The Physiology of Flowering in the Australian Paper Daisies *Helipterum roseum* and *Helichrysum bracteatum*

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The Physiology of Flowering in the Australian Paper Daisies *Helipterum roseum* and *Helichrysum bracteatum*

SUMMARY

Floral initiation and development in *Helipterum roseum* and *Helichrysum bracteatum* (Asteraceae) were investigated by scanning electron microscopy. The sequence of events in these two species was similar and occurred rapidly. Seven stages in apical development were identified, which were distinctive in both appearance and size. Stage 1 was a small vegetative meristem with between two and four leaf primordia. Stage 2 (also vegetative) was characterised by a doubling in both height and diameter. A doming of the apical meristem signified the commencement of stage 3 and at the appearance of the first involucral bracts (stage 4) the apex had further tripled in height and doubled in diameter. This was followed by the appearance of floret primordia (stage 5). By the time the inflorescence buds were visible to the naked eye (stage 6) several rows of florets were present, and at anthesis (stage 7) the capitulum was covered with florets.

The effects of photoperiod, temperature and plant age on floral initiation and inflorescence production in both species were investigated using controlled environment growth cabinets, glasshouse and field environments. Both species were quantitative long day plants, with floral initiation occurring sooner in long photoperiod and night-break conditions. Photoperiod affected time to floral initiation rather than floral development. Floral initiation in *Helipterum roseum* was

inhibited at 25 °C (photoperiod 12 h, light intensity 250 W m^{-2}).

Peaks in bloom production occurred during the spring and summer under Adelaide conditions, regardless of planting time in both glasshouse and outdoor environments. There was a tendency for inflorescence diameter of successive inflorescences of both species and corresponding stem length of successive blooms of *Helipterum roseum* to decline with time from anthesis of the apical inflorescence. Optimum production of top quality blooms of *Helipterum roseum* extended from October to January following planting between autumn and spring. Peak production of *Helichrysum bracteatum* between December and March could be expected following planting during autumn to spring. It is proposed that both species be considered for the fresh cut flower market in addition to the traditional drying and wiring, with *Helipterum roseum* marketed as single stems and *Helichrysum bracteatum* as sprays.

The effect of temperature on morphological development and cell-cycling in shoot apical meristems of *Helipterum roseum* during the floral transition was investigated. Apical development proceeded to anthesis (stage 7) at 20 °C, but rarely progressed beyond stage 2 at 25 °C. Morphological development was arrested at stage 3 when plants were transferred from 20 to 25 °C at stage 3, and delayed for a short period when transferred at stage 4. It was concluded that the apical meristem was committed to the production of an inflorescence at stage 4 and that developmental pathways were still optional at stage 3. The length of the cell-cycle and its component phases were determined by the percent-labelled-mitoses method using autoradiography and Nomarski interference microscopy, after labelling with tritiated

thymidine. The duration of the cell-cycle was constant in cells labelled during the pulse at stages 2, 3 and 4 at 20 °C (64, 41 and 47.5 h respectively) but varied in cells of meristems inhibited at stage 2 at 25 °C. It was concluded that the inhibition of floral initiation in *Helipterum roseum* at 25 °C was due, at least in part, to the variation in the duration of the cell-cycle within the cell population.

DECLARATION

I hereby declare that the work presented in this thesis has been carried out by myself and does not incorporate any material previously submitted for another degree in any university. To the best of my knowledge and belief, it does not contain any material previously written or published by another person, except where due reference is made in the text. I am willing to make the thesis available for photocopying and loan if it is accepted for the award of the degree.

November
1989

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CHAPTER 1. INTRODUCTION

1.1 The cut-flower industry

Floriculture has attracted much interest in Australia as a new and innovative export industry. The range of climatic conditions available, and location in the southern hemisphere gives Australia the opportunity to become a major supplier of out-of-season cut-flowers, foliage, and potted plants to world markets. Sales of single-stemmed flowers; roses, carnations and chrysanthemums dominate international trade. However, there is a demand for flowers which are uniquely Australian.

A review of international trade statistics for cut-flowers demonstrates the enormous value of the industry. In 1985, the Flower Council of Holland valued world trade in cut-flowers and potted plants at US$2.5 billion [1] (Pegrum 1988). The Netherlands is the major participant, importing and exporting flowers and plants worldwide, and accounting for 63 % of all exports of cut-flowers, and 51 % of house plants (The Economist 1987). The United States alone imported US$298 million worth of cut-flowers in 1985, primarily from the major exporting countries; The Netherlands, Colombia and Israel (Minnis 1986).

Australia presently has a market share of less than 0.5 % of world trade, although flower and foliage exports have increased dramatically. In 1980/81 exports were valued at A$2.9 million [2] and in 1987/88 this had risen to A$10.6 million (Pegrum 1988). During 1986/87 the value of sales of cut-flowers

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1. US$ United States dollar

2. A$ Australian dollar

in Australia (the most recent figures available) was A$50.67 million, an increase of 50 % on the previous survey conducted in 1983/84 (Australian Bureau of Statistics 1986-87). The state of Victoria was the largest producer in 1986/87 with A$20.3 million followed by New South Wales with A$9.9 million. Local consumption of cut-flowers is very low with the average Australian spending less than A$2 per head on fresh flowers per annum while in Europe this is closer to A$30 per head (Ragless 1980).

1.2 Manipulation of flowering

The flowering process is an important aspect of horticulture, since limitations to the initiation and development of flowers often adversely affect productivity. Controlled manipulation of flowering therefore offers potential advantages to horticulture, and particularly to floriculture. Some methods which have been successfully used to control the flowering response and floral development include manipulation of the environment (photoperiod, temperature, light intensity), chemical manipulation (growth retardants or promotors) and genetic improvement of plant stock through plant breeding.

The flowering response, floral development, propagation and post-harvest vase-life in traditional flower crops such as chrysanthemum, rose and carnation have been extensively studied (Halevy 1985, 1986). This has resulted in the development of techniques for the year-round production of these crops. The successful manipulation of growth and flowering via appropriate production procedures, ensures that market demand for cut-flowers

or potted plants is met. The timing of crops is particularly important where a speciality product is produced to coincide with a particular holiday season or festive day such as Christmas or Mothers' Day. The development of successful manipulative techniques depends upon a thorough understanding of the physiological principles underlying the flowering process.

The continued development of the Australian floriculture industry depends upon increased knowledge of the flowering response, the adaptation of improved methods of production for traditional and native cut-flowers, and the exploitation of the native flora.

1.3 *Helipterum roseum*

Species: *Helipterum roseum* (Hook.) Benth. (1867)

Tribe: Inuleae

Family: Asteraceae

The genus *Helipterum* comprises approximately 60 Australian and 40 South African species (Jessop and Toelken 1986). There is considerable taxonomic confusion surrounding this genus and it is presently under review (M. Lawrence, personal communication 1986).

Helipterum roseum (Plate 1.1) is an erect annual 15-40 cm high. The petal-like laminae of the inner involucral bracts are pink (dominant form) or white and less than 2.5 cm long. Pappus hairs are white although those of *Helipterum roseum* var.

nigropapposum are black. The achenes are covered with long soft white hairs (Grieve and Blackall 1975). *Helipterum roseum* is adapted to the arid inland plains area of Western Australia (Canberra Botanic Gardens 1976). Several cultivars and pure colour strains have been developed and are available from local and overseas seed merchants. It is often referred to by its obsolete generic name *Acrolinium* (Canberra Botanic Gardens 1976).

Limited information is available on the flowering and reproductive biology of *Helipterum*. Both *Helipterum manglesii* (Lindley) F. Muell. ex Benth. and *Helipterum craspedioides* W.K. Fitz. are quantitative long day plants with low temperature (15 °C) promotion of flowering in the latter (Ramaley 1934, Mott and McComb 1975b). The response of *Helipterum roseum* to photoperiod has not been conclusively established. Allard and Garner (1940) reported that this species "for the most part grew poorly under all light periods, but appears to be of long day-habit in some respects." Water stress increases the time to initiation of visible floral primordia and significantly reduces the number of seeds and seed viability in *Helipterum craspedioides* (Mott and McComb 1975a). Seeds of this species are normally produced in the spring and summer and germinate after following winter rains, with dormancy being broken by a prolonged period of storage at high temperature (Mott 1972).

Plate 1·1

Helipterum roseum (Hook.) Benth. (x 0·3)

1. Inflorescence bud (x 0·3)
2. Inflorescence at anthesis (x 0·3)
3. Magenta inflorescence (x 1)
4. Magenta inflorescence with black pappus (x 1)
5. Rose inflorescence (x 1)

Artist: Kerry Sharman 1989.

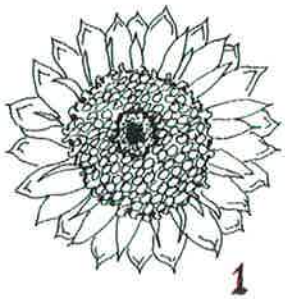
Helipterum roseum

Plate 1·2

Helichrysum bracteatum (Vent.) Andrews (x 0·5)

1. Inflorescence (x 1)
2. Inflorescence (longitudinal section) (x 3)
3. Hermaphrodite floret (x 6·25)

Artist: Kerry Sharman 1989.



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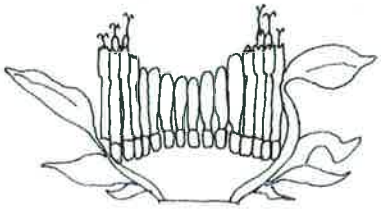

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Helichrysum bracteatum

1.4 Helichrysum bracteatum

Species: *Helichrysum bracteatum* (Vent.) Andrews (1805)

Tribe: Inuleae

Family: Asteraceae

The genus *Helichrysum* is represented by approximately 500 species in Eurasia, New Zealand, Australia, Madagascar and Africa. It is poorly understood and has ill-defined limits (Jessop and Toelken 1986). The genus is currently under review and the name *Helichrysum bracteatum*, for the present, refers to a species complex (M. Lawrence, personal communication 1986).

Helichrysum bracteatum (Plate 1.2) is an erect, finely scabrous-pubescent plant up to 60 cm high. The laminae of the conspicuous involucral bracts are white, bright yellow or reddish-brown. The achene is glabrous (Grieve and Blackall 1975). Forms of *Helichrysum bracteatum* may be found in all Australian states, and may be prostrate or upright, perennial or annual. Strains of *Helichrysum bracteatum* known as 'strawflowers' which bear globular inflorescences in many colours including maroon and bronze were developed in Europe early last century (Canberra Botanic Gardens 1972). Recently registered perennial cultivars include; *Helichrysum bracteatum* 'Diamond Head' and *Helichrysum bracteatum* 'Princess of Wales' (Canberra Botanic Gardens 1978, Butler 1986).

Investigative work on the chemical constituents of *Helichrysum* is well documented (Jakupovic *et al.* 1987), however there is limited information available on the flowering and

reproductive biology in the genus despite its ecological and economic importance. *Helichrysum cassinianum* Gaud. is a quantitative long day plant with retardation of floral initiation at temperatures above and below 20 °C (Mott and McComb 1975b). Floral initiation is also delayed when plants are water stressed, and significant reductions in seed number and viability are observed (Mott and McComb 1975a). Seeds of *Helichrysum cassinianum* require a period of 4 months at elevated temperatures to break dormancy followed by a period below 20 °C for germination (Mott 1972). Available cultural information relates to the production of dried flowers or to the growth of plants for amenity horticulture (Florists Review 1966, Hass 1985, Sciaroni 1971, Schaumann *et al.* 1987) rather than to the production of fresh cut-flowers.

1.5 Aims of project

1. To examine the morphological development of the apical meristem in *Helipterum roseum* and *Helichrysum bracteatum* from germination to anthesis by scanning electron microscopy, and investigate the breeding system of these species.

2. To investigate the environmental control of floral initiation and development in *Helipterum roseum* and *Helichrysum bracteatum*.

3. To determine the effect of temperature on (i) morphological development of the shoot apical meristem and (ii) cell-cycle duration during the floral transition, in *Helipterum roseum*.

4. To investigate inflorescence production in both *Helipterum*

roseum and *Helichrysum bracteatum* in two commercial growing conditions, and develop guidelines for the scheduling of these crops for optimum production.

CHAPTER 2. LITERATURE REVIEW

2.1 Floral induction

In this review, floral induction refers to the earliest changes that occur in plants in response to a stimulus eg. an environmental condition such as temperature or daylength, which occur prior to morphological changes.

2.1.1 Control by daylength

2.1.1.1 Photoperiodic response groups

The discovery that flowering could be controlled by daylength is largely attributed to the work of Garner and Allard (Evans 1969). They classified plants into photoperiodic response groups and introduced the terms "photoperiod" and "photoperiodism" (Garner and Allard 1920, 1923). Responses to photoperiod are divided into three groups; plants which do not respond to photoperiod are termed day neutral (DN) whilst plants referred to as absolute (qualitative) short day plants (SDP) or long day plants (LDP) have an obligate requirement for short days (SD) or long days (LD), respectively. Quantitative (facultative) SDP or LDP will produce flower buds in any daylength, but will do so earlier in SD or LD, respectively (Vince-Prue 1975, Salisbury 1982, Bernier *et al.* 1981a). It should however be emphasised that the classification of plants into groups is an indicator as to whether flowering is promoted when the daylength is increased or decreased, it is not based on a single critical daylength at which the plant will flower. The response of even absolute photoperiodic species may be profoundly modified by other

environmental factors such as temperature, nutrition and light flux and by factors such as plant age and genotype (Lang 1965, Salisbury 1982, Bernier *et al.* 1981a). Thus, the classification of plants into response groups is only valid under a given set of conditions.

2.1.1.2 Importance of dark and light periods

Photoperiodic responses depend, by definition, on the alternation of dark and light periods (Lang 1965). Plants respond to the duration and timing of these periods in the daily cycle (Vince-Prue *et al.* 1984). The work of Hamner and Bonner (1938) with *Xanthium*, a SDP, established the crucial role of the dark period. This plant will flower only when the dark period exceeds 8.5 h in a 24 h cycle. The same workers also discovered that an interruption of the otherwise inductive long night by a brief (1 min) period of light nullified the effect of the long night. This discovery was a significant event in the critical analysis of the photoperiodic response. Briefly interrupting the long night with a short period of low-intensity light will also prevent flowering in many other SDP, eg. *Pharbitis*, *Kalanchoe* and *Perilla* (Lang 1965, Vince-Prue 1975).

Light breaks may also promote flowering in some LDP when grown in SD eg. *Lolium*, *Sinapis* and *Fuschia* (Lang 1965, Vince-Prue 1975). These LDP usually require either night-breaks of several hours duration and/or continued treatment during several successive nights to promote flowering (Bernier *et al.* 1981a).

The effectiveness of a night-break varies according to

the time at which it is given. The time of maximum effectiveness in *Pharbitis* is close to 8 h in a 16 h dark period (Takimoto and Hamner 1961). However, coincidence with the middle of the dark period is not always the case, eg. *Coleus fredericii* is most sensitive to light interruptions after 4 h of a 16 h dark period (Vince-Prue 1975). The relationship between the time of maximum sensitivity to a night-break and night and/or day length is complex and leads to the question of the biological timing mechanism (Vince-Prue 1975), a topic which will be considered in subsequent sections.

In both SDP and LDP the light and dark periods interact and the effectiveness of a day (or night) of a certain length is dependent on the length of the associated night (or day), (Bernier *et al.* 1981a). The daily light periods are therefore also important. Short day plants favour an alternation of light and darkness for flower formation as shown by the work of Hamner (1940) with 'Biloxi' soybean. Light periods are not however essential for flowering as evidenced by the fact that plants belonging to various response groups may initiate flowers in total darkness eg. the SDP *Pharbitis* (Takimoto 1960) and the LDP *Sinapis* (Kinet *et al.* 1973). Furthermore some LDP eg. *Sinapis* will flower both in continuous darkness and continuous light (Bernier *et al.* 1981a).

2.1.1.3 Photoinduction

A brief exposure to a favourable daylength is sufficient to promote flower initiation of some plants even after plants are returned to unfavourable daylength conditions (Bernier *et al.*

1981a). The photoperiodic control of flower initiation has therefore an inductive character (Lang 1965) and is referred to as "photoperiodic induction" or "photoinduction" (Lang 1965, Bernier *et al.* 1981a).

2.1.1.4 Fractional induction

Fractional induction is the summation of inductive cycles despite the intercalation of non-inductive cycles (Vince-Prue 1975). It has been demonstrated in both LDP and SDP requiring more than one inductive cycle, that a summation of subthreshold inductive treatments is possible eg. the LDP *Beta vulgaris* and the SDP *Impatiens balsamina* (Lang 1965). Successful fractional induction depends upon many factors including the positioning and duration of non-inductive cycles and environmental influences such as temperature (Bernier *et al.* 1981a). Whether the summation takes place in the leaves or in the apical meristem is not clear, thus a unitary interpretation of the mechanism(s) involved is not yet available (Bernier *et al.* 1981a).

2.1.1.5 Permanence of the induced state

Generally suboptimal photoinduction is short lasting and plants revert to vegetative growth soon after transfer to non-inductive conditions. The simplest explanation for this is that one or more factors present after induction are gradually depleted or diluted during growth of the apical meristem such that the initial state of differentiation is lost (Bernier *et al.* 1981a). In some instances however the effect of the inductive period persists for long periods. For example, in the

SDP *Perilla*, flowers will be produced and seed set in continuous SD conditions. If however the plant is transferred to LD conditions after 10 to 20 SD, then the terminal inflorescence will still produce a few flowers with subsequent complete reversion to vegetative growth taking place within two months (Zeevart 1985). In the case of *Xanthium* the effect of minimal induction is even more persistent and plants continue to proceed towards flowering in non-inductive conditions for up to 6 months before reverting to vegetative growth (Lam and Leopold 1960). Only repeated decapitation and removal of young leaves results in earlier loss of the flowering habit (Lam and Leopold 1960). The persistence of the induced state in this species may be related to the phenomenon of "indirect induction". When an induced leaf of *Xanthium* is grafted to a non-induced plant, flowering results, and in addition other non-induced leaves on the receptor plant may in turn promote flowering when grafted to non-induced receptor plants (Zeevart 1958). Young leaves are therefore continually induced by old leaves and this may explain the continued flowering habit of this species upon transfer to non-inductive conditions (Vince-Prue 1975).

2.1.1.6 Interaction of environmental factors

Temperature

The fact that photoperiodic species do not flower in nature at an identical date year after year is usually attributed to changes in temperature (Bernier *et al.* 1981a). It is quite common to observe a change in the photoperiodic response type with a change in temperature conditions. For example, a plant

may have a LD requirement at one temperature and be DN at another. Virtually all possible combinations between photoperiodic response groups and temperature are known (Salisbury 1982). Small changes in temperature may suffice to alter the plant response. For example *Chamelaucium uncinatum* is an absolute SDP at 24/16 °C day/night but is a quantitative SDP at 20/10 °C (Shillo 1985).

In some cases the requirement for long or short days may be completely replaced by changes in temperature. For example, the SD requirement of *Begonia* may be replaced by a low temperature treatment (below 15 °C) (Bernier *et al.* 1981a) whilst a high temperature treatment (above 30 °C) may eliminate the requirement for LD in *Rudbeckia bicolor* (Murneek 1940). It is clear that plant responses to photoperiod must always be considered in close relationship to temperature.

Light intensity

Interactions between the duration of the light period and the photon flux density during this period are numerous amongst photoperiodic plants (Bernier *et al.* 1981a). In some cases daylength requirements are eliminated by high photon flux densities, as is the case with the LDP *Sinapis* (Bodson *et al.* 1977). They may also be modified, as in *Chrysanthemum*, a quantitative SDP, where flowering is promoted at higher light/shorter daylength combinations (Hughes 1973). Low photon flux densities generally delay flower initiation, although there are exceptions. For example, flowering in the SDP *Perilla* will proceed in continuous light of low intensity (de Zeeuw 1953).

Short periods of lower or higher photon flux density during SD treatment of *Chrysanthemum morofolium* can also delay or promote flowering, the effect being dependent on the timing of the treatments (Cockshull and Hughes 1971). Long day conditions which consist of a short period of high intensity light followed by an extended period of supplementary light at low irradiance (below the light compensation point) are often sufficient to induce flowering in some LDP, for example, *Dianthus caryophyllus* L. (Besemer 1980).

2.1.2 Perception of light and time measurement in photoperiodism

2.1.2.1 Perception of light

Photoperiodic sensitivity is a property of all plant parts, but as a general rule it is most effectively perceived by the leaves (Bernier *et al.* 1981a). Moreover, the flowering response may be obtained in both SDP and LDP by exposing as little as one leaf to inductive daylength conditions (Lang 1965, Vince-Prue 1975, Bernier *et al.* 1981a). The sensitivity of leaves to photoinduction is dependent on several factors. These include physiological age, as very young leaves are usually far less effective than expanded ones, position on the stem, and leaf area. As little as a few square centimeters from one sensitive leaf is sufficient to produce a significant response in some plants (Bernier *et al.* 1981a).

2.1.2.2 Phytochrome mechanism and action

The perception of light in photoperiodism is essentially attributed to the pigment "phytochrome" (Salisbury 1961, Briggs and Rice 1972, Bernier *et al.* 1981a). Borthwick *et al.* (1952) demonstrated in *Xanthium* that inhibition of flowering with red light (R) could be reversed with far-red irradiation (FR). They deduced that the photoreceptor, which they called "phytochrome", occured as two interconvertible forms, one with an absorption maximum in the red region of the visible spectrum (Pr 660 nm), the other predominantly absorbing far-red radiation (Pfr 730 nm) (Bernier *et al.* 1981a). Irradiating plants with natural light cycles phytochrome between the two forms Pr and Pfr, although complete photochemical reversion is impossible. In addition to the photochemical conversions there are also nonphotochemical reactions, or dark reversions of Pfr to Pr. This is supported by the fact that since white light acts like R, the pigment is predominantly in the Pfr form at the end of the light period. However, SDP and LDP become sensitive to R after several hours of darkness indicating that Pr is present in significant amounts (Vince-Prue 1975). The Pfr produced in response to R, is assumed to be the physiologically active form since relatively small amounts of Pfr produce a response (Bernier *et al.* 1981a). A general theory of the phytochrome system was developed based on these concepts and is shown schematically in Fig. 1. Red radiation would convert the pigment to the Pfr form and FR would change the pigment to Pr. Using red/far-red reversibility as a criterion, many plant responses to light, other than flowering in photoperiodically sensitive plants, have been shown to be under the control of the phytochrome system and include the germination

of light-sensitive seeds and de-etiolation of plant parts (Vince-Prue 1975).

Figure 2.1 Phytochrome mechanism and action.
(Adapted from Bernier *et al.* 1981a.)

2.1.2.3 Phytochrome and flowering

Short day plants

Two types of phytochrome reaction are distinguishable in the photoperiodic control of flowering in SDP. Initially there is a requirement for Pfr before the dark period, followed by the dark reaction in which the level of Pfr is depleted, after which floral induction proceeds. Secondly there is the night-break reaction which prevents flowering by interrupting the dark reaction.

- ***Promotive effects of Pfr***

At the end of the light period most of the phytochrome is present as Pfr and a brief exposure to FR at this time or at the beginning of the dark period will inhibit flowering. The promotive effects of Pfr on flowering were first shown in *Pharbitis* where the application of FR at the end of the photoperiod depressed flowering. This inhibition could also be

reversed by subsequent exposure to R (Takimoto and Hamner 1965). Pfr is therefore required for flowering and its removal early in the dark period inhibits flowering (Vince-Prue 1975).

- *Night-break reaction*

In many SDP, interrupting the inductive dark period with a brief exposure to light at a particular time will inhibit or prevent flowering. Red light has little effect on flowering at the end of the day or during the first few hours of darkness, however after several hours a night-break with R will inhibit flowering (Vince-Prue 1975). The Pfr formed in response to R is therefore inhibitory to flowering at this time. This implies that a reduction in the Pfr level after a certain period is required for floral induction. The time of maximum sensitivity to night-breaks varies between plants. In *Pharbitis nil* they are most effective 8-10 h after the end of a continuous photoperiod (Vince-Prue and Gressel 1985).

The inhibitory effect of R may be reversed if Fr is applied after R, and the reaction is repeatedly reversible (Takimoto and Hamner 1965). This phenomenon has been demonstrated in many SDP including, *Xanthium* (Borthwick *et al.* 1952) and *Chrysanthemum morifolium* (Cathey and Borthwick 1957). In *Xanthium* flowering is almost completely restored if 1-2 min of Fr is given immediately after R, but the treatment is less effective if a period of darkness is interposed. The effectiveness of Fr in reversing a night-break with R is often influenced by conditions during the photoperiod. For example, Fr reversal will not take place in *Pharbitis* if the photoperiod is extremely short (2 h),

or if the light intensity is low (Fredericq 1963).

Phytochrome may therefore have a dual action on flowering in SDP requiring a diurnal variation in the Pfr level. High levels of Pfr at the end of the day and during the beginning of the dark period are initially required, followed by the reduction of the Pfr level below some threshold level. If these reactions are interrupted by R or in some cases FR, floral induction fails.

Long day plants

Briefly interrupting the dark period by periods of light does not generally promote flowering in LDP. As a rule, much longer light exposures at higher light intensities are required for floral induction (Bernier *et al.* 1981a). Photoperiodic induction of flowering in LDP has not been as extensively studied as that in SDP (Deitzer 1984), however there is evidence to suggest that flowering responses in LDP are also governed by diurnal changes in the level of Pfr. LDP require a low Pfr level early in the daily cycle and a high Pfr concentration later in the night, as evidenced by the night-break effect (Vince-Prue 1975). For example, in *Lolium*, 8 h day extensions of low-intensity R marginally accelerate flowering compared with control plants in SD, but flowering is markedly promoted when the light extension is interrupted for a few hours with FR in the early part of the extension (Vince 1965). These results suggest that shortly after the end of a period in sunlight, a high concentration of Pfr in the leaf is inhibitory to flowering (Vince-Prue 1975). This dual response to Pfr is similar to that

already discussed for SDP, except that the sequence of promotion and inhibition by Pfr is reversed. A diurnal change in the level of Pfr is not however essential to flowering in LDP and floral induction will proceed with a mixture of R and FR irradiation (Bernier *et al.* 1981a). In fact, flowering in LDP often proceeds most rapidly in continuous light which contains a suitable mixture of R and FR (Vince-Prue 1975). It is therefore possible that an intermediatory state exists in which both low and high Pfr reactions can proceed (Bernier *et al* 1981a).

