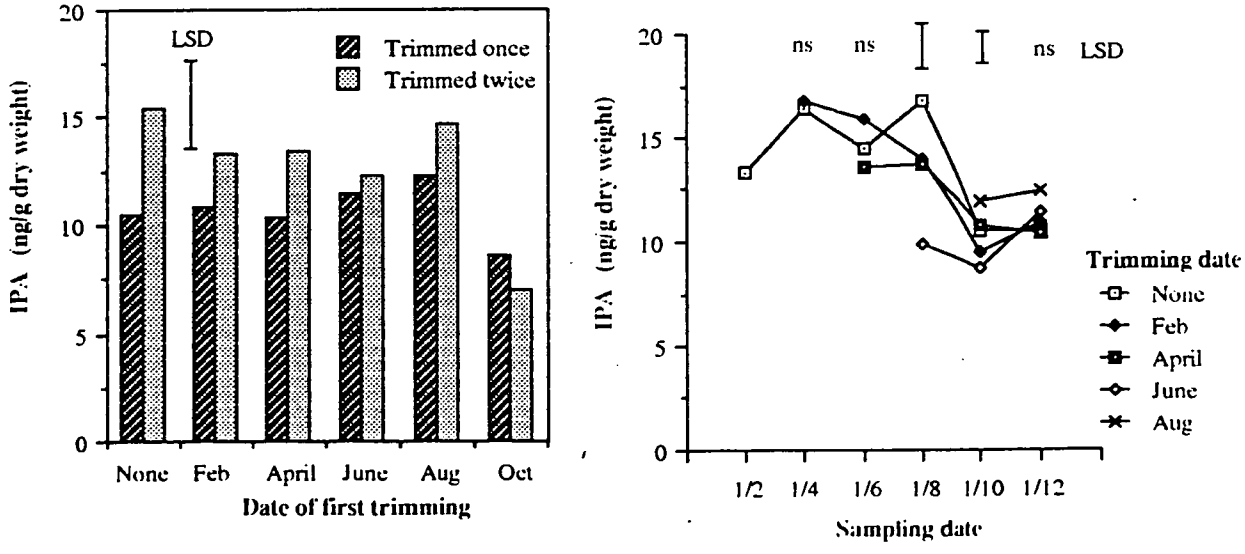


Figure IV.C.1.3.3.f



in plants trimmed once were not significantly different from untrimmed plants two months after the treatments were given. The treatments did, however, result in higher gibberellin concentrations later in the year; on 1/10 for the February, April and June trimming treatments and on 1/12 for the August trimming treatment.

Figure IV.C.1.3.3.b indicates that trimming stimulates an increase in auxin content in the mature leaves. Significant differences in auxin concentrations on 1/12 were observed between the plants trimmed once in February or once in April and those trimmed in February and November or April and November. The effect was smaller in magnitude when the first trimming was given at a later date. Any change in auxin content after the single trimming treatments was not evident two months after the treatment was given. It is possible that trimming stimulated changes in auxin content soon after the treatments were imposed but the auxin concentration had returned to levels similar to those in untreated plants within two months of being trimmed. The only significant differences between the auxin concentrations in untrimmed plants and plants trimmed once were the lower concentrations recorded on the 1/12 in the plants trimmed in April, June and October.

Significant differences in ABA concentrations were observed on 1/12 between the plants trimmed once and those trimmed on two occasions. In each case the second trimming in November resulted in a reduction in the ABA concentration compared with that of the plants trimmed once. Again the smallest difference was recorded when the first trimming was carried out in October, as seen in Figure IV.C.1.3.3.c. Each of the single trimming treatments resulted in lower ABA concentrations than in the untrimmed plants two months after trimming. Apart from this initial change in concentration, the ABA levels remained similar in plants trimmed once and untrimmed plants throughout the growing season.

The endogenous cytokinin changes after trimming are less pronounced than for the acidic hormones examined, but significant trends are evident. Trimming tended to result in an increase in cytokinin concentrations in the mature leaves on 1/12. Figure IV.C.1.3.3.d reveals this trend when the zeatin concentration of once- and twice-trimmed plants were examined on 1/12. Single trimming also tended to decrease cytokinin levels at earlier dates. This is evident for zeatin where a single trimming in June or August reduced the zeatin concentration at subsequent dates when compared with the untrimmed plants. Figure IV.C.1.3.3.e shows this trend for dihydrozeatin, with both the June and August trimming treatments reducing the dihydrozeatin

Figure IV.C.1.3.3.g - Endogenous gibberellin concentration in mature leaf and bud tissue of field grown CIG 3 plants between 15/8/'89 and 1/10/'90. Plants were trimmed after harvest on 10/1 as indicated by the arrow.

Figure IV.C.1.3.3.h - Endogenous indolylacetic acid concentration in mature leaf and bud tissue of field grown CIG 3 plants between 15/8/'89 and 1/10/'90. Plants were trimmed after harvest on 10/1 as indicated by the arrow.

Figure IV.C.1.3.3.i - Endogenous abscisic acid concentration in mature leaf and bud tissue of field grown CIG 3 plants between 15/8/'89 and 1/10/'90. Plants were trimmed after harvest on 10/1 as indicated by the arrow.

Figure IV.C.1.3.3.g

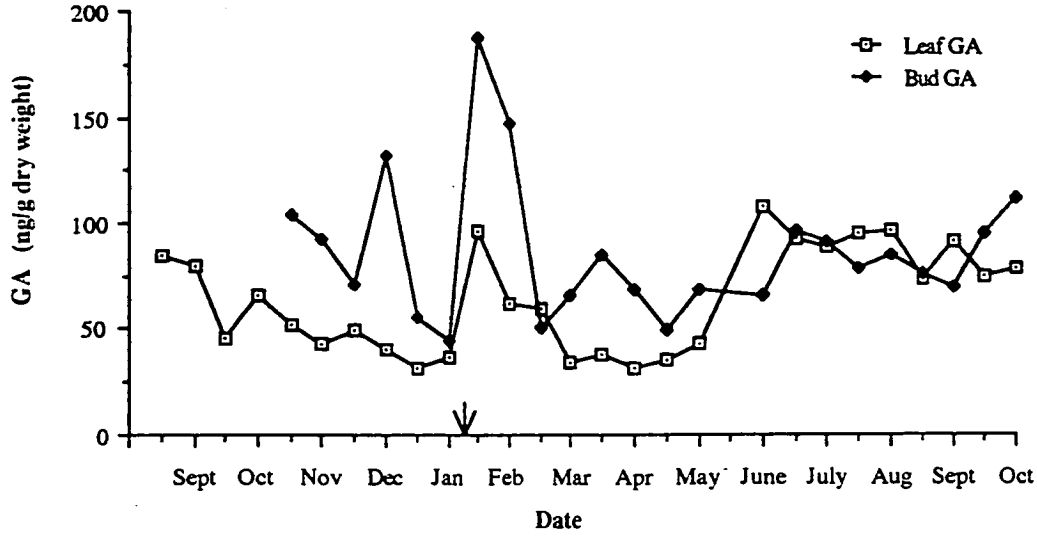


Figure IV.C.1.3.3.h

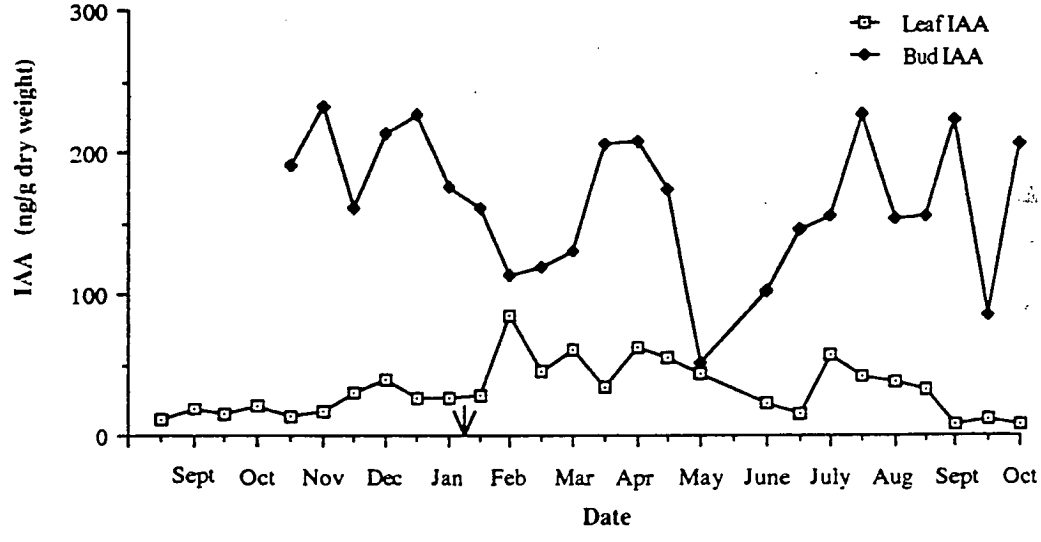


Figure IV.C.1.3.3.i

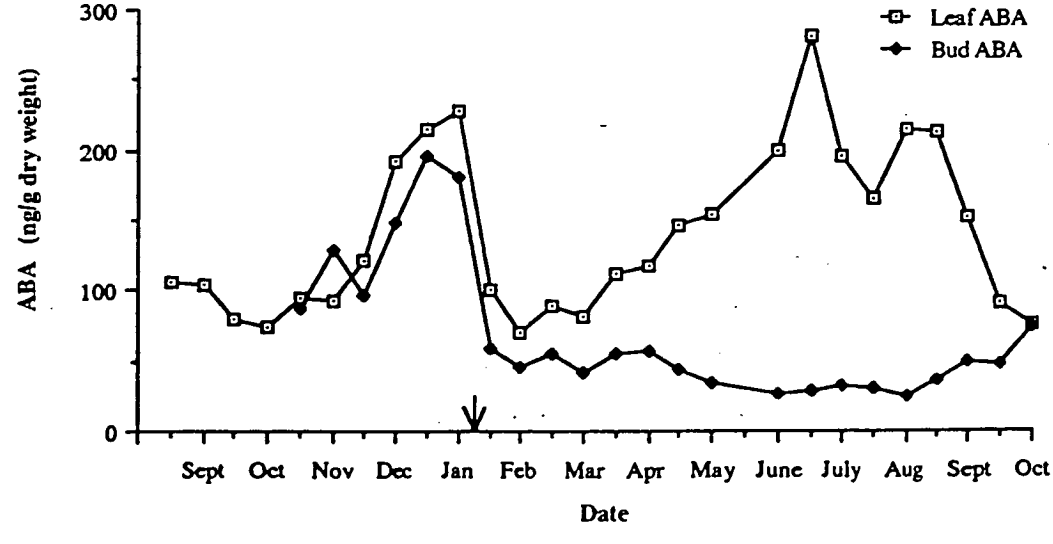


Figure IV.C.1.3.3.j - Endogenous zeatin concentration in mature leaf and bud tissue of field grown CIG 3 plants between 15/8/'89 and 1/10/'90. Plants were trimmed after harvest on 10/1 as indicated by the arrow.

Figure IV.C.1.3.3.k - Endogenous dihydrozeatin concentration in mature leaf and bud tissue of field grown CIG 3 plants between 15/8/'89 and 1/10/'90. Plants were trimmed after harvest on 10/1 as indicated by the arrow.

Figure IV.C.1.3.3.l - Endogenous isopentyladenosine concentration in mature leaf and bud tissue of field grown CIG 3 plants between 15/8/'89 and 1/10/'90. Plants were trimmed after harvest on 10/1 as indicated by the arrow.

Figure IV.C.1.3.3.j

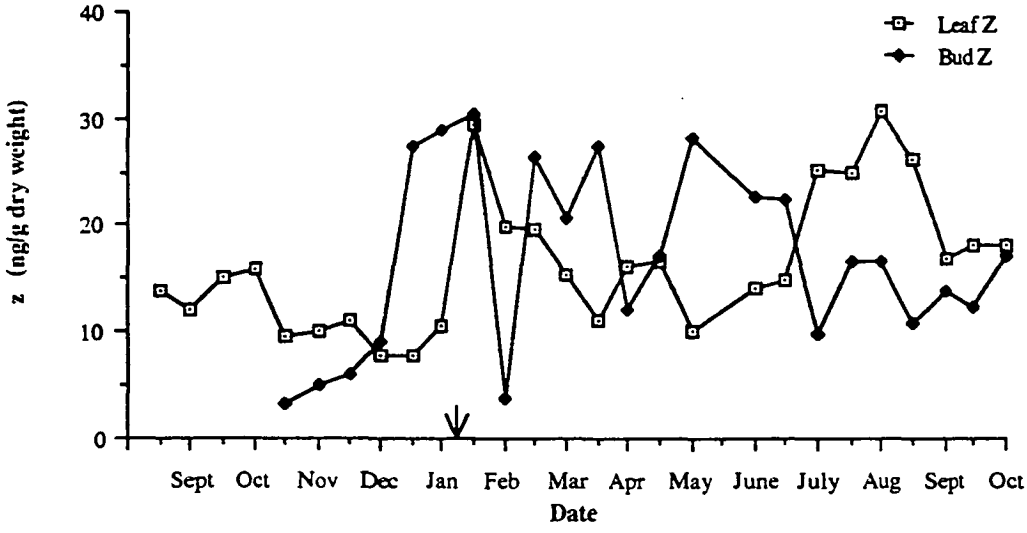


Figure IV.C.1.3.3.k

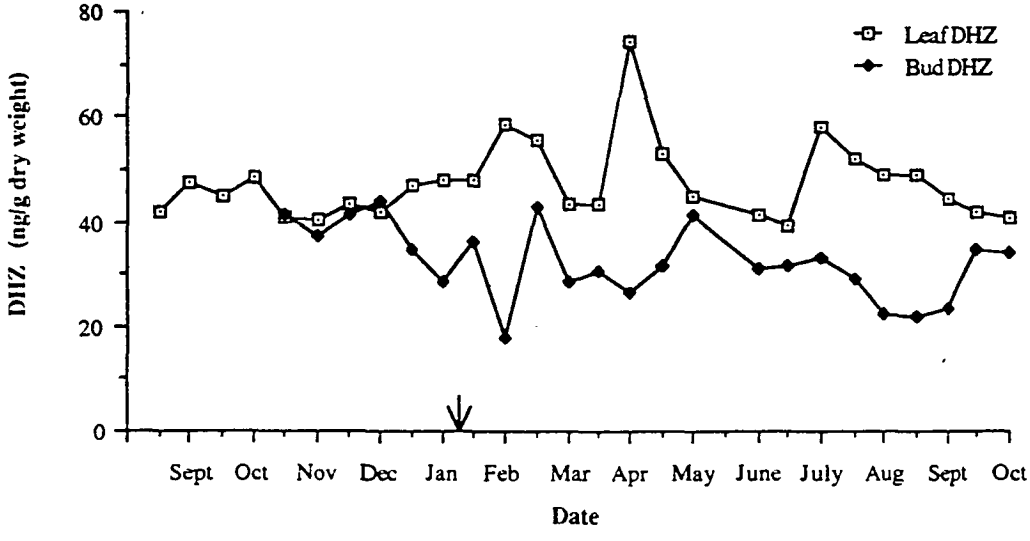
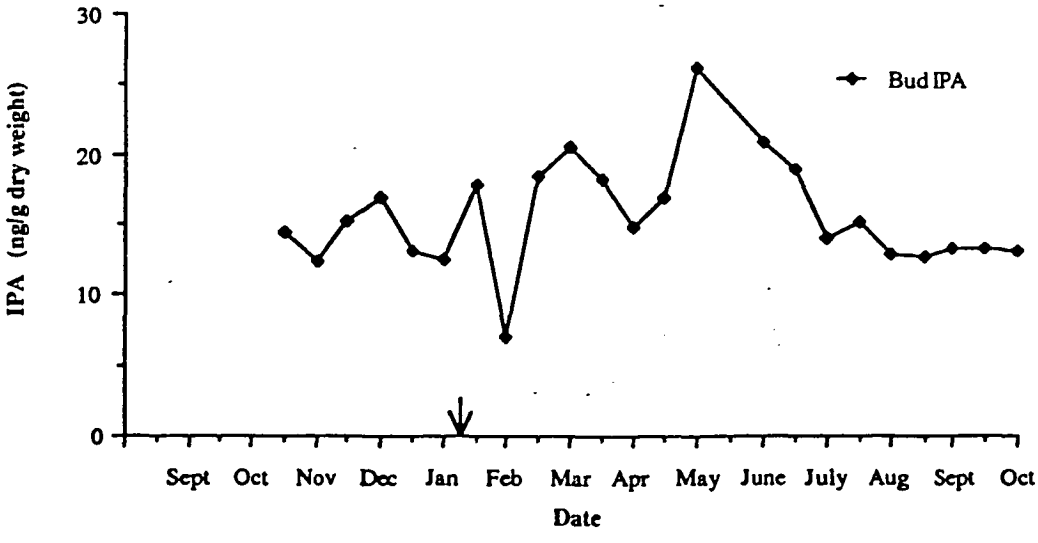


Figure IV.C.1.3.3.l



concentration on 1/8 and 1/10. An increase in dihydrozeatin after the second trimming was not evident except for the plants trimmed in February and untrimmed plants. The concentration of isopentyladenosine (Figure IV.C.1.3.3.f) did increase in each of the second trimming treatments except when the first of the two trimmings was carried out in October.

As mentioned previously, these results reflect the trends in hormonal status in plants as a whole but give no indication of localised fluxes in hormone concentrations.

The difference between hormone concentrations in mature leaf and bud samples throughout the year reinforces this point. Figure IV.C.1.3.3.g shows the fluctuations in gibberellin concentrations throughout the year in mature leaves and buds. The gibberellin concentration in mature leaves increased dramatically when the plants were trimmed in January, but declined thereafter and remained low until mid winter. The GA concentration remained high during the period of flower initiation and stem elongation and declined during later stages of flower development. In contrast, a peak in bud GA concentration was observed on 1/12. Bud GA concentration was at its maximum following trimming, decreased during autumn before rising again in winter and spring. The GA content in the buds remained high throughout the period of stem elongation.

Figure IV.C.1.3.3.h shows the changes in auxin concentration in the mature leaves and the buds. The IAA concentration in the buds during the period October to January was high whereas the concentration in the leaves was at its lowest during this period. After trimming, leaf IAA levels increased dramatically while bud levels decreased. During winter the IAA concentration decreased for a short period in the buds. A corresponding decline was observed in the leaf IAA levels after a 15 day lag period.

The ABA concentration during late flower development (November to January) was high in both leaf and bud samples. Trimming resulted in the concentration declining in both tissue types, with the concentration remaining low in bud samples for the remainder of the dormant season. In contrast, leaf ABA levels steadily rose during winter, peaking in mid-June, before declining in September and October.

The cytokinin concentrations did not fluctuate to the same extent as the acidic hormones during the flowering period, August to January. The only obvious trend during this period was the increase in bud zeatin concentration during late flower development in January. Each of the cytokinins levels were found to decline in bud samples taken on 1/2, probably as a result of trimming twenty days earlier. Bud

samples taken immediately after trimming did not show this decline, suggesting some lag phase in the buds' response. Leaf zeatin levels increased markedly after trimming while the dihydrozeatin concentration increased slightly. Both bud and leaf zeatin and bud isopentyladenosine levels tended to be higher in winter before declining around August - September. The dihydrozeatin concentration in the leaves peaked twice, in April and July, whereas the bud concentrations remained relatively constant through winter, declined in August and increased again in September. Each of the cytokinins were found in very low concentrations and it is possible that other physiologically active cytokinins may have been present in the tissue samples.

1.3.4 Glasshouse Trials

Each of the plants was inadvertently exposed to some degree of water stress during the course of the trial. This resulted in the death of several plants. Figure IV.C.1.3.4.a shows the harvest data, including the number of dead plants in each treatment. Each of the trimming treatments reduced the number of plants flowering and the number of flowers per flowering plant. Trimming at the late overblown stage (stage VII) or trimming plants twice resulted in the greatest decrease in flowering, both in terms of the number of flowers and the number of plants flowering. The effects resulting from trimming at the three different heights used in the trial were very similar.

The height at which the flower stems were trimmed determined the flowering response of the plant. Figure IV.C.1.3.4.b shows that the lower the stems were trimmed the less likely the plants were to continue floral development and the greater the delay in flowering. There was a strong interaction with daylength, with plants less likely to produce flowers after trimming when held in short-day conditions. This trend was also reflected in the percentage of axillary apices developing florally, vegetatively or not developing or aborting. In short days, vegetative development or shoot abortion was favoured while under long days floral development predominated if plants were trimmed above the third leaf on the flower stem and, equally, vegetative development predominated if stems were trimmed above the second leaf.

Treatment	Trimming height (cm)	Number of dead plants	Number of plants flowering	Flower number
No trimming	-	0	10	22.60
Stage 0	1	2	4	2.75
Stage 0	3	0	7	3.29
Stage 0	5	0	6	2.67
Stage IV	1	1	5	3.60
Stage IV	3	0	3	2.67
Stage IV	5	0	5	2.00
Stage VII	1	1	1	1.00
Stage VII	3	0	0	0.00
Stage VII	5	0	0	0.00
Twice	1	2	0	0.00
Twice	3	0	0	0.00
Twice	5	0	1	1.00

Figure IV.C.1.3.4.a - Effect of trimming height on flowering. The plants were trimmed when flowers reached stage 0, stage IV or stage VII of the flower development scale (Figure II.2.1.a) or twice (at stage 0 and again at the time of the stage VII trimming). The plants were trimmed at heights of 3cm, 2cm and 1cm above ground level. Flower number was expressed as the average number of flowers per flowering plant in each treatment.

Treatment	Number of plants flowering	Flowering time (days)	% axillary apices		
			Floral	Vegetative	Aborted
LD C	5	27	-	-	-
LD 3	4	52	52.3	43.9	3.8
LD 2	3	58	20.0	20.0	60.0
LD 1	3	58	7.1	57.2	35.7
SD C	5	29	-	-	-
SD 3	2	65	7.5	71.4	21.1
SD 2	0	-	0.0	79.3	20.7
SD 1	0	-	0.0	40.9	59.1

Figure IV.C.1.3.4.b - Effect of trimming flower stems on flowering. Plants were maintained in long day (LD) or short day (SD) conditions after trimming. The trimming heights were above the third leaf on the flower stem (3), above the second leaf (2), above the first leaf (1) or untrimmed controls (C). The state of the axillary apices on the cut flower stems at harvest was recorded, and the numbers expressed as a percentage of the total number of apices. The percentage of aborted buds includes buds which showed no signs of developing but had not actually aborted.

1.4 Discussion

Trimming can be used effectively to increase nursery yields of vegetatively divided splits. The greatest yield increases resulted when plants were trimmed on two occasions. This trend was also observed in three-year-old plants, where trimming once through the dormant season and again in November greatly reduced the flower yield when compared with untrimmed plants or plants trimmed once. Plants trimmed twice in the glasshouse trial also had lower flower numbers than untrimmed plants or plants trimmed once.

The time at which the plants were trimmed also influenced the yield of flowers. Trimming three-year-old plants in February, April, June or August significantly increased flower yields at harvest over untrimmed plants. This finding supports the practice of trimming plants to ground level after harvesting, but also suggests that it may be advantageous to trim plants in April, June or August to reduce lodging. Trimming in October or November may decrease flower yield, a desirable result if vegetative split production is planned. Trimming plants at a late stage of flower development in the glasshouse also reduced flower yields over earlier trimming treatments. Late trimming may not be the most effective trimming treatment for stimulating vegetative growth as plants must expend a large amount of assimilate for floral growth before the treatment is given.

Multiple trimming treatments would appear to be the best strategy for promoting vegetative growth in the field during the normal flowering period. When flower stems are first trimmed, axillary meristems on the remaining stem sections retain the potential to initiate floral development. As mentioned in Section IV.B.2.4, the vernalisation stimulus may be passed from a vernalised shoot tip to axillary meristems on that shoot even if the axillary meristems themselves are not vernalised. Provided that the other evocational requirements are satisfied, these axillary meristems will be initiated and floral development will result. The fact that short-day conditions in the glasshouse trial resulted in the inhibition of floral development of the axillary meristems on trimmed flower stems is a further indication that an adequate assimilate supply is one of the evocational requirements. As assimilate supply in the field during the normal flowering period is not likely to be limiting, axillary meristems on trimmed sections would be expected to initiate floral development. A second trimming may remove these developing flower stems, further stimulating vegetative growth. The higher flower

numbers on the October plus November trimmed plants compared with the other plants receiving treatments involving trimming on two occasions, while not being statistically significant, suggests that a period of greater than one month should be left between trimming treatments. This would allow the developing axillary shoots to reach a size where the second trimming is able to remove them.