Summary

The relative proportions of Pfr to the total quantity of phytochrome present is critical in determining plant responses. The photoreactions which control the induction of flowering in LDP and SDP are similar. Both SDP and LDP require high and low levels of Pfr for flowering, but the sequence in which high and low Pfr reactions take place is different in the two groups. In SDP the high Pfr concentration is required late in the light period or early in the dark period followed by a requirement for reduced levels later in the dark period. In LDP, low Pfr concentrations are required on transfer to darkness and higher concentrations are necessary later. The alternation of Pfr levels is not however an absolute requirement for flowering since floral induction will take place under some circumstances in both continuous light and darkness in both SDP and LDP. This suggests that both the high and low Pfr reactions can proceed at some intermediatory level (Vince-Prue 1975, Bernier *et al.* 1981a).

2.1.2.4 Time measurement

The two major models postulated for the accurate measurement of time by plants are the hour-glass timer and the endogenous rhythm. The first views the clock as an hour-glass which measures time by the accumulation or destruction of some unknown product. The second implicates the circadian rhythm (Vince-Prue 1975, Bernier *et al.* 1981a).

Hour-glass timer

This theory was formulated to account for the fact that in certain SDP it is not the duration of the light period which is the important factor in floral initiation but that of the dark period, and that flowering proceeds after exposure to a critical night length, independant of the associated light period (Vince-Prue 1975, Bernier *et al.* 1981a). It is suggested that an unknown substrate decays during the dark period until it falls below a threshold level at which point other reactions may start, leading ultimately to flower formation. Light would invert the hour-glass and reinitiate the process (Bernier *et al.* 1981a).

- *Phytochrome reversion*

It has been proposed that the dark reversion of phytochrome may act as an an hour-glass timer, with inductive reactions commencing when nearly all of the Pfr has reverted to Pr (Bernier *et al.* 1981a). The critical night length may then represent the time taken for the concentration of Pfr in the leaves to fall below a threshold value which no longer inhibits

flowering in SDP nor promotes flowering in LDP (Vince-Prue 1975).

Endogenous rhythms

The second approach to time measurement in photoperiodism incorporates endogenous rhythms. Bünning (1967) was the first to propose that circadian endogenous oscillations effect the measurement of time in photoperiodism. Three approaches have been used to investigate the existance of these rhythms; cycle length, light breaks in long dark periods and skeleton photoperiods.

Hamner and Takimoto (1964) showed that when constant photoperiods of 8 h were combined with dark periods of various durations, flowering in *Glycine* cv. 'Biloxi' was inhibited in cycle lengths of 36 and 60 h, while optimal flowering occurred in cycle lengths of 24, 48 and 72 h. A rhythmic response is clearly present.

Flowering in certain plants exposed to long dark periods interrupted by short periods of light, is related to the timing of the light breaks. Light breaks near the middle of the dark period promote flowering in the SDP soybean (Carpenter and Hamner 1963) and inhibit flowering in the LDP *Sinapis* whilst light interruptions at the beginning or end of the dark period will promote flowering in this species (Kinet 1972). Rhythmic flowering responses to light perturbations of long dark periods has not however been recorded in all species (Bernier *et al.* 1981a).

Some circadian rhythms can be entrained to skeleton

photoperiods which begin and end with a short light pulse, one coincident with the start and the other with the end of the complete photoperiod (Vince-Prue 1975, Bernier *et al.* 1981a). The flowering response in relation to skeleton photoperiods has so far been almost exclusively demonstrated in *Lemna* (Vince-Prue 1975). It is postulated that a photoperiodic rhythm of sensitivity to light begins on transfer to darkness and that flowering is inhibited when light falls at a particular light-sensitive phase of the rhythm (Vince-Prue 1975).

- *Phytochrome and circadian rhythms*

The involvement of phytochrome in circadian rhythms remains unresolved (Vince-Prue 1975, Bernier *et al.* 1981a). There is some evidence to suggest that a rhythm of sensitivity to a decreased Pfr/P ratio is present and that R reversion may set the phase of an endogenous rhythm (Bernier *et al.* 1981a).

Summary

Transitions from light to dark must be percieved by photoperiodic plants and phytochrome is a contender for such a function. The kinetics of phytochrome reversion in the flowering process however, remains unresolved due largely to the lack of an assay for induction in the leaf, as direct spectrophotometric measurements cannot, at present, be made in green leaves (Vince-Prue 1975, Bernier *et al.* 1981a). The two timers; hour-glass and circadian rhythm may not be mutually exclusive and a combination of both may be involved in the photoperiodic control of flowering (Bernier *et al.* 1981a).

2.2 Floral evocation

In this review, floral evocation is defined as the event or events in the apex required for commitment to initiate flower primordia. It is the first stage of morphological change to floral organ production following floral induction.

2.2.1 Macromorphological events at the shoot apex

Morphological events which occur at the shoot apex during the floral transition are regarded as symptoms of flowering. The precocious initiation of axillary buds, increased elongation of young internodes, changes in leaf size and form (bract formation), modified phyllotaxis, increased rate of initiation of primordia, and enlargement and doming of the meristem (Bernier *et al.* 1981b, Lyndon and Francis 1984).

2.2.1.1 Precocious initiation of axillary buds

Precocious initiation of axillary buds is an almost universal event of the floral transition, and seems to be associated with a loss of apical dominance (Lyndon and Francis 1984). The axillary buds form closer to the apex in prefloral shoot apices compared with vegetative ones and branch out, forming flowers or inflorescences (Thomas 1961). In *Sinapis* precocious axillary buds form before the meristem reaches the prefloral stage (Bernier *et al.* 1981b). Their formation is however, an insufficient component of evocation since spikelet primordia (precocious axillary buds) are formed in 'Winter Petkus' rye after GA3 (Gibberellic Acid) application without

further floral differentiation (Bernier *et al.* 1981b).

2.2.1.2 Stem elongation

Rapid internode elongation or "bolting" is considered by many workers to be the earliest indication of the reproductive transition. It is most obvious in plants which possess a rosette growth habit during the vegetative stage eg. *Rudbeckia* (Murneek 1940), but is also observed in caulescent plants eg. *Chenopodium amaranticolor* (Thomas 1961). Although stem elongation and flower initiation are usually associated processes they may also be separated in many plants by appropriate treatments. For example flowering may be achieved in the absence of bolting in the rosette LDP *Rudbeckia* by growth in SD at high temperature (Murneek 1940), and the application of GA3 can stimulate stem growth in many rosette LDP without inducing flowering (Vince-Prue 1975).

2.2.1.3 Leaf growth and phyllotaxis

In many plants the transition from vegetative to floral growth is marked by a change from the production of large vegetative leaves to smaller foliar appendages and bracts (Bernier *et al.* 1981b). Thomas (1961) however, reported that plants of *Chenopodium amaranticolor* exposed to inductive conditions initially displayed a stimulation of leaf growth and petiole elongation which was followed by a dramatic inhibition. A change to a higher order of phyllotaxis, coincident with flower initiation, may also be observed during evocation and is most spectacular in species bearing terminal flowers. For example the

onset of flowering in *Impatiens balsamina* is accompanied by a change in primordium arrangement from spiral to whorled (Battey and Lyndon 1984). Lyndon (1978) studied the positioning of subsequent primordia in the terminal flower of *Silene coeli-rosa* and proposed that the apical geometry of all of the primordia within the flower was ultimately traceable to the position of the sepals. There is no doubt that the geometry of the apical meristem changes at evocation, but these changes may not necessarily imply commitment to flower. This is suggested by the fact that applications of GA3 to vegetative meristems of *Xanthium* may produce floral phyllotactic changes without flower formation, and that reversions to vegetative growth are possible in many species (Bernier *et al.* 1981b).

2.2.1.4 Rate of primordia initiation

An increased rate of leaf initiation (or reduction in plastochron) is observed in many plants prior to floral initiation eg. the SDP *Kalanchoe* and the LDP *Sinapis* (Langer and Bussell 1964). It is suggested that this stimulation together with apical elongation is an essential step in the morphological sequence by which flowers are produced (Langer and Bussell 1964). The shortening of the plastachron also appears to coincide with the reduction in size of the primordia at initiation relative to the apical dome (Lyndon 1977, Battey and Lyndon 1984, Lyndon and Battey 1985). At the reproductive stage there is also an increase in the rate of primordia production, such as bracts and floral organs. Lyndon (1979) reported that the plastochron during sepal initiation of *Silene* was shorter than for leaf initiation, and tended to be even shorter during initiation of stamens and

petals. Flower primordia are also produced at a faster rate than leaf primordia in *Chrysanthemum* (Schwabe 1959). As a general rule, when a flower is formed the primordia of floral organs are smaller either relative to the meristem or in absolute size, thus permitting a closer arrangement of primordia which is essential for a change to higher orders of phyllotaxis (Lyndon and Francis 1984, Lyndon and Battey 1985).

2.2.1.5 Changes in meristem size and form

Vegetative meristems are commonly flattened domes but at the time of, or shortly preceeding, the initiation of the first flower primordia, a marked "doming" of the apical meristem can be observed. Enlargement of the apical dome results from an increasing proportion of tissue being allocated to the apical dome rather than to the primordium of each plastachron (Lyndon 1977, Lyndon and Battey 1985). This increase in height relative to width has been reported in plants of various response types including the SDP *Chrysanthemum* (Cathey and Borthwick 1957) and the cold requiring species *Lilium longiflorum* (de Hertogh *et al.* 1976). It is not however a universal event since the size of the apical dome decreases in some species prior to floral initiation eg. *Perilla* (Nougarède *et al.* 1964). Meristem enlargement may also occur without flower initiation and is particularly observed in plants grown in non-inductive conditions (Lance 1957). Later changes in shape and size are related to essential features of the reproductive structures eg. heightening is typical of cereals (Kirby 1974) and broadening of Asteraceae (Nougarède 1967). The diameter of the apical dome of the quantitative SDP *Chrysanthumum morifolium* at the transition to flowering

(appearance of first bract primordia) is 0.23 mm when grown in SD and only slightly larger, 0.26 mm when grown in LD, and each step in the further development of the inflorescence is also associated with a narrow range of apical sizes (Horridge and Cockshull 1979).

2.2.2 Histological events at the apical meristem

Shoot apical meristems are comprised of a number of distinct zones and the number, size and shape of these zones may differ dramatically between the vegetative and reproductive state. The shape of the meristem itself is governed by the directions of growth, as reflected in the planes of cell division and by the rate of cell division within each zone (Lyndon 1968, 1970). Progressive transformation of the vegetative meristem ultimately gives rise to the reproductive meristem (Bernier *et al.* 1981b).

2.2.2.1 Vegetative meristem

A typical zonation pattern for a vegetative meristem is shown in Fig. 2. Three zones are generally recognised: (i) the "central (axial) zone" which includes the axial portion of the tunica and the upper, central portion of the corpus; (ii) the "peripheral zone" which surrounds the central zone and includes the tunica and corpus cells located in the flanks of the meristem (all leaf primordia are derived from this region); (iii) the "pith-rib meristem" which lies below the central zone and constitutes the forerunner of the pith (Gifford 1963, Bernier *et al.* 1981b).

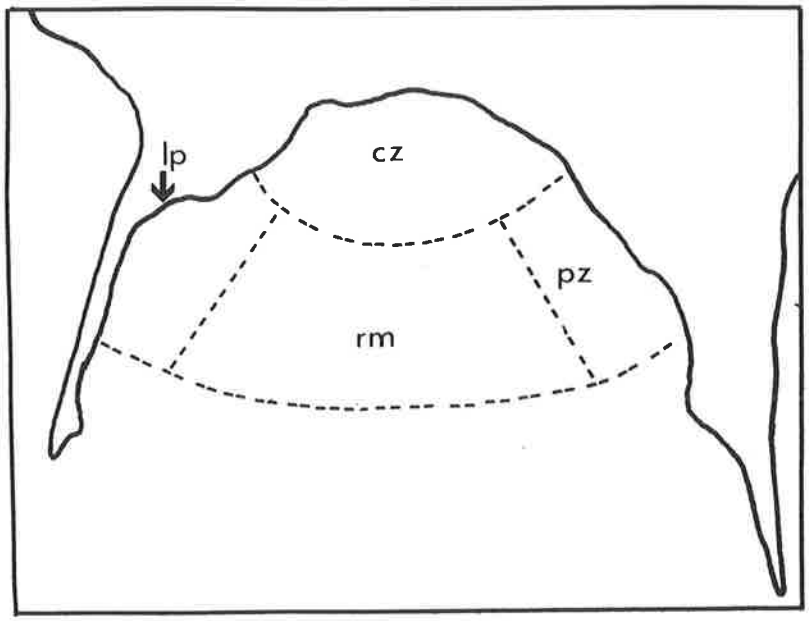

Figure 2.2 Vegetative meristem of *Chrysanthemum segetum*. Longitudinal section showing zonation pattern; central zone (cz) ringed by the peripheral zone (pz) from which leaf primordia (lp) are initiated, and the pith-rib meristem (rm) lying just below the central zone. (Adapted from Lance 1957.)

Gifford and Tepper (1962) reported that cells in the central zone of *Chenopodium album* contained lower concentrations of RNA than those of the peripheral zone and concluded that this probably reflected the mitotic activity of the cells in the various regions. Indeed central zone cells of *Chrysanthemum* are reported to divide at low frequency and have a long G1 (pre DNA synthesis) phase (Nougarède and Rembur 1978). The central zone is often referred to as the "méristème d'attente" by french workers who argue that the zone is relatively inactive during the vegetative stage and is "waiting" for the onset of reproductive development to become activated (Nougarède 1967). Other workers

however envisage these cells as "initials" which give rise to all other cells in the meristem (Gifford and Corson 1971).

2.2.2.2 Prefloral and floral meristem

A reorganisation of the vegetative meristem takes place during the floral transition and a "prefloral stage" is observed in many species. A universal feature of this stage is an increase in the rate of cell division in both the central and peripheral zones (Lyndon and Francis 1984). The prefloral stage is considered to be an essential feature of floral evocation since it is reported in a range of species with differing floral arrangements and is independent of photoperiod or vernalisation requirements (Bernier *et al.* 1981b).

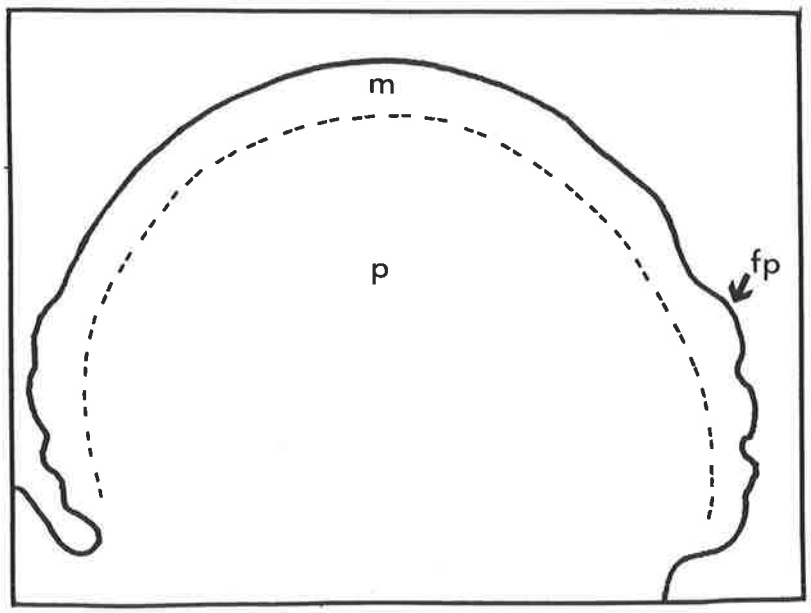

Figure 2.3 Floral meristem of *Chrysanthemum segetum*. Longitudinal section. A uniform meristematic mantle (m) covers the central core of parenchymatous cells (p) with floret primordia (fp) initiated on the flanks of the meristem. (Adapted from Nougarède 1967.)

Lance (1957) found that the first detectable change in the meristem of *Chrysanthemum segetum* was an increased activity in the cells of the central zone corpus. Cell activation then spread to all regions and the zonation typical of vegetative meristems was no longer detectable. This was followed by vacuolation and elongation of the cells in the pith rib meristem. The central parenchymatous core continued to increase and a layer of meristematic cells was formed over the entire surface, creating a uniform mantle of cells from which floret primordia were initiated (Fig. 3).

2.2.2.3 Intermediate meristem

Meristems of some photoperiodic species grown in unfavourable conditions for flowering attain a configuration which is "intermediate" between the vegetative and reproductive state ie. some of the changes observed in inductive conditions appear to be in progress in unfavourable photoperiodic conditions. Lance (1957) reported that the quantitative LDP *Chrysanthemum segetum* when grown in SD had a typical vegetative configuration during the first 14 plastochrons after which there was an increase in meristem size and cell number in all zones and increased mitotic activity in the central zone, resulting in the gradual elimination of the typical zonation pattern of the vegetative meristem. Mitotic activity and cell DNA content in both the central and peripheral zones of *Aster sinensis* are also higher in intermediate than vegetative meristems (Nougarède and Rembur 1979). Lance (1957) proposed that the intermediate stage was preparatory to flowering since meristems in the intermediate

condition reached the reproductive state sooner when transferred to inductive conditions, than typically vegetative meristems grown exclusively in inductive conditions. The intermediate structure is not however an obligatory step towards flowering since it is not observed in meristems exposed exclusively to inductive conditions (Nougarède *et al.* 1964). The exact role of the intermediate condition in the flowering process is unclear (Bernier *et al.* 1981b).

2.2.3 Molecular and ultrastructural events during the floral transition

A number of molecular events occurring during the floral transition are common to a variety of photoperiodically sensitive plants. These include; increased rate of RNA and protein synthesis, increased activity of several enzymes; invertase, phosphatase and ribonuclease, and an increase in respiratory substrates eg. soluble sugars (Bernier 1971, Miller and Lyndon 1977, Auderset *et al.* 1980, Bernier *et al.* 1981b, Lyndon and Francis 1984, Auderset *et al.* 1985).

Some of the earliest detectable ultrastructural changes in the LDP *Sinapis* and the SDP *Xanthium* include increased cellular, cytoplasmic, and nucleolar size, changed nucleolar structure and an increased number of mitochondria (Havelange and Bernier 1974, Havelange *et al.* 1974, Havelange 1980). These changes are considered to be essential for floral evocation due to their universality (Havelange 1980). The replacement of large vacuoles by an increased number of smaller vacuoles in the cells of evoked meristems is also observed in *Sinapis alba*, and implies

increased membrane synthesis (Havelange *et al.* 1974).

2.2.4 Cell division at the apical meristem

2.2.4.1 Synchronisation of the cell population

The cell population in meristems is generally regarded as asynchronous, ie. at any one time cells are in all phases of the mitotic cycle including G1 (pre-synthetic interphase), S (DNA synthetic phase), M (mitoses), and G2 (post-synthetic interphase). A dramatic rise in the number of mitotic figures present in the apical meristem is seen during the floral transition. Bernier *et al.* (1967) reported two peaks in the mitotic index of *Sinapis* following induction by a single LD. A rise in the mitotic activity began in the peripheral zone around 18 h after the start of the inductive LD, and 4 h later in the central zone, with a peak in both zones at 26 to 30 h. A second mitotic wave then peaked at 62 h when flower primordia were initiated. Between the two mitotic peaks there was a dramatic rise in the DNA synthesis index in both the peripheral and central zones which peaked at 38 h. Since the mitotic index increased prior to the DNA synthesis index, it was postulated that an early response to induction in *Sinapis* was the release of cells from the G2 phase, or post-DNA synthesis phase, into mitosis ie. shortening of G2. This was later confirmed by Jacqmard and Miksche (1971) using Feulgen cytophotometry. They established that vegetative meristems of *Sinapis* contained more nuclei with the 4C amount of DNA (G2 nuclei) than those with a 2C DNA content (G1 nuclei). There was also a major shift in nuclei from the 4C to the 2C condition in evoked meristems such that at

30 h, 65-70 % of the cell population was in G1. This correlates well with the completion of the first mitotic wave as reported by Bernier *et al.* (1967). Synchronisation of cell division in the shoot apex of *Silene coeli-rosa* (LDP requiring seven consecutive LD for induction) has also been observed during the eighth day after the beginning of LD treatment (Francis and Lyndon 1978 a,b, 1979). In this case an estimated 20 % of cells became synchronised in induced plants, and was associated with a shortening of both the G1 and G2 phases.

The occurrence of successive peaks in the mitotic index of apices undergoing floral induction suggests that synchronisation of cells into a particular phase of the cell-cycle may be an important feature of evocation in the shoot apex.

2.2.4.2 Rate of cell division

The cell doubling time within the meristem may be calculated from the accumulation of metaphases ie. the number of cells dividing per unit time, after colchicine treatment. Bodson (1975) using this technique reported that the cell doubling time in the LDP *Sinapis* was reduced in all regions of the apex, during the transition to flowering. This observation has since been made in several unrelated species and implies that an increased rate of cell division may be essential to floral evocation (Bernier *et al.* 1981b). A decrease in the cell doubling time may be due to a decrease in the length of the mitotic cycle and/or to an increased proportion of dividing cells.

The duration of the mitotic cycle has been investigated

using pulse labelling with tritiated thymidine by Francis and Lyndon (1978b). They concluded that the cell-cycle of *Silene* was shortened upon transfer to inductive LD, and was a result of a decrease in the length of G1 and perhaps S, whilst G2 and M remained constant.

Some investigations indicate that the assumption that all meristematic cells are cycling is valid. In the shoot meristem of *Chrysanthemum* for example there is no evidence that non-cycling cells are present since the cell-cycle duration is nearly identical to the cell doubling time (Nougarède and Rembur 1977). However, in the case of *Sinapis* there is evidence to suggest that both cycling and non-cycling cells are present within the meristem since the cell-cycle duration is about 2.5 times shorter than the cell doubling time (Gonthier *et al.* 1985). In this case cycling and non-cycling cells were randomly distributed throughout the meristem. Non-cycling cells have also been found in root meristems where they may be grouped in one particular zone, the quiescent centre (Clowes 1976, Taylor and Clowes 1978).

2.2.5 Sequence and nature of evocation

2.2.5.1 Sequence of evocation

It is difficult to determine which events are essential and which are incidental to flowering. Floral evocation has been classically viewed as a response to a single floral hormone which produces a specific event which then switches on a series of evocational changes (Bernier *et al.* 1981b). This hormone has yet to be identified. It is more likely that evocation is a

multisequential process since many evocational events appear to be unrelated and occur in different parts of the apex or in different cell components (Bernier *et al.* 1981b). The general occurrence of a multitude of events of evocation suggests that they are all necessary for flower initiation, although it has been possible to inhibit some of them without inhibition of flowering (Lyndon and Francis 1984). Evocational events may also occur in the absence of floral initiation, where plants subjected to suboptimal conditions may attain "partial evocation" and "intermediate" meristems may be formed in plants held in non-inductive conditions. This suggests that evocation has started in non-inductive conditions but cannot be completed because not all of the endogenous controlling factors are available. Clearly full evocation does not necessarily follow the activation of only one of the component sequences. This suggests that there is no single initial event which can set in motion all subsequent events (Bernier *et al.* 1981b). Meristems do however reach a point where they are irreversibly committed to initiate flowers, and this suggests that the different sequences interact at some stage of the floral transition (Bernier *et al.* 1981b). Events such as; the doming of the meristem, increased RNA synthesis in the meristem, increased rate of cell division, and ultrastructural changes are therefore necessary but not determining components of the evocational process.

2.2.5.2 Irreversible commitment to flowering

The "point of no return" marks the end of evocation during the transition of the meristem. At this time the meristem is irreversibly committed to flower. The point of no return

might occur either when the last individual sequence has reached its own point of no return or when the last essential interaction between sequences has occurred during the transition to flowering (Bernier *et al.* 1981b). This is in accordance with the concept that the meristem is capable of pursuing several developmental pathways before becoming inexorably committed to any one of them (Halperin 1978, Bernier *et al.* 1981b). In some species irreversible commitment of the meristem to flowering occurs in advance of the onset of flower initiation. The "point of no return" in meristems of *Chrysanthemum segetum* and *Xanthium* for example is reported to be the prefloral stage (Lance 1957, Salisbury 1955). However it has also been observed that meristems in some suboptimally induced plants may regain a vegetative function resulting in the production of vegetative-reproductive interphases. This suggests that the reproductive stage is not stable indefinitely and that developmental pathways remain open for some time. In some photoperiodic and cold requiring species given marginal, interrupted, or otherwise perturbed induction, an almost complete reversal to vegetative growth may be seen. Close scrutiny of these interphasic structures however reveals that complete reversion occurs only in a few cases. In most instances, the mixed structures are indicative of partial evocation or of a return of reproductive apices to some, but not all, aspects of vegetative growth. The greater the number of reappearing vegetative characters in these formations the more complete has been the apex reversion (Bernier *et al.* 1981b).