Except for ABA, the endogenous hormonal changes, if any, resulting from single trimming treatments were not evident two months after the treatments were imposed. Trimming reduced the ABA concentration in the leaves, possibly by removing the older leaf and stem tissue which is thought to be a major source of ABA. The trimming treatments also opened up the plant canopy, resulting in enhanced light interception. These two changes are both likely to stimulate plant growth.

As only changes in ABA concentration were observed two months after trimming, the differences in hormone concentrations between trimmed and untrimmed plants on 1/12 are not likely to be a direct result of the treatment. Changes in growth rate after trimming, resulting in larger plants and more developing shoots, may explain the differences. Higher gibberellin levels would be expected when a greater number of floral shoots develop as gibberellin levels have been seen to increase during floral development (Section IV.B.2.3.2). Similarly, low auxin contents in plants trimmed in April and June are consistent with the higher flower numbers observed with these treatments.

One month after each of the second trimming treatments were imposed, cytokinin levels in the leaves were higher than in plants trimmed once. Cytokinins are thought to be associated with cell division and so are present in actively growing tissue. If trimming stimulates vegetative growth, then higher cytokinin concentrations would be expected immediately after trimming. The early single trimming treatments favoured floral growth and so cytokinin levels in the leaves of vegetative shoots may decline while activity in the floral shoots increases.

The gibberellin concentration on 1/12 in plants trimmed twice was lower than in the corresponding single trimmed plants while the auxin concentration was higher. The differences were greatest when the time between the two trimmings was largest. Again these results are consistent with the results noted earlier, with lower gibberellin levels and higher auxin levels favouring vegetative growth, as seen after each second trimming. The flower yield differences were smallest between once and twice trimmed

plants when the first trimming was in October, and the hormone concentration differences were, as could be expected, also smaller between these than between the other treatments.

The hormone concentration changes throughout the year also indicate that the balance between auxin and gibberellin levels determines the flowering response. Gibberellin concentrations were high from June through to August in the leaves. Cold night temperatures may mediate the increase in GA levels in June, as noted in the vernalisation experiment (Section IV.B.2.3.2). Bud GA concentration increases in September when stem elongation begins and remains high to November, when elongation is completed. The peak in December may be due to GA production by one of the flower parts, such as the pollen, during flower development. The increase in GA concentration immediately after trimming was not observed in the trimming experiment, probably because samples were not taken until two months after each treatment in that trial.

The decline in leaf and bud auxin levels in May and June may be associated with flower initiation, as stem elongation was observed in the field soon after this time. ABA levels increase in the leaves over winter and may be related to the slow growth rate observed during this time. Bud levels remained low, suggesting that assimilates or some other essential metabolites are restricting growth rate. The rise in ABA concentration in December and January was probably associated with aging and senescence of the flower buds during this time.

The delay in changes in cytokinin concentration in response to trimming after harvest is of interest. The roots are generally accepted as being the plants main source of cytokinins and so interference to the transpiration rate caused by trimming may result in a lag phase before a response is observed. During the period of low transpiration following trimming, metabolism of cytokinins may reduce levels in the shoots, but as the transpiration rate increases as new leaves develop the cytokinin concentration may rise. Also the removal of shoots may directly affect cytokinin metabolism in the roots by altering the availability of carbohydrates, amino acids and other essential metabolites. As observed in earlier experiments, the changes in cytokinin levels do not appear to be strongly correlated with the morphological events associated with flowering. For this reason, overall cytokinin levels are not considered to play a significant role in mediating flower initiation and development.

IV.C.2 Growth Retardants

2.1 Introduction

The insecticidal constituents of pyrethrum are found in the flowers. As an aid to mechanical harvesting, it is important that the flowers form a uniform canopy to facilitate harvesting, thereby maximising the yield of flowers and pyrethrins. Failure to form a uniform canopy at harvest has proved to be a major problem in areas where local climatic conditions cause lodging of the flower stems. Some mechanical systems are not effective at harvesting lodged flowers and crop yield is therefore reduced as a certain percentage of the flowers are not picked.

The group of chemosynthetic plant growth regulators which interfere with the biosynthesis, translocation or metabolic conversion of plant hormones and restrict plant growth are termed growth retardants. These substances generally influence the balance between gibberellins and ethylene either by inhibiting the biosynthesis of gibberellin or by enhancing the ethylene level (Bruinsma, 1982). Growth retardants can inhibit stem elongation and promote thickening and strengthening of the stem. Gibberellins are considered to be the principal endogenous promoters of internode elongation, and so the growth retardant action of reducing the gibberellin concentration restricts stem elongation. Lodging can be reduced by growth retardant applications which increase stem strength and decrease stem height, reducing exposure to the effects of wind.

Two new classes of compounds with plant growth retardant activities have been widely studied in recent years. The substituted pyrimidines (e.g. ancymidol and flurprimidol) and the triazole derivatives (e.g. paclobutrazol) inhibit the early steps of *ent*-kaurene oxidation but not the later steps in the gibberellin biosynthetic pathway. Of the older growth retardants, chlormequat chloride has been most widely studied. It not only inhibits *ent*-kaurene biosynthesis but also sterol biosynthesis (Graebe, 1987). The newer growth retardants are considered more active and have a broader spectra of action than the older types of structures but have also been shown to influence abscisic acid and ethylene biosynthesis and metabolism (Buta and Spaulding, 1991. Grossmann *et al*, 1989). This lack of specificity must be kept in mind when the plant growth retardants are used to probe the effects of gibberellin biosynthesis inhibition in plants.

The aim of this study was to examine the use of growth retardants as anti-lodging

agents in the field. The effectiveness of the different classes of plant growth retardants was examined. The effect of timing of growth retardant applications and the endogenous changes resulting from these treatments were also studied to better understand the physiological significance of the observed changes in gibberellin concentration during flowering.

2.2 Materials and Methods

2.2.1 Screening of Growth Retardants

A large range of growth retardants is available commercially. Four growth retardants were chosen for testing of their effectiveness in reducing flower stem height and lodging in field grown pyrethrum. These growth retardants were :

Paclobutrazol ; [2RS,3RS]-1-(4-chlorophenyl)-4,4-dimethyl-1,2-(1 H-1,2,4-triazol-1-yl) pentan-3-ol , available as a liquid with 250g active ingredient per litre, commercial name 'Cultar'

Flurprimidol ; α-(1-methyl)-α-[4-(trifluromethoxy)phenyl]-5-pyrimidine methanol , available as a wettable powder with 50 percent active ingredient, commercial name 'EL-500'

Ancymidol ; α-cyclopropyl-α-[4-methoxyphenyl]-5-pyrimidine methanol , available as a liquid with 0.0264 percent active ingredient, commercial name 'A-rest'

Chlormequat chloride ; (2-chloroethyl)trimethylammonium chloride , available as a liquid with 100g active ingredient per litre, commercial name 'Cycocel'

The trial was conducted at both the Ouse and the Bushy Park sites using the five-year-old plantings of clonal material formerly used in a multilocation trial. The site conditions are described in Section III.1.2. Each plot contained eight pyrethrum clones and each clone was planted in four rows containing ten plants. Both sites were divided into four blocks containing one row of each clone planted in random order. The growth

retardants were applied to groups of twenty four plants, consisting of three plants of each of the eight clones. Three treatments could thus be applied in every block, with one plant of each clone left as an untreated control.

Growth retardants were applied at three different concentrations. 'Cultar', 'EL-500' and 'Cycocel' were applied at rates equivalent to 1 Kg active ingredient (a.i.) per hectare, 5 Kg a.i. ha^{-1} and 10 Kg a.i. ha^{-1}. The supply of 'A-rest' was limited and so it was applied at rates of 1 g a.i. ha^{-1} , 10 g a.i. ha^{-1} and 50 g a.i. ha^{-1}. Both 'A-rest' and 'EL-500' are substituted pyrimidines with similar physiological actions, and so it was considered that using the low dosages of 'A-rest' extended the concentration range examined for this class of growth retardant. Each treatment was applied as a foliar spray. Growth retardant solutions were made up to one litre which allowed spraying of plants to the point of incipient runoff. The solution concentrations were equivalent to 300 mg a.i. per litre (1 $Kg.ha^{-1}$), 1500 $mg.l^{-1}$ (5 $Kg.ha^{-1}$), 3000 $mg.l^{-1}$ (10 $Kg.ha^{-1}$), 0.3 $mg.l^{-1}$ (1 $g.ha^{-1}$), 3 $mg.l^{-1}$ (10 $g.ha^{-1}$), and 15 $mg.l^{-1}$ (50 $g.ha^{-1}$). Each treatment was applied to 24 plants so that, for example, the 1 $Kg.ha^{-1}$ treatment plants each received 12.5 mg active ingredient.

Treatments were applied at the Ouse site on 9/8/1988 and at the Bushy Park site on 10/8/1988. Individual treatments and clones were harvested when the majority of plants had reached flower development stage IV, two thirds of the disc florets open. The harvest data was recorded as described in Section III.3. Flower stem height and leaf canopy height were noted and the percentage of flowers between these heights recorded as the percentage of harvestable flowers. The total flower number, average flower weight, degree of lodging and pyrethrins content were measured and used to calculate the harvestable pyrethrins yield per plant. The pyrethrins data is shown in appendices IV.C.2.3.1.a and IV.C.2.3.1.b. The data from all clones were combined for each treatment for statistical analysis. Using different clones increased the variation for each measured parameter but as each treatment was given to the same number of plants of each clone, the mean treatment effects can be used to assess the growth retardants' effects over a range of plant material.

2.2.2 Timing of 'Cultar' Applications

'Cultar' and 'EL-500' were both shown to affect the flowering pattern of pyrethrum by the screening trial. 'Cultar' was chosen for further studies, despite the

fact that it was slightly less active than 'EL-500', because of availability and cost considerations.

The effect of timing of 'Cultar' applications was examined in 1989 at the Ouse trial plot planted in 1988. The trial was restricted to CIG 3 plants. The experiment was arranged in the form of randomised complete blocks with three replications of eight treatments. Treatments within each block were applied to twenty plants.

'Cultar' was applied at a rate equivalent to 4 Kg active ingredient per hectare in August (3/8), September (1/9), October (1/10) and November (2/11). Also, treatments of 3 $Kg.ha^{-1}$ in August plus 1 $Kg.ha^{-1}$ in October, 2 $Kg.ha^{-1}$ in August plus 2 $Kg.ha^{-1}$ in October and 1 $Kg.ha^{-1}$ in each of the four months were applied. The final treatment in each block was a control where no 'Cultar' was applied. The treatments were applied as foliar sprays. Each was made up to one litre and sprayed to the point of incipient runoff.

Flower stem height was recorded each month from August to December. Mature leaf samples were also taken for RIA analysis of plant growth regulator concentrations. Plants were harvested when the majority of flowers in each treatment block had reached flower development stage IV, two thirds of the disc florets open. The harvest data was recorded as described in Section III.3. Flower yield was estimated using a scale of zero to six, with six corresponding to the highest flower yield. The flower number of a representative plant from each treatment was calculated by recording the total flower weight and the weight of 25 average sized flowers. This data was used to validate the estimates described above.

A glasshouse trial was conducted in conjunction with the timing trial. Twelve CIG 3 plants were transferred to vernalising (6 °C night temperature), 12 hour daylength conditions (20-25 °C, 600-900 $\mu mol.m^{-2}.s^{-1}$ PFD) for one month before treatment. Four treatments were used : 'Cultar' (50mg Paclobutrazol per plant), 'Cultar' (50mg Paclobutrazol per plant) plus GA_3 (10mg per plant), GA_3 (10mg per plant), and an untreated control. Each treatment was applied as a foliar spray, with 50ml of solution applied to each plant. The treatments were replicated three times. The flowering time (time taken to reach flowering stage IV), flower stem height and flower number were recorded.

2.3 Results

2.3.1 Screening of Growth Retardants

Two months after the treatments were applied, clear differences in flower stem height were observed at both sites. Plate 31 shows the marked height reductions associated with the 'Cultar' treatments (5 $Kg.ha^{-1}$ and 10 $Kg.ha^{-1}$) at the Bushy Park site. All 'Cultar' and 'EL-500' treatments were effective in reducing flower stem height at this time at both the Bushy Park and Ouse sites.

The height reduction observed at harvest was less distinct, particularly at the Ouse site. The only mean to differ significantly from the control was the 'EL-500' 10 $Kg.ha^{-1}$ treatment mean. At the Bushy Park site, all of the 'EL-500' and 'Cultar' treatments caused significant decreases in flower stem height. Flower stem height was not affected by 'Cycocel' or 'A-rest' applications at either site. Plate 32 shows the height difference between 'Cultar' and 'Cycocel' treated plants at Bushy Park.

'EL-500' and 'Cultar' treatments also affected the foliage canopy structure. The leaves were observed to be thicker and greener after these growth retardant treatments. The leaf canopy height at Bushy Park was significantly reduced by each 'EL-500' treatment and by all but the 1 $Kg.ha^{-1}$ 'Cultar' applications. As with the flower stem height measurements, 'EL-500' proved to be the most effective of the growth retardants in reducing leaf canopy height. The 'Cycocel' and 'A-rest' treatments did not result in any significant differences from the untreated control plants for this or any of the other measured parameters except flower number.

The degree of lodging was significantly reduced by the 10 $Kg.ha^{-1}$ 'EL-500' treatment at Ouse and all the 'EL-500' treatments at Bushy Park. Plates 33 and 34 show the difference in the degree of lodging between control and 'EL-500' treated plants respectively at Ouse.The 5 $Kg.ha^{-1}$ and 10 $Kg.ha^{-1}$ 'Cultar' applications also significantly reduced lodging at Bushy Park. This reduction in lodging was correlated with an increase in the percentage of flowers which was harvestable. Significant differences were observed at Bushy Park with the 5 $Kg.ha^{-1}$ and 10 $Kg.ha^{-1}$ 'Cultar' treatments and the 1 $Kg.ha^{-1}$ and 5 $Kg.ha^{-1}$ 'EL-500' treatments. The 10 $Kg.ha^{-1}$ 'EL-500' treatment at Bushy Park caused a reduction in flower stem height to the extent that many of the flowers were below leaf canopy height and so were considered

Plate 31

Plate 32

Plate 31 - 'Cultar' treatments at Bushy Park, 2 months after application. Control plants are on the right of the block and 'Cycocel' treated plants on the left.

Plate 32 - 'Cultar' (foreground) and 'Cycocel treated plants at harvest.

Plate 33

Plate 34

Plate 33 - Lodging in an untreated plant at harvest (Ouse). Note that the flower stems have fallen away to reveal the crown of the plant.

Plate 34 - 'EL-500' treated plant (5 $Kg.ha^{-1}$) at Ouse. Lodging is restricted to the outer flower stems.

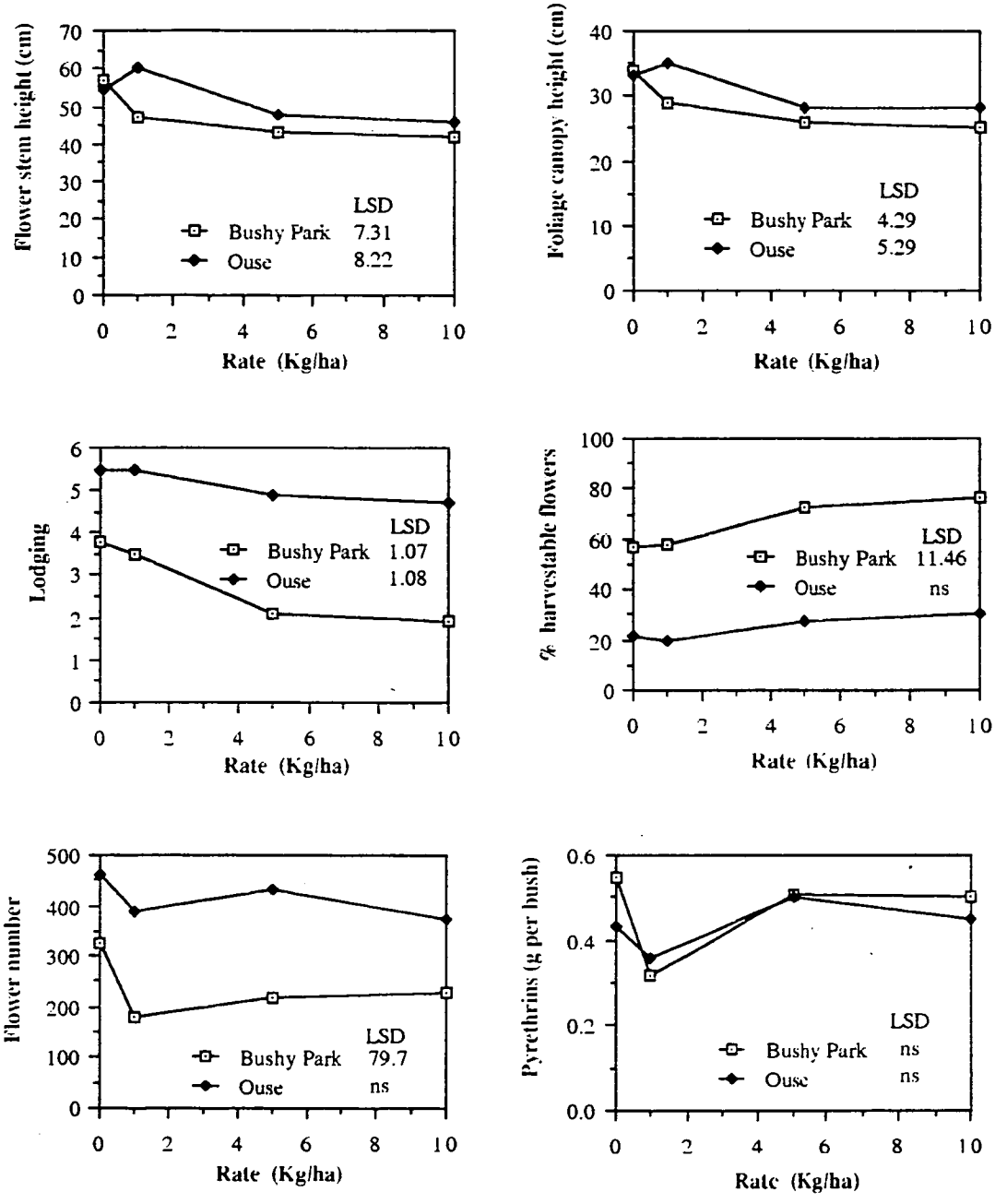


Figure IV.C.2.3.1.a - Effect of 'Cultar' on the flowering of pyrethrum.
The degree of lodging was rated on a scale of 1 to 6, with 6 indicating maximum lodging. The percentage of flowers above foliage canopy height were designated as harvestable. ns indicates that no statistically significant differences exist between the means.

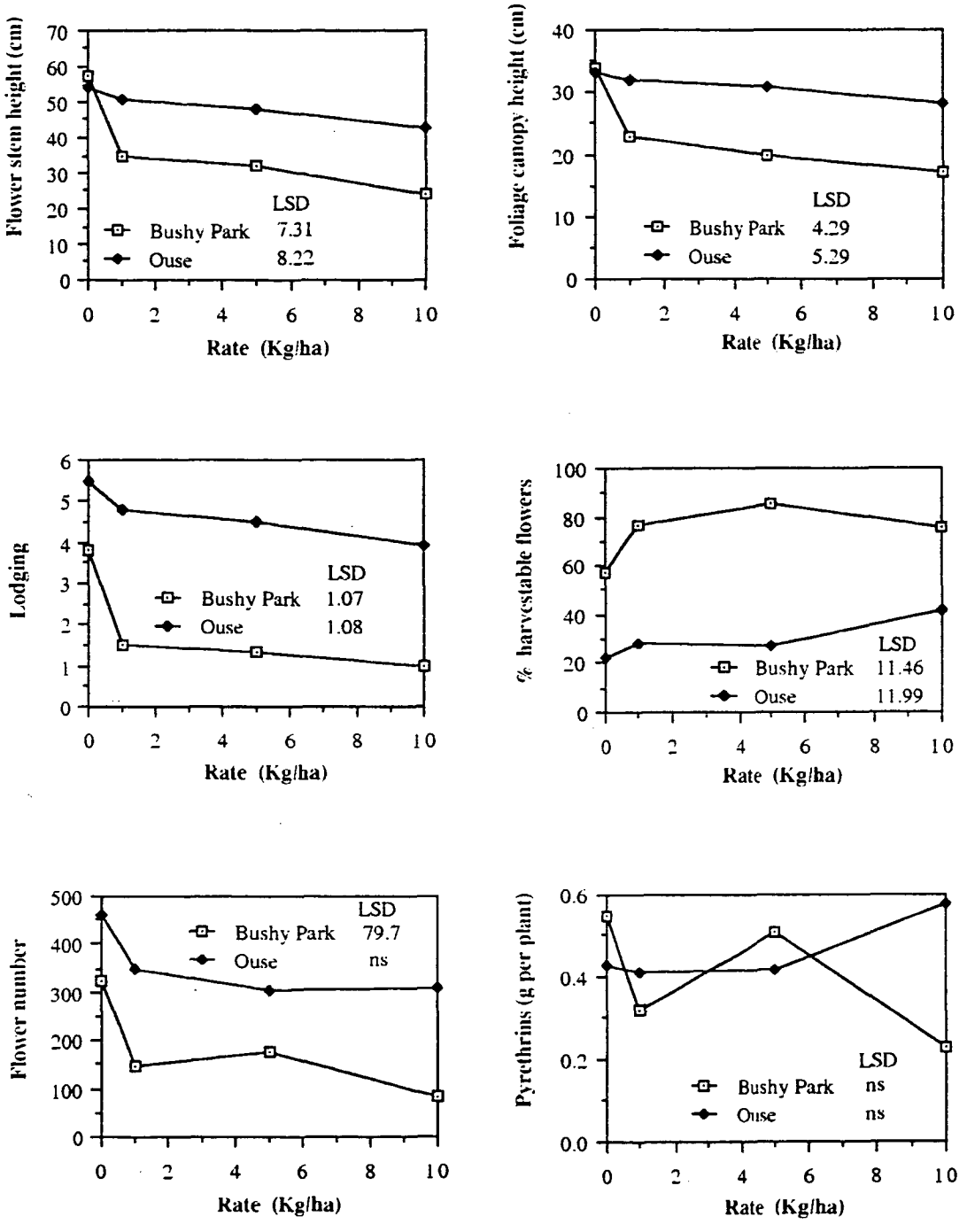


Figure IV.C.2.3.1.b - Effect of 'EL-500' on the flowering of pyrethrum. The degree of lodging was rated on a scale of 1 to 6, with 6 indicating maximum lodging. The percentage of flowers above foliage canopy height were designated as harvestable. ns indicates that no statistically significant differences exist between the means.

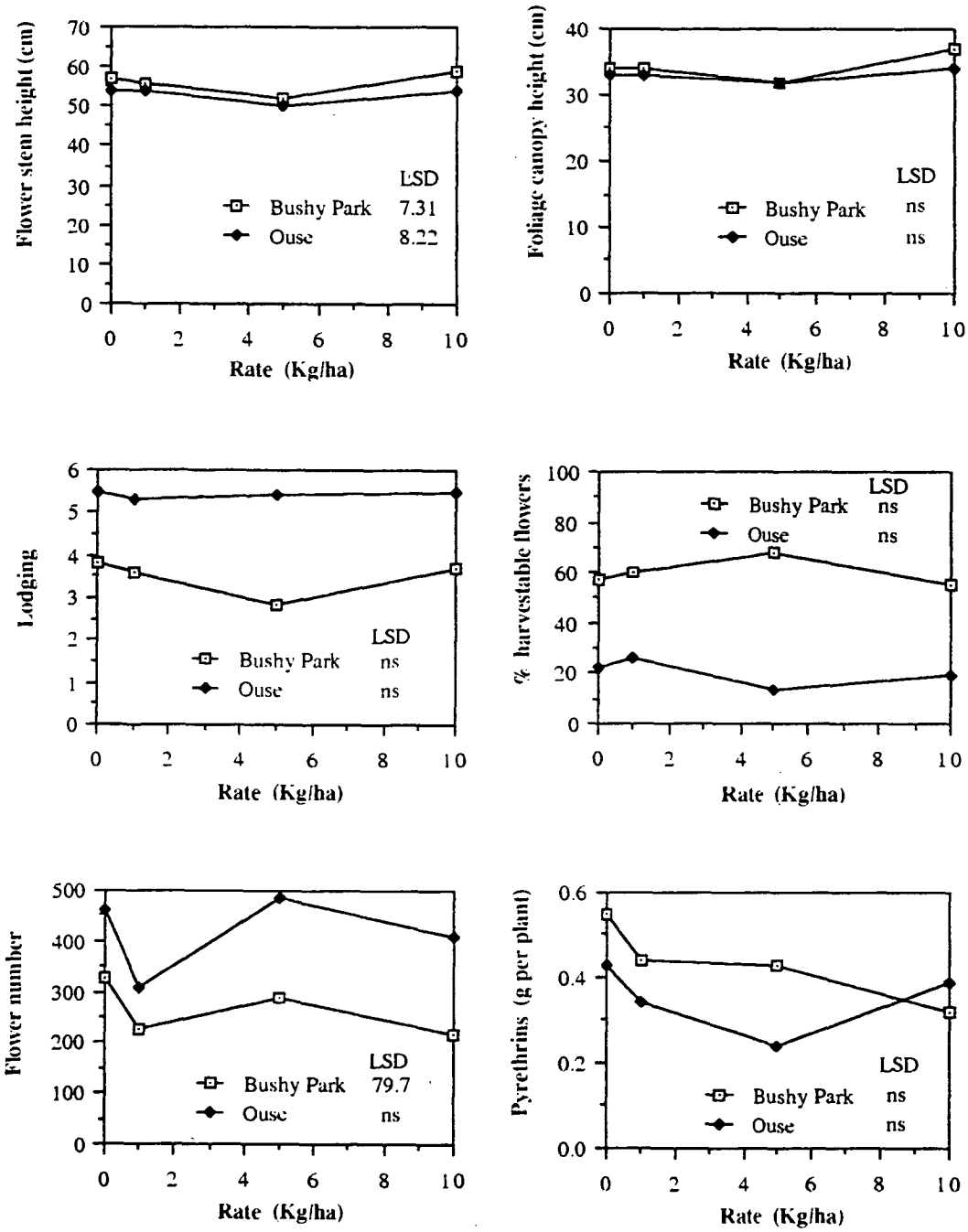


Figure IV.C.2.3.1.c - Effect of 'Cycocel' on the flowering of pyrethrum. The degree of lodging was rated on a scale of 1 to 6, with 6 indicating maximum lodging. The percentage of flowers above foliage canopy height were designated as harvestable. ns indicates that no statistically significant differences exist between the means.

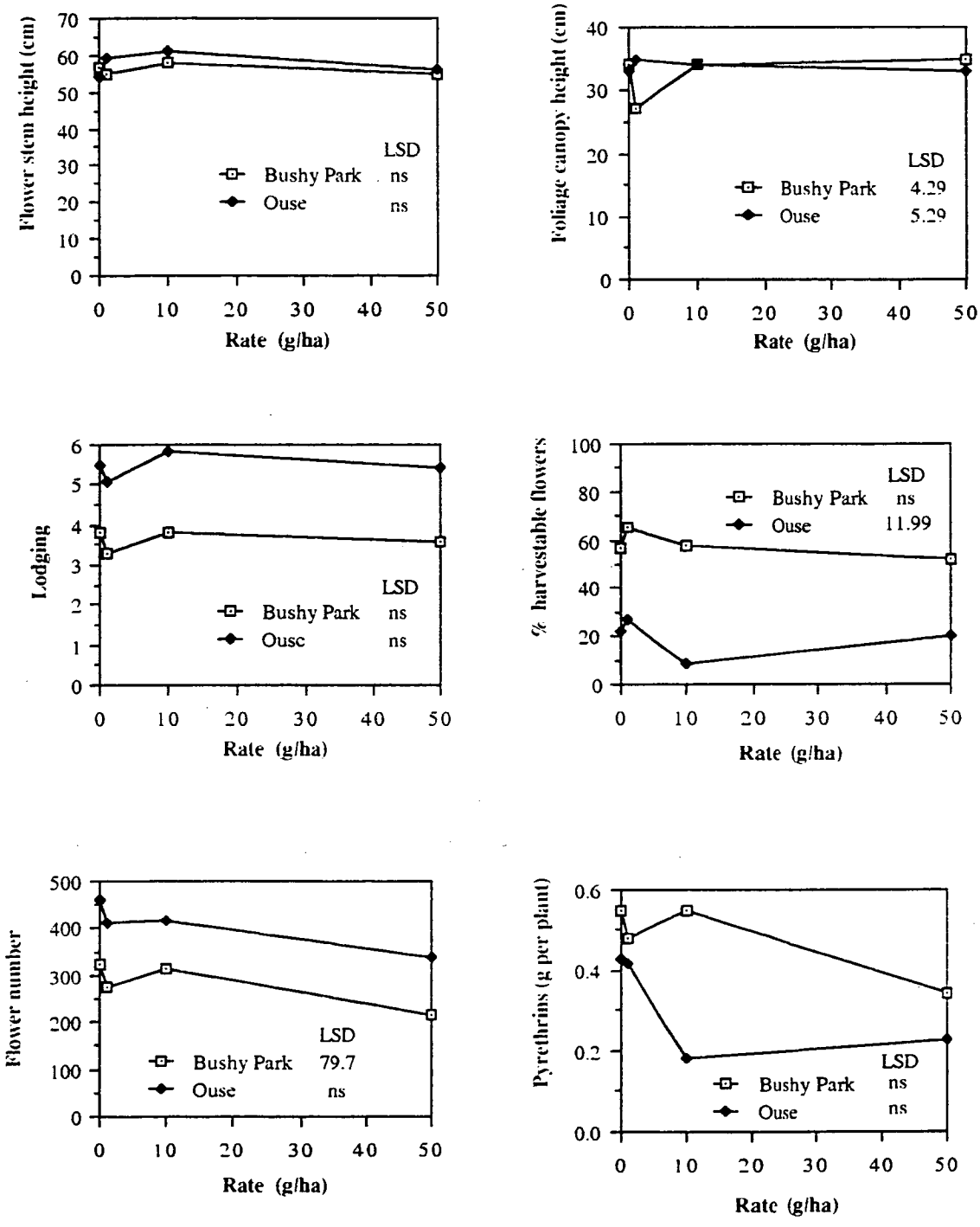


Figure IV.C.2.3.1.d - Effect of 'A-rest' on the flowering of pyrethrum.
The degree of lodging was rated on a scale of 1 to 6, with 6 indicating maximum lodging. The percentage of flowers above foliage canopy height were designated as harvestable. ns indicates that no statistically significant differences exist between the means.

unharvestable.

Flower number was significantly reduced by several of the growth retardants. Both 'A-rest' at 50 $g.ha^{-1}$ and 'Cycocel' at 1 $Kg.ha^{-1}$ and 10 $Kg.ha^{-1}$ caused a significant reduction in the average number of flowers per plant at Bushy Park. All the 'EL-500' and 'Cultar' treatments at the same site resulted in significant reductions in flower number. No significant differences were observed at Ouse. Unfortunately the experimental design incorporated the end plants from each clonal row as untreated controls, and part of the variation in flower number, and possibly all of the other measured parameters, may have been due to this as the end plants tended to be larger.

The pyrethrin content of flowers at both development stages IV and VI was not significantly affected by the growth retardant treatments. The marked differences in pyrethrin content between clones may have contributed to this finding. The 'EL-500' and 'Cultar' treatments did tend to increase pyrethrin content, but because of the large flower numbers recorded for control plants, the pyrethrin content per bush tended to be highest for the control treatment. Again no significant differences were observed in the pyrethrin content per bush measurements.

2.3.2 Timing of 'Cultar' Applications

Each of the 'Cultar' treatments significantly reduced flower stem height. The earliest applications and multiple application treatments resulted in the largest reductions in stem height. Figures IV.C.2.3.2.a, IV.C.2.3.2.b, IV.C.2.3.2.c and IV.C.2.3.2.d show the effects of 'Cultar' applications in August, September, October and November respectively. The period of most rapid stem elongation was during October. The August (3/8) and September (1/9) treatments restricted stem elongation during this period as well as in the months leading up to October, thereby resulting in greater stem height reductions than the later 'Cultar' applications. The October (1/10) treatment, applied when the flower stems had already reached leaf canopy height, caused the greatest restriction in stem elongation during the October period. The November (2/11) treatment resulted in a small but significant reduction in stem elongation during November. Stem elongation during November proceeded at the same rate as the control plants for each of the three earlier 'Cultar' application treatments.

The effects of the August (3 $Kg.ha^{-1}$) October (1 $Kg.ha^{-1}$), August (2 $Kg.ha^{-1}$) October (2 $Kg.ha^{-1}$) and the August to November (1 $Kg.ha^{-1}$) treatments are shown in

unharvestable.

Flower number was significantly reduced by several of the growth retardants. Both 'A-rest' at 50 $g.ha^{-1}$ and 'Cycocel' at 1 $Kg.ha^{-1}$ and 10 $Kg.ha^{-1}$ caused a significant reduction in the average number of flowers per plant at Bushy Park. All the 'EL-500' and 'Cultar' treatments at the same site resulted in significant reductions in flower number. No significant differences were observed at Ouse. Unfortunately the experimental design incorporated the end plants from each clonal row as untreated controls, and part of the variation in flower number, and possibly all of the other measured parameters, may have been due to this as the end plants tended to be larger.

The pyrethrin content of flowers at both development stages IV and VI was not significantly affected by the growth retardant treatments. The marked differences in pyrethrin content between clones may have contributed to this finding. The 'EL-500' and 'Cultar' treatments did tend to increase pyrethrin content, but because of the large flower numbers recorded for control plants, the pyrethrin content per bush tended to be highest for the control treatment. Again no significant differences were observed in the pyrethrin content per bush measurements.

2.3.2 Timing of 'Cultar' Applications

Each of the 'Cultar' treatments significantly reduced flower stem height. The earliest applications and multiple application treatments resulted in the largest reductions in stem height. Figures IV.C.2.3.2.a, IV.C.2.3.2.b, IV.C.2.3.2.c and IV.C.2.3.2.d show the effects of 'Cultar' applications in August, September, October and November respectively. The period of most rapid stem elongation was during October. The August (3/8) and September (1/9) treatments restricted stem elongation during this period as well as in the months leading up to October, thereby resulting in greater stem height reductions than the later 'Cultar' applications. The October (1/10) treatment, applied when the flower stems had already reached leaf canopy height, caused the greatest restriction in stem elongation during the October period. The November (2/11) treatment resulted in a small but significant reduction in stem elongation during November. Stem elongation during November proceeded at the same rate as the control plants for each of the three earlier 'Cultar' application treatments.

The effects of the August (3 $Kg.ha^{-1}$) October (1 $Kg.ha^{-1}$), August (2 $Kg.ha^{-1}$) October (2 $Kg.ha^{-1}$) and the August to November (1 $Kg.ha^{-1}$) treatments are shown in

Figure IV.C.2.3.2.a

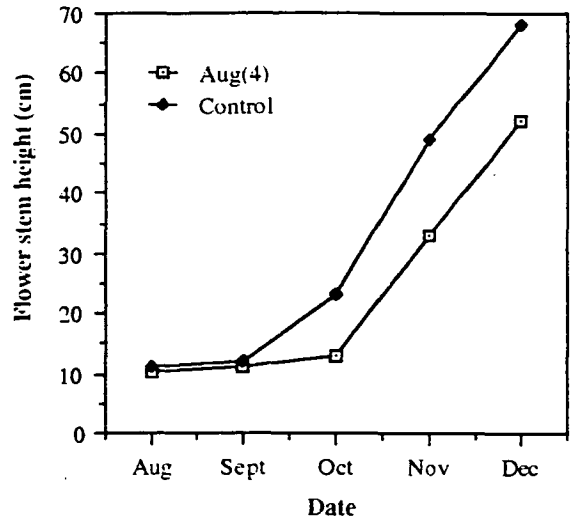


Figure IV.C.2.3.2.b

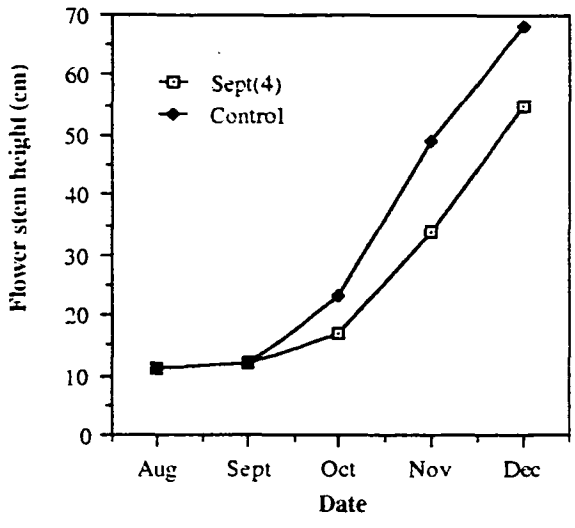


Figure IV.C.2.3.2.c

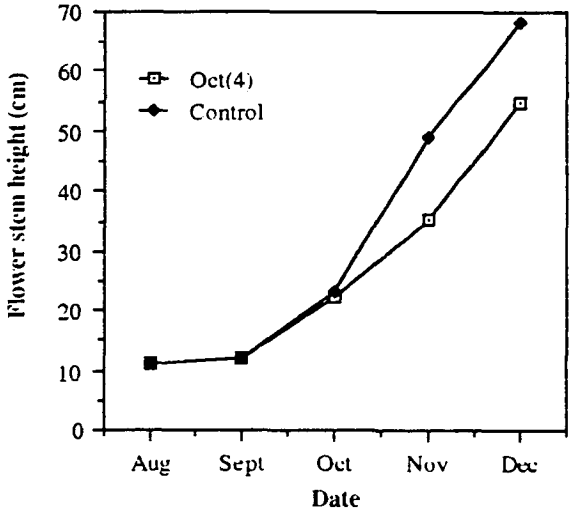


Figure IV.C.2.3.2.d

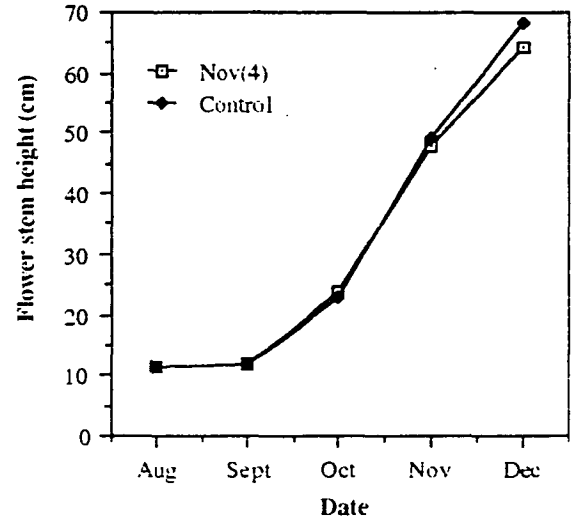


Figure IV.C.2.3.2.a - Comparison between flower stem heights of untreated, control plants and plants treated with 'Cultar' (at a rate equivalent to 4 $Kg.ha^{-1}$ active ingredient) in August. The differences between the means in October, November and December are statistically significant.

Figure IV.C.2.3.2.b - Comparison between flower stem heights of untreated, control plants and plants treated with 'Cultar' (4 $Kg.ha^{-1}$ active ingredient) in September. The differences between the means in October, November and December are statistically significant.

Figure IV.C.2.3.2.c - Comparison between flower stem heights of untreated, control plants and plants treated with 'Cultar' (4 $Kg.ha^{-1}$ active ingredient) in October. The differences between the means in November and December are statistically significant.

Figure IV.C.2.3.2.d - Comparison between flower stem heights of untreated, control plants and plants treated with 'Cultar' (4 $Kg.ha^{-1}$ active ingredient) in November. The differences between the means in December are statistically significant.

Figure IV.C.2.3.2.e

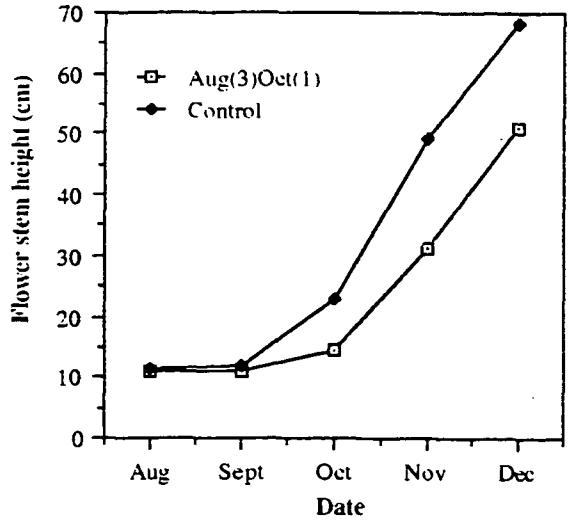


Figure IV.C.2.3.2.f

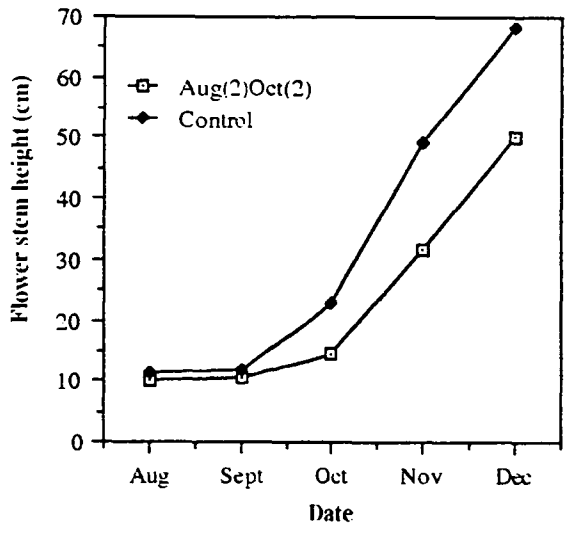


Figure IV.C.2.3.2.g

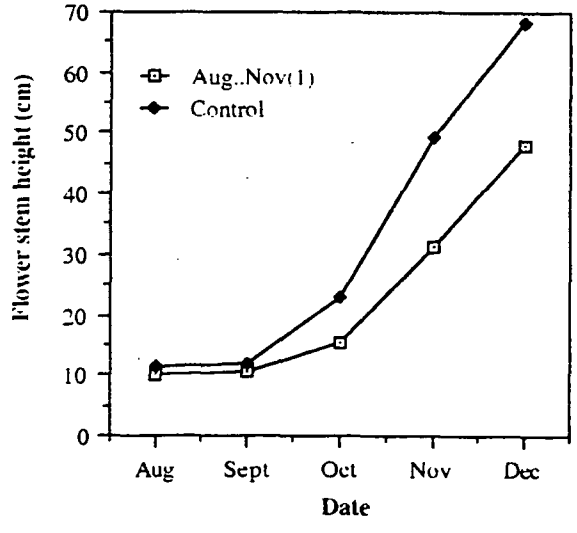


Figure IV.C.2.3.2.e - Comparison between flower stem heights of untreated, control plants and plants treated with 'Cultar' in August (3 $Kg.ha^{-1}$) plus October (1 $Kg.ha^{-1}$). The differences between the means in October, November and December are statistically significant.

Figure IV.C.2.3.2.f - Comparison between flower stem heights of untreated, control plants and plants treated with 'Cultar' in August (2 $Kg.ha^{-1}$) plus October (2 $Kg.ha^{-1}$). The differences between the means in October, November and December are statistically significant.

Figure IV.C.2.3.2.g - Comparison between flower stem heights of untreated, control plants and plants treated with 'Cultar' each month between August and October (1 $Kg.ha^{-1}$). The differences between the means in October, November and December are statistically significant.

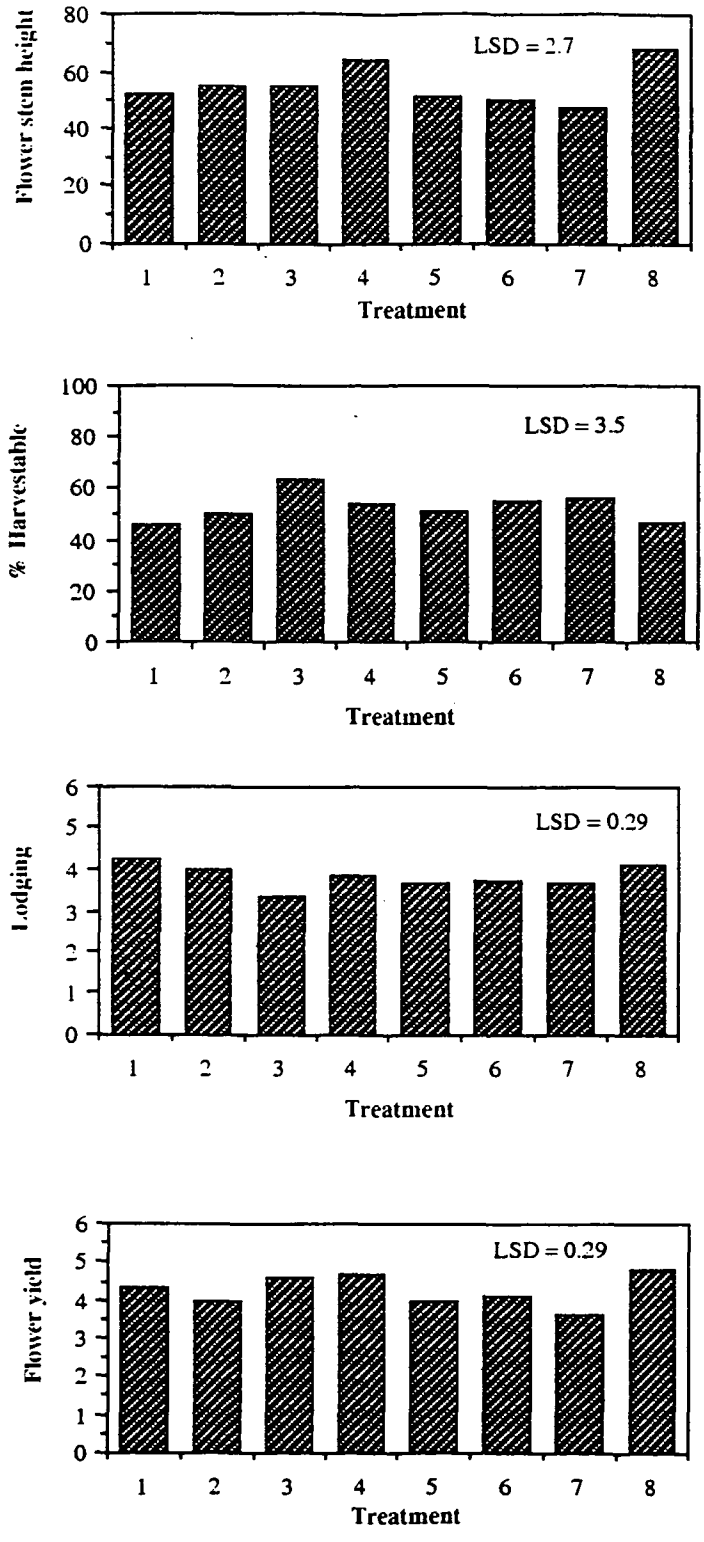


Figure IV.C.2.3.2.h - Effect of 'Cultar' treatments on flower stem height, percentage of flowers which were harvestable, degree of lodging and flower yield at harvest. The treatments were ; 1: August (4 Kg.ha^{-1}), 2: September (4 Kg.ha^{-1}), 3: October (4 Kg.ha^{-1}), 4: November (4 Kg.ha^{-1}), 5: August (3 Kg.ha^{-1}) plus October (1 Kg.ha^{-1}), 6: August (2 Kg.ha^{-1}) plus October (2 Kg.ha^{-1}), 7: August to November (1 Kg.ha^{-1}) and 8: untreated control. Both lodging and flower yield were estimated on a scale of 1 to 6.

Treatment	Flowering time (days)	Flower stem height (cm)	Flower number
'Cultar'	72.3	22.7	12.3
GA	53.7	72.3	14.7
'Cultar' + GA	54.3	65.7	21.0
Control	51.3	67.0	22.7
LSD	5.3	7.7	5.0

Figure IV.C.2.3.2.i - Effect of 'Cultar' (50mg) and GA_3 (10mg) applications on the flowering of pyrethrum. Flowering time was noted when the flowers reached Stage IV, but did not include the 28 days spent in inductive conditions before the treatments were applied.