Research concerning the phenomenon of apical reversion of the floral meristem is still very fragmentary. A number of authors have signalled its existence, however very little

morphological evidence and even less histological evidence is given to explain its occurrence. There is also considerable confusion in the literature as to whether the apical meristem itself has undergone a reversion from the reproductive to vegetative state or whether changes in the formation of axillary buds, are the subject of study. In the Asteraceae this situation would appear to be avoided since a terminal inflorescence is produced. However, Lance (1957) states "inflorescence reversion in Compositae (Asteraceae) is a rare event that is extremely difficult to produce experimentally".

Resumption of vegetative growth has been reported in the marginally induced SDP *Cosmos bipinnatus* (Asteraceae) after the formation of involucral bracts (Greulach 1942). These new shoots however exhibited many characteristic traits not found in purely vegetative shoots, such as abnormally short internodes, precocious branching, and alternate instead of opposite leaves.

Experimental reversion of the spikelet inflorescence is reported to be easily obtained in *Perilla* (Labiatae) and *Sinapis* (Cruciferae) upon transfer of suboptimally induced plants to unfavourable daylength regimes, and in *Cheiranthus cheiri* (Cruciferae) upon transfer to high temperature after thermoinduction at low temperature (Zeevart 1969, Bagnard *et al.* 1972, Diomaiuto-Bonnand 1972). Apical reversion in *Cheiranthus cheiri* is not accompanied by any structural change in the meristem, since it is apparently the same when in inductive or non-inductive conditions. It is clearly zonate and possesses a typical pith-rib meristem in both situations. It is questionable therefore as to whether the apical meristem itself has undergone a reversion or whether the authors are reporting changes in

axillary buds. On the other hand, in the case of *Sinapis* it is reported that reverted inflorescences keep a meristem that is structurally identical to a normal reproductive meristem characterised by the absence of the pith-rib meristem, a typical feature of the reproductive condition. Apical activity in reverted meristems of this species is retarded considerably, but this is not universal to reversions in other species. For example the terminal flower meristem of the SD plant *Impatiens balsamina* reverts to leaf initiation if plants are returned to LD during flower formation (Krishnamoorthy and Nanda 1968) but this reversion is not accompanied by a reversal in the increased apical growth rate, or change in phyllotaxis which accompany flowering (Battey and Lyndon 1984). The reverted apex is therefore a vegetative meristem that has a growth pattern like a flowering meristem (Battey and Lyndon 1986). It is postulated that reverted apices remain partially evoked since meristems which have reverted by transfer to LD conditions can be induced to re-flower immediately upon transfer to inductive SD conditions (Battey and Lyndon 1986).

2.3 Floral morphogenesis

In previous sections it was concluded that many events of floral evocation were universal. There is on the other hand great diversity in the structural changes which occur during inflorescence and flower morphogenesis.

2.3.1 Inflorescence morphology and flower development

There is a vast range of flower forms including solitary

and racemose types; raceme, spike, capitulum, corymb, umbel, cyme. Flower morphogenesis in many of these forms has been well documented using the dissecting and compound microscopes, and more recently the scanning electron microscope (S.E.M). Studies include the solitary flower of *Tulipa gesneriana* (Shoub and de Hertogh 1975) and *Citrus sinensis* (Lord and Eckard 1985,1987); the cyme of *Silene coeli-rosa* (Lyndon 1978); the spike of *Triticum aestivum* (Moncur 1981) and the capitulum of *Chrysanthemum morifolium* (Horridge *et al.* 1985). A detailed description of floral morphogenesis in all floral forms is beyond the scope of this review. Floral morphogenesis of the capitulum will however be considered in detail since it is characteristic of the Asteraceae.

2.3.1.1 The capitulum

The capitulum is a racemose inflorescence with florets initiated acropetally on an enlarged receptacle. It is simply a condensed shoot system developed from an apical meristem (Burtt 1971, Kinet *et al.* 1985). Each capitulum is surrounded by an involucre of bracts, within which many florets are mounted on a broadened receptacle, which simulates in appearance and function a single large flower (Blackmore and Toothill 1984). Capitula are often made up of two distinct types of florets: disc florets in which the corolla tube terminates in five short teeth; and ray florets, in which the corolla flattens out above a tubular base creating a strap or "ligule" (Cockshull 1985). When both types of floret are present, disc florets are found in the centre and ray florets are arranged around the edge giving the typical daisy flower (Blackmore and Toothill 1984). The involucre has several

functions: (i) protection of young flowers; (ii) protection of mature flowers by reclosure during unfavourable weather or at night; (iii) attraction of pollinators; (iv) protection of developing fruits; (v) release of ripe achenes, or their enclosure and dispersal (Burtt 1971). The death of the involucre is essential to release the interlocking of the involucral bracts and free the achenes enclosed within the head.

2.3.1.2 Sequence of initiation and development

Early stages in floral evocation of the Asteraceae up to the stage of receptacle formation have been described in section 2.2. The further development of the capitulum follows a general pattern with many variations in details, but is typically documented in *Chrysanthemum*. Cathey and Borthwick (1957) described a series of discrete developmental stages of the apex using the dissecting microscope and these have since been illustrated using scanning electron microscopy (Vermeer and Peterson 1979a, Cockshull 1985, Horridge *et al.* 1985). Development proceeds acropetally with numerous involucral bracts being initiated around the receptacle in a phyllotactic spiral. During each plastochron the primordia which develop as bracts do not increase in size as rapidly as developing leaf primordia. This results in the presence of numerous bract primordia of similar size in contrast to the difference in size between successive leaf primordia. Floret primordia initiation and development continues in a phyllotactic spiral beginning around the outer edge and proceeds acropetally such that florets reach anthesis earlier at the periphery than in the centre. Each floret also forms a centre of differentiation, producing floral

parts which are initiated acropetally in the order; sepals, petals, stamens, carpels (Horridge *et al.* 1985, Kinet *et al.* 1985). Florets are to be found in the axils of receptacular bracts in some members of the Asteraceae eg. *Helianthus annuus* (Schuster 1985) but these structures are absent from *Chrysanthemum* (Cockshull 1985). The central portion of the capitulum is gradually reduced in size as the tissue of the capitulum is committed to floret development, until the entire surface is covered with floret initials. The true apical meristem ceases to exist at this point (Horridge *et al.* 1985). Differentiation inside the floret primordium commences with the formation of a ring-shaped corona which eventually grows into the corolla tube (Kinet *et al.* 1985). Ray and disc florets may be distinquished at this time. Disc florets are initiated later than ray florets and have an actinomorphic tubular corolla resulting from the fusion of petal primordia. Ray florets have a zygomorphic ligulate corolla due to asymetrical growth of the corolla tube (Horridge *et al.* 1985, Kinet *et al.* 1985). Glandular trichomes may be observed on and between florets of *Chrysanthemum* (Vermeer and Peterson 1979a). Stamen initiation occurs at five points on the inner surface of the corolla tube, and the stamens are carried upwards as the tube elongates. At the same time pappus primordia may be observed on the outer surface of the floret. Carpel primordia arise at the base of the corolla tube with the formation of the ovary, style and stigma (Horridge *et al.* 1985, Kinet *et al.* 1985). *Chrysanthemum* ray florets are female, while disc florets are hermaphrodite and the proportion of each in the capitulum varies between cultivars (Cockshull 1985). Capitula in other members of the Asteraceae vary in composition eg. lettuce (*Lactuca*) is composed entirely

of hermaphrodite ray florets whilst male and female disc florets are found in separate heads of *Xanthium* (Kinet *et al.* 1985).

2.3.1.3 Reproductive biology in the Asteraceae

Self incompatibility or the failure of gametes from the same plant to form a viable embryo effectively enforces outbreeding in many plants (Brewbaker 1957). Members of the Asteraceae often exhibit a sporophytic type of incompatibility system (Heslop-Harrison 1975, Heslop-Harrison *et al.* 1975) ie. inhibition commonly occurs at the stigma surface so as to inhibit pollen germination or prevent pollen tube growth (Brewbaker 1957). In this system the behaviour of the pollen is imposed by the maternal genotype and as a result, all pollen grains from a given plant behave similarly (Brewbaker 1957). There is also evidence to suggest that sporophytic incompatibility is linked to trinucleate pollen grains such as are common in the Asteraceae (Brewbaker 1957).

Dichogamy, the presentation of pollen and stigmas within a plant at different times during the reproductive season, is a further outcrossing mechanism (Lloyd and Webb 1986). Both protandry (the presentation of pollen before that of the stigma) and protogyny (the presentation of stigmas before pollen) are found in the Asteraceae. In protandrous species possessing hermaphrodite florets, pollen is shed into the corrolla tube before the stigma is receptive and prior to style extension. In protogynous species, the marginal female florets open before the central hermaphrodite florets (Lloyd and Webb 1986).

Many members of the Asteraceae have a well developed

pollen presentation mechanism. This depends upon the delayed extension of the style within the corolla tube of the hermaphrodite protandrous florets. During style extension papillae or long hairs on the stigmatic branches sweep pollen out of the floret and present it to insect collectors well clear of the capitulum surface (Small 1915, van der Pijl 1978). The stigmatic lobes remain closely appressed during elongation so that the receptive stigmatic surfaces are not exposed for self-pollination. Some time after pollen presentation, when the flowers own pollen has been removed by insect foragers, the lobes diverge to expose the receptive surface of the stigma (Knox 1973). A vector is required both to remove pollen that is "presented" by the elongating stigma, and to transfer pollen to the receptive surface of the stigmatic lobes after they diverge. The aggregation of florets into capitula, and the presence of colourful involucral bracts suggests that foraging insects are likely vectors of pollen in the Asteraceae.

Pollen of members of the Asteraceae characteristically possess three germinal apertures (Wodehouse 1935). The grains are colporate, that is possessing furrows (colpi) which contain a germinal opening (pore) (Skvarla *et al.* 1977). Three major surface patterns are recognised: (i) psilate - having an almost smooth, relatively unadorned surface; (ii) echinate - with prominant spines; (iii) lophate - with the surface consisting of ridges and depressions (Skvarla *et al.* 1977). Besold (1971) and Leins (1971) examined nearly all described genera and some 800 species within the tribe Inuleae (including some species of *Helipterum* and *Helichrysum*) and concluded that pollen was echinate throughout the whole tribe.

2.3.2 Effect of light

2.3.2.1 Photoperiod

Flower development may have absolute or quantitative photoperiodic requirements depending on the species or cultivar (Kinet and Sachs 1984). Plants with an absolute requirement for a certain photoperiod will not develop flowers to maturity unless this parameter is provided ie. flower buds abort. In plants with a quantitative requirement, the rate of flower development is decreased and anthesis is delayed in unfavourable photoperiods (Kinet and Sachs 1984). In some plants the photoperiod requirement may change with the stage of development of reproductive organs (Vince-Prue 1975). Photoperiod requirements for initiation and development may also differ for the same species. Vince-Prue (1975) and Kinet *et al.* (1985) proposed the following five groups to encompass the photoperiodic responses of the flowering process.

1. Plants with the same photoperiodic requirement for initiation and development. Some cultivars of *Chrysanthemum morifolium* have a quantitative SD requirement for floral initiation and an absolute SD requirement for floret development (Schwabe 1951).

2. Plants with a photoperiodic requirement for initiation but which are DN for flower development. *Fuschia hybrida* is daylength indifferent for flower growth once initiation is completed in LD (Sachs and Bretz 1962).

3. Plants which are DN for initiation but which have a

photoperiodic requirement for flower development. *Bougainvillea* cv. 'San Diego Red' is a SDP for flower development but is DN for initiation (Hackett and Sachs 1968).

4. Plants with different photoperiodic requirements for initiation and flower development. *Delphinium ajacis* is a LDP for floral initiation whilst SD promote further development (Austin 1941).

5. Plants which are day neutral for both initiation and development.

Non-inductive photoperiods not only affect the rate of development and abortion of reproductive structures but may also induce reproductive abnormalities (Kinet and Sachs 1984, Kinet *et al.* 1985). Foliaceous bracts are frequently observed in capitula of Asteraceae when plants are transferred to unfavourable photoperiods after minimal induction (Kinet *et al.* 1985) eg. *Chrysantheumum morifolium* transferred to LD conditions (Schwabe 1951). The total reversion of the apical meristem to vegetative growth, upon transfer to non-inductive photoperiodic conditions, has already been covered in section 2.2.5.2 and will not be discussed again here except to reiterate that a given primordium within a reproductive structure is not committed at initiation, and that developmental pathways remain optional for some time (Kinet *et al.* 1985). Other abnormalities which commonly occur in *Chrysanthemum morifolium* include; floret distortion, formation of secondary inflorescences and the occurence of petaloid stamens (Cockshull 1985).

Photoperiod also influences sex expression in many

species eg. SD decrease the proportion of male to female flowers in *Cucurbita* (Nitsch *et al.* 1952) and enhance the female tendency in *Xanthium* (Léonard *et al.* 1981). The number of female ray florets in *Chrysanthemum morifolium* is increased by intercalating LD during the period of floret initiation, whilst the number of hermaphrodite disc florets is increased by SD (Post 1943). Appropriate light conditions are usually not required for the entire process of flower development but are essential for a limited time interval only (Kinet and Sachs 1984). Flower development may therefore be arrested by changes in photoperiod at any stage prior to some critical point after which daylength has no further influence (Kinet *et al.* 1985). For example, flower bud development in *Chrysanthemum* cv. 'Indian Summer' may be arrested by transfer to LD up to the stage of ovule formation (Schwabe 1951).

2.3.2.2 Light intensity

The time to anthesis and hence inflorescence development, is delayed by low light levels in many species. An example is the effect of light intensity on floral initiation and inflorescence development in the SDP *Chrysanthemum morifolium* cv. 'Bright Golden Anne' (Cockshull and Hughes 1971, 1972, Hughes and Cockshull 1971, Hughes 1973). Flowering in this species is earliest in high light/short day combinations (Hughes 1973). Receptacle formation is delayed and significantly more leaves are formed below the receptacle when the inductive SD treatment is conducted in low light at 31 J cm^{-2} day^{-1} compared to 125 J cm^{-2} day^{-1} (Cockshull and Hughes 1971). Floret development is retarded and the number of florets per capitulum reduced if

plants are transferred to low light conditions at the stage of floret formation (Cockshull and Hughes 1971). Transfer later, when further floret formation has ceased, does not affect flower development or delay anthesis. The flowering response is determined by the daily light total, and not by the irradiance since development proceeds at a similar rate in conditions of constant intensity as in conditions of fluctuating intensity, provided that the daily light totals are the same (Hughes and Cockshull 1971). Plants are capable of integrating the light received over two days. Hence low light intensity one day can be countered by high light intensity the next day (Cockshull and Hughes 1972).

Flower bud abortion in many species, including roses and some bulbous crops, is associated with low light intensity environments. Zieslin and Halevy (1975) reported that in 'Baccara' roses the percentage of flowers reaching anthesis decreased, and the percentage of blind shoots increased as the light intensity was reduced. Low light intensity or shading of *Gladiolus* decreases both the percentage of inflorescences and the number of florets per spike. The cultivar 'San Souci' is most sensitive at the four to six leaf stage, when the inflorescence is elongating and some florets are completely formed (Shillo and Halevy 1976). Sex expression may also be influenced by light intensity. For example, the number of female ray florets in *Chrysanthemum* cv. 'Bright Golden Anne' is increased with higher light intensities, and reduced if plants are transferred to lower light intensities at the onset of floret initiation, about 14 days after the start of SD (Cockshull and Hughes 1971).

Light intensity interacts with photoperiod in some

photoperiodic species. Bodson (1983) reported that flower bud abortion in SD in some *Azalea* cultivars could be prevented by increasing the irradiance. In *Bougainvillea* cv. 'San Diego Red', which is usually considered a SDP for flower development, high light not only promotes inflorescence development, but overrides the photoperiodic signal so that flowering is promoted in LD (Sachs and Hackett 1969).

2.3.3 Effect of temperature

Temperature may affect both the rate of floral development and the degree of floral abortion in certain species. Increases in temperature are generally reported to reduce the time to anthesis. For example, increasing day or night temperatures speed development in *Chrysanthemum* (Vince 1960, Lepage *et al.* 1984, van Ruiten and de Jong 1984). In the case of some bulbous species eg. *Tulipa*, a period of cold temperature is required before floral development can proceed at warm temperatures. In the latter stage, day/night temperatures above 18/14 °C advance anthesis (Dosser and Larson 1981). Very high temperatures are also known to prevent flowers from reaching anthesis. Floral abortion results in *Chrysanthemum* cv. 'Orange Bowl' when plants are transferred from 22/18 °C (day/night) to 30/26 °C (day/night) (Whealy *et al.* 1987). In a few species reducing temperature may also cause flower bud abortion. For example, the number of blind shoots in 'Bacarra' roses increases from zero to 13.6% with a reduction in night temperature from 18 to 14 °C with a day temperature of 28 °C (Zieslin and Halevy 1975). A decrease in soil temperature in the range 23 to 13 °C will also increase the incidence of flower bud atrophy in 'Baccara' roses

(Tsujita and Dutton 1983).

Warmer temperatures which have been shown to hasten anthesis in some plants may also have a detrimental effect on flower quality. This is evident in *Tulipa* where warm day/night temperatures (26/22 °C) produce smaller flowers and a greater incidence of stem topple, than cool temperatures (18/14 °C, day/night) (Dosser and Larson 1981). Bonaminio and Larson (1980) and Parups and Butler (1982) also demonstrated that reduced night temperatures improved stem length, flower diameter and fresh weight in several cultivars of *Chrysanthemum*, but the time to anthesis was delayed. On the other hand lowering night temperature from 18 to 13 °C during short day treatment of *Euphorbia pulcherima* had a deleterious effect on bract development. The bracts being delayed in maturity and reduced in number and size (Tsujita and Craig 1980).

The production of female flowers is favoured by low temperatures in many species eg. 'Acorn' squash, cucumber and gherkin (Nitsch *et al.* 1952). Male and female fertility is generally reduced by high temperature (Kinet *et al.* 1985).

Both temperature and photoperiod interact in their effect on flower development. The length of the photoperiod required for capitulum development in *Chrysanthemum* cv. 'White Wonder', 'Encore' and 'Snow' is increased with reduced mean daily temperature (Cathey 1954). Low temperature can therefore partly substitute for SD in these cultivars. In some cases changes in temperature may override the photoperiodic response. For example, in the SDP *Chamelaucium uncinatum* decreasing temperatures from 24/18 °C (day/night) to 20/14 °C (day/night)

promotes flower development in LD (Shillo *et al.* 1985). It should be noted that the temperatures which promote or inhibit the various stages of the flowering process are species and in some cases cultivar dependent.

CHAPTER 3. MORPHOLOGICAL ASPECTS OF FLORAL INITIATION AND DEVELOPMENT, AND REPRODUCTIVE BIOLOGY IN *Helipterum roseum* AND *Helichrysum bracteatum*

3.1 Introduction

There has been no detailed study of the floral development and reproductive biology of *Helipterum roseum* or *Helichrysum bracteatum*. Such studies in other plants, such as citrus (Lord and Eckard 1985, 1987) have contributed valuable information on the relationship between vegetative and reproductive development in relation to crop production. The aim of this study is to examine the development of the apical meristem from germination to anthesis by scanning electron microscopy, and to investigate the breeding system of these species. This will increase understanding of the reproductive process in relation to both their ecology and economic development.

3.2 Materials and methods

3.2.1 Plant material

Seeds of *Helipterum roseum* were obtained from Kings Park Botanic Gardens, Perth, Western Australia, and those of *Helichrysum bracteatum* from Black Hill Native Flora Nursery, Adelaide, South Australia. Both seed lots were originally collected from wild populations in Western Australia and had received no genetic improvement. The dominant form of *Helipterum roseum*, which bears inflorescences with magenta involucral bracts, was used in all experiments in this thesis where

selection was possible. Less than one percent of the *Helipterum roseum* seed lot was *Helipterum roseum* var. *nigropapposum*. Voucher specimens of a plant of each species have been deposited in the State Herbarium of South Australia (*Helipterum roseum* AD 98817248, *Helichrysum bracteatum* AD 98817240).

The following germination and planting procedures were used for all experiments reported in this thesis unless otherwise specified. Seeds of each species were germinated at 25 °C in petri dishes lined with moist filter paper. Seedlings of *Helipterum roseum* and *Helichrysum bracteatum* were planted seven and 14 days respectively after germination, when the two cotyledons had fully opened. Individual seedlings were planted into 15 cm pots containing a soil mix based on the University of California system (Baker 1957). The soil mix contained: 0.25 m^3 of coarse sand and 0.25 m^3 of peatmoss and the nutrients, potassium nitrate, 60 g; magnesite, 120 g; reverted super phosphate, 700 g; plaster of Paris, 460 g; potassium sulphate, 60 g; blood meal, 700 g; hydrated lime, 900 g. A liquid fertiliser (Thrive ® [1]) was applied monthly at the recommended rate (N:P:K, 31:4.57:8.71). Potted plants were placed in treatment environments on the day of planting. All experimental work reported in this thesis was conducted at the Waite Agricultural Research Institute, Glen Osmond, South Australia, Meteorological Station No. 023031, 34° 56′ S, 138° 35′ E. Experiment 1: floral initiation and development and experiment 2: reproductive biology, were commenced on 4 December 1986 and 1 July 1988 respectively. Plants were grown in a glasshouse with an average

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1. ® Trade Mark

daily temperature during the sampling period of 22.4 ± 1.7 °C [2] (Experiment 1) and 20.7 ± 1.4 °C (Experiment 2). The glasshouse was heated by hot water pipes and cooled by evaporative cooling. The average temperature was maintained with an automatic fan connected to a thermostat control. There was no supplementation to natural light. The glasshouse was whitewashed during the summer months to reduce extremes in temperature. Pots were arranged to achieve a plant spacing of 20 cm between rows and within rows.

3.2.2 Experiment 1: floral initiation and development

3.2.2.1 Scanning electron microscopy

One hundred and twenty seedlings were planted. Two plants were selected daily from day one of planting and the terminal apices were dissected with the aid of a stereo microscope. The leaves and some involucral bracts were removed where appropriate to facilitate viewing of the apical meristem. The dissected apices were immediately fixed in 3 % glutaraldehyde in 0.025 M phosphate buffer and 0.5 % caffeine for 24 h. The tissue was then dehydrated in a graded ethanol series (15,30,50,75,100%), critical-point dried, mounted on stubs and coated with gold (44 nm thickness) before observation with a scanning electron microscope (S.E.M.) (Cambridge stereoscan 250 MK3) operated at 20 kV. For later stages, florets were dissected from the developing capitulum and fixed individually. Fresh and dehydrated pollen were mounted on separate stubs with

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2. ± standard error

double-sided tape and coated with gold.

3.2.2.2 Measurements

The stage of apical development of each sample was recorded after viewing with the S.E.M. The number of days from planting to the first appearance of each stage was recorded. The height and diameter of the apical meristem were measured from micrographs taken from a minimum of three primordia within each developmental stage. Measurements of diameter were taken from the widest point on or above the line connecting the axils of the two most recently formed leaf or involucral bract primordia. Measurements of height were taken along the line perpendicular to the width, passing through the apex at its summit. Measurements included floret primordia when present.

The height of the apical meristem at inflorescence anthesis was determined by bisecting the receptacle of 10 replicates of each species. The diameter of the apical inflorescence (including the involucral bracts) was measured to the nearest millimetre on 10 randomly selected replicates of each species. The mean length of the longest involucral bract was then derived as the difference between the diameter of the apical inflorescence including the involucral bracts and the diameter of the floret area. Ten florets from each of three apical inflorescences of both species were removed at floret anthesis. Floret length (including the ovary) was measured using a stereo microscope with a calibrated eyepiece. The size of dehydrated pollen grains was established from micrographs by measuring the length along the longest axis of five randomly selected grains of

each species.

Plant height and diameter of the rosette plant *Helichrysum bracteatum* and plant height only of caulescent *Helipterum roseum* were measured with a ruler on a minimum of 10 replicates at each stage of apical development. Plant height was the length of the apical shoot, and plant diameter the breadth measured across the two longest leaves.

3.2.3 Experiment 2: reproductive biology

3.2.3.1 Experimental design

The breeding system of both *Helipterum roseum* and *Helichrysum bracteatum* was investigated by a series of four treatments.

Treatment 1. Tagged but otherwise untreated to test for seed set under glasshouse conditions.

Treatment 2. Bagged but otherwise untreated to test for requirement of insect vectors.

Treatment 3. Self pollination followed by bagging to test for self-compatibility.

Treatment 4. Cross pollination followed by bagging to test for cross-compatibility.

Four capitula from each of four plants of both species were included in each treatment.

Foreign pollen was excluded by placing a 14.0 x 10.5 cm white paper bag over the inflorescence, secured with a plastic twist tie. Entire capitula were used, the female florets of

Helichrysum bracteatum were not removed as they comprised less than 1 % of the total number of florets. Self and cross pollination was performed by hand with a small brush, in the latter case pollen was transferred from three plants used exclusively as a pollen source to the treated inflorescence. Both self and cross pollination commenced at anthesis of the inflorescence and continued until the last floret reached anthesis.

3.2.3.2 Measurements

Capitula were harvested into individual envelopes when the involucral bracts reflexed, just prior to seed ejection. The total number of florets, number of viable seed and the percentage seed set were recorded for each inflorescence. In these experiments fertile seed of *Helichrysum bracteatum* was plump and dark brown while sterile seed was shrivelled and white. Fertile seed of *Helipterum roseum* was swollen and firm unlike sterile seed which was withered and soft.

Seed diameter and length (not including the pappus) were measured using a stereo microscope with a calibrated eyepiece, on ten randomly selected seeds of each species. Mean seed weight was calculated from the average weight of three lots of 50 seeds.