Figures IV.C.2.3.2.e, IV.C.2.3.2.f and IV.C.2.3.2.g respectively. In each case stem elongation was significantly reduced during September, October and November. The graphs of the August (3) October (1) and August (2) October (2) treatments are almost identical, indicating that the maximum physiological response had been achieved by both August applications and indicating that lower concentrations of 'Cultar' could have been used to achieve a similar response. Spreading the applications of 'Cultar' over four months resulted in the maximum height reduction recorded. This suggests that the effectiveness of 'Cultar' declines between two and four months after application as the same total amount of 'Cultar' was used in each treatment.

August (3/8) and September (1/9) 'Cultar' applications did not reduce lodging at harvest. The October (1/10) treatment was most effective in reducing the degree of lodging while the November (2/11) treatment and each of the multiple application treatments also significantly reduced lodging, as seen in Figure IV.C.2.3.2.h. The percentage of flowers judged to be harvestable showed a similar trend. The important effect of the growth retardant which influences the percentage of flowers which are harvestable appears to be in reducing lodging and not simply reducing flower stem height. The overall flower number was reduced by each of the treatments except for the October and November 'Cultar' applications. The timing of growth retardant applications is obviously vital in determining the effectiveness of the treatment in altering the flower canopy structure to facilitate harvesting.

The morphological effects of 'Cultar' treatment are reversible by GA_3 application. Figure IV.C.2.3.2.i shows that 'Cultar' treatment delayed flowering, restricted stem elongation and lowered the number of flowers produced while 'Cultar' plus GA_3 did not affect flowering. GA_3 alone slightly increased flower stem height compared with untreated plants although the difference was not statistically significant. This indicates that the plants endogenous gibberellin levels are optimal for stem elongation during flowering. GA_3 application also reduced the total flower number and it was observed that fewer flowers per flower stem were initiated after GA_3 treatment.

2.3.3 Endogenous Hormonal Changes

The endogenous gibberellin and abscisic acid concentrations in mature leaf samples taken from plants in the timing of 'Cultar' application experiment were determined by RIA. Figures IV.C.2.3.3.a and IV.C.2.3.3.b show the significant

Figure IV.C.2.3.3.a

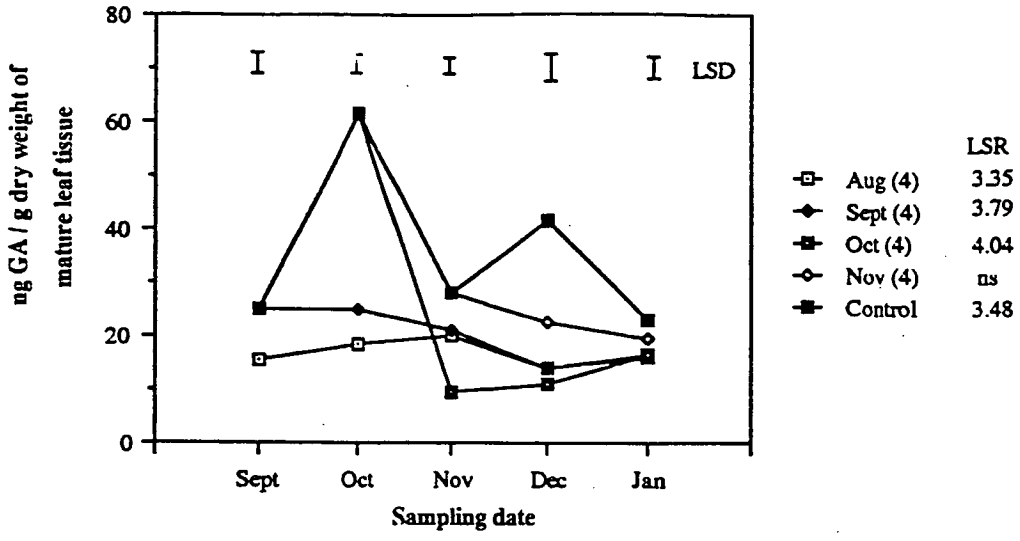


Figure IV.C.2.3.3.a - Effect of 'Cultar' applications on the gibberellin concentration in mature leaf samples. 'Cultar' (4 Kg.ha^{-1} active ingredient) was applied in August, September, October or November. The controls were not treated with the growth retardant.

ns = no statistically significant differences between the means.

Figure IV.C.2.3.3.b

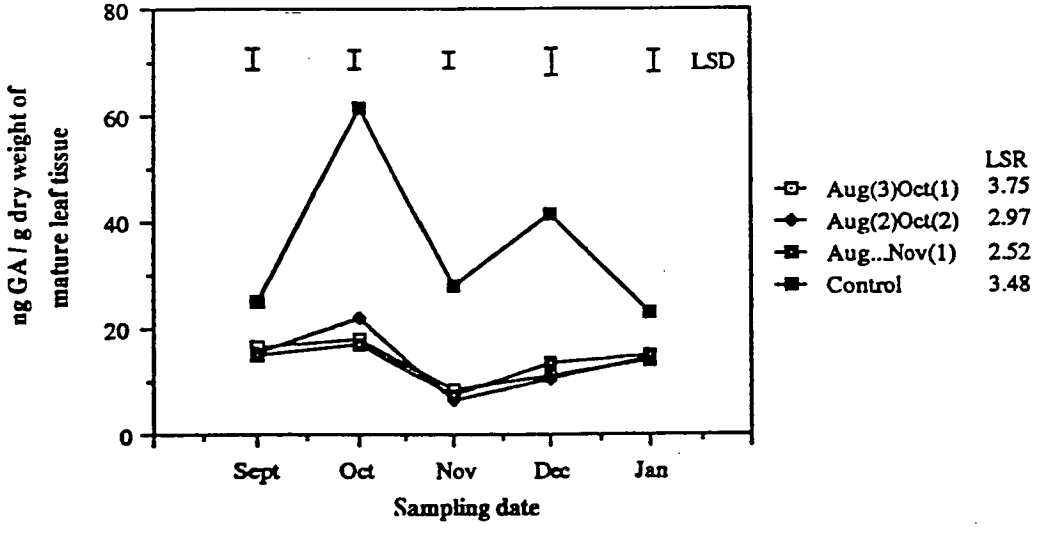


Figure IV.C.2.3.3.b - Effct of 'Cultar' applications on the gibberellin concentration in mature leaf samples. 'Cultar' was applied in August (3 Kg.ha^{-1}) plus October (1 Kg.ha^{-1}), August (2 Kg.ha^{-1}) plus October (2 Kg.ha^{-1}) or each month between August and November (1 Kg.ha^{-1}). The controls were not treated with the growth retardant.

Figure IV.C.2.3.3.c

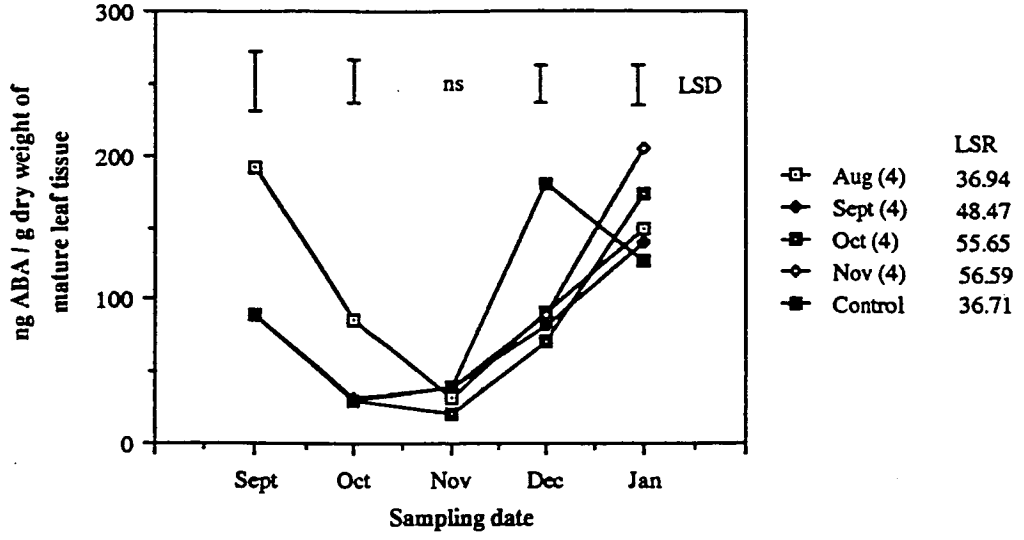


Figure IV.C.2.3.3.c - Effect of 'Cultar' applications on the abscisic acid concentration in mature leaf samples. 'Cultar' (4 Kg.ha^{-1} active ingredient) was applied in August, September, October or November. The controls were not treated with the growth retardant.

ns = no statistically significant differences between the means.

Figure IV.C.2.3.3.d

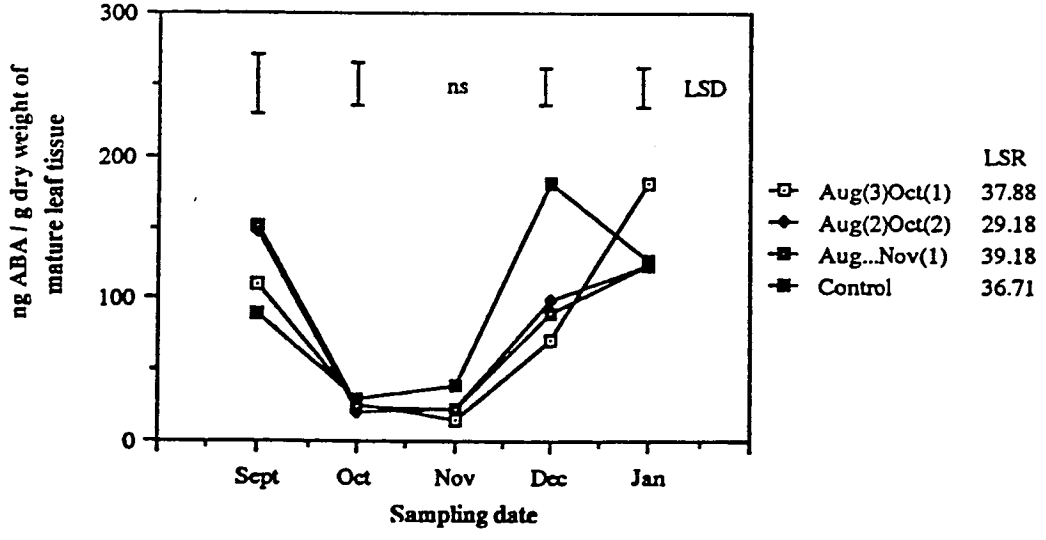


Figure IV.C.2.3.3.d - Effct of 'Cultar' applications on the abscisic acid concentration in mature leaf samples. 'Cultar' was applied in August (3 Kg.ha^{-1}) plus October (1 Kg.ha^{-1}), August (2 Kg.ha^{-1}) plus October (2 Kg.ha^{-1}) or each month between August and November (1 Kg.ha^{-1}). The controls were not treated with the growth retardant.

ns = no statistically significant differences between the means.

decline in gibberellin concentration associated with each of the treatments. The gibberellin concentration in untreated plant tissue was highest at the start of October, and is likely to have mediated the rapid stem elongation observed during October. Gibberellin levels are also high in December but decline by the beginning of January. Very little stem elongation was observed during December, indicating that high gibberellin levels at this time mediate processes associated with floral differentiation and development. Gibberellins produced by the flower buds during November may be responsible for the stem elongation observed during this month and could explain the similar rates of elongation observed in treated and untreated plants at this time.

Applications of 'Cultar' in August at rates of 1 $Kg.ha^{-1}$, 2 $Kg.ha^{-1}$, 3 $Kg.ha^{-1}$ and 4 $Kg.ha^{-1}$ caused similar declines in gibberellin concentration at the beginning of September. The increased effectiveness of multiple applications totalling 4 $Kg.ha^{-1}$ compared with single applications at 4 $Kg.ha^{-1}$ appears to be due to maintaining low gibberellin concentrations over a longer period of time. The ability of 'Cultar' to interfere with the biosynthesis and metabolism of gibberellin decreased by approximately two months after application, but continued to have some effect for the duration of the experiment as the gibberellin concentration in the control plants was always higher than in the treated plants.

The treatments also influenced the biosynthesis or metabolism of abscisic acid. Figures IV.C.2.3.3.c and IV.C.2.3.3.d reveal that the ABA concentration in untreated plants was lowest during October and November when stem elongation was most rapid. 'Cultar' did not significantly influence the ABA concentration during this time, the exception being the August (4 $Kg.ha^{-1}$) treatment which caused an increase in ABA concentration in September and October. The greatest effect was seen in December when all the treatments caused a significant decline in ABA levels. In January this trend was reversed, with the October (4 $Kg.ha^{-1}$), November (4 $Kg.ha^{-1}$) and August (3 $Kg.ha^{-1}$) October (1 $Kg.ha^{-1}$) treatments all significantly increasing ABA concentration. ABA appeared to be involved with the late flower development processes rather than the stem elongation process, and the observed effects of 'Cultar' on ABA concentration was an indirect result of the growth retardants action in delaying flower development.

2.4 Discussion

'Cycocel' and 'A-rest' did not affect the flowering of pyrethrum at the concentrations studied. 'A-rest', a substituted pyrimidine compound, had no effect at the rates used (1, 10 and 50 $g.ha^{-1}$) but could reasonably be expected to alter flowering if used at a higher concentration as 'EL-500', another substituted pyrimidine, dramatically altered the flowering pattern when applied at rates of 1, 5 and 10 $Kg.ha^{-1}$. 'Cultar' and 'EL-500' were both effective in reducing flower stem height and lodging at harvest at the Bushy Park site. The highest 'EL-500' concentration (10 $Kg.ha^{-1}$) was the only treatment to significantly reduce flower stem height at the Ouse site. The plants at Ouse were larger than those at Bushy Park and flowered more profusely, as can be seen by the higher yield of flowers at this site. The large number of flower stems and the exposed nature of this site contributed to the extensive lodging observed. The fact that both 'Cultar' and 'EL-500' applications caused a noticeable reduction in flower stem height two months before harvest suggests that higher rates of these growth retardants or application at a later date may have resulted in effective control of flower stem height and lodging at the site at harvest time.

'EL-500' applications resulted in the greatest morphological responses at the Bushy Park site. Flower stem height was reduced by the 10 $Kg.ha^{-1}$ treatment to the point where a large percentage of flowers were below the foliage canopy height and therefore unharvestable. The 'Cultar' treatments at 5 $Kg.ha^{-1}$ and 10 $Kg.ha^{-1}$ were most effective in controlling lodging without decreasing the harvestable flower yield. The total flower yield was reduced by each of the growth retardant treatments which effectively controlled flower stem height and lodging. In the case of the 'EL-500' treatments, the desirable effects of reduced lodging and increased percentage of harvestable flowers were outweighed by the reduction in total flower number.The results from the timing of 'Cultar' application trial indicate that the time of application of the growth retardant is important in determining the total flower number at harvest. It is necessary to establish the optimum rates to be applied as well as the optimum time of application to effectively increase harvestable yield by controlling lodging.

Application of 'Cultar' just prior to the period of rapid stem elongation in October effectively reduced stem height and lodging without significantly reducing the total flower number. Earlier applications of 'Cultar', whether as single application treatments or multiple application treatments, significantly reduced the total number of flowers at

harvest. The August and September applications (4 $Kg.ha^{-1}$) did not reduce lodging even though flower stem height was significantly reduced. This suggests that stem strengthening during the period of rapid stem elongation in October and November is the morphological effect of the October application which reduced lodging rather than shortening of the flower stem. The growth retardant treatments in the screening trial may also have been more effective if applied at the start of October rather than early August.

The total flower number at harvest was reduced by early 'Cultar' applications but not by the October and November applications. As flower initiation may still be occurring during August and September, particularly initiation of lateral shoots on developing flower stems, the physiological effects of 'Cultar' application must include inhibition of one or more of the evocational processes.

Flower stem elongation appears to be mediated by gibberellins. The morphological effects of 'Cultar' are reversible by GA_3 application, and endogenous gibberellin levels are significantly lowered by 'Cultar' treatments which lead to reduced stem elongation. Gibberellins may also mediate one or more of the evocational processes as early 'Cultar' applications appear to inhibit flower initiation. GA_3 application also appeared to inhibit initiation of lateral shoots on flower stems, indicating that a certain concentration of gibberellins may be required for evocation or that gibberellins may only be required for a specific step or steps in the evocational sequence and may in fact be inhibitory if present at other stages. Flower development is also slowed by growth retardant treatments as flowering time is delayed. This may be a direct result of the endogenous chemical changes produced by the growth retardant application or an indirect result of other physiological changes produced by the treatment.

The increased ABA levels observed during the latter stages of flower development in 'Cultar' treated plants may be causal to or a consequence of the delay in flower development. 'Cultar' applications have been noted to decrease abscisic acid levels in wheat seedlings soon after application (Buta and Spaulding, 1991). No similar trend was observed in pyrethrum in the samples taken one month after application, indicating that the fluctuations in ABA concentration in December were a result of other physiological effects of 'Cultar' indirectly influencing ABA metabolism.

Growth retardants have been shown to effectively reduce lodging and increase

the percentage of harvestable flowers in pyrethrum when applied immediately before the period of rapid internode elongation in October. This effect is a result of increased stem strength and decreased stem height caused by the inhibition of gibberellin biosynthesis. Gibberellins may also mediate one or more of the evocational processes, and so growth retardant applications during flower initiation may result in reduced flower yields at harvest. Growth retardants are potentially useful tools to increase harvestable flower yields, but may prove too expensive for widespread commercial application.

V. General Discussion

Floral initiation, at its most fundamental level, involves a change in gene expression which ultimately leads to the development of reproductive structures. Apical cells begin to express a set of genes that are not expressed in the vegetative phase of growth, and the vegetative growth pattern is eliminated. This change in gene expression in the meristem may be the primary cause of evocation or a consequence of the particular molecular changes which constitute evocation. Evocation is essentially a molecular and cellular process, with the physiological changes occurring as a consequence of or causal to the changes in genetic expression triggering changes at the higher levels of organisation. Limits to quantitative preparatory and analytical techniques have meant that until recently the majority of flowering research has centered on these higher order morphological and physiological changes and the environmental stimuli which trigger them. This has provided a framework for each of the species examined upon which future genetic, molecular and cellular studies can be based. This study has endeavoured to define such a framework for *T. cinerariaefolium* into which future findings may be included to gain an understanding of the complete flowering process.

Pyrethrum seedlings, tissue cultured clonal material and vegetatively divided splits from mature plants exhibit a period of juvenile-like growth in which they are not competent to respond to normally inductive treatments. The juvenile-like phase lasts until the plant has reached a minimum size or stage of development, but does not depend on chronological age. This finding is in agreement with published data on the juvenile phases of several other woody and herbaceous species. The commitment to form flowers is correlated with the number of nodes produced by the meristem in tobacco (Singer and McDaniel, 1986), and a minimum number of nodes must be present for flower initiation in raspberry and black currant (Schwabe and Al-Doori, 1973). The attainment of meristem competence in pyrethrum is linked to the release of axillary shoots from apical dominance. The number of nodes produced by the apical meristem is correlated with this release, and so a relationship exists between the number of nodes on the main shoot axis and competence to flower. As in *D. grandiflora* (Cockshull, 1976), a minimum number of leaves must be present on the flowering stem for flowering to occur. This number is increased when the plant is grown under 'non-inductive' or marginally inductive conditions.

While the attainment of a minimum node or leaf number is often critical for the expression of meristem competence, the cellular and molecular changes at the transition are not as obvious. Genetic studies of *Pisum* have revealed a number of genes controlling the length of the juvenile phase (Murfet, 1985), and it is likely that a complex interaction of several processes controls meristem competence. This may include restriction of floral expression by insufficient photosynthetic capacity and/or other metabolic limitations in the leaves, correlative influences such as apical dominance or root activity, or intrinsic differences between cells in juvenile and competent meristems.

Apical dominance appears to play a major role in the juvenile response of pyrethrum as only axillary meristems released from apical dominance may be induced to flower. Similar indeterminate flowering has been described for wild cherries (Webster and Shepherd, 1984) and roses (Cockshull and Horridge, 1977) following release from apical dominance, while axillary meristems in most cold-requiring perennials flower automatically when released from apical dominance. While the apical meristem of cold-requiring *D. grandiflora* cultivars can be induced to flower, decapitation of non-vernalised plants results in accelerated inflorescence initiation on distal, lateral shoots (Vince and Mason, 1954). Also, devernalisation of axillary meristems occurs when plants are grown, after a chilling treatment, in conditions which favour strong apical dominance (Cockshull, 1985). Thus apical dominance also appears to play a regulatory role in the flowering of cold-requiring chrysanthemum cultivars.

The mechanism of action of apical dominance is thought to involve both assimilate partitioning and the plant growth regulators. Most of the evidence indicates that correlative inhibition of lateral bud growth involves a deficiency in the lateral buds of some essential factor or factors for their development. It seems probable that the lateral buds lack both an adequate supply of cytokinins and assimilates, and that auxin produced by the apical bud and transported basipetally influences assimilate distribution and plant growth regulator translocation to maintain this correlative inhibition of lateral bud growth.

The auxin concentration in pyrethrum seedlings was observed to increase during early growth, corresponding to the increase in the growth rate of the terminal shoot apex. While overall plant auxin levels did not decrease significantly at the time of release of lateral shoots from apical dominance, it is likely that the concentration in the vicinity of the lateral shoot would have declined as the distance between it and the

terminal apex increased. No significant changes in cytokinin concentration on a whole plant basis were observed but again it is possible that localised fluctuations near the lateral shoot could have occurred. The data presented indicates that the correlative influence of apical dominance mediates the juvenile condition in pyrethrum but sheds little light on the mechanisms on a molecular level.

Gibberellins may also play a role in maintaining juvenility in seedlings. Schwabe and Al-Doori (1973) concluded that juvenility in blackcurrants is mediated by gibberellins produced by the roots, with the proximity of the shoot tips to the roots determining meristem competence. The gibberellin concentration in pyrethrum seedlings declines with age, remaining at a level below 20 ng/g dry weight of mature leaf tissue in non-inductive conditions after the juvenile period is completed. However, high gibberellin concentrations cannot be the sole factor preventing meristem competence as inductive conditions stimulate increased gibberellin levels. Therefore high gibberellin levels must be linked to other physiological processes if they are involved in maintaining juvenility. Alternatively, the high gibberellin concentrations observed during the juvenile phase are not involved with juvenility but may be linked to the rapid growth rate of the seedlings and the lack of senescing tissue present during this period of growth.

Pyrethrum meristems become competent to flower a short time after release from apical dominance, while older axillary meristems lose their competence. In a similar way the axillary meristems of *D. grandiflora* released from apical dominance by decapitation of the terminal shoot are not immediately competent to respond to inductive conditions (De Jong, 1981b), while the axillary shoots of *Pharbitis* lose their meristematic competence as the shoot ages (Owens and Paolillo, 1986). The short lag period between release from apical dominance and becoming competent to flower may correspond to the time required for the apex to reach the critical size required for flower initiation (discussed later). The juvenile-like condition observed in physiologically older axillary meristems may be related to physiological limitations or intrinsic changes to the meristematic cells through aging. It was noted that the loss of competence of each lateral shoot meristem occurred after the release from apical dominance of new axillary shoots on the lateral shoot. The sink strength of the shoot may thus be decreased as the new axillaries compete for assimilates or other factors essential for floral evocation. It is also possible that the increased auxin concentration associated with shoot growth, and responsible for apical dominance within the shoot, is inhibitory to floral evocation.

Radioimmunoassay and *in vitro* studies of isolated meristematic tissue are warranted to extend our knowledge in this area.

The first macromorphological sign of flower initiation is the elongation of the internodes below the meristem. Normal floral development then proceeds through the series of floral development stages proposed by Head (1966). The timing of the macroscopic floral development stages was found to vary under different environmental conditions, and under optimal conditions to closely follow those reported by Bhat and Menary (1984). Stem elongation was first observed twenty-five days after plants were transferred to inductive conditions. Elongation may precede the end of evocation, the point at which the meristem is irreversibly committed to generative growth, as the vegetative, rosette growth pattern was observed in several shoots after a short period of stem elongation had occurred. Stem elongation does not always precede the point of floral commitment, particularly under marginally inductive conditions. Thus, while stem elongation always accompanies flowering, it may not be an integral component of the evocational sequence.