3.3 Results

3.3.1 Experiment 1

The sequence of floral initiation and development was

similar for both *Helipterum roseum* (Figs 1-24) and *Helichrysum bracteatum* (Figs 25-47). At the earliest stages observed the terminal apex had two leaf primordia that were larger than the meristem (Fig. 1). The vegetative meristem continued to enlarge and produced leaf primordia in a spiral arrangement (Figs 2, 25). This was followed by doming of the apical meristem (Figs 3, 26) and the initiation of involucral bracts (Figs 4, 27, 28). The bracts were smaller than the leaf primordia, had a more pointed profile and developed into flattened structures. The initiation of involucral bracts was followed by the production of a few primordia on the receptacle which appeared to divide after initiation. These divisions were observed in both *Helipterum roseum* (Figs 5, 6) and *Helichrysum bracteatum* (Figs 29, 30). The lower portion of the primordium developed into an involucral bract and the upper portion developed into a floret primordium. Thereafter all developing primordia were potentially fertile florets (Figs 7, 31). By the time the inflorescences were obvious to the naked eye considerable development of the involucral bracts had occurred (Fig. 8) but floret initiation was not complete. The involucral bracts grew up and enclosed the capitulum. In the outer florets, perianth, anther and pappus hair development had commenced (Figs 9, 32). The pappus hairs developed from the receptacle of the epigynous flower (Figs 10, 32, 35, 40). The florets developed centripetally (Fig. 11) such that outer florets were more developed than inner florets. They arose in a genetic spiral on the capitulum and ultimately developed over the entire surface (Fig. 33).

Multicellular trichomes developed at the tips of the perianth in both species (Fig. 34) but were obscured by papillae

Figures 3.1-3.24 Apical development of *Helipterum roseum*. Figures 3.1-3.17 are scanning electron micrographs.

Fig. 3.1 Apical meristem at stage 1, small vegetative meristem, showing two leaf primordia (l). The cotyledons have been removed. Scale: 80 µm.

Fig. 3.2 Apical meristem at stage 2, large vegetative meristem, showing leaf primordia in a spiral arrangement (l). Several leaves have been removed. Scale: 40 µm.

Fig. 3.3 Apical meristem at stage 3, doming of meristem. Scale: 80 µm.

Fig. 3.4 Apical meristem at stage 4, initiation of involucral bracts (b). Scale: 100 µm.

Fig. 3.5 Apical meristem at stage 5, initiation of florets, showing developing involucral bracts (b), dividing primordia (p) and floret primordia (f). Several rows of involucral bracts have been removed. Scale: 100 µm.

Fig. 3.6 Developing primordia on the receptacle (apex top left) showing division of primordia (p). Scale: 40 µm.

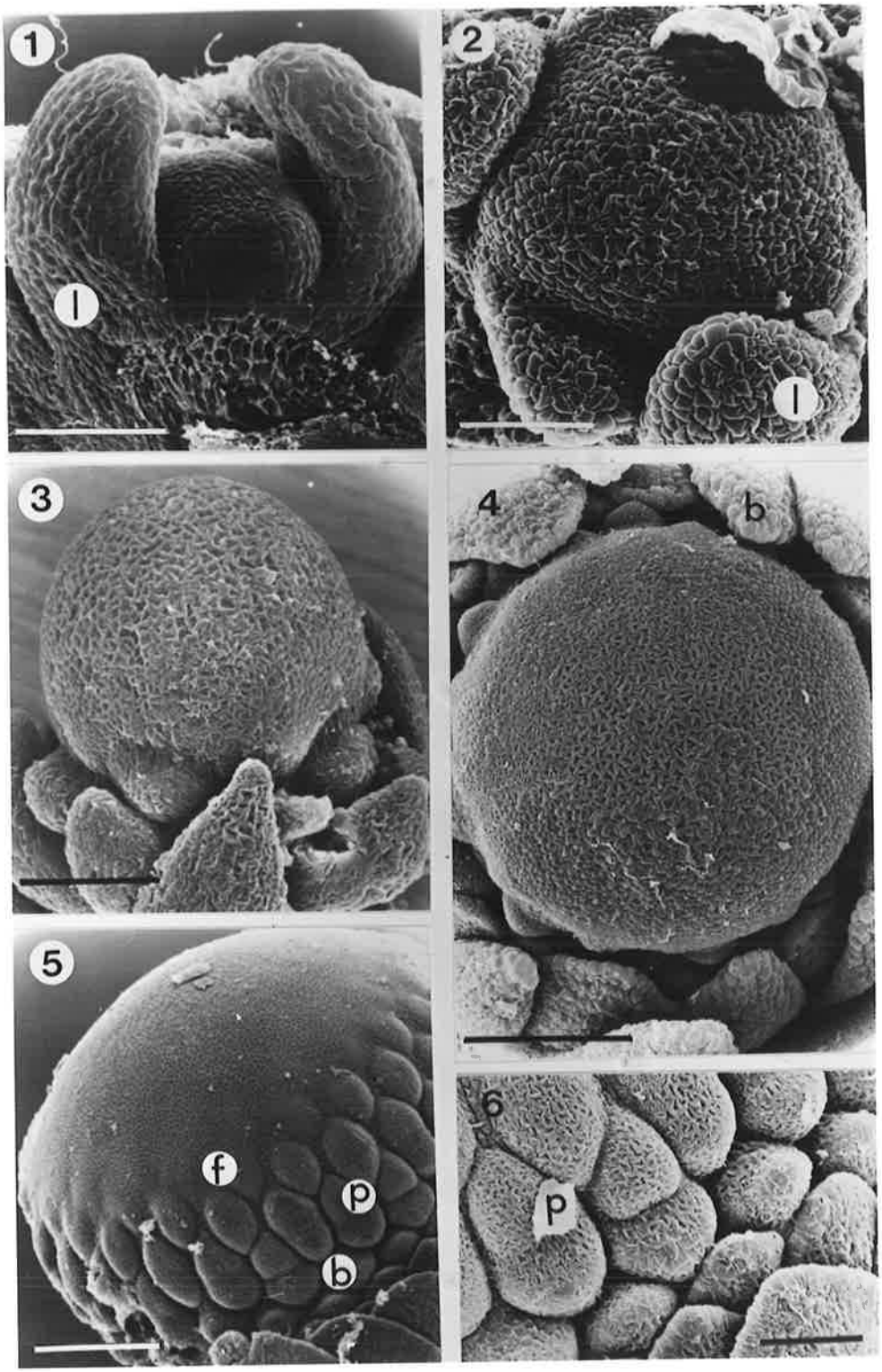

1
l
2
l
3
4
b
5
f
p
b
6
p

Fig. 3.7 Further development of primordia on the receptacle (apex top right) showing the development of the lower portion of the dividing primordium into an involucral bract (b) and the upper portion into a floret (f). Note the appearance of the two in the same plane, indicating that they arose from the same primordium. Scale: 50 µm.

Fig. 3.8 Apex at stage 6, macroscopic appearance of inflorescence buds, showing the involucral bracts (b) enclosing the developing florets (f). Scale : 800 µm.

All of the involucral bracts have been removed on the following samples to facilitate viewing of the apex.

Fig. 3.9 Developing capitulum showing continued initiation and differentiation of florets (f) and pappus hairs (pa). Scale: 400 µm.

Fig. 3.10 Excised florets showing pappus hairs (pa). Scale: 100 µm.

Fig. 3.11 Further development of the capitulum (apex top left) showing centripetal floret initiation and development; central florets soon after initiation (1), perianth development (2), anther development (3), continued perianth development and enclosure of the developing anthers (4). Scale: 100 µm.

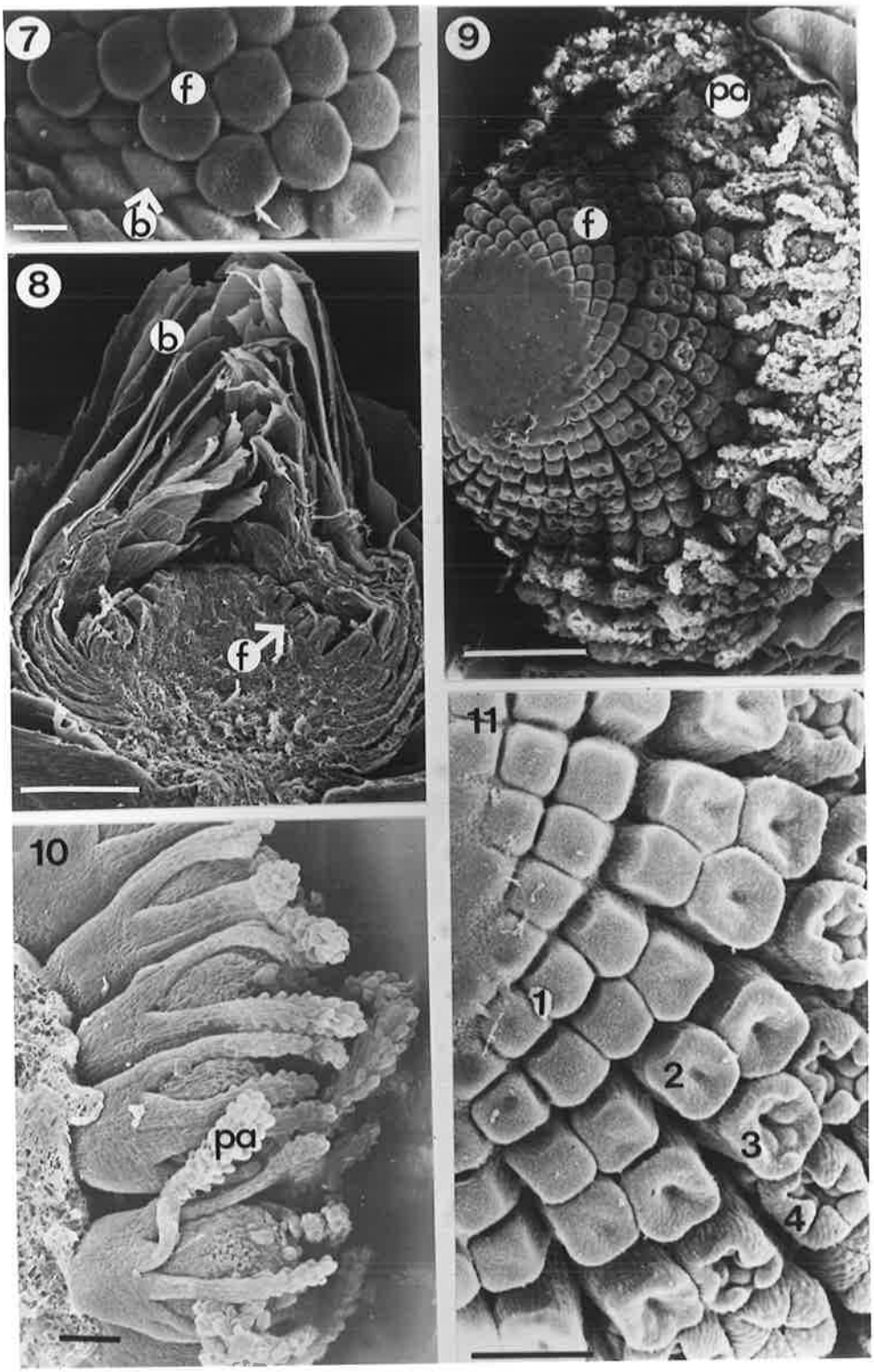

7
f
b
8
b
f
9
pa
f
10
pa
11
1
2
3
4

Fig. 3.12 Individual floret (apex top right) showing plumose pappus hairs (pa) and the elongated perianth (pe). Scale: 400 µm.

Fig. 3.13 Plumose pappus (pa). Scale: 100 µm.

Fig. 3.14 Tip of an individual floret at anthesis showing papillae (pp) and protruding anthers (a). Scale: 100 µm.

Fig. 3.15 Hydrated, colporate pollen grain showing echinate surface and operculum (op). Equatorial view. Scale: 5.0 µm.

Fig. 3.16 Hermaphrodite floret showing extended papillate stigma (st) with reflexed lobes and elongated papillae (pp) at the tips. Scale: 200 µm.

Fig. 3.17 Ovary bisected longitudinally, showing the hairs covering the ovary wall (o). Scale: 400 µm.

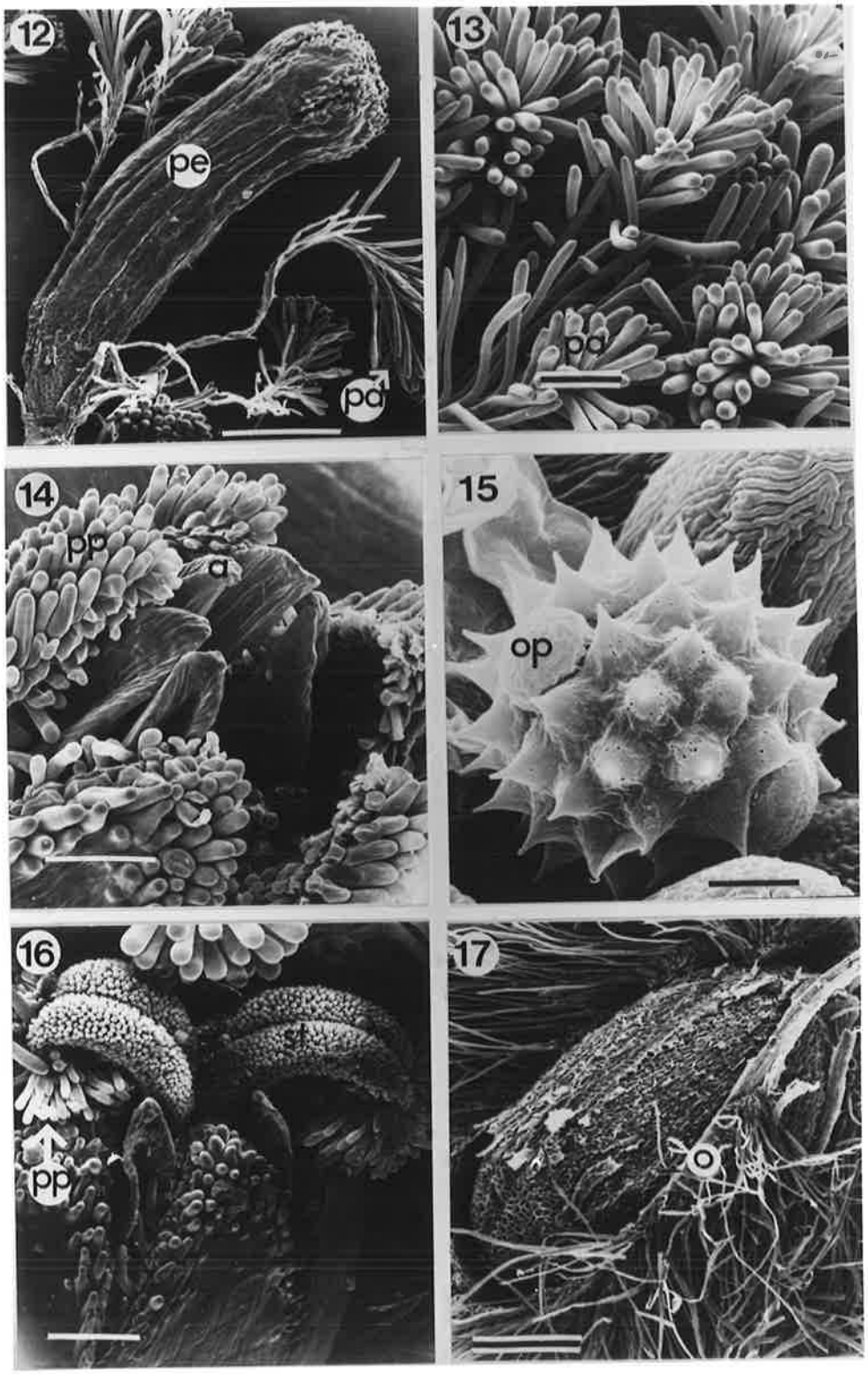

12
pe
pd
13
pa
14
pp
a
15
op
16
pp
17
o

Fig. 3.18 Inflorescence at anthesis showing the reflexed involucral bracts (b) surrounding the capitulum covered with florets (f). Note the presence of small involucral bracts dispersed amongst the peripheral florets. Scale: 40 mm.

Fig. 3.19 Abnormal inflorescence showing proliferation of bract scales and thickened peduncle (arrow). Growing conditions; daylength 12 h, temperature 20 $\pm$ 2.0 $^{\circ}$C, light intensity 250 W m^{-2}. Scale: 20 mm.

Fig. 3.20 Abnormal growth showing reversion to vegetative growth after the production of bract scales (arrow). Growing conditions; daylength 16 h, temperature 20 $\pm$ 2.0 $^{\circ}$C, light intensity 250 W m^{-2}. Scale: 20 mm.

Fig. 3.21 Plant at stage 1, small vegetative meristem. Scale: 5.5 mm.

Fig. 3.22 Plant at stage 3, doming of meristem. Scale: 45 mm.

Fig. 3.23 Plant showing macroscopic appearance of inflorescence buds (arrow), stage 6. Scale: 100 mm.

Fig. 3.24 Plant at anthesis, stage 7. Scale: 105 mm.


18
b
f


21


19


20


22


24
10a


23
12a

Figures 3.25-3.47 Apical development of *Helichrysum bracteatum*. Figures 3.25-3.40 are scanning electron micrographs.

Fig. 3.25 Apical meristem at stage 2, large vegetative meristem, showing leaf primordia in a spiral arrangement (1). Several leaves have been removed. Scale: 100 µm.

Fig. 3.26 Apical meristem at stage 3, doming of meristem. Scale: 40 µm.

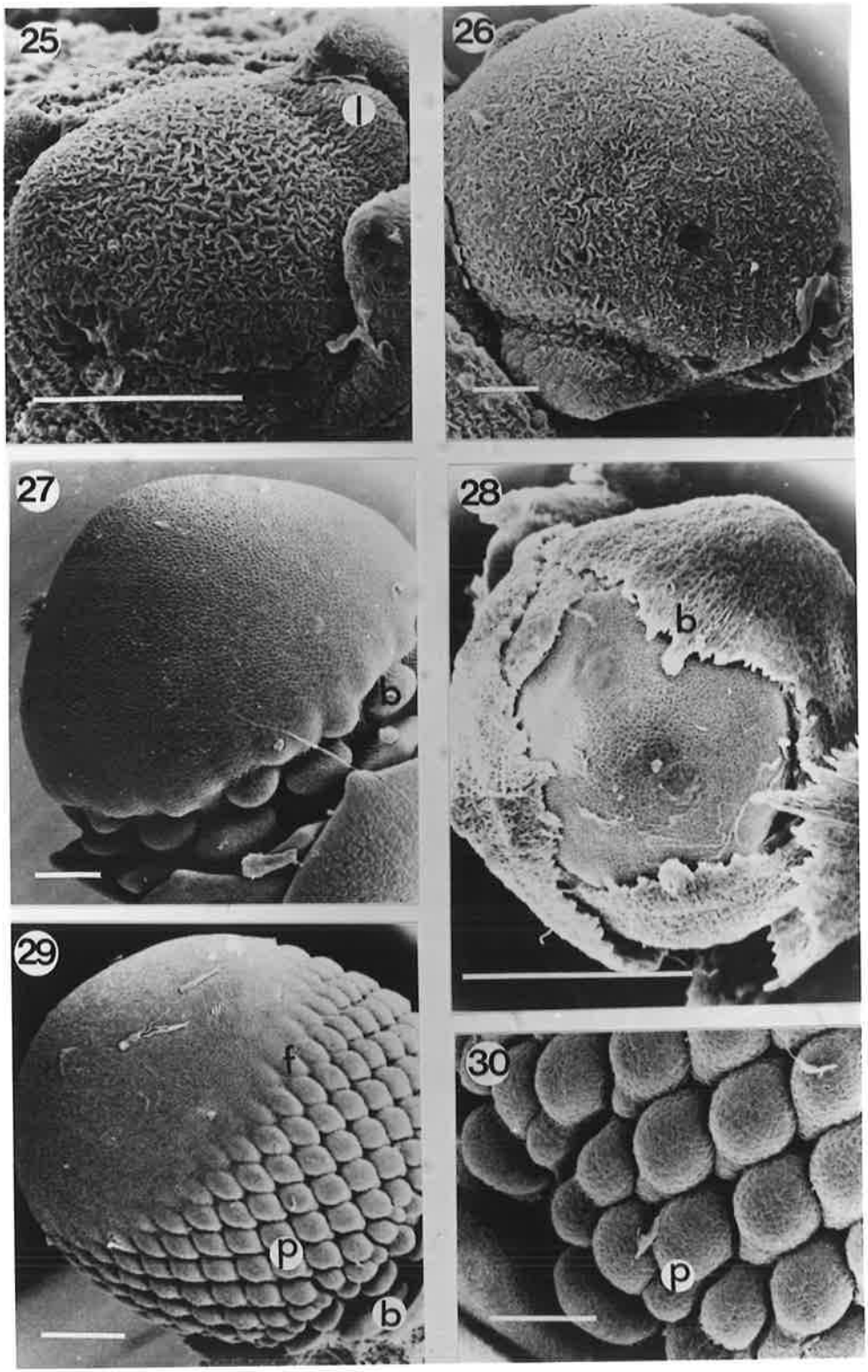
Fig. 3.27 Apical meristem at stage 4, initiation of involucral bracts (b). Scale: 100 µm.

Fig. 3.28 Apical meristem enclosed by involucral bracts (b). Scale 400 µm.

All of the involucral bracts have been removed on the following samples to facilitate viewing of the apex.

Fig. 3.29 Apical meristem at stage 5, initiation of florets, showing developing involucral bracts (b), dividing primordia (p) and floret primordia (f). Scale: 200 µm.

Fig. 3.30 Developing primordia on the receptacle (apex top right) showing division of primordia (p). Scale: 40 µm.


25
l
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b
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b
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p

Fig. 3.31 Apical meristem at stage 6, macroscopic appearance of inflorescence buds, showing developing florets (f). Scale: 400 µm.

Fig. 3.32 Floret on capitulum showing perianth (pe), anther (a) and the beginning of pappus development (arrow). Scale: 20 µm.

Fig. 3.33 Capitulum (apex centre bottom) covered with florets. Scale: 200 µm.

Fig. 3.34 Tip of an individual floret showing glandular trichomes (t) on the surface of the perianth (pe). Scale: 100 µm.

Fig. 3.35 Individual floret showing barbellate pappus hairs (pa) and the inferior ovary (o). Scale: 400 µm.

Fig. 3.36 Barbellate pappus (pa). Scale: 40 µm.

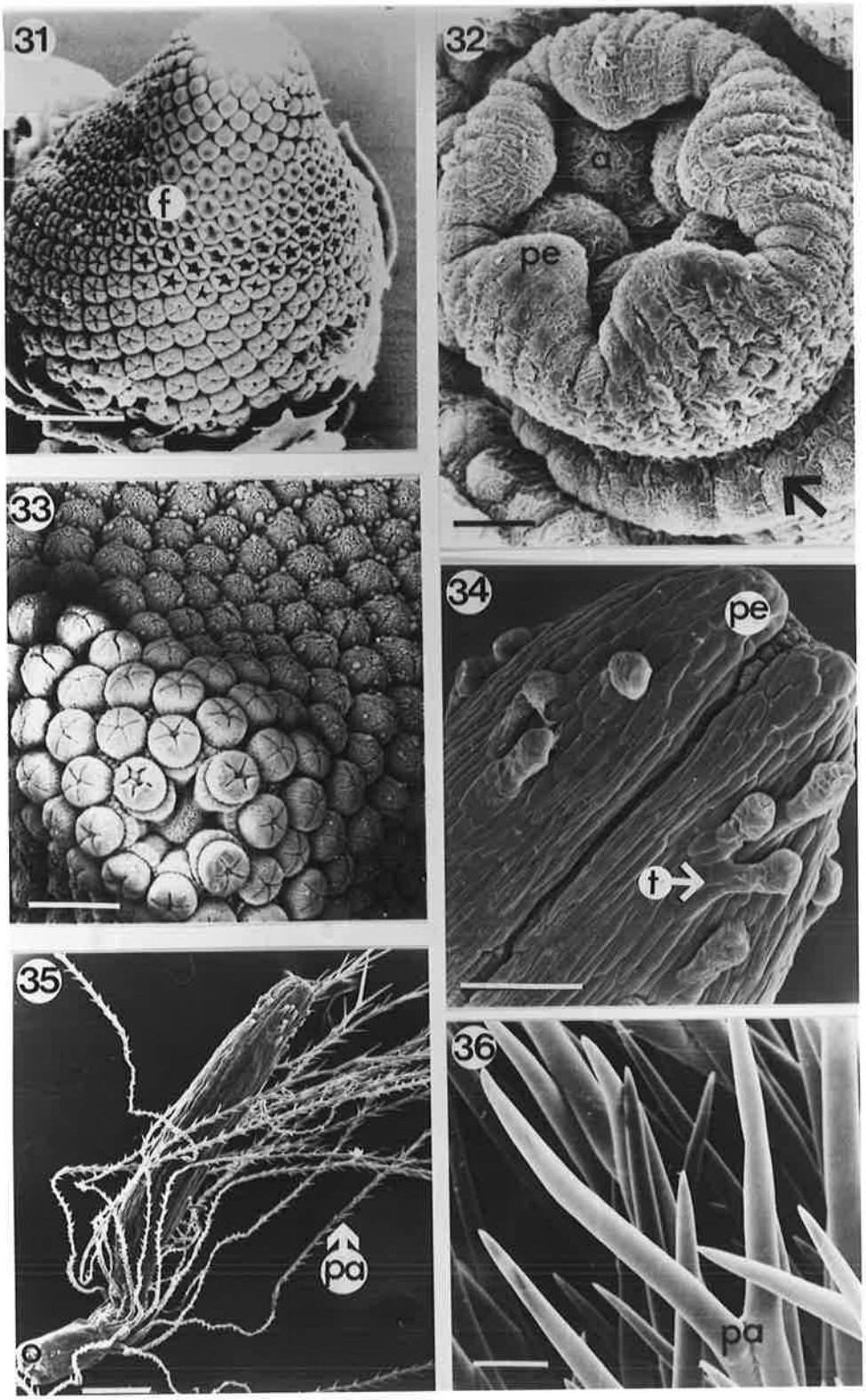
31
f
32
a
pe
33
34
pe
f
35
pa
36
pa

Fig. 3.37 Dehydrated, colporate pollen grain showing echinate surface and colpi (c). Equatorial view. Scale: 10 µm.

Fig. 3.38 Unisexual female floret showing extended style (s) and perianth (pe). Scale: 400 µm.

Fig. 3.39 Hermaphrodite floret showing the papillate stigma (st). Note the absence of elongated papillae at the tips of the lobes. Scale: 200 µm.

Fig. 3.40 Glabrous ovary showing pappus hairs (pa) arising from the junction of the receptacle and the corolla. Scale: 200 µm.

Fig. 3.41 Light micrograph of the capitulum at anthesis of the inflorescence (apex centre left), showing outer florets at anthesis (1) and inner florets at varying stages of development (2). Scale: 0.7 mm.

Fig. 3.42 Inflorescence at anthesis showing reflexed involucral bracts (b) and an outer ring of florets at anthesis (f). Scale: 20 mm.

Fig. 3.43 Inflorescence at seed ejection showing reflexed involucral bracts (b) and displaced florets (arrow). Scale: 10 mm.

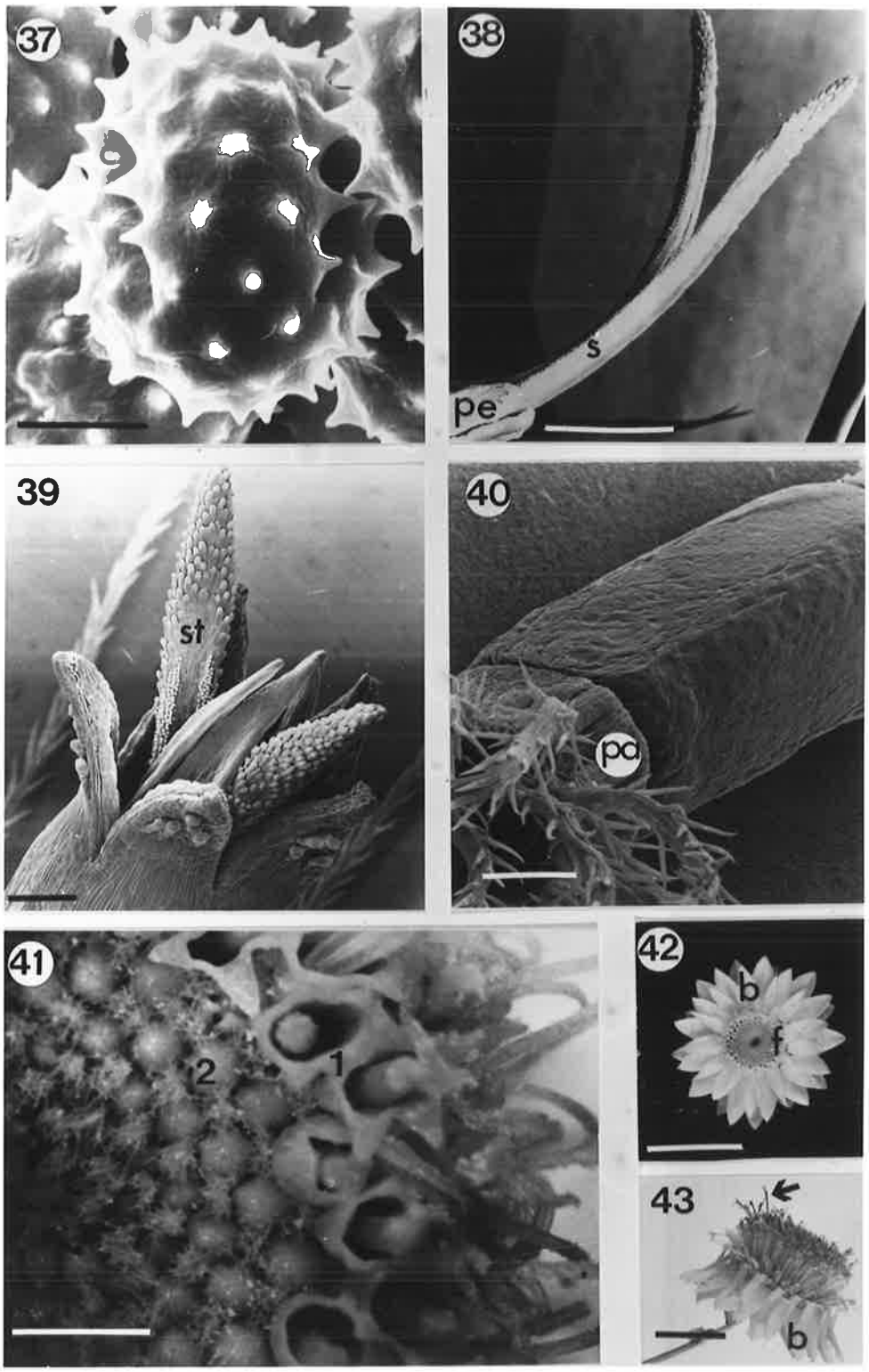
37
9
38
s
pe
39
st
40
pa
41
2
1
42
b
f
43
b

Fig. 3.44 Plant at stage 2, large vegetative meristem. Scale: 21.5 mm.

Fig. 3.45 Plant at stage 3, doming of meristem. Scale: 55.5 mm.

Fig. 3.46 Plant showing macroscopic appearance of inflorescence buds (arrow), stage 6. Scale: 46.0 mm.

Fig. 3.47 Plant at anthesis, stage 7. Scale: 75 mm.

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47

which developed at the tips of the perianth parts in *Helipterum roseum* (Figs 12, 14). The pappus hairs were distinctive for each species. *Helipterum roseum* had plumose pappus hairs that were white (occasionally black) in colour (Figs 12, 13) whereas those of *Helichrysum bracteatum* were barbellate with a few longer barbs at the tips (Figs 35, 36) and were bright yellow. The pappus hairs were particularly dense in *Helipterum roseum* (Fig. 13) and completely covered the developing florets. The perianth elongated at floret anthesis and raised the tip of the floret above the pappus hairs. The flowers of both species were protandrous with anther dehiscence preceding style extension and separation of the stigma lobes. At anthesis of the floret the tips of the perianth separated and the anthers protruded and dehisced (Fig. 14).

The pollen of both species was colporate with three germination apertures. The characteristic surface was spined (echinate) with many exine micropores (Figs 15, 37). Pollen shape was similar for both species in the dehydrated and hydrated state; oval in the former (Fig. 37) and spherical in the latter (Fig. 15). Dome like opercula (aperture caps) were observed in hydrated pollen grains of *Helipterum roseum* (Fig. 15). Pollen grains of *Helipterum roseum* were smaller than those of *Helichrysum bracteatum* with a mean polar diameter (including the spines) in the dehydrated state of 25.9 ± 0.54 µm [3] compared with 31.6 ± 0.54 µm. All florets of *Helipterum roseum* were bisexual while *Helichrysum bracteatum* had one to two rows of female florets (Fig. 38) that consisted only of perianth and

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3. ± standard error

pistil.

During the maturation process the pappus hairs developed further and the style extended, pushing pollen from the already dehisced anthers onto the surface of the floret. The stigma protruded from the perianth (Figs 16, 39) and the stigmatic lobes diverged to reveal the papillate surface of the stigma. The lobes of *Helipterum roseum* were reflexed with longer papillae forming a fringe at the tips (Fig. 16). Those of *Helichrysum bracteatum* did not reflex; the papillae were marginal, larger towards the apex but did not form a fringe (Fig. 39). Pollen became caught in the papillae on both the stigma and the perianth. In both species the ovary was inferior. The ovary wall of *Helipterum roseum* was hairy (Fig. 17) while that of *Helichrysum bracteatum* was glabrous (Fig. 40).

The florets reached anthesis centripetally so that a range of developmental stages was present in the mature inflorescence (Fig. 41). Individual florets of *Helipterum roseum* were shorter at anthesis than those of *Helichrysum bracteatum* with a mean length (including the ovary) of 3.2 ± 0.1 mm [4] in the former compared with 7.3 ± 0.08 mm in the latter. This difference was also evident at very early stages of development (Figs 12, 35).

The apical meristem of both species increased in height and diameter throughout development and seven distinct developmental stages were defined (Table 1). The first stage was that of a small vegetative meristem and endured for approximately

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4. ± standard error

Table 3.1 Stages of development and accompanying changes in mean height (µm) and diameter (µm) of the apical meristem in *Helipterum roseum* and *Helichrysum bracteatum*

Values presented are means and standard errors of a minimum of three replicates. n.a., not available; sufficient examples of the apical meristem could not be isolated at this early stage due to the rosette growth habit of *Helichrysum bracteatum*.

	Helipterum roseum				*Helichrysum bracteatum*			
Stage of development	Height (µm)	Diameter (µm)	First appearance (days from planting)	Fig. no.	Height (µm)	Diameter (µm)	First appearance (days from planting)	Fig. no.
1. Small vegetative meristem	17.7 ± 2.9	56.0 ± 3.8	1	1,21	n.a.	n.a.	1	-
2. Large vegetative meristem	31.1 ± 4.0	84.5 ± 5.5	13	2	82.7 ± 6.9	182.2 ± 12.3	38	25,44
3. Doming of meristem	75.5 ± 43.6	136.0 ± 4.8	29	3,22	127.5 ± 18.0	229.8 ± 37.8	57	26,45
4. Initiation of involucral bracts	221.8 ± 49.2	268.4 ± 31.1	39	4	360.3 ± 31.5	565.7 ± 37.9	66	27,28
5. Initiation of florets	523.1 ± 88.4	531.1 ± 78.1	42	5-7	592.2 ± 16.8	824.8 ± 21.1	69	29,30
6. Macroscopic appearance of apical inflorescence bud	1168.5 ± 18.8	1473.1 ± 378.4	50	8-13, 23	1370.0 ± 114.0	1371.7 ± 114.0	83	31-36, 46
7. Anthesis of the apical inflorescence	5.6×10^{3} ± 0.3	7.2×10^{3} ± 0.4	66	14-16, 18,24	6.6×10^{3} ± 0.2	7.6×10^{3} ± 0.5	121	37-39, 41,42, 47

12 days in *Helipterum roseum*. Stage 2 was also vegetative. The apical meristem had enlarged, doubling in height and diameter. Doming of the apex occurred at the transition to stage 3, when the meristem appeared more rounded and bulbous and there was an accompanying increase in both height and diameter. When the first involucral bracts began to develop (stage 4) the apical meristem had further tripled in height and the diameter had doubled. Numerous bracts were produced during a brief period of three days in both *Helipterum roseum* and *Helichrysum bracteatum*. When the first floret primordia appeared (stage 5) the meristem in *Helipterum roseum* was more domed than that of *Helichrysum bracteatum*. By the time the apical inflorescence bud was visible to the naked eye (stage 6) the apical meristem had broadened in *Helipterum roseum* resulting in a flattened profile, whilst that of *Helichrysum bracteatum* was conical. Anthesis of the apical inflorescence (stage 7) occurred when the capitulum was covered with florets. The height and diameter of the apical meristem of the two species were comparable at this stage. *Helipterum roseum* required approximately 24 days to complete floret initiation (stages 5 and 6) whilst *Helichrysum bracteatum* required a longer period of approximately 52 days.

The mean diameter of the apical inflorescence, including the involucral bracts, was greater for *Helipterum roseum* (37.6 ± 0.3 mm [5]) than for *Helichrysum bracteatum* (30.6 ± 0.1 mm) due to the greater length of the involucral bracts of *Helipterum roseum* (Table 2). The involucral bracts reflexed at anthesis of the inflorescence and exposed the florets (Figs 18, 42). There

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5. ± standard error

Table 3.2 Dimensions of the apical inflorescence (including involucral bracts) of *Helipterum roseum* and *Helichrysum bracteatum* at anthesis of the inflorescence

Values presented are means and standard errors of 10 replicates

Inflorescence diameter (including involucral bracts) (mm)	Diameter of the floret area (mm)	Length of the longest involucral bract (mm)
	Helipterum roseum	
37.6 ± 0.3	7.2 ± 0.1	15.2 ± 0.1
	Helichrysum bracteatum	
30.6 ± 0.1	7.6 ± 0.1	11.5 ± 0.1

was a progression in the size, shape and colour of the outer to the inner involucral bracts in both species. The outer bracts of *Helipterum roseum* were short and grey merging to long, narrow magenta-coloured structures and those of *Helichrysum bracteatum* were short and white merging to longer, buttercup yellow structures. This was followed by a gradual reduction in the size of mature involucral bracts and the presence of involucral bracts dispersed amongst the peripheral florets (Fig. 18). Floral abnormalities including a thickened peduncle with increased numbers of bract scales (Fig. 19) and reversions to vegetative growth after the production of a few bract scales (Fig. 20) were observed in a minority of plants of *Helipterum roseum* only.

The early vegetative stage (stage 1) of development in *Helipterum roseum* corresponded to a small seedling which was 10.0 ± 0.0 mm [6] in height (Fig. 21) (*Helichrysum bracteatum*; height 3.7 ± 0.9 mm, diameter 9.5 ± 5.3 mm). Plants of *Helipterum roseum* reached a height of 52.5 ± 10.2 mm during stage 2, large vegetative meristem (*Helichrysum bracteatum*; height 5.5 ± 2.0 mm, diameter 80.5 ± 10.2 mm) (Fig. 44). The transition to flowering (stage 3) commenced when plants of *Helipterum roseum* had reached a height of 98.2 ± 6.7 mm (*Helichrysum bracteatum*; height 22.7 ± 1.3 mm, diameter 293.5 ± 18.7 mm) with four to six well developed laterals (Figs 22, 45). Flower buds were not visible to the naked eye until the plant had reached a height of 443.3 ± 38.2 mm with numerous laterals (Fig. 23) (*Helichrysum bracteatum*; height 232.7 ± 89.2 mm, diameter 395.8 ± 18.7 mm, Fig. 46). Anthesis of the apical inflorescence was reached when the plant had attained a

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6. mean ± standard error

height of 609.5 ± 41.8 mm (Fig. 24) (*Helichrysum bracteatum*; height 667.7 ± 21.7 mm, diameter 410.4 ± 8.3 mm, Fig. 47).

3.3.2 Experiment 2

Sexual maturity (stage 7) in *Helipterum roseum* and *Helichrysum bracteatum* was reached 66 and 121 days after planting, respectively (Table 1). The percentage seed set in capitula of *Helipterum roseum* and *Helichrysum bracteatum* following each of four treatments to test for breeding systems is shown in Table 3. Cross pollination, treatment 4, produced the greatest number of seeds in both *Helipterum roseum* and *Helichrysum bracteatum* with 22.8 % seed set in the former and 63.7 % in the latter. Seed set was insignificant following treatments 1 and 2 indicating that pollen vectors are required for pollen transfer. Both species set a small number of seeds following self pollination, treatment 3. Following fertilisation and maturation the involucral bracts of both species reflexed and the seeds were ejected (Fig. 43). The seeds of *Helipterum roseum* were twice the diameter and weight of those of *Helichrysum bracteatum* (Table 4).

3.4 Discussion

The sequence of floral initiation and development is similar in the two species *Helipterum roseum* and *Helichrysum bracteatum*. These processes are also essentially similar to those described in other members of the Asteraceae including *Chrysanthemum* (Cathey and Borthwick 1957, Vermeer and Peterson 1979a, Horridge *et al.* 1985) and *Enceliopsis* (Sanders and Clark

Table 3.3 Observations on the breeding system of *Helipterum roseum* and *Helichrysum bracteatum*

Treatments: 1, tagged but otherwise untreated; 2, bagged but otherwise untreated; 3, self pollinated and bagged; 4, cross pollinated and bagged. Values presented are means and standard errors of 16 replicates.

Treatment	Number of florets/ capitulum, at anthesis	Number of viable seed/ capitulum	Seed set/ capitulum (%)
	Helipterum roseum		
1	255.2 ± 2.0	0.2 ± 0.2	0.07
2	247.0 ± 28.4	0.0	0.00
3	262.9 ± 20.0	11.7 ± 5.8	4.45
4	212.0 ± 16.6	48.4 ± 17.3	22.80
	Helichrysum bracteatum		
1	371.7 ± 33.0	0.0	0.00
2	326.3 ± 58.3	0.0	0.00
3	385.2 ± 25.6	5.5 ± 2.6	1.40
4	376.5 ± 27.7	240.0 ± 11.5	63.70

Table 3.4 Seed diameter and length (without pappus) (mm) and seed weight (including pappus) (mg) of *Helipterum roseum* and *Helichrysum bracteatum*

Values presented are means and standard errors of (a) 10 replicates and (b) three lots of 50 seeds

Species	Seed diameter (mm) (a)	Seed length (mm) (a)	Seed weight (mg) (b)
Helipterum roseum	2.25 ± 0.05	3.85 ± 0.01	3.1 ± 0.01
Helichrysum bracteatum	1.05 ± 0.05	3.25 ± 0.05	1.7 ± 0.06

1987) both of which produce ray florets in addition to disc florets. Asymmetrical development of the perianth is responsible for the development of the ray florets in *Chrysanthemum* (Horridge *et al.* 1985). *Helipterum roseum* and *Helichrysum bracteatum* have only disc florets and the perianth develops uniformly in all florets both hermaphrodite and unisexual. The showy coloured bracts of *Helipterum roseum* and *Helichrysum bracteatum* are involucral bracts and are never fertile.

Glandular trichomes similar to those investigated by Vermeer and Peterson (1979a,b) in *Chrysanthemum* are present in both species and develop at the tips of the perianth parts and occasionally at the base of the perianth. In *Chrysanthemum* they act as secretory organs for what appears to be a terpenoid (Vermeer and Peterson 1979b). They may have a function in producing floral odour. The initiation of trichomes in *Helipterum roseum* and *Helichrysum bracteatum* commences on peripheral florets when several rows of florets are present but before floret initiation on the receptacle is complete. There are no receptacular trichomes like those present between the florets in *Chrysanthemum* (Vermeer and Peterson 1979a) in either species. It was noted that elongation of the perianth surrounding the stamens and style occurred very late (shortly before anthesis) in both species. This phenomenon was reported by Leins and Erbar (1987) for a number of members of the Asteraceae.

Both species are protandrous, and the features of style extension and long papillae at the tips of the stigmatic lobes and perianth in *Helipterum roseum* may play a part in pollen-presentation (Small 1915, van der Pijl 1978). All these

mechanisms place pollen on the surface of the capitulum where it is collected, either intentionally or unintentionally, by foraging insects. Insect vectors appear to be required for successful pollen transfer in both species as shown by the breeding system experiment, and by the fact that the pollen grains are large and sticky and do not readily become airborne. The structure of the pistil in both species conforms to that described in other members of the Inuleae (Small 1915). Pollen grains from both species possess three germinal apertures, as is characteristic of the Asteraceae (Wodehouse 1935) and the opercula observed in *Helipterum roseum* are also found in other Asteraceae including *Helianthus annuus* (Iwanami *et al.* 1988). The echinate pollen wall is also present in *Helichrysum davenportii* and other members of the tribe Inuleae (Skvarla *et al.* 1977). Distinguishing features between the two taxa in addition to the difference in pollen grain size, include the shape and colour of the pappus hairs, the structure of the stigma, and the decoration of the ovary wall. The white plumose pappus hairs of *Helipterum roseum* form a dense mat which covers the developing florets, while the yellow barbed pappus hairs of *Helichrysum bracteatum* do not.

Seed production in both species appears to be controlled by a self-incompatibility mechanism, as is characteristic of other members of the Asteraceae (Heslop-Harrison 1975). The low seed number compared with initial floret number in both species is consistent with seed size and the spacial constraints imposed by the size of the capitulum and the surrounding involucre. In both species, cross-pollination resulted in a seed crop which filled the capitulum area, which enlarged little if at all

following anthesis. Similar spacial constraints to seed number have been reported to be a feature of a number of other plant groups eg. *Banksia* (George 1984). The presence and arrangement of pappus hairs suggest that the seeds produced by both species are adapted for wind dispersal (Sheldon and Burrows 1973). The heavier seeds of *Helipterum roseum* may be further aided in wind dispersal by the presence of hairs on the ovary wall. The withering of the involucre and subsequent reflexion of the involucral bracts is essential to the release of the seeds and is a common feature of species posessing capitula (Burtt 1971).

One of the most interesting findings of this study is the observation of dividing floret primordia on the apical meristem. A few rows of developing primordia divide into two unequal segments in both taxa. The lower portion of the primordium develops into a small involucral bract, whilst the upper portion develops into a floret. In the majority of cases a single row of primordia divide, thus providing a distinct border between the involucral bracts and the florets. Occasionally two to four rows of dividing primordia are present. In these instances the small lower portion of the divided primordium either ceases further development or a small, slender, involucral bract is formed. Involucral bracts are occasionally observed amongst the peripheral florets of both *Helipterum roseum* and *Helichrysum bracteatum*. Schuster (1985) examined the work of Marc and Palmer (1981) with *Helianthus annuus* and highlighted that ridges on the periphery of the receptacle in this species were divided into two parts by a furrow. The pyramid-shaped portion near the edge developed into a floret bract and the "drop" shaped part nearer the centre developed into a floret. The composite capitulum is

considered in evolutionary terms to be a condensed shoot (Burtt 1971) and the involucral bracts, modified leaves (Schuster 1985). It is possible therefore that the dividing primordia observed in *Helipterum roseum* and *Helichrysum bracteatum* are examples of axillary buds (florets) each subtended by a leaf (involucral bract). The floret bracts found in *Helianthus annuus* are in fact occasionally reduced to green leaves so that the capitulum appears to be covered with moss (Schuster 1985).

The apical meristem proceeds systematically through a series of well defined, yet progressive stages from the vegetative to the floral state. The meristem undergoes dramatic changes in configuration punctuated by the appearance of leaf, involucral bract and floret primordia. Each step in the development of the inflorescence is associated with increases in the size of the apical dome. Horridge and Cockshull (1979) reported that inflorescence initiation and development in *Chrysanthemum* was closely related to apex size. In *Helipterum roseum* and *Helichrysum bracteatum* this series of events occurs rapidly and is completed when the plant is small. Sexual maturity (stage 7) is attained within two to four months from germination in *Helipterum roseum* and *Helichrysum bracteatum* respectively, with seed set and dispersal occurring within one year. This is in accordance with their ephemeral nature and arid ecological habitat.

Defining the point at which the apical meristem is committed to flower initiation has been a matter of contention amongst various authors. Stage 3, doming of the apical meristem, was defined by Popham and Chan (1952) to be the point of transition for *Chrysanthemum morifolium* whilst Lance (1957)

proposed that stage 3 corresponded to a young "prefloral" meristem in *Chrysanthemum segetum* with commitment to the reproductive state occurring in older prefloral meristems, at the initiation of involucral bracts (corresponding to stage 4 of the morphological scale for *Helipterum roseum*). Horridge and Cockshull (1979) similarly concluded that reproductive commitment in *Chrysanthemum* occurred at stage 4 and more precisely when the first involucral bract was initiated. The apical meristem of *Helipterum roseum* may develop a few rows of sessile, scarious bracts and still be capable of reverting to the vegetative condition, producing numerous leaves before once again approaching the floral state, stage 4 (Fig. 20). Once involucral bracts with coloured papery laminae are produced, the apical meristem appears to be committed to the formation of a capitulum.

CHAPTER 4. EFFECTS OF PHOTOPERIOD, TEMPERATURE AND PLANT AGE ON FLORAL INITIATION AND DEVELOPMENT IN *Helipterum roseum* AND *Helichrysum bracteatum*

4.1 Introduction

The discovery by Allard and Garner (1940) that flowering responses in some plants could be controlled by manipulating the length of the light and dark period in each daily cycle has played an important role in the development of many ornamental species, the most notable example being *Chrysanthemum morifolium*. Flowering is promoted in this quantitative short day plant by manipulating the daylength, and prevented or delayed by the use of intermittent periods of low intensity light during long periods of darkness (Kofranek 1980). Temperature also plays a significant role in floral initiation and development in this species. High temperatures delay floral initiation and development and increase the incidence of floral abnormalities (Cathey 1954, Cathey and Borthwick 1957, Whealey *et al.* 1987).

The successful development of Australian native species as cut-flower crops or flowering pot plants is dependent upon the development of techniques to extend the natural flowering season and to ensure flower quality. Limited information is available on the flowering response of *Helichrysum* and *Helipterum*. *Helichrysum cassinianum*, *Helipterum manglesii* and *Helipterum craspedioides* are quantitative long day plants, with low temperature promotion of flowering in *Helipterum craspedioides* (Ramaley 1934, Mott and McComb 1975b). The aim of this section is to investigate the environmental control of floral initiation and development in *Helipterum roseum* and *Helichrysum bracteatum*.

4.2 Materials and methods

4.2.1 Environmental conditions

The environmental conditions used are shown in Table 1. All controlled environment growth cabinets were illuminated with four, G.E.C. Lucalux high pressure sodium lamps (400 W) and five, Philips TL 65-80 W fluorescent tubes. The night-break treatment involved the addition of one hour of low intensity light provided by three incandescent globes (60 W) in the middle of the dark period following an eight hour day. The low light intensity treatment employed the same mix of lights as the other treatments with the addition of a shade cloth screen to reduce the light intensity by 60 %. This treatment received approximately the same photon flux density during a 20 h photoperiod as did the 8 h photoperiod treatment. Eighteen plants of each species were used in all treatments conducted in controlled environment growth cabinets (ie. 36 plants per cabinet) with the exception of the 16h photoperiod treatment where an additional two cabinets containing only *Helipterum roseum* were used. The glasshouse treatment is that previously described in section 3.2.1 where 120 plants of *Helipterum roseum* were planted on 4 December 1986.