The first morphological sign associated with floral evocation in pyrethrum is enlargement and doming of the apical meristem. Activation of the central zone of the meristem is common to most species during evocation and results in the loss of the zonate pattern typical of many vegetative meristems. The irreversible commitment to flowering in pyrethrum occurs after the first bract is initiated on the apical dome. As in *D. grandiflora* (Horridge and Cockshull, 1979) and other Asteraceae species (Sharman and Sedgley, 1988), the point of floral commitment is characterised by a critical apical size. An absolute size requirement is not a universal determinant of flower formation as the meristem of *Impatiens* does not change size during initiation (Lyndon and Battey, 1985) while the meristem of *Humulus* becomes smaller (Thomas and Schwabe, 1970).

Flower development in pyrethrum proceeds through a similar series of developmental stages as in *D. grandiflora.* Involucral bracts are initiated around the rim of the apical dome which rapidly enlarges to form a receptacle. Floret primordia are initiated around the rim of the receptacle after between twenty-six and thirty-eight bracts have been initiated. Floret initiation proceeds centripetally until the entire receptacle is covered. Floret differentiation, which may begin before the receptacle is completely covered, proceeds until the perianth is present on all florets and is followed by differentiation of the reproductive organs.

Radioimmunoassay and *in vitro* studies of isolated meristematic tissue are warranted to extend our knowledge in this area.

The first macromorphological sign of flower initiation is the elongation of the internodes below the meristem. Normal floral development then proceeds through the series of floral development stages proposed by Head (1966). The timing of the macroscopic floral development stages was found to vary under different environmental conditions, and under optimal conditions to closely follow those reported by Bhat and Menary (1984). Stem elongation was first observed twenty-five days after plants were transferred to inductive conditions. Elongation may precede the end of evocation, the point at which the meristem is irreversibly committed to generative growth, as the vegetative, rosette growth pattern was observed in several shoots after a short period of stem elongation had occurred. Stem elongation does not always precede the point of floral commitment, particularly under marginally inductive conditions. Thus, while stem elongation always accompanies flowering, it may not be an integral component of the evocational sequence.

The first morphological sign associated with floral evocation in pyrethrum is enlargement and doming of the apical meristem. Activation of the central zone of the meristem is common to most species during evocation and results in the loss of the zonate pattern typical of many vegetative meristems. The irreversible commitment to flowering in pyrethrum occurs after the first bract is initiated on the apical dome. As in *D. grandiflora* (Horridge and Cockshull, 1979) and other Asteraceae species (Sharman and Sedgley, 1988), the point of floral commitment is characterised by a critical apical size. An absolute size requirement is not a universal determinant of flower formation as the meristem of *Impatiens* does not change size during initiation (Lyndon and Battey, 1985) while the meristem of *Humulus* becomes smaller (Thomas and Schwabe, 1970).

Flower development in pyrethrum proceeds through a similar series of developmental stages as in *D. grandiflora.* Involucral bracts are initiated around the rim of the apical dome which rapidly enlarges to form a receptacle. Floret primordia are initiated around the rim of the receptacle after between twenty-six and thirty-eight bracts have been initiated. Floret initiation proceeds centripetally until the entire receptacle is covered. Floret differentiation, which may begin before the receptacle is completely covered, proceeds until the perianth is present on all florets and is followed by differentiation of the reproductive organs.

Each stage in the development of the apical meristem is associated with a narrow range of apical diameter, indicating that inflorescence initiation and development are closely related to apex size. This relationship between apex size and stage of development was observed under a range of environmental conditions, including flower buds formed in 'non-inductive' conditions through an autonomous induction process. The arrested development of flower buds under 'non-inductive' conditions is similar to the 'crown' or 'break' bud conditions described for *D. grandiflora* (Cockshull and Horridge, 1980). The arrested state may simply represent a meristem at a normal stage which cannot develop further as the assimilate supply has been partitioned to other plant parts. The role of assimilates in the flowering of pyrethrum is further implicated by the relationship between apical size and stage of development. As each stage of development is characterised by a narrow range of apical sizes, the timing of the stages must be related to the assimilate supply to the developing apices.

Floral development in pyrethrum proceeds in a similar sequence irrespective of environmental conditions so long as the conditions are conducive for growth. Flowering can be regarded as the result of autonomous induction in 'non-inductive' conditions and enhanced by the vernalisation stimulus in 'inductive' conditions. Bernier (1988) asserts that the difference between autonomous, facultative and absolute species is only one of degree, with the progression to flowering being part of a normal sequence under varying degrees of environmental regulation. Manipulation of external stimuli can alter many species' responses, changing the threshold values for other environmental factors. Different environmental factors must be influencing different steps amongst the various promotory and inhibitory processes occurring in the different plant organs and as such the importance of the different organs changes according to the external conditions.

While flowering of pyrethrum occurs eventually under 'non-inductive' conditions through the autonomous induction process, vernalising conditions stimulate rapid flower initiation and development. Night temperatures of less than 18 °C are required to provide the vernalisation stimulus, with two weeks at 6 °C or three weeks at 12 °C being the minimum vernalisation requirement under short days and day temperatures of 20 - 30 °C. Pyrethrum displays a quantitative response to vernalisation, with longer periods in vernalising conditions providing the plant with a greater inductive stimulus leading to larger numbers of flowers, longer flower stems and more rapid flower initiation and development. Marginally inductive periods, where initiation is observed

to occur after less than the stated minimum durations of vernalisation, provide a sufficient stimulus to promote flower initiation but the delayed timing of initiation as well as the reduced flower stem height observed suggests that some or all of the physiological processes involved in initiation and development are proceeding at a reduced rate.

Both day temperature and photon flux density conditions can modify the effects of vernalisation in pyrethrum. Low photon flux density conditions, regardless of day temperature, retard flower initiation, either by decreasing the rate of, or by inhibiting one or more of, the processes in the evocational sequence. Flower development is similarly retarded by low photon flux density conditions. High day temperature conditions combined with low photon flux can result in a devernalisation-like effect where the plants are incapable of responding to otherwise inductive, vernalising conditions. A true devernalisation effect may also occur under these conditions, with the vernalisation stimulus being reversed by a later high temperature, low photon flux treatment. Transfer from vernalising conditions to devernalising conditions can result in a return to vegetative growth if the plants are moved before the end of floral evocation, the point of irreversible commitment to floral development. After this time, devernalising conditions inhibit floral development resulting in arrested bud development and eventually flower bud abortion.

Daylength has a quantitative effect on both flower initiation and development in pyrethrum, with both processes being promoted by long days. The inhibitory effect of short days on the flowering process appears to be mediated through the restriction of photosynthate production and not via the phytochrome reactions. In this regard pyrethrum must be considered a day-neutral species as its daylength reaction is to the daily light integral and not to photoperiod.

The inhibitory effects on flowering of reduced daily light integrals and low photon flux density conditions indicates that an adequate level of photosynthetic assimilates is required for floral evocation. An adequate assimilate supply to the shoot apical meristem is an essential component in the evocational processes of most if not all plants (Bernier *et al*, 1981b). Partitioning of assimilates within the plant and particularly within the apical bud is proposed as the mechanism controlling the photosynthetic effects. While increased carbohydrate levels in the apical bud during initiation have been reported in both photoperiodic and cold-requiring plants, examples

have also been cited where no changes occur. For instance, the assimilate supply to the apex in *Pharbitis* remains unchanged during evocation (Ogawa and King, 1991) and in *Bougainvillea* gibberellin treatments inhibit flowering without significantly lowering the level of soluble solids in the bud (Ramina, Hackett and Sachs, 1979). In these cases it is obvious that factors other than assimilates are required for flowering, and that if assimilate supply is mediating evocational processes then its distribution within the apical bud is critical and not its overall levels.

While the nutrient diversion hypothesis cannot account for all of the diverse flowering responses recorded, assimilate supply does appear to control one or more of the evocational processes in the majority of plants as well as affecting the further development of the flower buds. In Asteraceae species such as *D. grandiflora*, assimilate supply plays a major role in floral evocation. Charles-Edwards *et al* (1979) developed a mathematical model for transition between the vegetative and reproductive development of chrysanthemum in which flowering is almost entirely governed by photosynthesis, which promotes apical dome growth, and an inhibitor, which controls primordial initiation and, indirectly, flowering. The pattern of apical development in pyrethrum is almost identical to that of *D. grandiflora*, with a similar relationship between apical size and stage of floral development, suggesting a similar mediating role for assimilates in the flowering of pyrethrum.

A change in the pattern of assimilate distribution during floral evocation and flower development in pyrethrum was revealed through autoradiography. The sink strength of axillary shoots was observed to increase prior to the end of evocation, and became the dominant sink for radiolabelled assimilates as floral development progressed. The increase in sink activity of the axillary buds occurred before the end of initiation, supporting the hypothesis that the partitioning of assimilates to the axillary meristems is an essential part of the evocational sequence.

The change in assimilate distribution pattern during flowering is complicated by the correlative influence of apical dominance. The release of axillary shoots from apical dominance is accompanied by an increase in the sink strength of the axillary shoots. The observed increase in the ability of the axillary shoots to compete for assimilates may be involved in the evocational process or may simply be associated with the shoot's release from apical dominance. However, it was noted in the juvenility experiments that axillary shoots were not competent to respond to inductive stimuli for a short period after release from apical dominance, while ^{14}C-labelled assimilate was

not observed to accumulate in the axillary buds of fed shoots until just prior to floral determination. Thus the axillary buds were released from apical dominance and displayed a period of growth before becoming significant sinks for assimilates. This indicates that a supply of assimilates over and above that required for the release of axillary shoots from apical dominance is necessary for floral evocation.

One of the effects of vernalisation is to alter the assimilate distribution pattern to favour the axillary meristems. Devernalising conditions have the opposite effect, reducing the sink strength of axillary buds or preventing the translocation of assimilates to them. The reduced growth rate and abortion of flower buds under these conditions can be explained by this reduced assimilate supply. Assimilate supply may also mediate the daylength response as shorter days result in a reduced accumulation of assimilate by the plant. Thus a simple model of assimilate partitioning to the axillary apices during and after floral evocation can explain many of the observed flowering responses of pyrethrum.

Unfortunately the process of floral evocation is more complex than this simple model. While it is evident that an adequate assimilate supply to the axillary meristems is an essential component of evocation, it is not the sole factor necessary for the flowering response. Terminal meristems are strong sinks for assimilates during vegetative growth and yet they are never evoked to flower. Also the devernalisation effect is more pronounced under 25 °C day temperature conditions than under lower day temperature conditions, yet the rate of net CO_2 assimilation peaks at 25 °C. Therefore the inhibition of flower initiation observed at 25 °C cannot be attributed solely to a reduction in photosynthesis. Obviously other endogenous factors must be mediating one or more of the reactions in the evocational sequence.

Vernalising conditions stimulate an increase in free gibberellins in pyrethrum. This increase becomes more pronounced as the duration of vernalisation increases and may mediate some of the physiological processes which result in the plant's quantitative response to vernalisation. The gibberellin concentration rises before the end of evocation, indicating that some of the processes mediated by gibberellins may be involved in floral evocation. This assertion is supported by the growth retardant data as application of 'Cultar' during floral evocation decreased the gibberellin concentration and resulted in a corresponding decrease in the flower number at harvest.

While gibberellins have been implicated as playing a role in the floral evocation of many if not all plants, the extent to which they can influence flowering varies with

species. Exogenous gibberellins can elicit a flowering response in many LDP and cold-requiring rosette plants grown in non-inductive conditions (Pharis and King, 1985). Recent studies, however, have shown that gibberellin applications may be inhibitory in certain species and in other species may promote or inhibit flower initiation depending on the prevalent environmental conditions (Bernier *et al*, 1981b). The reaction of SDP is equally complex, ranging from promotive to markedly inhibitory or apparently unreactive. The apparent complexity of the flowering response to exogenous gibberellins may be at least partially due to the complex nature of endogenous gibberellin metabolism. It is thought that rapid activation and inactivation of biologically active gibberellins through reversible conjugation (Rood and Pharis, 1986) may be the regulatory mechanism which influences the levels of the specific gibberellins involved in floral evocation.

Apart from the possible mediating role for gibberellins in one or more of the evocational processes in pyrethrum, the rise in gibberellin levels during flowering appears to influence flower stem elongation. Gibberellins are thought to play a major role in regulating internode elongation, with GA_1 demonstrated as being the active gibberellin in several species (Reid, 1990) while GA_4 is the active endogenous gibberellin in at least one species (Nakayama *et al*, 1991). The antibodies used during the radioimmunoassay of gibberellin concentrations in this study detected gibberellins A_1, A_3 and A_{20}, so changes in the concentrations of individual gibberellins were not monitored. However the overall gibberellin concentration increased during vernalisation resulting in an increase in internode elongation, indicating that the overall concentration reflected the changes in concentration of the active gibberellin mediating internode elongation. Gibberellin levels may be suppressed by the application of the growth retardant 'Cultar', which results in a decrease in flower stem height. The effect of 'Cultar' may be reversed by exogenous GA_1 application. Marginally inductive conditions, where the duration of vernalisation is less than that required for the rapid initiation and development of flowers, could thus be expected to stimulate a less than optimal increase in gibberellin levels, resulting in the large percentage of shortened flower stems observed under these conditions.

Devernalising conditions result in a reduction in gibberellin concentration to levels below that of unvernalised plants. As stated above, assimilate partitioning away from the axillary meristems forms only part of the inhibitory effect of devernalising conditions. It is possible that devernalising conditions may be influencing other

components of the evocational sequence by reducing the gibberellin concentration in the plant.

Auxin is implicated in the flowering of pyrethrum by its role in the correlative influence of apical dominance in suppressing lateral shoot growth. Floral evocation is associated with the loss of apical dominance due at least partially to a decrease in auxin concentration. IAA levels decline in pyrethrum under vernalising conditions, and this may be involved in the outgrowth of lateral shoots whose meristems are competent to respond to the other evocational processes stimulated by vernalisation. The reported increase in flower yields after application of the auxin transport inhibitor TIBA (2,3,5-Triiodobenzoic acid) (Mohandass, 1986) may be due to the action of TIBA in preventing the basipetal transport of auxin from the shoot tip, thus reducing apical dominance and enhancing lateral bud outgrowth.

The role of auxin during flower initiation may not be limited to the control of apical dominance as a decline in auxin concentration is commonly observed during flower initiation in a number of different species where the release of lateral shoots from apical dominance is not necessary for flower initiation (Bernier *et al*, 1981b). This is the case in the cold-requiring chrysanthemum cultivar 'Sunbeam', where auxin levels increase after vernalisation but decline rapidly thereafter (Tompsett and Schwabe, 1974). Auxins have generally been reported as inhibitory to flowering in chrysanthemum, and as release from apical dominance is not required for floral evocation in this species any effect of auxin must be on the evocational processes. Plants treated with ethylene, a potent inhibitor of flowering, also contain higher levels of auxin than untreated plants (Tjia, Rogers and Hartley, 1969) suggesting that the two hormones may be operating in the same inhibitory process.

Low photon flux densities, which restrict floral evocation and development in pyrethrum, appear to prevent the decline in auxin concentration usually associated with vernalising conditions. Apical dominance is enhanced by low photon flux conditions (Martin, 1987), and in pyrethrum this effect appears to be mediated through enhanced auxin levels and reduced photosynthate partitioning to the axillary apices. These processes may be linked as IAA-induced assimilate transport has been demonstrated in other species (Bowen and Wareing, 1971).

No evidence was found during this study of a role for the cytokinins or abscisic acid in the flowering of pyrethrum. However a possible role for these hormones cannot be ruled out as all assays were performed on mature leaf samples and not on other

isolated organs. Localised fluxes of hormones, particularly in the region of the lateral buds during release from apical dominance, have not been examined and may reveal the physiological processes involved during flower initiation. Cytokinins have been implicated in the release of lateral buds from apical dominance, stimulating bud outgrowth in several species (Seidlova and Krekule, 1977. Ali and Fletcher, 1970), as well as generally being considered as promoters of floral initiation. Cytokinin applications have been shown to produce several other responses which are typical components of evocation, including an increased rate of leaf production (Miginiac, 1978) and increased mitotic activity in meristems (Bernier *et al*, 1981b). Abscisic acid has also been demonstrated to influence flowering, with both inhibitory and promotive effects observed (Bernier *et al*, 1981b). Further studies with pyrethrum may reveal a role for these hormones in the flowering process, with localised changes in concentrations, changes in tissue sensitivity or the presence of specific biologically active hormones which were not assayed explaining the lack of positive results in the assays already undertaken.

Manipulation of the flowering response by field management practices can be achieved through an understanding of the processes of floral evocation and flower development. The application of growth retardants to reduce stem elongation is an effective treatment to reduce lodging as the action of the growth retardant is in reducing gibberellin concentration. Gibberellins mediate the stem elongation process and therefore the concentration of gibberellins may be manipulated to induce the desired length of stem elongation. The optimum time of application was found to be just prior to the period of maximum stem elongation. This resulted in no reduction in the number of flowers at harvest and yet significant decreases in flower stem height and the degree of lodging. Application of growth retardants to reduce stem elongation by decreasing gibberellin concentrations should not be made during floral evocation as the gibberellins may play a role in this process.

Trimming may also be used to control flowering. Multiple trimming treatments appear to the best strategy for promoting vegetative growth during the normal flowering period. When flower stems are first trimmed, axillary meristems on the remaining stem sections retain the potential to initiate floral development as the vernalisation stimulus is passed to meristems formed on the initially vernalised shoot. Multiple trimming can remove these stems as they develop and, provided vernalising conditions no longer

prevail, vegetative growth is promoted. Also, removing mature leaves reduces the plants photosynthetic capacity and may therefore reduce the assimilate levels available for floral development, promoting abortion of any flower buds present. These finding are in agreement with Glover's (1955) observation that defoliation during the growing season reduces flower yield.

Adoption of the field management practices outlined in this study has helped in the establishment of pyrethrum cultivation as a viable agricultural enterprise in Tasmania. The knowledge gained on the flowering behaviour of the plant may in the future provide further methods of manipulating the flowering response in order to aid in the successful commercial exploitation of the crop. This study has also defined the physiological changes involved in the flowering process, providing a framework upon which future genetic, molecular and cellular studies can be based. New advances in quantitative preparatory and analytical techniques offer the opportunity to explore the early events in the flowering process and are already providing breakthroughs in our understanding of flowering. Knowledge of the complex series of interacting mechanisms controlling the flowering of *T. cinerariaefolium* is essential if these breakthroughs are to be translated into a fuller understanding of flowering in this species. The environmental conditions, including vernalisation, photon flux density, day temperature and the daily light integral, have all been shown to influence evocational processes. Several possible endogenous controlling factors have been identified in this study and this data has been combined with relevant data from similar species to provide an acceptable interpretation of the interacting environmental and physiological factors. The current level of activity in the field of floral evocation promises to deliver significant advances in our understanding of flowering and may produce data on the genetic and molecular as well as physiological aspects of flowering which will provide further insight into the flowering of pyrethrum. It is hoped that this study may go some way towards contributing to our knowledge of morphogenesis and thus to our eventual understanding of the complex series of events and interactions which govern the flowering process.

VI Bibliography

Adamec,L., Machackova,I., Krekule,J. and Novakova,M. (1989). Electric current inhibits flowering in the short-day plant *Chenopodium rubrum* L. J.Plant Physiol. 134 : 43-46.

Ali,A.A. and Fletcher,R.A. (1971). Hormonal interaction in controlling apical dominance in soybeans. Can.J.Bot. 49 : 1727-1731.

Alvord,E.D. (1976). The effects of irrigation and nitrogen applications on yield and quality of pyrethrum in the highveld. Rhodesia Agric.J. 73 (5) : 127-132.

Amrhein,N., Forreiter,C., Kionka,C., Skorupka,H. and Tophof,S. (1986). Metabolism, and its compartmentation, of 1-aminocyclopropane-1-carboxylic acid in plant cells. in Conjugated Plant Hormones. Structure, Metabolism and Function. Schreiber,K., Schutte,H.R. and Sembdner,G. (Eds.). VEB Deutscher Verlag der Wissenschaften, Berlin. pp 102-110.

Anderson,N.O. (1987). Reclassifications of the Genus *Chrysanthemum* L. HortScience 22 (2) : 313.

Anon. (1978). Pyrethrum: from a new ITC study. Pyrethrum Post 14 (4) : 103-105.

Araki,T. and Komeda,Y. (1990). Electrophoretic analysis of florally-evoked meristems of *Pharbitis nil* Choisy cv. Violet. Plant Cell Physiol. 31 (1) : 137-144.

Baier,M. and Hartung,W. (1991). Movement of abscisic acid across the plasma membrane of phloem elements of *Plantago major*. J.Plant Physiol. 137 : 297-300.

Barendse,G.W.M., Croes,A.F., Van Ende,G., Bosveld,M. and Creemers,T. (1985). Role of hormones of flower bud formation in thin-layer explants of tobacco. Biol.Plant. 27 : 408-413.

Beckley,V.A. (1950). The spectrophotometric estimation of the pyrethrins. Pyrethrum Post 2 (1) : 23-24.

Bhat,B.K. and Menary,R.C. (1979). Scanning electron microscopic study of oil glands in pyrethrum flowers. Pyrethrum Post 15 (1) : 11-15.

Bhat,B.K. and Menary,R.C. (1984a). Pyrethrum production in Australia: Its past and present potential. J.Aust.Inst.of Agric.Sci. 50 : 189-192.

Bhat,B.K. and Menary,R.C. (1984b). Registration of Hypy pyrethrum. Crop Sci. 24 : 619-620.

Bhat,B.K.,Menary,R.C. and Pandita,P.N. (1985). Population improvement in pyrethrum (*Chrysanthemum cinerariaefolium* Vis.). Euphytica 34 : 613-617.

Bernier,G. (1986). A quantitative study of the morphological changes in the apical bud of *Sinapis alba* in transition to flowering. Arch.Int.Physiol.Biochem. 94 : 36.

Bernier,G. (1988). The control of floral evocation and morphogenesis. Ann.Rev.Plant Physiol.Plant Mol.Biol. 39 : 175-219.

Bernier,G., Kinet,J-M. and Sachs,R.M. (1981a). The Physiology of Flowering, Vol. I. CRC Press, Boca Raton.

Bernier,G., Kinet,J-M. and Sachs,R.M. (1981b). The Physiology of Flowering, Vol. II. CRC Press, Boca Raton.

Bismuth,F. and Miginiac,E. (1984). Influence of zeatin on flowering in root forming cuttings of *Anagallis arvensis* L. Plant Cell Physiol. 25 : 1073-1076.

Bodson,M. (1977). Changes in the carbohydrate content of the leaf and the apical bud of *Sinapis* during transition to flowering. Planta 135 : 19-23.

Bodson,M. (1985). *Sinapis alba* in CRC Handbook of Flowering, Vol. IV. Halevy,A.H. (Ed.). CRC Press, Boca Raton. pp 336-354.

Bodson,M., Bernier,G., Kinet,J-M., Jacqmard,A. and Havelange,A. (1978). Flowering of *Sinapis* as influenced by different treatments acting on photosynthetic activity. in Photosynthesis and Plant Development. Marcelle,R., Clijsters,H. and Van Poucke,M. (Eds.). Dr W. Junk, The Hague. pp 37-82.

Bodson,M., King,R.W., Evans,L.T. and Bernier,G. (1977). The role of photosynthesis in flowering of the long day plant *Sinapis alba*. Aust.J.Plant Physiol. 4 : 467-468.

Borkovec,V. and Prochazka,S. (1989). The effect of cycloheximide on IAA-stimulated transport of ^{14}C-ABA and ^{14}C-sucrose in long pea epicotyl segments. Plant Growth Regul. 8 : 1-9.

Borthwick,H.A. and Cathey,H.M. (1962). Role of phytochrome in control of flowering in chrysanthemum. Bot.Gaz. 123 : 155-162.

Bourne,R.K. and Rosenthal,M.W. (1976). Summary of attempts to obtain radio-actively labelled pyrethrum extract. Pyrethrum Post 13 (4) : 127-130.

Bowen,M.R. and Wareing,P.F. (1971). Further investigations into hormone-directed transport in stems. Planta 65 : 139-156.

Bowman,J.L., Smyth,D.R. and Meyerowitz,E.M. (1989). Genes directing flower development in *Arabidopsis*. The Plant Cell 1 : 37-52.

Brewer,J.G. (1974). Incompatibility relationship in pyrethrum (*Chrysanthemum cinerariaefolium* Vis.). Euphytica 23 : 45-47.

Brewer,J.G. and Henstra,S. (1974). Pollen of pyrethrum (*Chrysanthemum cinerariaefolium* Vis.). Fine structure and recognition reaction. Euphytica 23 : 657-663.