4.2.2 Measurements

Data were collected for all experimental treatments conducted in controlled environment growth cabinets twice weekly from day one after planting on a minimum of 10 plants of each species for a period of 147 days. The time to first appearance of the apical inflorescence bud visible to the naked eye (macroscopic appearance) and to anthesis of the apical

Table 4.1 Environmental conditions for the study of the flowering response of *Helichrysum bracteatum* and *Helipterum roseum*

	Photoperiod (h)	Average daily temperature (°C)	Light intensity (Wm^{-2})	Experiment number
Controlled environment growth cabinets	8	20.0 ± 2.0	250 constant	1, 3
	12	20.0 ± 2.0	250 constant	1, 2, 3
	16	20.0 ± 2.0	250 constant	1, 3
	20	20.0 ± 2.0	250 constant	1
	12	15.0 ± 2.0	250 constant	2, 3
	12	25.0 ± 2.0	250 constant	2, 3
	8 + 1 (night break)	20.0 ± 2.0	250 + 50 (night break)	1
	20	20.0 ± 2.0	100 constant	1, 3
Glasshouse	14.3 ± 0.1	22.4 ± 2.3	178.0 ± 49.8	1
Field	13.4 ± 1.0	18.8 ± 3.6	441.1 ± 72.0	3

Experiment number
1. Effect of photoperiod on floral initiation and development
2. Effect of temperature on floral initiation and development
3. Effect of time from anthesis of the apical inflorescence on inflorescence size

± standard error

inflorescence were recorded (days). At anthesis of the apical inflorescence the following measurements were taken; (i) plant height from the soil surface to the inflorescence, (ii) plant diameter across the two longest leaves (recorded for the rosette plant *Helichrysum bracteatum* only), (iii) number of nodes to the apical inflorescence, (iv) mean internode length (derived) and (v) diameter of the apical inflorescence including the involucral bracts. The number of inflorescences reaching anthesis during 147 days was recorded, as in the majority of treatments the plants had completed their life cycle by this time. In those which had not, the number of unopened inflorescences was also recorded and added to the number which had undergone anthesis to give the total inflorescence number. All inflorescences were removed from the plant as they reached anthesis and their diameter recorded. At 84 days, five plants were harvested for determination of above ground plant dry weight.

Two plants from the glasshouse treatment and five plants from the 16 h photoperiod treatment were selected daily and on alternate days respectively from day one after planting. The apices were dissected and prepared for scanning electron microscopy as described in section 3.2.2.1. The stage of apical development of each meristem was recorded according to the scale given in Table 3.1: stage 1, small vegetative meristem; stage 2, large vegetative meristem; stage 3, doming of meristem; stage 4, initiation of involucral bracts; stage 5, initiation of florets; stage 6, macroscopic appearance of apical inflorescence bud; stage 7, anthesis of the apical inflorescence. The time (days) taken from planting to the first observation of an apical meristem at each developmental stage was recorded for each environment.

4.2.3 Statistical analysis

The effect of photoperiod and temperature on growth and flowering for each species was determined by a one-way analysis of variance for each growth parameter measured.

4.3 Results

4.3.1 Effect of photoperiod

The effects of photoperiod were studied under controlled environment growth cabinet and glasshouse conditions (Table 1). Floral initiation and development in both *Helipterum roseum* and *Helichrysum bracteatum* was affected by photoperiod (Table 2). Both species reached the stage of macroscopic appearance of inflorescence buds and anthesis of the inflorescence sooner at longer photoperiods. Changes in photoperiod also affected the duration of early stages of development. A longer photoperiod reduced the time taken to reach all the microscopic stages of apical development from stage 2 onwards in *Helipterum roseum* (Fig. 1). The addition of a one-hour night-break of low intensity incandescent light during a long dark period significantly reduced the time to macroscopic appearance of inflorescence buds in both species. This stage was also attained significantly sooner in the low light intensity treatment than in the 8 h photoperiod treatment which received approximately the same photon flux density. Plant height at anthesis of the apical inflorescence was reduced at longer photoperiods in the caulescent *Helipterum roseum* and increased minimally in the rosette *Helichrysum bracteatum*. Plant diameter in the latter species was reduced due to leaf necrosis. The number of nodes to

Table 4.2 Effect of photoperiod on flowering in *Helipterum roseum* and *Helichrysum bracteatum*

Values presented are means and standard errors of a minimum of 10 replicates

	Helipterum roseum							*Helichrysum bracteatum*						
	Photoperiod (h)				Low light intensity	Night break	Level of significance	Photoperiod (h)				Low light intensity	Night break	Level of significance
	8	12	16	20				8	12	16	20			
Time to macroscopic appearance of inflorescence buds (days from planting)	60.9 ±2.0	32.1 ±0.9	31.5 ±0.9	19.7 ±0.5	27.7 ±1.4	45.2 ±0.8	**	73.1 ±3.7	58.0 ±0.9	45.2 ±2.6	33.7 ±0.8	37.2 ±1.5	62.7 ±3.7	**
Time from macroscopic appearance to anthesis of apical inflorescence (days)	25.2 ±2.6	30.2 ±1.3	22.6 ±1.9	19.0 ±0.8	21.0 ±2.0	42.1 ±1.9	**	23.2 ±1.3	28.0 ±1.8	24.8 ±1.5	25.3 ±1.2	24.7 ±1.1	34.7 ±1.3	**
Time to anthesis of apical inflorescence (days from planting)	86.1 ±3.8	62.4 ±1.9	54.1 ±2.2	38.8 ±1.2	48.7 ±2.8	87.3 ±2.0	**	96.3 ±4.3	86.0 ±1.0	70.0 ±3.4	59.0 ±1.3	61.9 ±1.7	97.4 ±3.5	**
Plant height at anthesis of apical inflorescence (mm)	476.5 ±38.9	465.9 ±28.3	448.3 ±20.5	305.5 ±13.1	469.5 ±28.5	643.5 ±36.0	**	335.6 ±17.7	319.3 ±2.1	356.0 ±27.3	371.9 ±18.6	423.8 ±28.8	553.8 ±45.1	**
Plant diameter at anthesis of apical inflorescence (mm)	-	-	-	-	-	-		300.0 ±16.8	300.7 ±9.9	238.5 ±5.8	235.8 ±5.8	322.3 ±17.3	375.4 ±11.0	**
Number of nodes to apical inflorescence	114.9 ±12.1	83.5 ±4.1	72.4 ±3.8	38.5 ±1.9	77.1 ±5.3	89.2 ±3.2	**	43.7 ±1.8	35.3 ±0.5	32.9 ±2.3	25.3 ±1.6	31.9 ±1.2	33.4 ±2.1	**
Internode length at anthesis of apical inflorescence (mm)	4.4 ±0.3	5.6 ±0.2	6.1 ±0.2	8.1 ±0.4	6.2 ±0.4	7.4 ±0.5	**	7.8 ±0.6	9.0 ±0.3	11.0 ±0.8	14.8 ±0.8	13.6 ±1.2	17.1 ±1.6	**
Number of inflorescences reaching anthesis by day 147 from planting	57.1 ±8.9	20.8 ±4.6	60.8 ±8.6	50.3 ±5.6	12.3 ±1.1	36.2 ±3.8	**	25.1 ±5.3	32.9 ±0.8	47.6 ±4.0	44.5 ±3.2	14.0 ±1.1	14.0 ±2.1	**
Total number of inflorescences	85.4 ±10.2	20.8 ±4.6	67.5 ±3.5	50.3 ±5.5	12.3 ±1.2	44.8 ±4.2	**	63.4 ±7.5	67.1 ±1.2	47.6 ±4.0	44.5 ±3.2	14.0 ±1.1	14.8 ±2.1	**
Diameter of apical inflorescence at anthesis (mm)	41.4 ±1.7	52.1 ±1.5	43.6 ±0.3	43.3 ±1.3	44.4 ±2.0	47.2 ±1.6	**	30.5 ±2.0	34.9 ±1.2	32.2 ±2.2	33.1 ±0.8	36.7 ±0.7	32.8 ±2.0	n.s.
Plant dry weight at 84 days from planting(g)	12.5 ±1.2	25.0 ±2.8	29.2 ±3.5	36.2 ±3.5	14.8 ±1.3	12.8 ±2.5	**	10.0 ±0.8	17.0 ±0.8	35.5 ±4.9	31.2 ±1.6	14.4 ±1.1	11.0 ±0.5	**

** $P < 0.01$; n.s., not significant

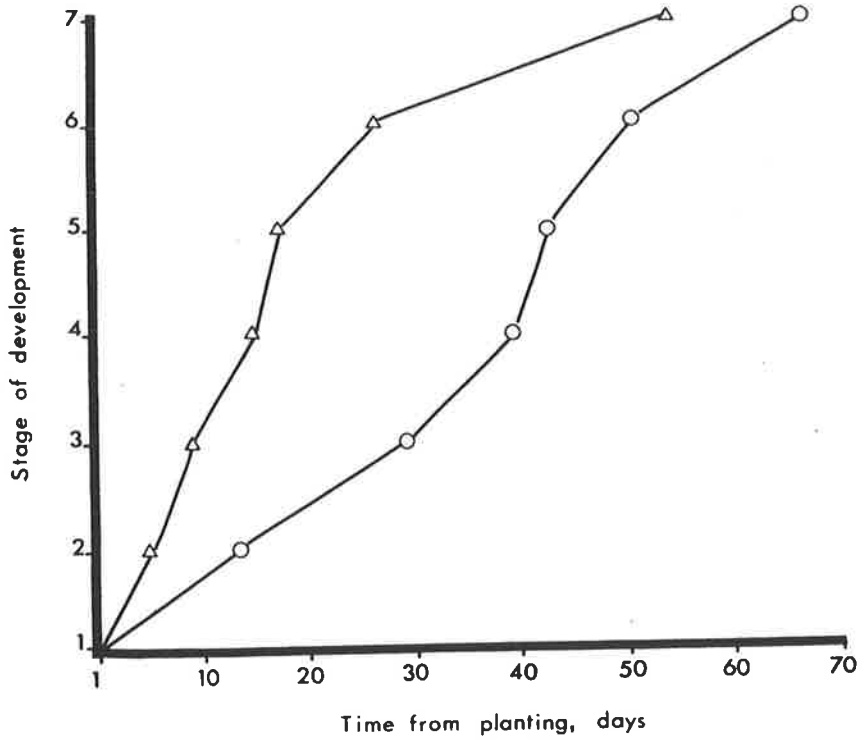


Figure 4.1

Effect of photoperiod on time to floral initiation in *Helipterum roseum* under two environmental conditions. △ Controlled environment, constant photoperiod 16 h, light intensity 250 W m^{-2}, average daily temperature 20.0 ± 2.0 °C. ○ Glasshouse, average photoperiod 14.3 ± 0.1 h, light intensity 178.0 ± 49.8 W m^{-2}, average daily temperature 22.4 ± 2.3 °C. Stage of development of apical meristem: 1, small vegetative meristem; 2, large vegetative meristem; 3, doming of meristem; 4, initiation of involucral bracts; 5, initiation of florets; 6, macroscopic appearance of apical inflorescence bud; 7, anthesis of the apical inflorescence. Values presented are number of days to first appearance of each stage of apical development.

the apical inflorescence was reduced and the internode length slightly increased at longer photoperiods in both species. Increasing photoperiod significantly increased the number of inflorescences reaching anthesis by day 147 in *Helichrysum bracteatum* but not in *Helipterum roseum*. The total number of inflorescences produced per plant was significantly increased at shorter photoperiods in both species. The diameter of the apical inflorescence at anthesis was not significantly affected by photoperiod in either species, with the exception of the 12 h photoperiod treatment of *Helipterum roseum* which produced larger inflorescences. Plants of both species grown in longer photoperiods had a higher dry weight.

4.3.2 Effect of temperature

The effects of temperature in a 12 h photoperiod were studied using controlled environment growth cabinets (Table 1). Temperature affected both floral initiation and development in *Helipterum roseum* and floral development only in *Helichrysum bracteatum* in the photoperiod and light conditions tested (Table 3). Floral initiation and development in *Helipterum roseum* was inhibited at 25°C and delayed at 15°C. The time to macroscopic appearance of inflorescence buds in *Helichrysum bracteatum* was not affected by temperature in the range tested but the time to anthesis was significantly reduced with increasing temperature. Increasing temperature reduced plant height and diameter only in *Helichrysum bracteatum*. The number of nodes to the apical inflorescence was not affected by temperature in either species and decreased internode length accompanied increases in temperature in *Helichrysum bracteatum*. The coolest temperature

Table 4.3 Effect of temperature on flowering in *Helipterum roseum* and *Helichrysum bracteatum*

Values presented are means and standard errors of a minimum of 10 replicates

	Helipterum roseum				*Helichrysum bracteatum*			
	Temperature (°C)			Level of significance	Temperature (°C)			Level of significance
	15	20	25		15	20	25	
Time to macroscopic appearance of inflorescence buds (days from planting)	42.8 ± 0.8	32.1 ± 0.9	-	**	60.2 ± 1.0	58.0 ± 0.9	60.0 ± 4.1	n.s.
Time from macroscopic appearance to anthesis of apical inflorescence (days)	40.9 ± 5.1	30.2 ± 1.3	-	**	39.8 ± 1.5	28.0 ± 1.8	19.5 ± 1.0	**
Time to anthesis of apical inflorescence (days from planting)	83.7 ± 5.9	62.4 ± 1.9	-	**	99.9 ± 3.8	86.0 ± 1.0	79.5 ± 4.5	*
Plant height at anthesis of apical inflorescence (mm)	480.8 ± 44.7	465.9 ± 28.3	-	n.s.	385.0 ± 23.3	319.0 ± 2.1	204.3 ± 22.3	**
Plant diameter at anthesis of apical inflorescence (mm)	-	-	-	-	313.0 ± 15.9	300.7 ± 9.9	248.6 ± 14.1	*
Number of nodes to apical inflorescence	74.5 ± 5.5	83.5 ± 4.1	-	n.s.	39.3 ± 3.2	35.3 ± 0.5	38.0 ± 2.7	n.s.
Internode length at anthesis of apical inflorescence (mm)	6.5 ± 0.5	5.6 ± 0.1	-	n.s.	10.4 ± 1.0	9.0 ± 0.3	5.5 ± 0.6	**
Number of inflorescences reaching anthesis by day 147 from planting	33.6 ± 4.5	20.8 ± 4.6	0.0	**	25.7 ± 3.6	32.9 ± 0.8	27.9 ± 3.4	n.s.
Total number of inflorescences	42.0 ± 2.7	20.8 ± 4.6	0.0	**	48.7 ± 3.4	67.1 ± 1.3	27.9 ± 3.4	**
Diameter of apical inflorescence at anthesis (mm)	51.7 ± 3.6	52.1 ± 1.5	-	n.s.	31.2 ± 1.5	34.8 ± 1.4	27.8 ± 1.1	*
Plant dry weight at 84 days from planting (g)	5.7 ± 1.0	25.0 ± 2.8	17.7 ± 1.6	**	15.0 ± 6.5	17.0 ± 0.7	17.9 ± 0.4	n.s.

(15°C) increased the number of inflorescences produced within 147 days in *Helipterum roseum* but had no effect on *Helichrysum bracteatum*. The total number of inflorescences produced per plant was greatest at 15°C in *Helipterum roseum* and at 20°C in *Helichrysum bracteatum*. Temperature had little effect on the diameter of the apical inflorescence in *Helichrysum bracteatum* and no effect on *Helipterum roseum*. Plant dry weight in *Helipterum roseum* was greatest at 20°C whilst that of *Helichrysum bracteatum* was unaffected by temperature.

4.3.3 Effect of time from anthesis of the apical inflorescence on diameter of axillary inflorescences

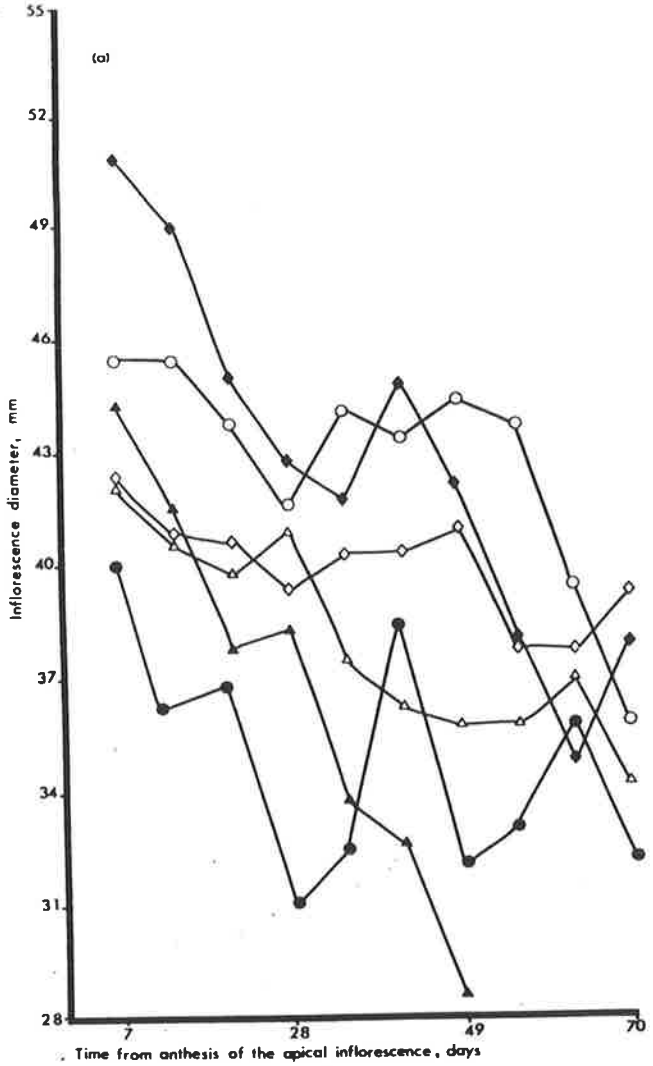
In controlled environments, the diameter of axillary inflorescences, produced sequentially after the apical inflorescence, declined in both species and under all photoperiod (Figs 2a, 2b) and temperature (Figs 3a, 3b) treatments investigated with the exception of the night-break and 15°C treatments of *Helichrysum bracteatum*. Diameter of sequential inflorescences decreased less at cooler temperatures in both species but there was no trend in the effect of photoperiod.

4.4 Discussion

Both *Helipterum roseum* and *Helichrysum bracteatum* are quantitative long day plants, with more rapid floral initiation following the production of fewer nodes under increasing daylength. Thus the time of flowering of these species can be dictated by imposed cultural conditions. However, even though the time to flowering can be reduced by long days, such treatment

Figure 4.2

Effect of time from anthesis of the apical inflorescence on mean diameter (mm) of all inflorescences at anthesis in (a) *Helipterum roseum* and (b) *Helichrysum bracteatum* at different photoperiods: ◇ 8, ◆ 12, △ 16, ● 20 h, temperature 20 °C, light intensity 250 W m^{-2} ; ○ night-break, photoperiod 8 h plus 1 h incandescent light, temperature 20 °C, light intensity 250 W m^{-2} and 50 W m^{-2}; ▲ low light, photoperiod 20 h, temperature 20 °C, light intensity 100 W m^{-2}. There was a significant effect of time from anthesis of the apical inflorescence on inflorescence diameter at $P<0.01$ for each treatment except *Helichrysum bracteatum* night-break treatment (Fig. 4.2b) which was not significant.


(a)
55
52
49
46
43
40
37
34
31
28
Inflorescence diameter, mm
7
28
49
70
Time from anthesis of the apical inflorescence, days

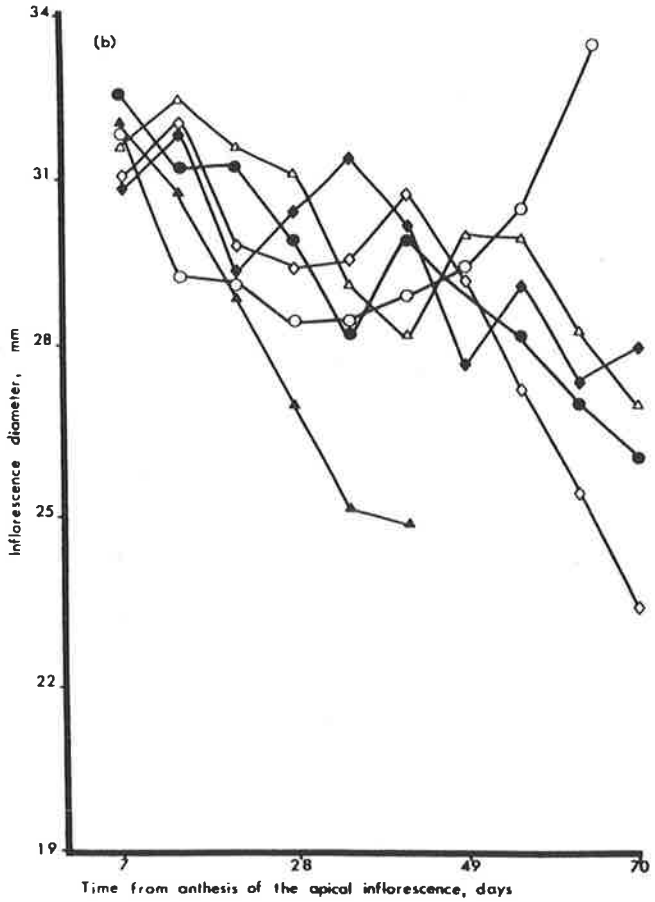
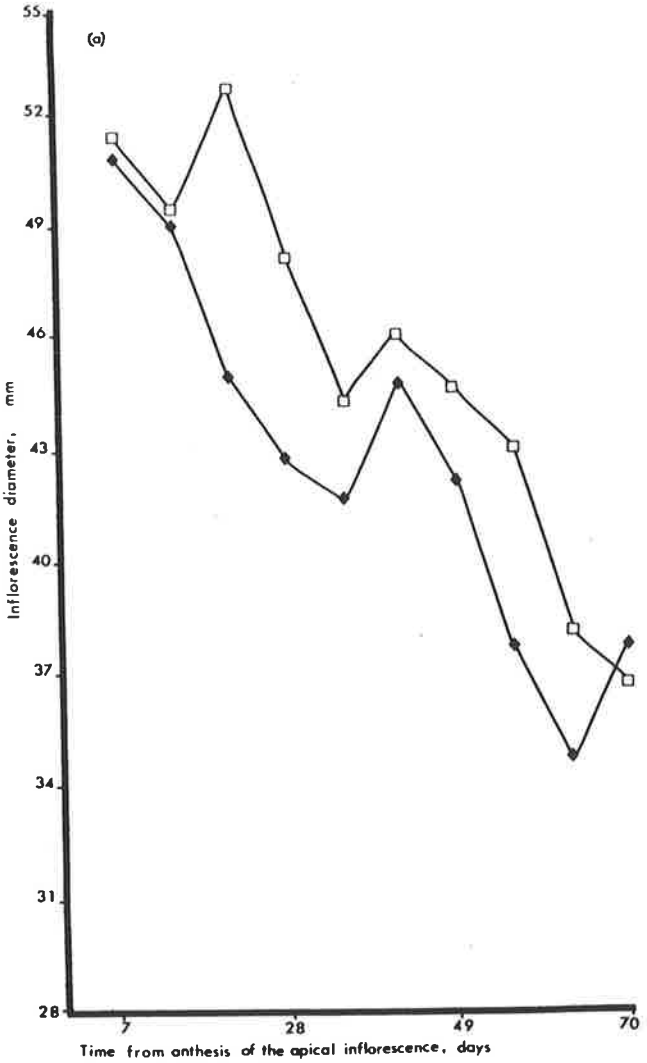

(b)
34
31
28
25
22
19
Inflorescence diameter, mm
7
28
49
70
Time from anthesis of the apical inflorescence, days

Figure 4.3

Effect of time from anthesis of the apical inflorescence on mean diameter (mm) of all inflorescences at anthesis of (a) *Helipterum roseum* and (b) *Helichrysum bracteatum* at different temperatures; □ 15, ◆ 20, ■ 25 °C, photoperiod 12 h, light intensity 250 W m^{-1}. There was a significant effect of time from anthesis of the apical inflorescence on inflorescence diameter at $P<0.01$ for each treatment except *Helichrysum bracteatum* 15 °C treatment (Fig. 4.3b) which was not significant.


(a)
55
52
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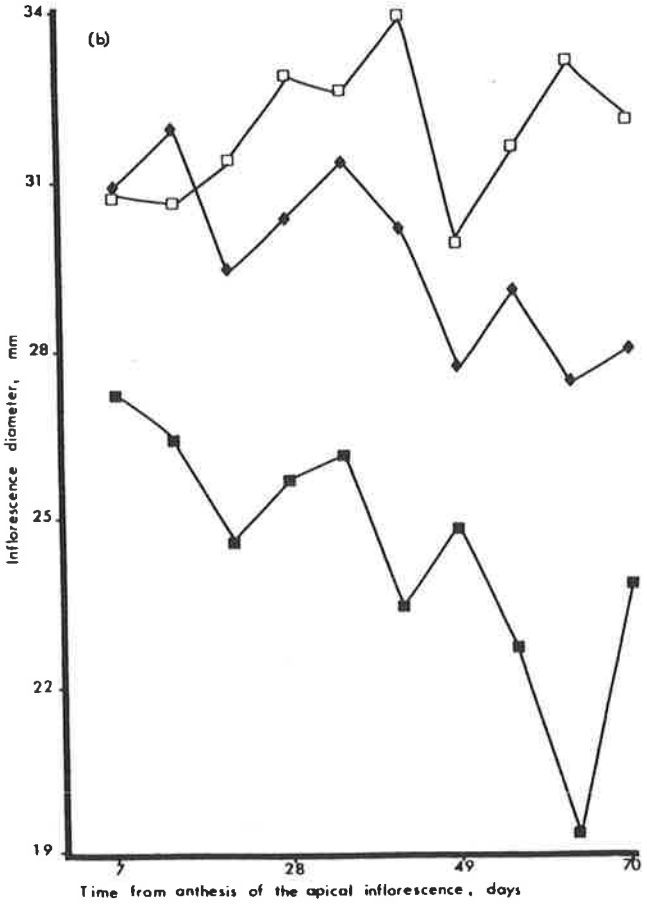
46
43
40
37
34
31
28
Inflorescence diameter, mm
7
28
49
70
Time from anthesis of the apical inflorescence, days


(b)
34
31
28
25
22
19
Inflorescence diameter, mm
7
28
49
70
Time from anthesis of the apical inflorescence, days

may not be economically advantageous, as conditions which delay floral initiation ie. short photoperiod and low temperature produce the greatest number of inflorescences over the shortest period. Increased flower productivity of carnation, a quantitative long day plant, under non-inductive photoperiodic conditions is thought to be due to increased branching (Heins *et al.* 1979). Both *Helichrysum bracteatum* and *Helipterum roseum* bear terminal inflorescences on apical and lateral shoots, hence the increased number of inflorescences at short photoperiods may be due to increased lateral production. Adequate light levels are important for maintaining inflorescence number in inductive long day conditions in both species. Low light conditions in inductive photoperiodic conditions are similarly reported to reduce inflorescence number in *Chrysanthemum* species (de Jong 1986). Temperature control is of particular importance in the cultivation of *Helipterum roseum*, and artificial cooling may be needed for bloom production in areas which experience high temperatures. Parups and Butler (1982) have shown that inflorescence numbers in some *Chrysanthemum* cultivars can be increased if temperatures are reduced for part of the night from the standard 16 °C to 10 °C.

These results confirm and extend previous work with the *Helipterum* and *Helichrysum* genera (Ramaley 1934, Allard and Garner 1940, Mott and McComb 1975b). There are now three species of *Helipterum* and two of *Helichrysum* which have been shown to be quantitative long day plants with additional low temperature (15°C) promotion of flowering in two species of *Helipterum* and one of *Helichrysum*. These responses may therefore be a common feature of these genera.

Diameter of sequential inflorescences produced after the apical inflorescence declined with increasing plant age, although consistently larger inflorescences were produced over time at a temperature of 15 °C. The production of consistently larger inflorescences at cooler treatment temperatures may be due to the constant day/night temperature regime. Vince (1960) reported that inflorescence diameter of chrysanthemums was increased by low night temperature and was primarily determined by temperature after the buds became visible macroscopically. A suitable compromise for successful commercial production would be to grow *Helipterum roseum* and *Helichrysum bracteatum* plants under moderately long photoperiod conditions (16 h) at a temperature of 15-20 °C to maintain inflorescence numbers and size throughout the life of the plant. Under 16 h days floral initiation is minimally delayed and potential inflorescence production is maintained at 80 % of the maximum recorded in this study in *Helipterum roseum* and at 75 % in *Helichrysum bracteatum*. Although both species respond to night-break treatment, the large reduction in inflorescence numbers renders the conditions used in this experiment inappropriate for commercial practice. Further experimentation is needed to determine if variation in the timing, length or intensity of the night-break may increase inflorescence number. Unacceptable reduction in inflorescence diameter starts to occur 40-50 days from the onset of flowering, indicating that this periodicity should be adopted for the commercial turnover of flowering plants for optimal bloom quality.

CHAPTER 5. THE INHIBITION BY TEMPERATURE OF FLORAL DEVELOPMENT IN THE SHOOT APICAL MERISTEM OF *Helipterum roseum*

5.1 Introduction

Temperature is reported to affect floral initiation and morphological development of the apical meristem in many species (Bernier *et al.* 1981a,b, Halevy 1985, 1986, Kinet *et al.* 1985). In *Helipterum roseum* a constant temperature of 25 °C with a photoperiod of 12 h and light intensity of 250 W m^{-2} inhibits flowering (Chapter 4).

An increase in mitotic activity of shoot apical meristems is commonly observed during the transition to flowering (Bernier *et al.* 1981b) and is consistent with an increase in the rate of cell division, ie. a decrease in the length of the cell-cycle, in *Silene* (Francis and Lyndon 1979), *Helianthus annuus* (Marc and Palmer 1984), *Sinapis alba* (Gonthier *et al.* 1987) and *Chrysanthemum* (Nougarède *et al.* 1987). Temperature is known to influence cell division in root meristems by altering the duration of the component phases of the cell-cycle (Francis and Lyndon 1979). The cell-cycle in *Pisum sativum* roots is reduced with increased temperature and the same trend is apparent for *Helianthus annuus* roots (Gudkov *et al.* 1974 cited in Francis and Barlow 1988, Burholt and van't Hof 1971). However according to Francis and Barlow (1988) "nothing is known about the relationship between temperature and the cell-cycle in shoot meristems".

It has been established that morphological development in the shoot apical meristem of *Helipterum roseum* proceeds through a

series of well defined stages from germination to anthesis (Chapter 3) and that perturbations in this sequence occur at high temperature (25 °C) (Chapter 4). The aim of this section is to determine the mechanism of this inhibition of flowering by investigating (a) the morphological development of the shoot apical meristem and (b) the duration of the cell-cycle and its component phases at the shoot apical meristem, during the floral transition.

5.2 Materials and methods

5.2.1 Part A: effect of temperature on morphological development of the shoot apical meristem

5.2.1.1 Experiment 1: determination of time-scale of apical development under controlled environment conditions at 20 °C

Thirty six plants of *Helipterum roseum* were placed in each of two controlled environment growth cabinets at 20 °C constant, photoperiod 12 h and light intensity 250 W m^{-2}. Growth cabinet specifications are given in section 4.2.1. Five apices were selected at random every three days from day two after planting and prepared for scanning electron microscopy as described in section 3.2.2.1. The morphological stage of each meristem was recorded according to the scale given in Table 3.1. The number of days after planting (range and mean) to the appearance of morphological stages 2, 3 and 4 was calculated.

5.2.1.2 Experiment 2: effect of high temperature (25 °C) on shoot apical development

This experiment was designed to determine whether high

(25 °C) temperature inhibited shoot apical development at stage 3 (doming of meristem) (trial 1) or stage 4 (appearance of involucral bracts) (trial 2). In both trials, seventy eight plants of *Helipterum roseum* were placed in each of two constant temperature conditions, 20 or 25 °C, with a photoperiod of 12 h and a light intensity of 250 W m^{-2}. Four controlled environment growth cabinets were used in each trial (two cabinets per environment).

Three plants were selected at random every three days from day 19 after planting (trial 1) and day 22 after planting (trial 2) (ie. prior to the first appearance of stages 3 and 4 recorded in experiment 1) from one cabinet in each environment. Apical meristems were dissected and prepared for scanning electron microscopy as previously described. The morphological stage of each meristem was recorded using the scale given in Table 3.1. When all three samples at 20 °C were at stage 3 (trial 1, 22 days after planting) or stage 4 (trial 2, 28 days after planting), 30 plants from each environment were exchanged between the two temperature conditions. Four temperature treatments were therefore imposed in each trial.

Treatment 1. Constant 20 °C

Treatment 2. Constant 25 °C

Treatment 3. Transfer from 20 to 25 °C

Treatment 4. Transfer from 25 to 20 °C

When all plants in treatment 1 (constant 20 °C) had reached stage 6 (macroscopic appearance of the apical inflorescence bud), five plants in each treatment were selected

and the apical meristems dissected. In previous experiments (Chapters 3, 4) all apices which reached stage 6 were subsequently observed to reach stage 7 (anthesis). A further five apical meristems were selected twice more at weekly intervals. Apices which had not produced a macroscopic inflorescence bud were observed by scanning electron microscopy. The stage of morphological development of each apex was recorded. The remaining 15 plants in each treatment were assessed at 119 days after planting (in previous experiments plants had completed stage 7, anthesis of the apical inflorescence, even in unfavourable environments by this time) and the number of plants producing normal and deformed apical blooms was recorded.

5.2.2 Part B: effect of temperature on the duration of the cell-cycle and its component phases in the shoot apical meristem

5.2.2.1 Experimental design

This experiment was designed to investigate the effect of constant temperature conditions; 20 °C (trial 1) and 25 °C (trial 2) on the length of the cell-cycle, at the shoot apical meristem. Cell-cycle duration was determined in trial 1 at days 16, 22 and 28 after planting (corresponding to morphological stages 2, 3 and 4 respectively) and in trial 2 at days 22 and 28 after planting (stage 2). Based on experience gained in the SEM studies of Chapters 3, 4 and 5 Part A, the stage of apical development was determined on the living plant with the aid of a stereo microscope, following removal of the apical leaves.

5.2.2.2 Growth conditions

Five hundred and four plants of *Helipterum roseum* were planted in trial 1 and 336 in trial 2. Seedlings were planted two per 12.5 cm pot and placed in controlled environment growth cabinets (42 pots per cabinet) in one of two constant temperature conditions 20 °C (trial 1) or 25 °C (trial 2), both with a photoperiod of 12 h and light intensity of 250 W m^{-2}.

5.2.2.3 Estimation of the cell-cycle duration

Treatment

Cell-cycle duration was estimated by the pulse-labelled-mitoses method. In essence a pulse of tritiated thymidine is applied to a cell population, cells are sampled at intervals thereafter, and the proportion of mitoses (metaphases) which are labelled are counted by autoradiography (Quastler and Sherman 1959). Tritium is incorporated into the DNA synthesised at the time of labelling and persists through subsequent divisions.

Seventy seven plants of uniform size and appearance were chosen on each day of treatment. Apical meristems from seventy plants were exposed by removal of approximately 10 surrounding leaves with the aid of a stereo microscope. Sixty three meristems were treated with [6-^{3}H]thymidine ([6-^{3}H]TdR) and seven with water. A further seven plants were left as untreated controls. The leaves were not removed from these plants and they received neither [6-^{3}H]TdR nor water. Small cotton plugs

impregnated with [6-^{3}H]TdR (0.37 MBq ml^{-1}; specific activity 910 GBq mmol^{-1}) or water were placed on the exposed meristems. Tween-20 at a concentration of 0.1 % was added to treatment solutions to improve uptake. The cotton plugs were removed after 2 h and meristems thoroughly washed with water before insertion of fresh cotton plugs moistened with water (these were renewed after 12 h in the longer sampling periods). Each meristem was covered from treatment to sampling with an 8.0 x 5.0 cm polyethylene bag secured with a snap fastener and supported by a plastic stake as shown in Fig. 1. Plants were returned to controlled environments (20 °C, trial 1 or 25 °C, trial 2) after treatment.

The effect of exposing the apical meristem and applying a cotton plug impregnated with water, as described above, on the subsequent development of the apex was also tested on a separate batch of plants. Five plants of *Helipterum roseum* were treated on each of days 16, 22 and 28 after planting at 20 °C and on days 22 and 28 after planting at 25 °C. A further five plants in each environment received no treatment (control). Plants were returned to controlled environments after treatment. The time (days after planting) to the appearance of macroscopically visible apical inflorescence buds (stage 6) was recorded.

Sampling

Seven meristems labelled with [6-^{3}H]TdR were dissected with the aid of a stereo microscope three, six, 12, 24, 36, 48, 60, 72 and 84 h after the beginning of the labelling period. Seven meristems treated with water and seven untreated controls

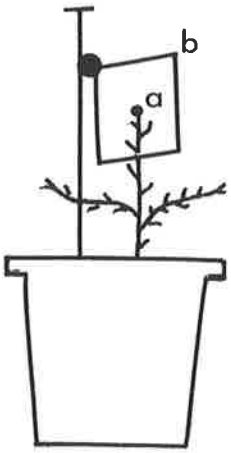


Figure 5.1

Estimation of the cell-cycle duration in *Helipterum roseum*. The apical meristem (a) is protected by a polyethylene bag (b) during treatment with [6-^{3}H] thymidine.

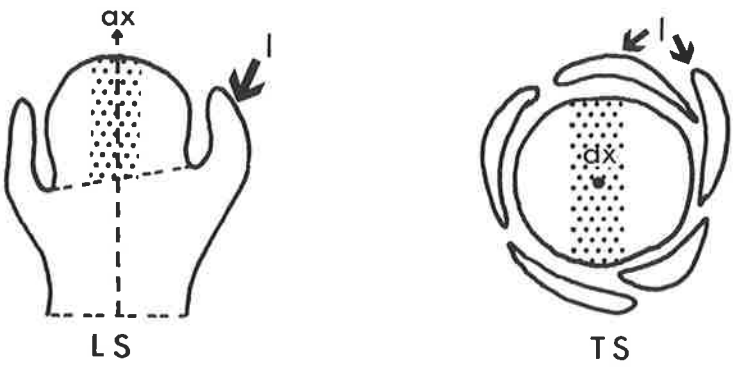


Figure 5.2

Longitudinal (LS) and transverse (TS) sections through the shoot apical meristem, at stage 3, showing sampling region (shaded), central axis (ax) and leaf primordia (l).

were collected 24 h after the beginning of the labelling period. The stage of morphological development of each meristem was recorded according to the scale given in Table 3.1. Meristems were fixed in formalin-propionic acid-50 % ethanol (5:5:90) and vacuum infiltrated overnight at 20 °C. They were dehydrated in a graded ethanol series, 2 h in each of two changes of methoxy ethanol, ethanol, propanal and butanol; infiltrated for 2 h in a butanol:GMA (glycol methacrylate) monomer mix (1:1) [1]; followed by two washes of two days duration in GMA. Specimens were embedded in GMA-filled gelatin capsules, and polymerised at 60 °C for 2 days (Feder and O'Brien 1968). Meristems were longitudinally sectioned at a thickness of 2.0 µm using an ultra-microtome, and 24 of the most axial sections (Fig. 2) were placed on microscope slides.

Light microscope autoradiography

Specimens were prepared for light microscope autoradiography following the method of Kopriwa and Leblond (1962). Sample slides were coated with Ilford L4 nuclear research emulsion in gel form (diluted 1:1 with water) using an Avarlaid semi-automatic coating machine; dried vertically for 1 h; placed in light-tight boxes, and exposed for seven days at 4 °C. Autoradiographs were developed in D170 developer [2] at 18°C for 8 min and fixed for 3 min in 24 % sodium thiosulphate solution.

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1. The GMA monomer was composed of 93 ml 2-hydroxyethyl methacrylate, 7 ml polyethylene glycol 400 and 0.6 g benzoyl perioxide

2. The D170 developer was composed of 25.0 g sodium sulphite (anhydrous), 1.0 g potassium bromide, 4.5 g 2,4-diaminophenol hydrochloride, 1000 ml water, pH 7.1

Sections were stained in 0.05 % toluidine blue O [3] for 1 min and mounted in Histoclad ® before viewing with a Zeiss Axiophot microscope using Nomarski interference optics at 100 x magnification.

5.2.2.4 Measurements

The percent of labelled mitoses in the ten most axial sections of each meristem was recorded. Alternate sections were scored, to eliminate the possibility of counting a single mitotic figure twice in successive sections. Only mitoses occurring in the region above the last formed involucral bracts or leaf primordia were considered (Fig. 2). Pulse-labelled-mitoses (PLM) curves for meristems at stages 2, 3 and 4 in trial 1 and stage 2 (22 and 28 days) in trial 2 were constructed using the mean per cent of labelled mitoses, calculated from three meristems, at each sampling time. The duration of the cell-cycle (T) and its component phases; pre-synthetic interphase (G1), the DNA synthetic phase (S), mitosis (M) and post-synthetic interphase (G2), were determined from these PLM curves according to the principles developed by Quastler and Sherman (1959) and described by Mitchison (1971). The earliest samples collected after a pulse of tritiated thymidine contain no labelled mitoses (Fig 3). The proportion of labelled mitoses then rises to a peak as the cells which were in S at the time of the pulse come through to division. Following this peak, there is a trough as the cells originally in G1 come to the end of their cycle. The second

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3. The toluidine blue O was composed of 0.1 g toluidine blue O in benzoate buffer; 0.29 g sodium benzoate, 0.25 g benzoic acid, 200 ml water, pH 4.5

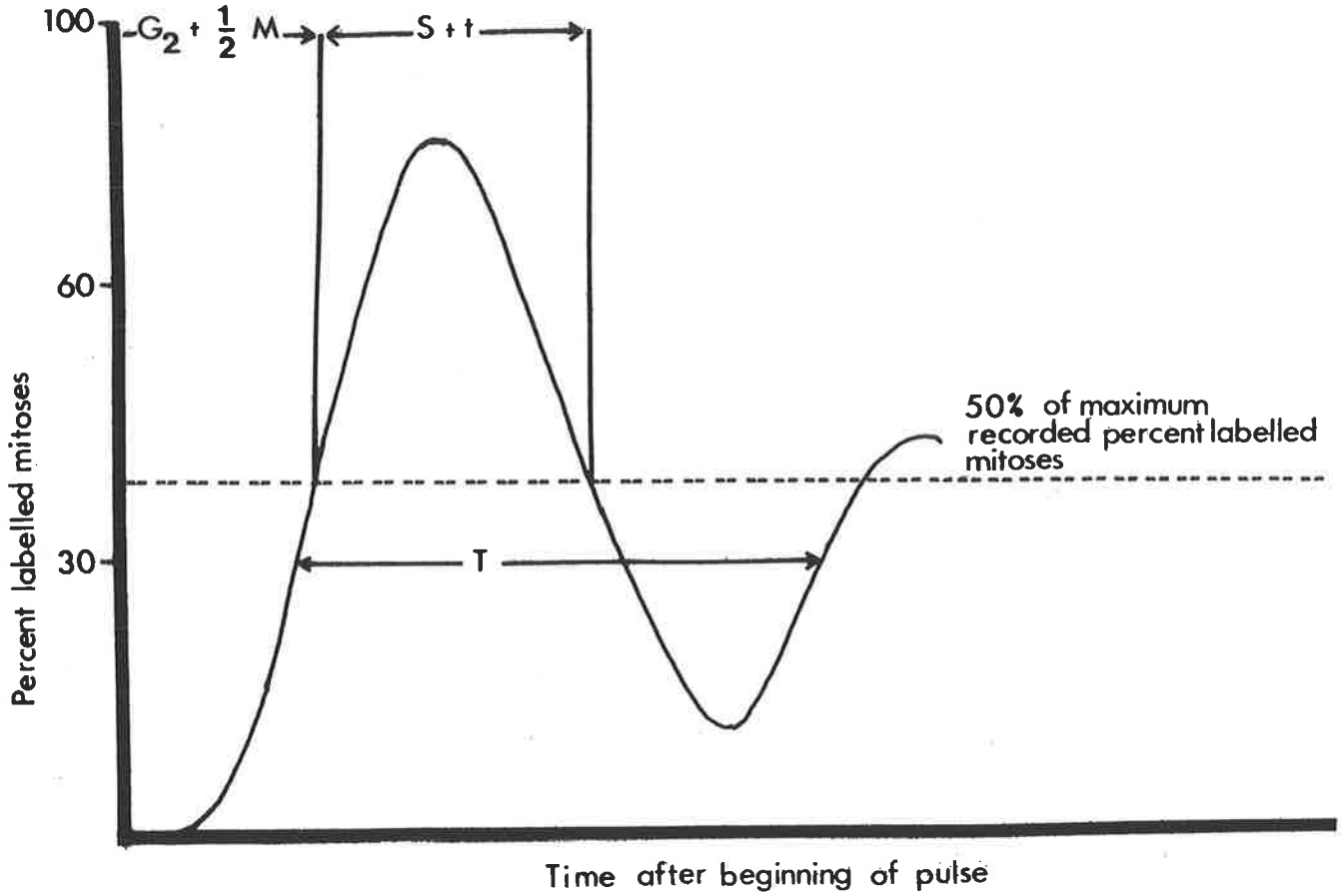


Figure 5.3

Diagram of percent labelled mitoses (metaphases) in successive cell samples after a pulse of tritiated thymidine (Hypothetical case, adapted from Quastler and Sherman 1959 and Mitchison 1971.)

T = Duration of the cell-cycle

S = Duration of the DNA synthetic phase

G2 = Duration of post-synthetic interphase

M = Duration of mitoses

t = Duration of pulse

The duration of G1 (pre-synthetic interphase) is derived using the formula $(G1 + \frac{1}{2}M) = T - S-(G2 + \frac{1}{2}M)$

cycle will then show a similar wave but the peak will be lower due to the spread in cycle times. The average S period plus labelling time is taken as the interval between the 50 % points of the maximum percent labelled mitoses on the first ascending and descending limbs of the PLM curve (Fig. 3). The time between the start of the pulse and the first of these points is taken as G2 +½M. Half the mitotic time is added to G2 because the mitoses are scored in metaphase. Thus the cells must pass through G2 and prophase before they are scored, and prophase lasts for roughly half the time of mitosis. The total cycle time (T) is the interval between two similar points in the first and second cycle. The duration of the cell-cycle (T) may be calculated as the duration between two successive peaks of the PLM curve (Quastler and Sherman 1959, van't Hof 1965, Gonthier *et al.* 1987) or as the duration between similar points on two successive ascending limbs of the PLM curve (Cattaneo *et al.* 1961, van't Hof 1966, Price and Bachmann 1976, Gonthier *et al.* 1985). The latter method of derivation was used for *Helipterum roseum* shoot apices, using the 30 % intercepts on the two successive ascending portions of the curve (shown in Fig. 3). The duration of G1 +½M is obtained by difference (G1 +½M) = T-S-(G2+½M).

5.3 Results

5.3.1 Part A

5.3.1.1 Experiment 1

Morphological stages 2, 3 and 4 of shoot apical development were observed in *Helipterum roseum* at 11-20, 20-26 and 26-32 days after planting, respectively, in controlled

environment conditions at 20°C (Table 1).

5.3.1.2 Experiment 2

The morphological development of the shoot apical meristem was affected by temperature (Tables 2, 3). Apical meristems in both trials, developed to stages 6 and 7 at constant 20°C but rarely progressed beyond stage 2 at constant 25°C. Morphological development was arrested at stage 3 when plants were transferred from 20 to 25°C at stage 3, and delayed for a short period when transferred at stage 4. Apical development in the latter proceeded to stage 7 in the majority of plants but resulted in the production of abnormal blooms (Figs 3.19, 3.20). Abnormalities included; reduced number and length of involucral bracts, crinkled involucral bracts, a thickened peduncle with increased numbers of bud scales, reduced number of florets on the receptacle, and reversions to vegetative growth after the production of a few bract scales. Transfer of plants from 25 to 20°C at both stages 3 and 4 resulted in very few apices reaching anthesis. Abnormal inflorescences were produced only in treatments 2 and 3, of both trials.

5.3.2 Part B

The removal of leaves surrounding the shoot apical meristem and application of cotton plugs impregnated with water did not affect subsequent floral initiation and development at 20°C (Table 4). Both control and treated plants at 25°C continued to produce similar numbers of leaves (Table 4).

Table 5.1 Time (days after planting) to appearance of morphological stages 2, 3 and 4 in shoot apical meristems of *Helipterum roseum* at constant temperature (20 °C), photoperiod (12 h) and light intensity (250 W m^{-2})

Morphological stage	Range	Mean ± s.e.
2	11–20	16.3 ± 1.1
3	20–26	25.7 ± 0.8
4	26–32	29.3 ± 0.8

Table 5.2 Effect of temperature on morphological development of the apical meristem and the number of normal and abnormal inflorescences of *Helipterum roseum* (Experiment 2, trial 1) as measured by transfer of plants between 20 and 25 °C at stage 3 (22 days after planting)

Days after planting	Days after transfer	Treatment * 1	2	3	4
		Mean stage of apical development (± s.e.)			
19		2.3 ±0.3	2.0 ±0.0	-	-
22		3.0 ±0.0	2.0 ±0.0	-	-
43	21	6.0 ±0.0	2.2 ±0.2	3.4 ±0.9	4.2 ±0.4
50	28	6.0 ±0.0	2.8 ±0.6	2.2 ±0.2	4.2 ±0.8
57	35	6.4 ±0.2	2.0 ±0.0	2.0 ±0.0	4.8 ±0.8
		Number of plants with inflorescences (of 15)			
119	97	15	2	2	4
		Number of plants with abnormal inflorescences (of 15)			
119	97	0	2	2	0

* Treatment
1. Constant 20 °C
2. Constant 25 °C
3. Transfer from 20 to 25 °C
4. Transfer from 25 to 20 °C

Table 5.3 Effect of temperature on morphological development of the apical meristem and the number of normal and abnormal inflorescences of *Helipterum roseum* (Experiment 2, trial 2) as measured by transfer of plants between 20 and 25 °C at stage 4 (28 days after planting)

Days after planting	Days after transfer	Treatment * 1	2	3	4
		Mean stage of apical development (± s.e.)			
25		3.6 ±0.3	2.0 ±0.0	-	-
28		4.0 ±0.0	2.0 ±0.0	-	-
48	20	6.1 ±0.2	2.2 ±0.2	4.0 ±0.9	4.6 ±0.9
55	27	6.4 ±0.2	2.0 ±0.0	6.4 ±0.2	4.8 ±0.8
62	34	6.4 ±0.2	2.0 ±0.8	6.4 ±0.2	6.0 ±0.0
		Number of plants with inflorescences (of 15)			
119	91	15	1	8	2
		Number of plants with abnormal inflorescences (of 15)			
119	91	0	1	6	0

* Treatment
1. Constant 20 °C
2. Constant 25 °C
3. Transfer from 20 to 25 °C
4. Transfer from 25 to 20 °C

Table 5.4 Effect of removing leaves surrounding the shoot apical meristem and the application of a cotton plug impregnated with water, on subsequent apical development under two constant temperature conditions

Values presented are means and standard errors of a minimum of three replicates.