Brown,P.H. (1988). The role of mature leaves in the flowering of pyrethrum (*Chrysanthemum cinerariaefolium* Vis.). Honours Thesis, University of Tasmania.

Bruinsma,J. (1982). Plant growth regulators in field crops. in Chemical Manipulation of Crop Growth and Development. McLaren,J.S. (Ed.). Butterworths, London.

Buban,T. and Faust,M. (1982). Flower bud initiation in apple trees : internal control and differentiation. Hort.Rev. 4 : 174-203.

Buta,J.G. and Spaulding,D.W. (1991). Effect of Paclobutrazol on abscisic acid levels in wheat seedlings. J.Plant Growth Regul. 10 : 59-61.

Calvert,A. (1969). Studies on the post-initiation development of flower buds of tomato (*Lycopersicon esculentum*). J.Hortic.Sci. 44 : 117-126.

Cathey,H.M. (1969). *Chrysanthemum morifolium* (Ramat.) Hemsl. in The Induction of Flowering. Evans,L.T. (Ed.). Macmillan, Melbourne. pp 268-290.

Cathey,H.M. and Borthwick,H.A. (1957). Photoreversibility of floral initiation in chrysanthemum. Bot.Gaz. 119 : 71-76.

Cathey,H.M. and Stuart,N.W. (1958). Growth and flowering of *Chrysanthemum morifolium* Ramat. as affected by time of application of gibberellic acid. Proc.Am.Soc.Hortic.Sci. 71 : 547-554.

Chacko,E.K., Kohli,R.R. and Randhawa,G.S. (1974). Investigations on the use of (2-chloroethyl) Phosphonic acid (Ethephon, CEPA) for the control of biennial bearing in mango. Sci.Hortic. 2 : 389-398.

Chan,A.P. (1950). The development of crown and terminal flower buds of *Chrysanthemum morifolium*. Proc.Am.Soc.Hortic.Sci. 55 : 461-466.

Charles-Edwards,D.A., Cockshull,K.E., Horridge,J.S. and Thornley,J.H.M. (1979). A model of flowering in *Chrysanthemum*. Ann.Bot. 44 : 557-566.

Chen,C-M., Ertl,J.R., Leisner,S.M. and Chang,C-C. (1985). Localisation of cytokinin biosynthetic sites in pea plants and carrot roots. Plant Physiol. 78 : 510-513.

Cleland,C.F. and Ajami,A. (1974). Identification of the flower-inducing factor isolated from aphid honeydew as being salicylic acid. Plant Physiol. 54 : 904-906.

Cleland,C.F. and Tanaka,O. (1979). Effect of daylength on the ability of salicylic acid to induce flowering in the long-day plant *Lemna gibba* G3 and the short-day plant *Lemna paucicostata* 6746. Plant Physiol. 64 : 421-424.

Cleland,C.F. and Zeevaart,J.A.D. (1970). Gibberellins in relation to flowering and stem elongation in the long day plant *Silene armeria.* Plant Physiol. 46 : 392-400.

Cockshull,K.E. (1972). Photoperiodic control of flowering in chrysanthemum. in Crop Processes in Controlled Environments. Rees,A.R., Cockshull,K.E., Hand,D.W. and Hurd, R.G. (Eds.). Academic Press, London. pp 235-250.

Cockshull,K.E. (1976). Flower and leaf initiation by *Chrysanthemum morifolium* Ramat. in long days. J.Hortic.Sci. 51 : 441-450.

Cockshull,K.E. (1979). Effects of irradiance and temperature on flowering of *Chrysanthemum morifolium* Ramat. in continuous light. Ann.Bot. 44 : 451-460.

Cockshull,K.E. (1985). *Chrysanthemum morifolium.* in CRC Handbook of Flowering, Halevy,A.H. (Ed.). CRC Press Inc., Boca Raton, Florida. pp 238-257.

Cockshull,K.E. and Horridge,J.S. (1977). Apical dominance and flower initiation in the rose. J.Hortic.Sci. 52 : 421-427.

Cockshull,K.E. and Horridge,J.S. (1978). 2-chloroethylphosphonic acid and flower initiation by *Chrysanthemum morifolium* Ramat. in short days and in long days. J.Hortic.Sci. 53 : 85-90.

Cockshull,K.E. and Horridge,J.S. (1980). Further evidence of a relationship between size of the *Chrysanthemum* shoot apex and inflorescence development. Ann.Bot. 46 : 125-127.

Cockshull,K.E., Horridge,J.S. and Langton,F.A. (1979). Ethephon and the delay of early budding in chrysanthemums. J.Hortic.Sci. 54 : 337-338.

Cockshull,K.E. and Hughes,A.P. (1967). Distribution of dry matter to flowers in *Chrysanthemum morifolium.* Nature 215 : 780-781.

Cockshull,K.E. and Hughes,A.P. (1972). Flower formation in *Chrysanthemum morifolium*: the influence of light level. J.Hortic.Sci. 47 : 113-127.

Contant,R.B. (1976). Pyrethrum, in Evolution of Crop Plants. Simmonds,N.W. (Ed.). Longman, London pp. 33-36.

Cooke,A.R. (1954). Changes in free auxin content during the photoinduction of short-day plants Plant Physiol. 29 : 440-444.

Cottrell,J. Dale,J.E. and Jeffcoat,B. (1981). Development of the apical dome of barley in response to treatment with gibberellic acid. Plant Sci.Lett. 22 : 161-168.

Cutting,J.C., Lishman,A.W., Hofman,P.J. and Wolstenholme,B.N. (1986). Plant growth substance trends in developing avocado fruit as determined by radioimmunoassay. Acta Hortic. 175 : 285-289.

Cutting,J.C., Lishman,A.W., Vander Hoven,A. and Wolstenholme,B.N. (1983). Development of a radioimmunoassay for the cytokinin isopentyladenosine. Crop Production 12 : 133-135.

Deitzer,G.F. (1987). Photoperiodic processes : induction, translocation and initiation. in Manipulation of Flowering, Atherton,J.G. (Ed.). Butterworths, London. pp 241-253.

De Jong,J. (1978). Selection for wide temperature adaptation in *Chrysanthemum morifolium* (Ramat.) Hemsl. Neth.J.Agric.Sci. 26 : 110-118.

De Jong,J. (1981a). The difference between chrysanthemum plants raised from seeds or cuttings in their response to light intensity. Acta Hortic. 125 : 31-36.

De Jong,J. (1981b). Effects of irradiance and juvenility on the selection of chrysanthemums. Euphytica 30 : 493-500.

De Jong,J. (1989a). Analysis of components controlling early flowering of chrysanthemum at low temperature. Euphytica 40 : 121-126.

De Jong,J. (1989b). The flowering of *Chrysanthemum morifolium* seedlings and cuttings in relation to seasonal fluctuation in light. Sci.Hortic. 41 (1-2) : 117-124.

Dorffling,K., Kettner,J., Naumann,R. and Tietz,D. (1986). Metabolites and conjugates of abscisic acid. in Conjugated Plant Hormones. Structure, Metabolism and Function. Schreiber,K., Schutte,H.R. and Sembdner,G. (Eds.). VEB Deutscher Verlag der Wissenschaften, Berlin. pp 249-261.

El-Antably,H.M.M. and Wareing,P.F. (1966). Stimulation of flowering in certain short-day plants by abscisin. Nature 210 : 328-329.

Evans,L.T. (1969). The Induction of Flowering. Macmillan, Melbourne.

Evans,L.T. (1975). Daylength and Flowering of Plants. Bengamin. Melno Park, California.

Firn,R.D. (1986). Growth substance sensitivity : The need for clearer ideas, precise terms and purposeful experiments. Physiol.Plant. 67 : 267-272.

Friend,D.J.C., Deputy,J. and Quedado,R. (1978). Photosynthetic and photomorphogenetic effects of high photon flux densities on the flowering of two long day plants, *Anagallis arvensis* and *Brassica campestris*. in Photosynthesis and Plant Development. Marcelle,R., Clijsters,H. and Van Poucke,M. (Eds.). Dr W. Junk, The Hague. pp 59-72.

Furuta,T. and Kiplinger,D.C. (1955). Chronological age of cuttings, a factor influencing the spray formation of pompom chrysanthemums. Proc.Am.Soc.Hortic.Sci. 66 : 383-385.

Gilmour,S.J., Zeevaart,J.A.D., Schwenen,L. and Graebe,J.E. (1986). Gibberellin metabolism in cell-free extracts from spinach leaves in relation to photoperiod. Plant Physiol. 82 : 190-195.

Glover,J. (1955). Chilling and flower bud stimulation in Pyrethrum (*Chrysanthemum cinerariaefolium*). Ann.Bot. 19 : 138-148.

Graebe,J.E. (1987). Gibberellin biosynthesis and control. Ann.Rev.Plant Physiol. 38 : 419-465.

Graves,C.J., Adams,P. and Winsor,G.W. (1977). Some effects of indol-3-ylacetic acid on the rates of initiation and development of flower buds of *Chrysanthemum morifolium*. Ann.Bot. 41 : 747-753.

Grossmann,K., Hauser,C., Sauerbrey,E., Fritsch,H., Schmidt,O. and Jung,J. (1989). Plant growth retardants as inhibitors of ethylene production. J.Plant Physiol. 134 : 538-543.

Halevy,A.H. (1984). Light and autonomous induction. in Light and the Flowering Process.Vince-Prue,D., Thomas,B. and Cockshull,K.E. (Eds.). Academic Press, London. pp 65-73.

Halevy,A.H., Spiegelstein,H. and Goldschmidt,E.E. (1991). Auxin inhibition of flower induction of *Pharbitis* is not mediated by ethylene. Plant Physiol. 95 : 652-654.

Hall,D.O. and Rao,K.K. (1989). Photosynthesis. Edward Arnold, London.

Hand,D.W. (1979). Effects of propylene on 'Polaris' chrysanthemums. Flor.Rev. 163 (4237) : 18-19.

Harada,H. and Nitsch,J.P. (1959). Changes in endogenous growth substances during flower development. Plant Physiol. 34 : 409-415.

Harris,M.J. and Outlaw,W.H.Jr. (1990). Histochemical technique : A low-volume, enzyme-amplified immunoassay with sub-fmol sensitivity. Application to measurement of abscisic acid in stomatal guard cells. Physiol.Plant. 78 : 495-500.

Hayes,P.M. and Patrick,J.W. (1985). Photosynthate transport in stems of *Phaseolus vulgaris* L. treated with gibberellic acid, indole-3-acetic acid or kinetin. Planta 166 : 371-379.

Head,S.W. (1966). A study of the insecticidal constituents in *Chrysanthemum cinerariaefolium*. (2) Their distribution in the plant. Pyrethrum Post 8 (4) : 32-37.

Hertel,R. (1986). Two comments on auxin transport : The uptake/efflux-mechanism and the problem of adaptation. in Plant Growth Substances 1985, Bopp,M. (Ed.). Springer-Verlag, Berlin. pp 214-217.

Hofman,P.J. (1990). Abscisic acid and gibberellins in the fruitlets and leaves of 'Valencia' orange in relation to fruit growth and retention. Sci.Hortic. 42 : 257-267.

Hofman,P.J., Fentonby-Smith,B.C. and Van Staden,J. (1986). The development of ELISA and RIA for cytokinin estimation and their application to a study of lunar periodicity in *Ecklonia maxima* (Osbeck) Paperif. J.Plant Physiol. 122 : 455-466.

Hofman,P.J., Forsyth,C. and Van Staden,J. (1985). A radioimmunoassay for dihydrozeatin and dihydrozeatin riboside, and its application to a study of the *in vitro* metabolism of dihydrozeatin by soybean callus. J.Plant Physiol. 121 : 1-12.

Horgan,R. (1985). Cytokinin biosynthesis and metabolism.in Plant Growth Substances, Bopp,M. (Ed.). Springer-Verlag, Berlin. pp 92-98.

Horridge,J.S. and Cockshull,K.E. (1979). Size of the *Chrysanthemum* shoot apex in relation to inflorescence initiation and development. Ann.Bot. 44 : 547-556.

Horridge,J.S. and Cockshull,K.E. (1989). The effect of the timing of a night-break on flower initiation in *Chrysanthemum morifolium* Ramat. J.Hortic.Sci. 64 (2) : 183-188.

Hughes,A.P. and Cockshull,K.E. (1971a). The effects of light intensity and carbon dioxide concentration on the growth of *Chrysanthemum morifolium* cv. Bright Golden Anne. Ann.Bot. 35 : 899-914.

Hughes,A.P. and Cockshull,K.E. (1971b). A comparison of the effects of diurnal variation in light intensity with constant light intensity on growth of *Chrysanthemum morifolium* cv. Bright Golden Anne. Ann.Bot. 35 : 927-932.

Hussain,T. and Ram,P. (1976). Effect of NPK fertilization on tillering, flower bud formation and fresh flower yield of pyrethrum crop. Pyrethrum Post 13 (3) : 89-91.

Ikahu,J.M. and Ngugi,C.W. (1989). Investigations into yield losses in some pyrethrum clones through picking of flowers at improper stage of development. Pyrethrum Post 17 (2) : 56-59.

Jackson,D.I. and Sweet,G.B. (1972). Flower initiation in temperate woody plants. Hort.Abstracts 42 : 9-24.

Jeffcoat,B. and Cockshull,K.E. (1972). Changes in the levels of endogenous growth regulators during development of the flowers of *Chrysanthemum morifolium*. J.Exp.Bot. 23 : 722-732.

Kadman-Zahavi,A. and Ephrat,E. (1973). Effect of red and far-red illuminations at the end of short days and their interaction with night-break illuminations, on flowering of *Chrysanthemum morifolium* plants. Plant and Cell Physiol. 14 : 409-411.

Kadman-Zahavi,A. and Yahel,H. (1971). Phytochrome effects in night-break illuminations on flowering of *Chrysanthemum*. Physiol.Plant. 25 : 90-93.

Kandasamy,D., Oblisami,G., Mohandass,S. and Santhanakrishan,R. (1986). Influence of VA-mycorrhizal innoculation on the growth of pyrethrum in nursery. Pyrethrum Post 16 (3) : 81-83.

Kandeler,R. (1985). Lemnaceae. in CRC Handbook of Flowering, Vol III. Halevy,A.H. (Ed.). CRC Press, Boca Raton. pp 251-279.

Karlsson,M.G., Heins,R.D., Erwin,J.E., Berghage,R.D., Carlson,W.H. and Biernbaum,J.A. (1989a). Temperature and photosynthetic photon flux influence chrysanthemum shoot development and flower initiation under short-day conditions. J.Am.Soc.Hortic.Sci. 114 (1) : 158-163.

Karlsson,M.G., Heins,R.D., Erwin,J.E., Berghage,R.D., Carlson,W.H. and Biernbaum,J.A. (1989b). Irradiance and temperature effects on time of development and flower size in chrysanthemum. Sci.Hortic. 39 : 257-267.

Karlsson,M.G., Heins,R.D., Erwin,J.E. and Berghage,R.D. (1989c). Development rate during four phases of chrysanthemum growth as determined by preceding and prevailing temperatures. J.Am.Soc.Hortic.Sci. 114 (2) : 234-240.

Kelly,A.J., Zagotta,M.T., White,R.A., Chang,C. and Meeks-Wagner,D.R. (1990). Identification of genes expressed in the tobacco shoot apex during the floral transition. The Plant Cell 2 : 963-972.

Khurana,J.P., Tamot,B.K. and Maheshwari,S.C. (1988). Floral induction in a photoperiodically insensitive duckweed, *Lemna paucicostata* LP6. Role of glutamate, aspartate and other amino acids and amides. Plant Physiol. 86 : 904-907.

Kinet,J-M., Bodson,M., Jacqmard,A. and Bernier,G. (1975). The inhibition of flowering by abscisic acid in *Sinapis alba* L. Z.Pflanzphysiol. 77 : 70-74.

Kinet,J-M., Sachs,R.M. and Bernier,G. (1985). The Physiology of Flowering, Vol. III. CRC Press, Boca Raton.

King,R.W. (1991). Early events in the flowering process : key issues from a recent workshop. Aust.J.Plant Physiol. 18 : 435-437.

King,R.W. and Evans,L.T. (1991). Shoot apex sugars in relation to long-day induction of flowering in *Lolium temulentum* L. Aust.J.Plant Physiol. 18 : 121-135.

King,R.W., Evans,L.T. and Firn,R.D. (1977). Abscisic acid and xanthoxin contents in the long-day plant *Lolium temulentum* L. in relation to daylength. Aust.J.Plant Physiol. 4 : 217-223.

Kirby,E.J.M. (1974). Ear development in spring wheat. J.Agric.Sci.,Camb. 82 : 437-447.

Kofranek,A.M. (1980). Cut chrysanthemums. in Introduction to Floriculture. Larson,R.A. (Ed.). Academic Press, New York. pp 1-45.

Kofranek,A.M. and Halevy,A.H. (1974). Minimum number of short days for production of high quality standard chrysanthemums. HortScience 9 : 543-544.

Kozaki,A., Takeba,G. and Tanaka,O. (1991). A polypeptide that induces flowering in *Lemna paucicostata* at a very low concentration. Plant Physiol. 95 : 1288-1290.

Krekule,J. (1979). Stimulation and inhibition of flowering. in La Physiologie de la Floraison. Champagnat,P., Jacques,R. (Eds.): CNRS, Paris. pp 19-57.

Krekule,J. and Seidlova,F. (1977). Effects of exogenous cytokinins on flowering of the short-day plant *Chenopodium rubrum* L. Biol.Plant. 19 : 142-149.

Kroll,U. (1962). Pyrethrum, Kenya's insecticidal cash crop. World Crops 14 : 232-235.

Kroll,U. (1963). The effect of fertilizers, manures, irrigation and ridging on the yield of pyrethrum. East Afr.Agric.and For.J. 28 : 139-145.

Kroll,U. (1964). Effect of mean temperature on the content of pyrethrins in flowers of *Chrysanthemum* (Pyrethrum) *cinerariaefolium*. Nature 202 : 1351-1352.

Larrieu,C. and Bismuth,F. (1985). *Scrophularia alata* and *S. venalis*. in CRC Handbook of Flowering, Vol. IV, Halevy,A.H. (Ed.).. CRC Press, Boca Raton. pp 283-290.

Lehmann,H. (1986). Synthesis and metabolism of abscisic acid conjugates. in Conjugated Plant Hormones. Structure, Metabolism and Function. Schreiber,K., Schutte,H.R. and Sembdner,G. (Eds.). VEB Deutscher Verlag der Wissenschaften, Berlin. pp 262-266.

Lejeune,P., Kinet,J-M. and Bernier,G. (1988). Cytokinin fluxes during floral induction in the LDP *Sinapis alba* L. Plant Physiol. 86 : 1095-1098.

Lenton,J.R. (1984). Are plant growth substances involved in the partitioning of assimilates to developing reproductive sites? Plant Growth Reg. 2 : 267-276.

Letham,D.S. and Palin,L.M.S. (1983). The biosynthesis and metabolism of cytokinins. Ann.Rev.Plant Physiol. 34 : 163-197.

Lilov,D. and Zozikova,E. (1986). Changes in the bound auxins in relation to the plant generative processes. in Conjugated Plant Hormones. Structure, Metabolism and Function. Schreiber,K., Schutte,H.R. and Sembdner,G. (Eds.). VEB Deutscher Verlag der Wissenschaften, Berlin. pp 61-68.

Lim,R. and Tamas,I.A. (1989). The transport of radiolabeled indoleacetic acid and its conjugates in nodal stem segments of *Phaseolus vulgaris* L. Plant Growth Regul. 8 (2) : 151-164.

Linstrom,R.S. and Asen,S. (1967). Chemical control of the flowering of *Chrysanthemum morifolium* Ram. I. Auxin and flowering. Pro.Am.Soc.Hortic.Sci. 90 : 403-408.

Lozhnikova,V., Machachova,I., Eder,J., Dudko,N., Krekule,J. and Chailakhyan,M.Kh. (1989). Gradient of free indole-3-acetic acid level in the day-neutral tobacco var. Trapezond with normal and reversed gradient of flowering. Plant Physiol.Biochem. 27 (1) : 127-129.

Lumsden,P.J. and Vince-Prue,D. (1984). The perception of dusk signals in photoperiodic time-measurement. Physiol.Plant. 60 : 427-432.

Lyndon,R.F. and Battey,N.H. (1985). The growth of the shoot apical meristem during flower initiation. Biol.Plant. 27 : 339-349.

Machackova,I., Pospiskova,M. and Krekule,J. (1990). Further studies on the inhibitory action of direct electric current on flowering in the short-day plant *Chenopodium rubrum* L. J.Plant Physiol. 136 : 381-384.

Machackova,I., Ullmann,J., Krekule,J. and Stock,M. (1986). Metabolism of 1-aminocyclopropane-1-carboxylic acid (ACC) in photoperiodic induction of flowering in *Chenopodium rubrum* L. in Conjugated Plant Hormones. Structure, Metabolism and Function. Schreiber,K., Schutte,H.R. and Sembdner,G. (Eds.). VEB Deutscher Verlag der Wissenschaften, Berlin. pp 111-117.

Maeng,J. and Khudairi,A.K. (1973). Studies on the flowering mechanism in *Lemna*. I. Amino acid changes during flower induction. Physiol.Plant. 28 : 264-270.

Marc,J. and Gifford,R.M. (1984). Floral initiation in wheat, sunflower and sorghum under carbon dioxide enrichment. Can.J.Bot. 62 : 9-14.

Marme,D. (1977). Phytochrome : membranes as possible sites of primary action. Ann.Rev.Plant Physiol. 28 : 173-198.

Martin,G.C. (1987). Apical dominance. J.Hortic.Sci. 22 : 824-833.

Matthysse,A.G. and Scott,T.K. (1984). Functions of hormones at the whole plant level of organisation. in Encyclopedia of Plant Physiology, New Series Vol. 10, Scott,T.K. (Ed.). Springer-Verlag, Berlin. pp. 218-243.

McDaniel,C.N. (1980). Influence of leaves and roots on meristem development in *Nicotiana tabaccum* L.cv. Wisconsin 38. Planta 148 : 462-467.

McDaniel,C.N. and Hsu,F.C. (1976). Position-dependent development of tobacco meristems. Nature (London) 259 : 564-565.

McDaniel,C.N., Sangrey,K.A. and Singer,S.R. (1989). Node counting in axillary buds of *Nicotiana tabacum* cv. Wisconsin 38, a day-neutral plant. Am.J.Bot. 76 (3) : 403-408.

Melzer,S., Majewski,D.M. and Apel,K. (1990). Early changes in gene expression during the transition from vegetative to generative growth in the long-day plant *Sinapis alba.* The Plant Cell 2 : 953-961.

Menhenett,R. (1979). Effects of growth retardants, gibberellic acid and indol-3-ylacetic acid on stem extension and flower development in the pot chrysanthemum (*Chrysanthemum morifolium* Ramat.). Ann.Bot. 43 : 305-318.

Menhenett,R. (1981). Interactions of the growth retardants daminozide and piproctanyl bromide, and gibberellins A_1, A_3, A_{4+7}, A_5 and A_{13} in stem extension and inflorescence development in *Chrysanthemum morifolium* Ramat. Ann.Bot. 47 : 359-369.

Menhenett,R. (1982). Interactions of daminozide, 2,2'-dipyridyl and gibberellins A_3, A_9 and A_{20}, in stem extension in *Chrysanthemum morifolium* Ramat. : an indication that daminozide may not inhibit the responses to GA_9 or GA_{20} by blocking gibberellin hydroxylation. Plant Growth Reg. 1 (1) : 31-36.

Metzger,J.D. and Zeevaart,J.A.D. (1982). Photoperiodic control of gibberellin metabolism in spinach. Plant Physiol. 69 : 287-291.

Miginiac,E. (1978). Some aspects of regulation of flowering : role of correlative factors in photoperiodic plants. Bot.Mag., Spec.Iss. 1 : 159-173.

Miginiac,E. and Sotta,B. (1985). Organ correlations affecting flowering in relation to phytohormones. Biol.Plant. 27 : 373-381.

Mnzava,N.A. and Mpina,G.A. (1988a). The effects of gibberellic acid and 6-benzylamino purine on growth, flowering and pyrethrin content of pyrethrum (*Chrysanthemum cinerariaefolium* Vis.). Pyrethrum Post 16 (4) : 132-138.

Mnzava,N.A. and Mpina,G.A. (1988b). Effects of silver nitrate on flower growth, senescence and pyrethrin content of pyrethrum (*Chrysanthemum cinerariaefolium* Vis.). Pyrethrum Post 16 (4) : 128-131.

Mohandass,S. (1986). Effect of some growth regulants on growth and flowering behaviour in pyrethrum (*Chrysanthemum cinerariaefolium* Vis.). Madras Agric.J. 73 (5) : 281-283.

Mohandass,S., Sampath,V. and Gupta,R. (1986). Response of ambient temperatures on production of flowers in pyrethrum at Kodaikanal (India). Acta Hortic. 188 : 163-168.

Moore,T.C. (1989). Biochemistry and Physiology of Plant Hormones, Second Edition. Springer-Verlag, New York.

Moore,T.C. (1990). Apparent endogenous circadian rhythm in *ent*-kaurene biosynthesis. J.Plant Growth Regul. 9 : 185-187.

Moore,T.C. and Moore,J.A. (1991). Induction of *ent*-kaurene biosynthesis by low temperature in dwarf peas. J.Plant Growth Regul. 10 : 91-95.

Mortensen,L.M. and Stromme,E. (1987). Effects of light quality on some greenhouse crops. Sci.Hortic. 33 : 27-36.

Mullins,M.G. (1970). Hormone-directed transport of assimilates in decapitated internodes of *Phaseolus vulgaris* L. Ann.Bot. 34 : 897-909.

Murfet,I.C. (1977). Environmental interaction and the genetics of flowering. Ann.Rev.Plant Physiol. 28 : 253-278.

Murfet,I.C. (1985). *Pisum sativum*. in CRC Handbook of Flowering. Vol.IV. Halevy,A.H. (Ed.). CRC Press, Boca Raton. pp. 97-126.

Muturi,S.N., Parlevliet,J.E. and Brewer,J.G. (1969). Ecological requirements of pyrethrum. Part I: A general review. Pyrethrum Post 10 (1) : 24-28.

Nakayama,M., Yamane,H., Murofushi,N., Takahashi,N., Mander,L.N. and Seto,H. (1991). Gibberellin biosynthetic pathway and the physiologically active gibberellin in the shoot of *Cucumis sativus* L. J.Plant Growth Regul. 10 : 115-119.

Napier,R.M. and Venis,M.A. (1990). Receptors for plant growth regulators : Recent advances. J.Plant Growth Regul. 9 : 113-126.

Napp-Zinn,K. (1987). Vernalization - environmental and genetic regulation. in Manipulation of Flowering, Atherton,J.G. (Ed.). Butterworths, London. pp. 123-132.

Ngugi,C.W. and Ikahu,J.M. (1989). The response of pyrethrum (*Chrysanthemum cinerariaefolium* Vis.) to phosphorous and nitrogen fertilizers. Pyrethrum Post 17 (2) : 70-73.

Noble,R. and Ellis,S.R. (1990). Variation in chrysanthemums grown five to a pot: the influence of light. J.Hortic.Sci. 65 (2) : 177-183.

Nougarede,A. (1967). Experimental cytology of the shoot apical cells during vegetative growth and flowering. Int.Rev.Cytol. 21 : 203-351.

Ogawa,Y. and King,R.W. (1990). The inhibition of flowering by non-induced cotyledons of *Pharbitis nil.* Plant Cell Physiol.31 (1) : 129-135.

Ottaro,W.G.M. (1977). The relationship between ploidy level and certain morphological characteristics of *Chrysanthemum cinerariaefolium* Vis. Pyrethrum Post 14 (1) : 10-11.

Owens,V. and Paolillo,D.J.Jr. (1986). Effect of ageing on flowering of the axillary buds of *Pharbitis nil.* Am.J.Bot. 72 : 882-887.

Papafotiou,M. and Schwabe,W.W. (1990). Studies on the long-day inhibition of flowering in *Xanthium* and *Kalanchoe.* Physiol.Plant. 80 : 177-184.

Parlevliet,J.E. (1970). The effect of rainfall and altitude on the yield of pyrethrins from pyrethrum flowers in Kenya. Pyrethrum Post 10 (3) : 20-25.

Parups,E.V. (1980). Gibberellins in photoperiodically treated chrysanthemum cv. Improved Indianapolis White. Phyton. 39 : 121-126.

Patrick,J.W. (1976). Hormone-directed transport of metabolites. in Transport and Transfer Processes in Plants, Wardlaw,I.F.and Passioura,J. (Eds.). Academic Press, New York. pp 433-446.

Patrick,J.W. (1982). Hormonal control of assimilate transport. in Plant Growth Substances. Wareing,P.F. (Ed.). Academic Press, London. pp 669-678.

Patrick,J.W. and Mulligan,D.R. (1989). Gibberellic-acid-promoted transport of assimilates in stems of *Phaseolus vulgaris.* Effects of assimilate accumulation by the sink. Ann.Bot. 63 : 581-587.

Patrick,J.W. and Wareing,P.F. (1973). Auxin-promoted transport of metabolites in stems of *Phaseolus vulgaris* L. Some characteristics of the experimental transport systems. J.Exp.Bot. 24 : 1158-1171.

Paulet,P. (1985). *Cichorium intybus* and *C. endiva.* in CRC Handbook of Flowering, Vol IV. Halevy,A.H. (Ed.). CRC Press, Boca Raton. pp 265-271.

Pengelly,W.L. (1985). Validation of immunoassays. in Plant Growth Substances. Bopp,M. (Ed.). Springer-Verlag, Berlin. pp 35-43.

Pharis,R.P. (1972). Flowering of *Chrysanthemum* under non-inductive long days by gibberellins and N^6-benzyladenine. Planta 105 : 205-212.

Pharis,R.P. and King,R.W. (1985). Gibberellins and reproductive development in seed plants. Ann.Rev.Plant Physiol. 36 : 517-568.

Phillips,I.D.J. (1975). Apical dominance. Ann.Rev.Plant Physiol. 26 : 341-367.

Pierard,D., Jacqmard,A., Bernier,G. and Salmon,J. (1980). Appearance and disappearance of proteins in the shoot apical meristem of *Sinapis alba* in transition to flowering. Planta 150 : 397-405.

Popham,R.A. and Chan,A.P. (1952). Origin and development of the receptacle of *Chrysanthemum morifolium*. Am.J.Bot. 39 : 329-339.

Poulhe,R., Arnaud,Y. and Miginiac,E. (1984). Ageing and flowering of the apex in young *Bidens radiata*. Physiol.Plant. 62 : 225-230.

Prasad,T.K., Hosokawa,Z. and Cline,M.G. (1989). Effects of auxin, auxin transport inhibitors and mineral nutrients on apical dominance in *Pharbitis nil*. J.Plant Physiol. 135 : 472-477.

Rajeswara Rao,B.R., Singh,S.P. and Prakasa Rao,E.V.S. (1983). N,P and K fertilizer studies in pyrethrum (*Chrysanthemum cinerariaefolium*). J.Agric.Sci.,Camb. 100 : 509-511.

Ramina,A., Hackett,W.P. and Sachs,R.M. (1979). Flowering in *Bougainvillea*. A function of assimilate supply and nutrient diversion. Plant Physiol. 64 : 810-813.

Rand,P.J. (1990). Water relations in pyrethrum (*Tanacetum cinerariaefolium* L.). Honours Thesis, University of Tasmania.

Reid,J.B. (1990). Phytohormone mutants in plant research. J.Plant Growth Regul. 9 : 97-111.

Roberts,N.J., Menary,R.C. and Hofman,P.J. (1991). Plant growth substances in *Boronia megastigma* Nees. during flowering. J.Hortic.Sci. 66 (3) : 327-334.

Rodrigues Pereira,A.S. (1962). Physiological experiments in connection with flower formation in wedgewood *Iris*. Acta Bot. 11 : 97-138.

Rogers,M.N., Hartley,D.E. and Tjia,B.O.S. (1969). Ethylene prevents normal photoperiodic response in *Chrysanthemum morifolium*. Flor.Rev. 143 (3716) : 19-21.

Rood,S.B. and Pharis,R.P.(1986). Evidence for reversible conjugation of gibberellins in higher plants. in Conjugated Plant Hormones. Structure, Metabolism and Function. Schreiber,K., Schutte,H.R. and Sembdner,G. (Eds.). VEB Deutscher Verlag der Wissenschaften, Berlin.pp 183-190.

Sachs,R.M. (1977). Nutrient diversion ; an hypothesis to explain the chemical control of flowering. HortScience 12 (3) : 220-222.

Sachs,R.M. (1987). Roles of photosynthesis and assimilate partitioning in flower initiation. in Manipulation of Flowering, Atherton,J.G. (Ed.). Butterworths, London. pp 317-340.

Sachs,R.M., Kofranek,A.M. and Kubota,J. (1980). Radiant energy required for the night break inhibition of floral initiation is a function of daytime light input in chrysanthemum. HortScience 15 : 609-610.

Sachs,R.M., Kofranek,A.M. and Shyr,S.Y. (1967). Gibberellin-induced inhibition of floral initiation in *Fuchsia.* Am.J.Bot. 54 : 921-929.

Saha,S., Nagar,P.K. and Sircar,P.K. (1985). Changes in cytokinin activity during flower development in *Cosmos sulphureus* Cav. Plant Growth Reg. 3 : 27-35.

Salisbury,F.B. (1955). The dual role of auxin in flowering. Plant Physiol. 30 : 327-334.

Sastry,K.P. and Singh,S.P. (1990). The effect of phosphorous and potassium application on the flower yield of pyrethrum. Pyrethrum Post 17 (4) : 130-132.

Sastry,K.P., Singh,S.P., Dinesh,K. and Saleem,S.M. (1987). Effect of soil pH on the growth and yield of pyrethrum *Chrysanthemum cinerariaefolium* Vis. Pyrethrum Post 17 (1) : 24-25.

Sawney,R. and Cumming,B.G. (1975). Non-photosynthetic light requirement for the development of flowers in *Chenopodium rubrum*, a short-day plant. Can.J.Bot. 53 : 512-516.

Schwabe,W.W. (1950). Factors controlling flowering of the chrysanthemum. I. The effects of photoperiod and temporary chilling. J.Exp.Bot. 1 :329-343.

Schwabe,W.W. (1954). Factors controlling flowering of the chrysanthemum. IV. The site of vernalization and translocation of the stimulus. J.Exp.Bot. 5 : 389-400.

Schwabe,W.W. (1955). Factors controlling flowering of the chrysanthemum. V. Devernalization in relation to high temperature and low light intensity treatments. J.Exp.Bot. 6 : 435-450.

Schwabe,W.W. (1957). Factors controlling flowering of the chrysanthemum. VI. Devernalization by low-light intensity in relation to temperature and carbohydrate supply. J.Exp.Bot. 8 : 220-234.

Schwabe,W.W. (1959). Some effects of environment and hormone treatment on reproductive morphogenesis in the chrysanthemum. J.Linn.Soc.Lond.Bot. 56 : 254-261.

Schwabe,W.W. (1970). The possible role of plant hormones in the devernalisation of the *Chrysanthemum.* in Cellular and Molecular Aspects of Floral Induction. Bernier,G. (Ed.). Longman, London. pp 358-365.

Schwabe,W.W. and Al-Doori,A.H. (1973). Analysis of a juvenile-like condition affecting flowering in the black currant (*Ribes nigrum*). J.Exp.Bot. 24 (82) : 969-981.

Seidlova,F. and Krekule,J. (1977). Effects of kinetin on growth of the apical meristem and floral differentiation in *Chenopodium rubrum* L. Ann.Bot. 41 : 755-763.

Sharman,K.V. and Sedgley,M. (1988). Floral initiation and development in *Helipterum roseum* (Hook.) Benth. and *Helichrysum bracteatum* (Vent.) Andrews (Asteraceae). Aust.J.Bot. 36 : 575-587.

Shindy,W.W. and Weaver,R.J. (1967). Plant regulators alter translocation of photosynthetic products. Nature 24 : 1024-1025.

Shindy,W.W., Kliewer,W.M. and Weaver,R.J. (1973). Benzyladenine-induced movement of ^{14}C-labelled photosynthate into roots of *Vitis vinifera.* Plant Physiol. 51 : 345-349.

Singer,S.R. and McDaniel,C.N. (1986). Floral determination in the terminal and axillary buds of *Nicotiana tabacum* L. Developm.Biol. 118 : 587-592.

Singer,S.R. and McDaniel,C.N. (1987). Floral determination in internode tissues of day-neutral tobacco first occurs many nodes below the apex. Pro.Natl.Acad.Sci.USA 84 : 2790-2792.

Smulders,M.J.M., Jaussen,G.F.E., Croes,A.F., Barendse,G.W.M. and Wullems,G.J. (1988). Auxin regulation of flower bud formation in tobacco explants. J.Exp.Bot. 39 : 451-459.

Smulders,M.J.M., Van de Ven,E.T.W.M., Croes,A.F. and Wullems,G.J. (1990). Metabolism of 1-naphthaleneacetic acid in explants of tobacco : evidence for the release of free hormone from conjugates. J.Plant Growth Regul. 9 : 27-34.

Stanley,C.J. and Cockshull,K.E. (1989). The site of ethephon application and its effect on flower initiation and growth of chrysanthemum. J.Hortic.Sci. 64 (3) : 341-350.

Stoddart,J.L. (1976). Phytochrome and gibberellins. Nature 261 : 454-455.

Suge,H. (1974). Nature of ethylene inhibition of flowering in *Pharbitis nil.* in Plant Growth Substances, 1973. Hirakawa Publ., Tokyo. pp 960-966.

Takeno,K. and Cleland,C.F. (1990). Comparison of gibberellin-like substances fom cotyledons and phloem exudate collected from cotyledons of *Pharbitis nil* in relation to photoperiodic flowering. Plant Growth Regul. 9 (3) : 227-236.

Tamas,I.A., Schlossberg-Jacobs,J.L., Lim,R., Friedman,L.B. and Barone,C.C.(1989). Effect of plant growth substances on the growth of axillary buds in cultured stem segments of *Phaseolus vulgaris* L. Plant Growth Regul. 8 (2) : 165-183.

Tanaka,T. (1967). Studies on the regulation of chrysanthemum flowering with special reference to plant regulators. I. The inhibiting action of non-induced leaves on floral stimulus. J.Jpn.Soc.Hortic.Sci. 36 : 339-347.

Thimann,K.V., Sachs,T. and Mathur,S.N. (1971). The mechanism of apical dominance in *Coleus*. Physiol.Plant. 24 : 68-72.

Thomas,G.G. and Schwabe,W.W. (1970). Apical morphology in the hop (*Humulus lupulus*) during floral initiation. Ann.Bot. 34 : 849-859.

Thomas,T.H. (1982). Plant Growth Regulator Potential and Practice. BCPC Publications, Lavenham Press Ltd, Great Britain.

Tjia,B.O.S., Rogers,M.N. and Hartley,D.E. (1969). Effects of ethylene on morphology and flowering of *Chrysanthemum morifolium* Ramat. J.Am.Soc.Hortic. Sci. 94 : 35-39.

Tompsett,P.B. and Schwabe,W.W. (1974). Growth hormone changes in *Chrysanthemum morifolium*. Effects of environmental factors controlling flowering. Ann.Bot. 38 : 269-285.

Tran Thanh Van,K. (1980). Control of morphogenesis by inherent and exogenously applied factors in thin cell layers. Int.Rev.Cytol., Suppl. 11A : 175-194.

Trewavas,A.J. (1983). Plant growth substances - metabolic fly wheels for plant development. Cell Biology International Reports 7 : 569-575.

Trewavas,A.J. (1986). Understanding the control of plant development and the role of growth substances. Aust.J.Plant Physiol. 13 : 447-457.

Tucker,D.J. and Mansfield,T.A. (1973). Apical dominance in *Xanthium strumarium*. J.Exp.Bot. 24 : 731-740.

Tuikong,A.R. (1984). Pyrethrum breeding in Kenya; A historical account. Pyrethrum Post 15 (4) : 113-117.

Umemoto,T. (1971). Effect of chlorogenic acid on flower production in long-day duckweed, *Lemna gibba* G3. Plant Cell Physiol. 12 : 165-169.

Van den Ende,H. and Zeevaart,J.A.D. (1971). Influence of daylength on gibberellin metabolism and stem growth in *Silene armeria*. Planta 98 : 164-176.

Van Rijn,P.J. (1974). The production of pyrethrum. Tropical Abstracts 29 (4) : 237-244.

Van Ruiten,J.E.M. and De Jong,J. (1984). Speed of flower induction in *Chrysanthemum morifolium* depends on cultivar and temperature. Sci.Hortic. 23 : 287-294.

Venis,M.A. (1985). Hormone Binding in Plants. Longman Inc., New York.

Vince,D. (1955). Some effects of temperature and daylength on flowering in the chrysanthemum. J.Hortic.Sci. 30 : 34-42.

Vince and Mason (1954). Acceleration of flowering in non-vernalised *Chrysanthemum* by removal of apical sections of the stem. Nature 174 : 842-843.

Vince-Prue,D. (1975). Photoperiodism in plants. McGaw-Hill, London.

Vince-Prue,D. (1978). Effect of photoperiod and phytochrome in flowering : Time measurement. in La Physiologie de la Floraison. Champagnat,P. and Jacques,R. (Eds.). CNRS, Paris.pp 91-127.

Vince-Prue,D. and Lumsden,P.J. (1987). Inductive events in the leaves : time measurement and photoreception in the short-day plant *Pharbitis nil*. in Manipulation of Flowering, Atherton,J.G. (Ed.). Butterworths, London. pp. 255-268.

Vince-Prue,D., Thomas,B. and Cockshull,K.E. (Eds.) (1984). Light and the Flowering Process. Academic Press, New York.

Wallensiek,S.J. (1968). Flower induction through the roots of *Silene armeria* L. Acta Bot. 17 : 5-8.

Walton,D.C. (1980). Biochemistry and physiology of abscisic acid. Ann.Rev.Plant Physiol. 31 : 453-489.

Wareing,P.F. (1987). Juvenility and cell determination. in Manipulation of Flowering. Atherton,J.G. (Ed.). Butterworths, London. pp 83-92.

Wareing,P.F., Horgan,R., Henson,I.E. and Davis,W. (1977). Cytokinin relations in the whole plant. in Plant Growth Regulation. Pilet,P-E. (Ed.). Springer-Verlag, Berlin. pp. 147-153.

Webster,A.D. and Shepherd,U.M. (1984). The effect of summer shoot tipping and root stock on the growth, floral bud production, and yield and fruit quality of young sweet cherries. J.Hortic.Sci. 59 : 175-182.

Weier,T.E., Stocking,C.R., Barbour,M.G. and Rost,T.L. (1982). Botany: An introduction to plant biology, Sixth Edition. John Wiley and Sons Inc., New York.

Weise,A.H. and Seeley,J.G. (1964). Translocation of the floral stimulus in *Chrysanthemum*. Proc.Am.Soc.Hortic.Sci. 85 : 574-583.

Whealy,C.A., Nell,T.A., Barrett,J.E. and Larson,R.A. (1987). High temperature effects on growth and floral development of chrysanthemum. J.Am.Soc.Hortic.Sci. 112 (3) : 464-468.

Williams,M.W. (1972). Induction of spur and flower bud formation in young apple trees with chemical growth retardants. J.Am.Soc.Hortic.Sci. 97 : 210-212.

Winney,R. (1973). The biological performance of synthetic pyrethroids. Pyrethrum Post 12 (1) : 2-11.

Yang,S.F. and Hoffman,N.E. (1984). Ethylene biosynthesis and its regulation in higher plants. Ann.Rev.Plant Physiol. 35 : 155-189.

Yeang,H.Y. and Hillman,J.R. (1984). Ethylene and apical dominance. Physiol.Plant. 60 : 275-282.

Zeevaart,J.A.D. (1976). Physiology of flower formation. Ann.Rev.Plant Physiol. 27 : 321-348

Zeevaart,J.A.D. and Boyer,G.L. (1987). Photoperiodic induction and the floral stimulus in *Perilla*. in Manipulation of Flowering. Atherton,J.G. (Ed.). Butterworths, London. pp 269-277.

Zeevaart,J.A.D., Brede,J.M. and Cetas,C.R. (1977). Translocation patterns in *Xanthium* in relation to long day inhibition of flowering. Plant Physiol. 60 : 747-753.

Zeevaart,J.A.D. and Creelman,R.A. (1988). Metabolism and physiology of abscisic acid. Ann.Rev.Plant Physiol.Plant Mol.Biol. 39 : 439-473.

Zito,S.W., Zeig,R.G. and Staba,E.J. (1983). Distribution of pyrethrins in oil glands and leaf tissue of *Chrysanthemum cinerariaefolium*. Planta Medica 47 : 205-207.

Zucker,M., Nitsch,C. and Nitsch,J.P. (1965). The induction of flowering in *Nicotiana*. II. Photoperiodic alteration of the chloogenic acid concentration. Am.J.Bot. 52 : 271-277.

Appendix IV.B.1.a

Treatment (week of transfer)	Leaf number at transfer	Shoot number at transfer	Flowering time (days)	Vernalisation period (days)	Leaf number	Shoot number	Number of secondary shoots	Flowering position
0	3.6	1.2	50.2	50.2	30.4	5.6	4.6	1.0
1	5.4	1.6	49.8	42.8	31.6	5.8	4.8	1.2
2	9.8	2.8	47.6	33.6	31.6	6.0	5.0	1.4
3	12.0	3.2	55.0	34.0	36.8	7.4	4.8	1.0
4	16.0	4.6	61.4	33.4	56.2	11.4	7.0	1.2
5	20.0	5.4	67.8	32.8	70.8	14.2	9.8	1.8
6	28.6	7.2	75.4	33.4	94.0	19.6	13.4	1.2
7	37.2	9.0	84.0	35.0	146.4	23.2	15.8	1.4
8	50.8	13.2	89.4	33.4	201.2	30.6	19.6	1.8
9	61.6	15.6	94.6	31.6	296.6	32.2	22.4	2.0
LSD	5.60	1.50	4.33	4.33	35.45	5.16	3.57	ns

Appendix IV.B.1.a - Physical measurements of tissue cultured plants at the point of transfer to inductive (vernalising) conditions and at the point of flower initiation (first visible sign of stem elongation), including the number of days between the commencement of the experiment and flower initiation and the number of days spent in inductive conditions before flower initiation. Shoot number includes the main apical shoot, while flower position refers to the first secondary shoot to show signs of stem elongation. Analysis of variance tables were calculated for each measurement and LSD values calculated where significant differences were indicated.

Appendix IV.B.1.b

Treatment (week of transfer)	Leaf number at transfer	Shoot number at transfer	Flowering time (days)	Vernalisation period (days)	Leaf number	Shoot number	Number of secondary shoots	Flowering position
0	5.5	1.0	64	64	31.3	5.5	4.5	1.3
1	4.5	1.0	66	59	26.0	5.8	4.8	1.0
2	5.3	2.3	67	53	29.0	6.3	5.3	1.3
3	5.8	1.3	74	53	29.5	6.8	5.8	1.5
4	7.5	2.8	64	36	32.3	6.7	5.7	1.0
5	8.3	2.3	79	44	40.0	9.5	5.5	1.0
6	9.8	2.8	79	37	40.8	12.5	6.0	1.3
7	11.0	3.0	87	38	71.0	20.5	13.0	1.8
8	12.5	3.5	95	39	75.8	20.0	8.8	1.0
9	19.0	4.5	98	35	106.0	23.8	9.0	1.3
10	20.0	6.8	101	31	119.0	19.8	10.0	1.0
11	24.0	9.0	110	33	130.3	28.8	11.8	1.3
12	25.8	8.3	118	34	169.8	32.0	14.8	1.0
LSD	8.79	3.01	22.40	23.12	47.41	12.25	4.04	ns

Appendix IV.B.1.b - Physical measurements of vegetatively divided splits at the point of transfer to inductive (vernalising) conditions and at the point of flower initiation (first visible sign of stem elongation), including the number of days between the commencement of the experiment and flower initiation and the number of days spent in inductive conditions before flower initiation. Shoot number includes the main apical shoot, while flower position refers to the first secondary shoot to show signs of stem elongation. Analysis of variance tables were calculated for each measurement and LSD values calculated where significant differences were indicated.

Appendix IV.B.1.c

Treatment (month of transfer)	Leaf number at transfer	Shoot number at transfer	Flowering time (days)	Vernalisation period (days)	Leaf number	Shoot number	Number of secondary shoots	Flowering position
0	1.0	1.0	262.1	262.1	20.3	5.4	4.4	1.4
1	2.4	1.0	269.0	239.0	20.1	5.4	4.4	1.1
2	3.9	1.3	258.5	197.5	18.5	5.8	4.8	1.6
3	5.2	1.3	266.3	174.3	18.9	5.6	4.6	1.6
4	7.8	2.0	272.0	150.0	24.1	5.6	4.6	1.4
5	10.9	2.6	264.4	111.4	20.6	5.4	4.4	1.4
6	14.6	3.1	242.0	58.0	28.2	7.5	7.5	1.3
7	17.0	6.0	258.0	44.0	29.0	7.8	7.8	1.9
8	22.9	5.9	283.4	38.4	52.1	14.0	11.9	1.7
LSD	5.21	1.63	20.66	20.65	6.99	1.52	1.40	ns

Appendix IV.B.1.c - Physical measurements of seedlings at the point of transfer to inductive (vernalising) conditions and at the point of flower initiation (first visible sign of stem elongation), including the number of days between the commencement of the experiment and flower initiation and the number of days spent in inductive conditions before flower initiation. Shoot number includes the main apical shoot, while flower position refers to the first secondary shoot to show signs of stem elongation. Analysis of variance tables were calculated for each measurement and LSD values calculated where significant differences were indicated.

Appendix IV.B.1.d

Treatment (month of transfer)	Sampling date							Flowering date
	9-Mar	9-May	9-Jun	9-Jul	9-Aug	9-Sep	9-Oct	
Control	34.8	22.3	15.8	17.4	20.8	13.7	9.1	-
January	-	-	50.0	22.9	18.6	12.2	17.7	6-Oct
February	-	-	37.8	19.8	25.1	15.8	18.5	28-Sep
March	-	-	34.9	29.8	20.6	19.5	12.0	23-Sep
April	-	-	37.8	20.3	11.2	15.2	12.3	30-Sep
May	-	-	29.7	18.2	22.0	18.3	14.3	1-Oct
June	-	-	-	17.2	20.6	17.8	13.2	28-Sep
July	-	-	-	-	14.9	14.9	12.8	6-Sep
August	-	-	-	-	-	10.9	14.2	25-Sep
September	-	-	-	-	-	-	13.5	16-Oct

Appendix IV.B.1.d - Endogenous gibberellin concentrations in mature leaf samples of seedlings. Control plants were maintained in non-inductive conditions while plants under inductive conditions were moved from the non-inductive to the inductive conditions on the dates indicated. Concentrations are in ng GA per g dry weight of mature leaf tissue.

Appendix IV.B.1.e

Treatment (month of transfer)	Sampling date							Flowering date
	9-Mar	9-May	9-Jun	9-Jul	9-Aug	9-Sep	9-Oct	
Control	160.3	212.1	294.3	216.7	176.6	204.8	209.4	-
January	-	-	217.2	280.9	237.2	223.7	148.0	6-Oct
February	-	-	199.9	223.1	205.6	130.1	108.1	28-Sep
March	-	-	149.8	143.2	168.4	189.5	114.9	23-Sep
April	-	-	124.9	177.1	194.7	202.7	119.2	30-Sep
May	-	-	154.4	132.9	100.2	139.8	92.1	1-Oct
June	-	-	-	145.9	122.9	129.7	85.3	28-Sep
July	-	-	-	-	148.5	130.9	107.2	6-Sep
August	-	-	-	-	-	174.9	130.8	25-Sep
September	-	-	-	-	-	-	83.8	16-Oct

Appendix IV.B.1.e - Endogenous auxin concentrations in mature leaf samples of seedlings. Control plants were maintained in non-inductive conditions while plants under inductive conditions were moved from the non-inductive to the inductive conditions on the dates indicated. Concentrations are in ng IAA per g dry weight of mature leaf tissue.

Appendix IV.B.1.f

Treatment (month of transfer)	Sampling date 9-Mar	9-May	9-Jun	9-Jul	9-Aug	9-Sep	9-Oct	Flowering date
Control	177.4	125.4	112.1	152.7	153.1	119.1	136.4	-
January	-	-	121.1	111.9	153.7	133.0	179.1	6-Oct
February	-	-	106.9	124.3	156.9	141.0	158.0	28-Sep
March	-	-	136.6	145.3	143.6	180.1	175.6	23-Sep
April	-	-	109.1	155.1	110.1	155.4	155.6	30-Sep
May	-	-	128.1	129.7	120.0	123.6	164.4	1-Oct
June	-	-	-	122.3	157.1	159.3	136.6	28-Sep
July	-	-	-	-	124.4	142.4	145.0	6-Sep
August	-	-	-	-	-	132.6	129.4	25-Sep
September	-	-	-	-	-	-	174.7	16-Oct

Appendix IV.B.1.f - Endogenous abscisic acid concentrations in mature leaf samples of seedlings. Control plants were maintained in non-inductive conditions while plants under inductive conditions were moved from the non-inductive to the inductive conditions on the dates indicated. Concentrations are in ng ABA per g dry weight of mature leaf tissue.

Appendix IV.B.1.g

Treatment (month of transfer)	Sampling date							Flowering date
	9-Mar	9-May	9-Jun	9-Jul	9-Aug	9-Sep	9-Oct	
Control	9.7	15.5	8.3	8.7	9.1	5.7	11.7	-
January	-	-	8.8	7.7	11.4	8.8	8.0	6-Oct
February	-	-	9.0	9.1	9.1	12.2	7.8	28-Sep
March	-	-	13.8	13.1	13.9	9.1	6.6	23-Sep
April	-	-	14.3	8.9	6.7	7.9	8.2	30-Sep
May	-	-	13.4	9.5	11.5	9.5	4.6	1-Oct
June	-	-	-	7.7	9.2	7.8	3.0	28-Sep
July	-	-	-	-	7.5	7.0	7.4	6-Sep
August	-	-	-	-	-	10.5	4.7	25-Sep
September	-	-	-	-	-	-	5.3	16-Oct

Appendix IV.B.1.g - Endogenous zeatin concentrations in mature leaf samples of seedlings. Control plants were maintained in non-inductive conditions while plants under inductive conditions were moved from the non-inductive to the inductive conditions on the dates indicated. Concentrations are in ng Z per g dry weight of mature leaf tissue.

Appendix IV.B.1.h

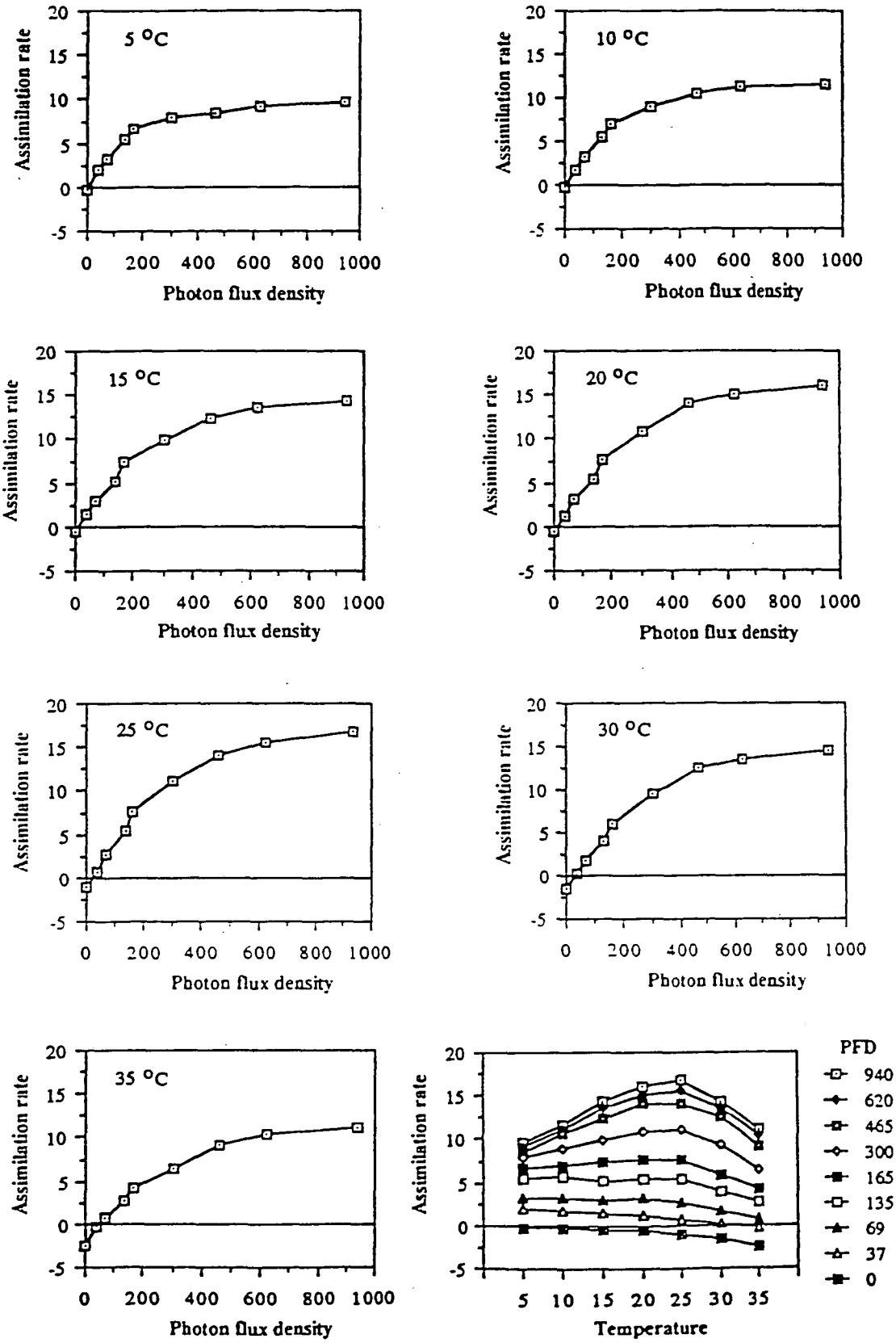
Treatment (month of transfer)	Sampling date							Flowering date
	9-Mar	9-May	9-Jun	9-Jul	9-Aug	9-Sep	9-Oct	
Control	30.1	35.3	33.2	34.2	29.6	39.6	27.8	-
January	-	-	31.7	28.4	39.2	26.4	25.1	6-Oct
February	-	-	29.6	28.0	35.6	35.6	30.4	28-Sep
March	-	-	37.8	36.2	45.0	37.2	29.6	23-Sep
April	-	-	37.5	38.1	30.6	32.9	30.9	30-Sep
May	-	-	36.0	37.6	41.9	37.6	27.4	1-Oct
June	-	-	-	30.6	42.2	36.0	24.0	28-Sep
July	-	-	-	-	35.0	29.4	32.8	6-Sep
August	-	-	-	-	-	30.3	24.1	25-Sep
September	-	-	-	-	-	-	29.8	16-Oct

Appendix IV.B.1.h - Endogenous dihydrozeatin concentrations in mature leaf samples of seedlings. Control plants were maintained in non-inductive conditions while plants under inductive conditions were moved from the non-inductive to the inductive conditions on the dates indicated. Concentrations are in ng DHZ per g dry weight of mature leaf tissue.

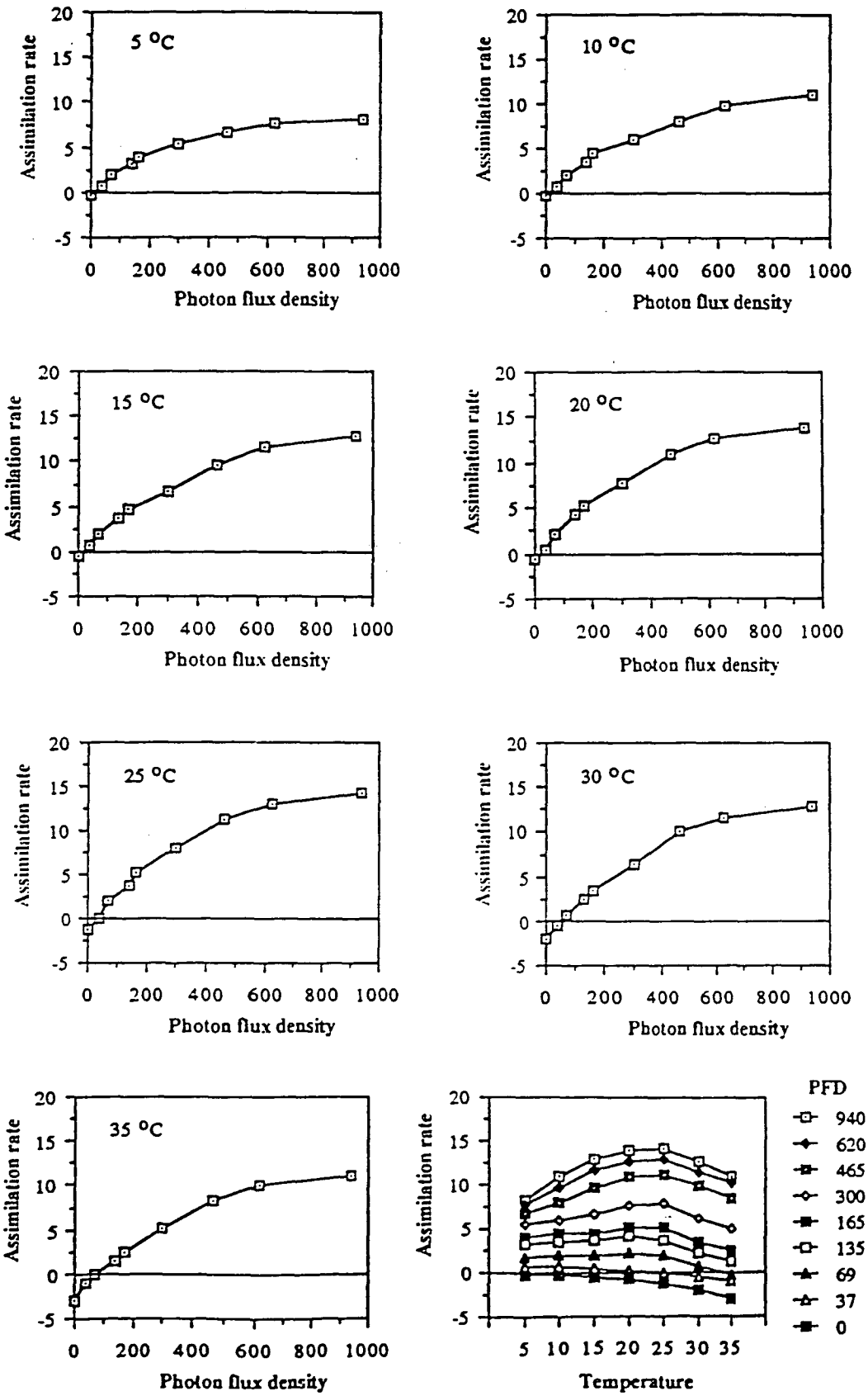
Appendix IV.B.1.i

Treatment (month of transfer)	Sampling date							Flowering date
	9-Mar	9-May	9-Jun	9-Jul	9-Aug	9-Sep	9-Oct	
Control	14.4	30.6	8.3	8.6	9.7	16.6	15.4	-
January	-	-	10.2	8.9	12.5	9.2	16.0	6-Oct
February	-	-	15.4	9.1	14.0	13.3	12.0	28-Sep
March	-	-	11.5	11.6	18.5	14.3	5.9	23-Sep
April	-	-	14.5	12.6	10.1	13.2	14.3	30-Sep
May	-	-	11.7	8.3	15.5	12.4	3.4	1-Oct
June	-	-	-	2.9	11.3	8.1	2.4	28-Sep
July	-	-	-	-	5.8	2.4	10.8	6-Sep
August	-	-	-	-	-	3.2	3.5	25-Sep
September	-	-	-	-	-	-	3.6	16-Oct

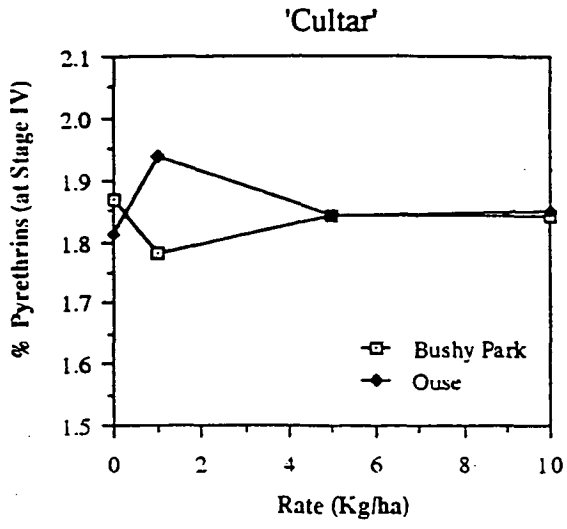
Appendix IV.B.1.i - Endogenous isopentyladenosine concentrations in mature leaf samples of seedlings. Control plants were maintained in non-inductive conditions while plants under inductive conditions were moved from the non-inductive to the inductive conditions on the dates indicated. Concentrations are in ng IPA per g dry weight of mature leaf tissue.

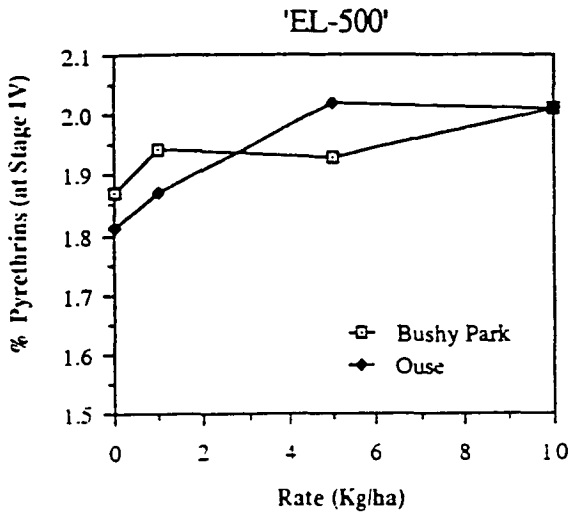


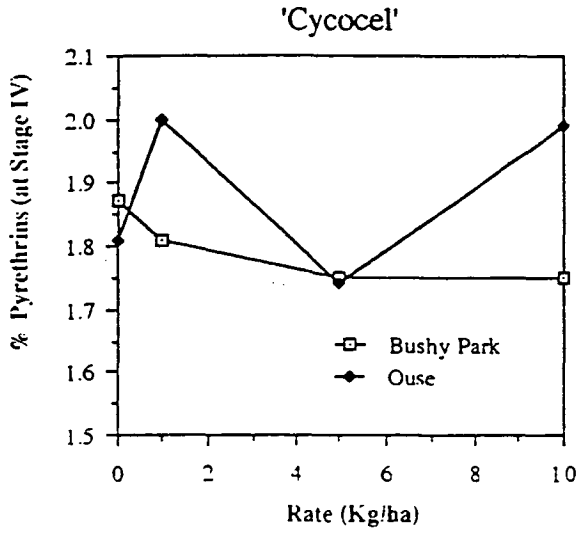
Appendix IV.B.3.3.a - Net CO_2 assimilation rate temperature and PFD response curves for mature leaves. Assimilation rate and PFD are measured in $\mu mol.m^{-2}.s^{-1}$

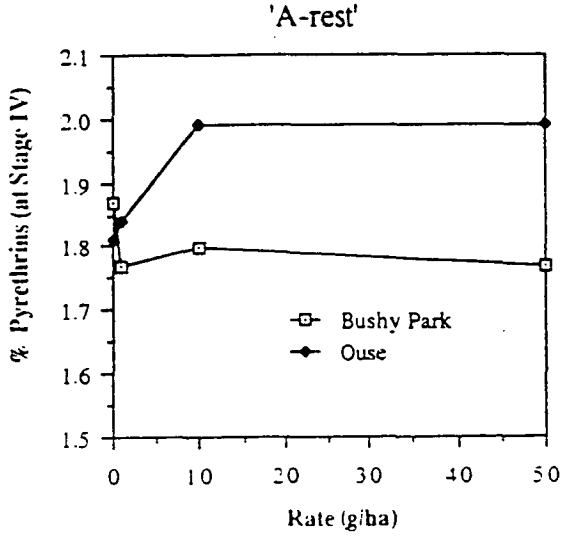


Appendix IV.B.3.3.b - Net CO_2 assimilation rate temperature and PFD response curves for immature leaves. Assimilation rate and PFD are measured in $\mu mol.m^{-2}.s^{-1}$

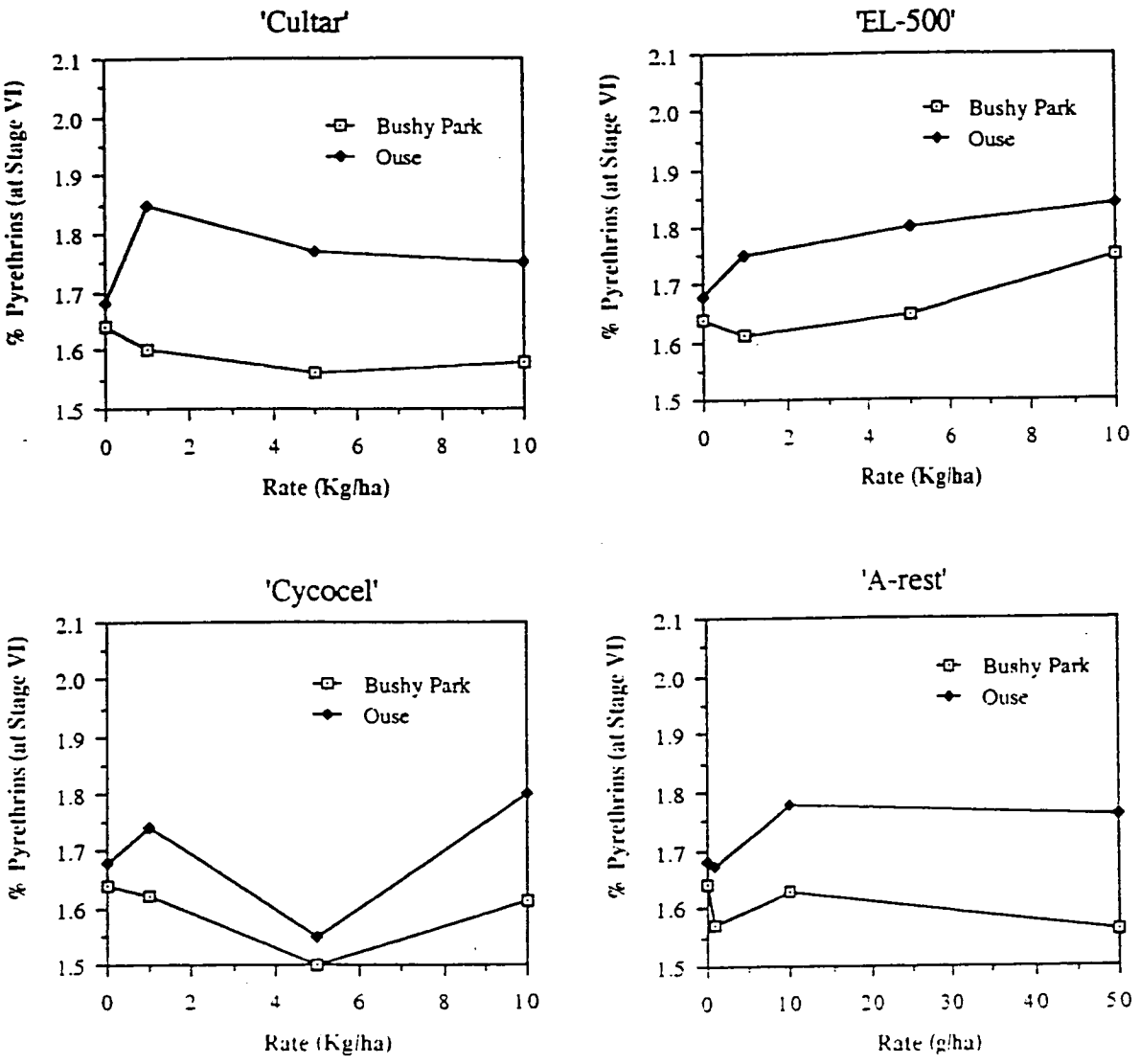








Appendix IV.C.2.3.1.a - Effect of growth retardants on pyrethrin content. Flowers were picked at floral development stage IV for pyrethrin analysis. The pyrethrin content is expressed as a percentage on a dry weight basis. No statistically significant differences between means were observed.



Appendix IV.C.2.3.1.b - Effect of growth retardants on pyrethrin content. Flowers were picked at floral development stage VI for pyrethrin analysis. The pyrethrin content is expressed as a percentage on a dry weight basis. No statistically significant differences between means were observed.