Temperature (°C)	Treatment time (days after planting)	Morphological stage	Time to stage 6 # (days after planting)
20	16	2.0 ± 0.0	68.3 ± 8.3
	22	3.0 ± 0.0	61.6 ± 2.9
	28	4.0 ± 0.0	61.0 ± 2.9
	control	-	58.0 ± 1.0
25	22	2.0 ± 0.0	*
	28	2.0 ± 0.0	*
	control	-	*

Stage 6, macroscopic appearance of apical inflorescence bud

* Plants grown at constant 25°C did not produce apical inflorescence buds but both control and treated plants continued to produce similar numbers of leaves

[6-^{3}H]TdR was incorporated into the nuclei of apical cells of *Helipterum roseum* and labelled and unlabelled nuclei were easily identified (Figs 5.4 - 5.7). The total number of metaphases (labelled and unlabelled) present in the sampled area of shoot apical meristems at stages 2, 3 and 4 (trial 1, 20 °C) and stage 2 (trial 2, 25°C), 24 h after the beginning of the labelling period, was not affected by the physical removal of leaves surrounding the meristem and the application of [6-^{3}H]TdR (Table 5).

5.3.2.1 Trial 1, 20°C

The duration of the cell-cycle and its component phases in the shoot apical meristem of *Helipterum roseum* was estimated from PLM curves at stages 2, 3 and 4 (Fig. 8). The duration of the cell-cycle in the vegetative meristem (stage 2) was 64 h (Table 6). Cell-cycle duration was shortened by 36 % to 41 h during stage 3 (doming of meristem) and then lengthened by 14 % to 47.5 h during stage 4 (appearance of involucral bracts). The shorter cell-cycle at stage 3 was due to the reduced duration of G1, S and G2 phases. The greatest reduction occurred in G2 +½M, followed by G1 +½M and then S. Lengthening of G2 +½M was primarily responsible for the increase in cell-cycle duration observed at stage 4 compared with stage 3. The maximum proportion of mitoses labelled (peak of PLM curve) increased from 50 % at stage 2 to 76 and 83 % at stages 3 and 4 respectively.

Figures 5.4 to 5.7 Light microscope autoradiographs of longitudinal sections of shoot apical meristems of *Helipterum roseum* at stage 2, after labelling with [6-^{3}H] thymidine.

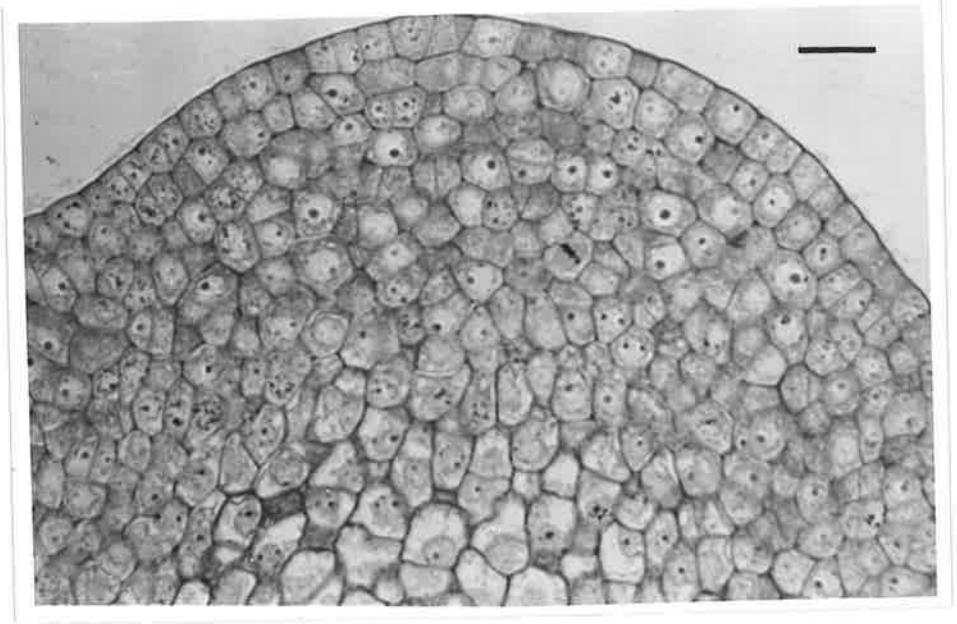


Figure 5.4 Shoot apical meristem sampled 36 h after labelling. Scale: 22 µm.

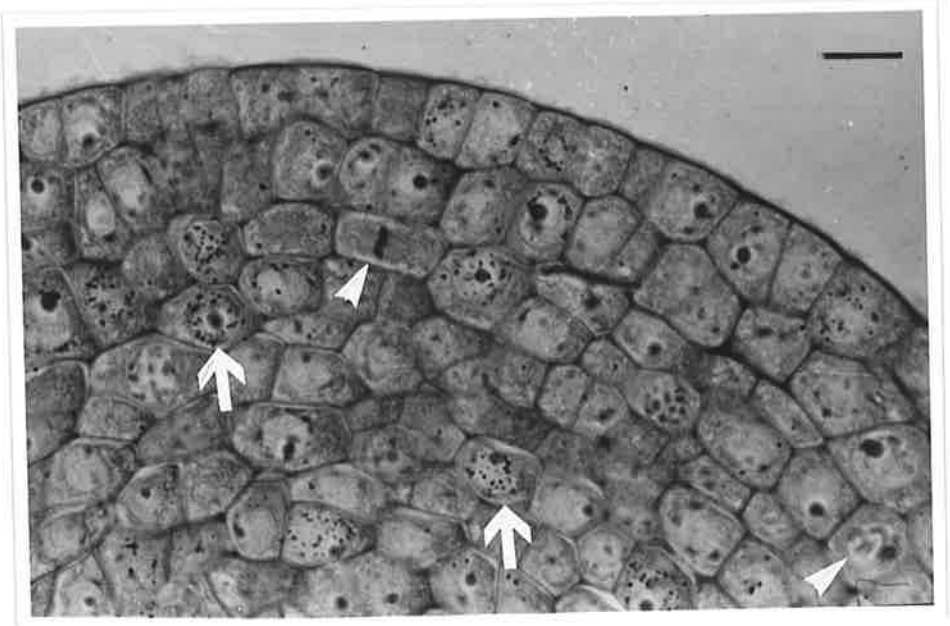


Figure 5.5 Shoot apical meristem sampled 12 h after labelling showing sites of DNA synthesis (⇨) and unlabelled metaphases (▷). Scale: 11 µm.

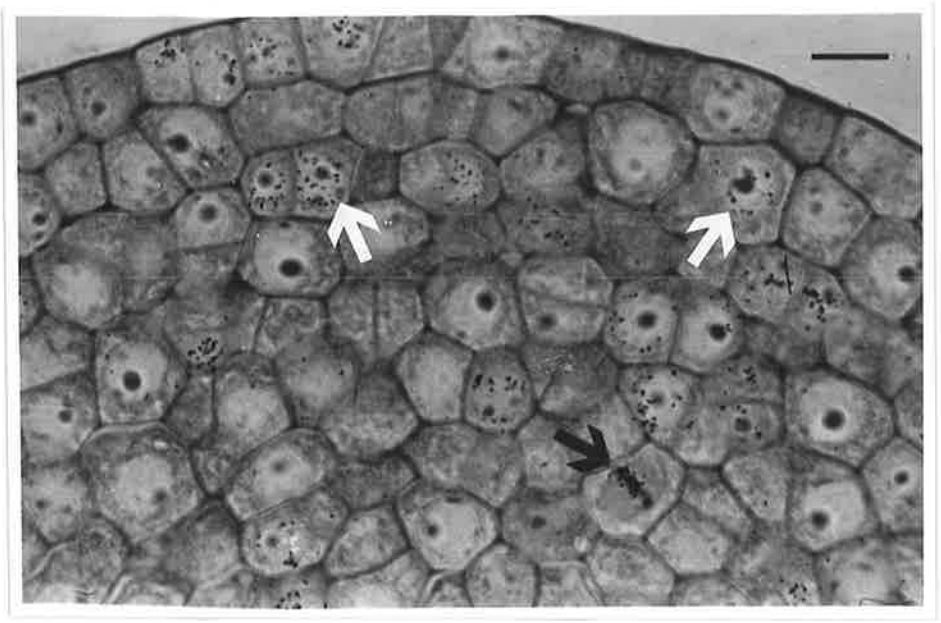


Figure 5.6 Shoot apical meristem sampled 36 h after labelling showing sites of DNA synthesis (⇨) and labelled metaphase (➔). Detail of Figure 5.4. Scale: 11 µm.

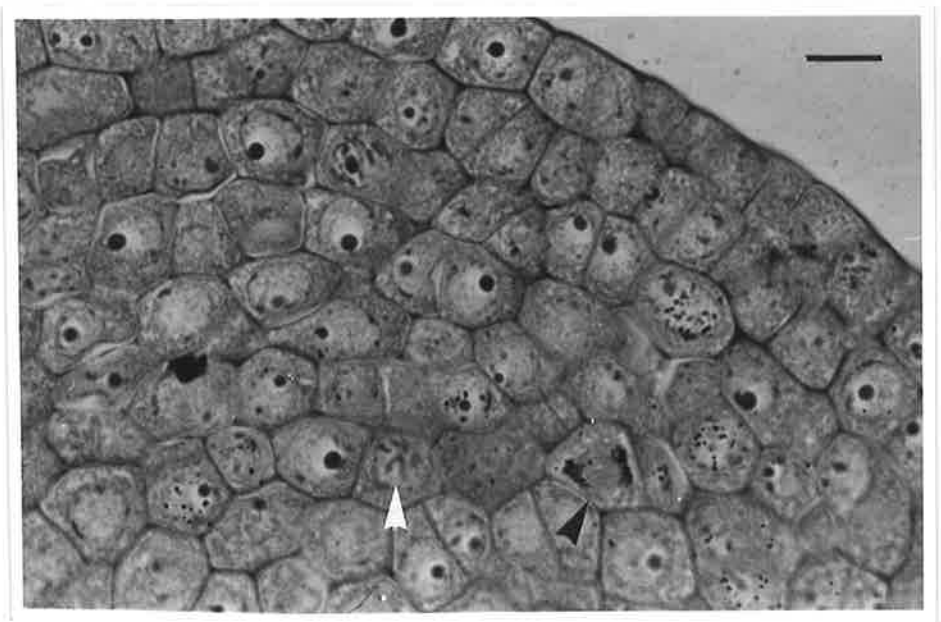


Figure 5.7 Shoot apical meristem sampled 60 h after labelling showing unlabelled metaphase (▷) and labelled anaphase (►). Scale: 11 µm.

Table 5.5 Number of metaphases present in shoot apical meristems of *Helipterum roseum* at stages 2, 3 and 4 (trial 1) and stage 2, 22 and 28 days after planting (trial 2), 24 h after the beginning of the labelling period

Values presented are means and standard errors of three replicates.

Treatment	Morphological stage				
	20 °C (Trial 1)			25 °C (Trial 2)	
	2	3	4	2 (22 days)	2 (28 days)
Untreated control	47.3 ± 5.9	30.6 ± 3.5	19.6 ± 3.9	30.3 ± 2.4	16.7 ± 2.9
Treated (water)	35.0 ±11.5	24.3 ± 5.0	12.0 ± 2.1	24.3 ± 1.7	11.3 ± 3.0
Treated ([6-^{3}H]TdR)	41.0 ± 6.2	25.3 ± 2.2	11.5 ± 1.8	35.6 ± 6.1	10.0 ± 1.6

Figure 5.8

Percent labelled mitoses after the beginning of a 2 h pulse of [6- ^{3}H]thymidine in shoot apical meristems of *Helipterum roseum* at (a) stage 2 (large vegetative meristem), (b) stage 3 (doming of meristem) and (c) stage 4 (appearance of involucral bracts), at 20 °C

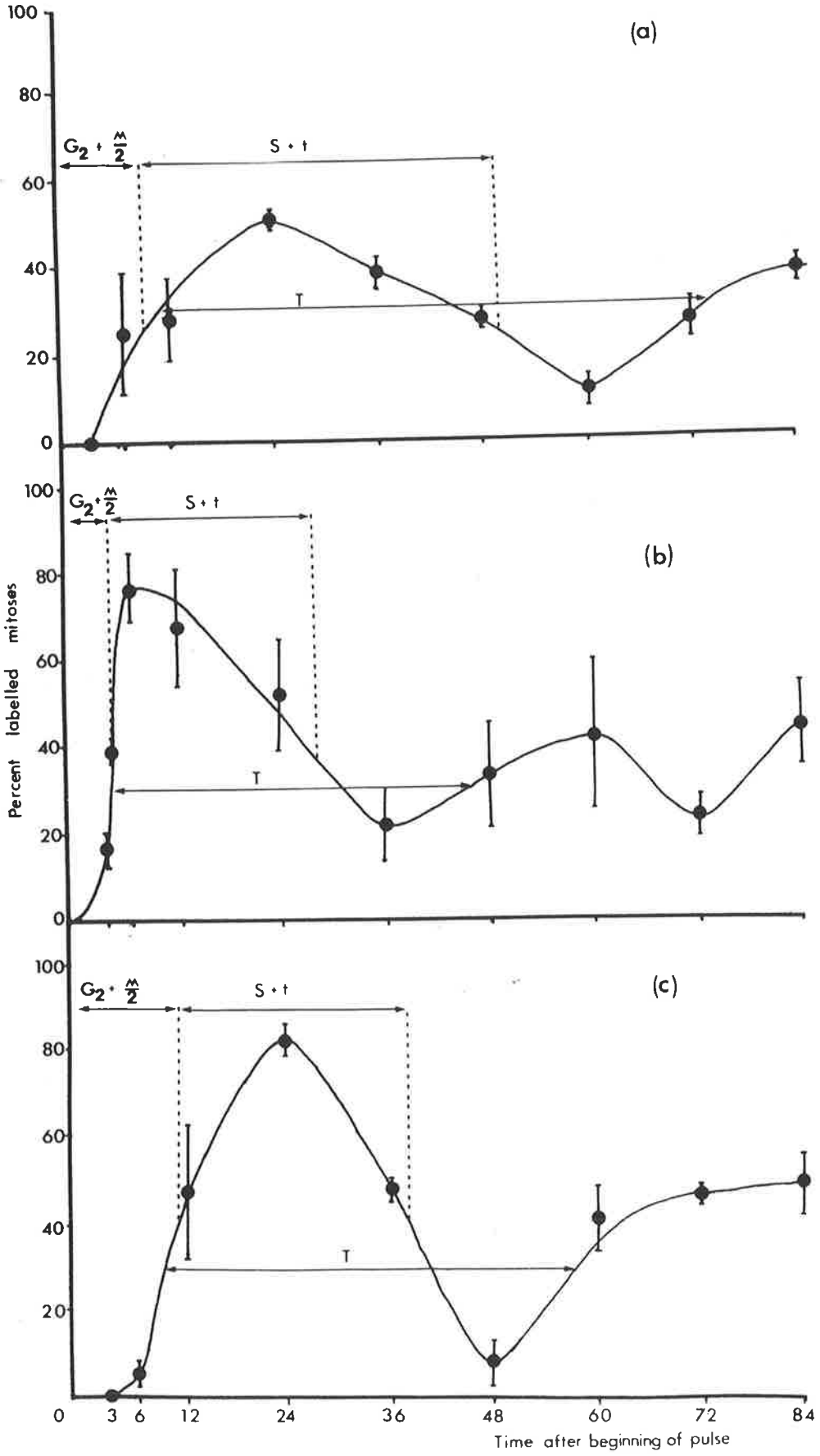

(a)
(b)
(c)
$G_2 + \frac{M}{2}$
S + t
T
Percent labelled mitoses
Time after beginning of pulse
0
20
40
60
80
100
3
6
12
24
36
48
60
72
84

Table 5.6 Duration (h) of the cell-cycle and its component phases, estimated from percent-labelled-mitoses curves (Figs 8, 9), in shoot apical meristems of *Helipterum roseum* at two temperatures

Temperature	Morphological stage	Length of cell-cycle	Pre-DNA synthetic phase	DNA synthetic phase	Post-DNA synthetic phase
(°C)	(Mean ± s.e.)	(T)	(G1+½M)	(S)	(G2+½M)
20	2.0 ± 0.0	64.0	27.0	39.0	9.0
20	3.0 ± 0.0	41.0	15.0	23.5	4.0
20	4.0 ± 0.0	47.5	11.0	25.5	11.0
25	2.0 ± 0.0 (22 days)	*			
25	2.0 ± 0.0 (28 days)	*			

* Steady-state cell-cycling was not observed in shoot apical meristems of *Helipterum roseum* at 25°C and no PLM curves were obtained

5.3.2.2 Trial 2, 25°C

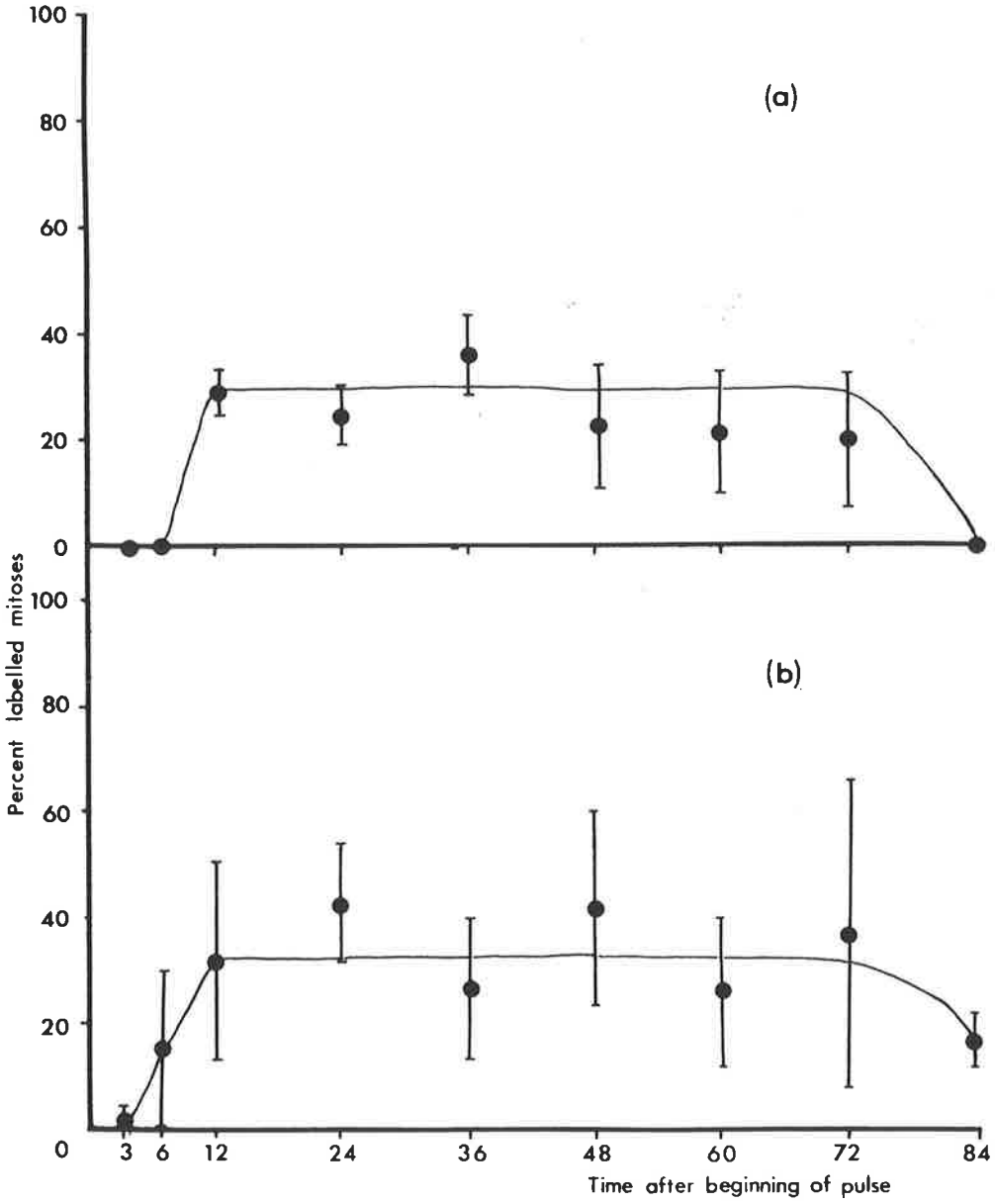
PLM curves were not obtained for meristems labelled at stage 2 (22 and 28 days after planting) at 25 °C (Fig 9). Following low rates of incorporation at 3 and 6 h after labelling, the maximum proportion of labelled mitoses was maintained at approximately 30 % until a reduction at 84 h.

5.4 Discussion

This study has shown that temperature influences both floral initiation and development in *Helipterum roseum*. Morphological development of the apical meristem in constant 20°C conditions proceeds from the vegetative to the reproductive state via a series of well defined stages (see Chapter 3). This sequence is interrupted in constant 25 °C conditions, and the meristem rarely proceeds beyond stage 2, the large vegetative meristem. A few plants do initiate flower buds at 25°C and this variability is probably due to natural genetic variation within the seedling population. Complete inhibition of floral initiation by constant high temperature is a feature of many plants which require vernalisation (a low temperature treatment) before they are induced to initiate flower primordia eg. *Brassica oleracea* var. *gemmifera* (Brussel sprouts) and *Matthiola incana* (stock) (Lang 1965). *Helipterum roseum* does not, however, have a requirement for vernalisation since floral initiation takes place in constant 20°C conditions. There are few reported examples of complete inhibition of floral initiation by high temperature in plants which do not require vernalisation. Floral initiation in *Senecio cruentus* cvs 'Master' and 'Gmunder zwerg'

Figure 5.9

Percent labelled mitoses after the beginning of a 2 h pulse of [6- ^{3}H]thymidine in shoot apical meristems of *Helipterum roseum* at stage 2 (large vegetative meristem) (a) 22 days after planting and (b) 28 days after planting, at 25 °C


100
80
60
40
20
0
(a)
100
80
60
40
20
0
(b)
Percent labelled mitoses
3
6
12
24
36
48
60
72
84
Time after beginning of pulse

is inhibited when constant temperature conditions exceed 18 or 21 °C respectively (Strømme and Hildrum 1985) and *Chrysanthemum frutescens* will not initiate flower buds if night temperatures exceed 20 °C (Konishi and Azuma 1975). Retardation of floral initiation by high temperature is, on the other hand, a feature of many plants. For example, floral initiation in *Silene* is delayed as the temperature rises in the range 13 to 25 °C (Lyndon 1977). A similar response is observed in carnation at temperatures above 10 °C (Bunt and Cockshull 1985) and in *Perilla* at temperatures above 32 °C (Vince-Prue and Gressel 1985).

Inhibition of floral initiation by high temperature in *Helipterum roseum* is dependent on the morphological stage of the meristem during the high temperature treatment. Floral initiation is reduced if the temperature rises from 20 to 25 °C when the apical meristem is at stage 4 (appearance of involucral bracts) but is inhibited if the perturbation occurs during stage 3 (doming of meristem). Stage 3 is therefore more sensitive to the change in temperature. These observations indicate that the apical meristem of *Helipterum roseum* is committed to the production of an inflorescence at stage 4 but that developmental pathways are still optional at stage 3. Thus not all of the events of floral evocation, which are required to commit the shoot apex to flower, are present at stage 3, and these meristems are therefore only partially evoked. Stage 2 apices were observed at 28 and 35 days after transfer from 20 to 25 °C at stage 3 (Table 2). It is possible that this may represent a reversion of the apical meristem from stage 3 to stage 2 under the high temperature conditions of 25 °C. This may occur via perturbations in cell-cycle duration in the apical meristem.

Alternatively it may indicate a sampling problem due to inherent variability in the stage of the apical meristem in the seedling population (Table 1). Nevertheless, the fact that apices were arrested following transfer at stage 3, but that approximately half of the plants flowered following transfer at stage 4 indicates that stage 3 is more sensitive than stage 4 to the 25 °C conditions. This confirms work in other members of the Asteraceae. Lance (1957) concluded that the "point of no return" in meristems of *Chrysanthemum segetum*, *Aster sinensis* and *Leucanthemum parthenium* occurred during the last part of the prefloral stage, when the first involucral bracts were initiated (corresponding to stage 4 of the morphological scale for *Helipterum roseum*). Such meristems continued development towards the reproductive state even when the plants were subsequently placed in unfavourable photoperiodic conditions.

High temperature also affects floral development in *Helipterum roseum*. Normal blooms were produced in 20 °C growing conditions, whilst a greater number of abnormal blooms with an increased number of bud scales, thickened peduncle, twisted involucral bracts and reduced floret number, were observed when plants were transferred to 25 °C conditions at either stage 3 or stage 4. The production of abnormal blooms or a high incidence of flower abortion is observed after high temperature treatment in many flower crops. Floral abortion in *Chrysanthemum* cv. 'Orange Bowl' results when plants are transferred from 22/18 °C (day/night) to 30/26 °C (day/night) (Whealy *et al.* 1987) and flower quality is reduced due to small flowers and distorted stems in *Tulipa* when day/night temperatures are raised from 18/14 °C to 26/22 °C (Dosser and Larson 1981).

Relatively few apical inflorescences were produced by plants held at 25 °C, either throughout development, or when transferred to 25 °C at stage 3 of the apical meristem. This suggests that the 25 °C temperature conditions may be inhibiting the attainment of the floral state at an early stage of development. Lance (1957) proposed that meristems of *Chrysanthemum segetum* and *Aster sinensis* held continuously in unfavourable photoperiodic conditions attained an "intermediate" configuration somewhere between the vegetative and prefloral state and that this condition was preparatory to flowering. The results with *Helipterum roseum* suggest that there is no intermediate configuration in *Helipterum roseum*, and that the capacity to flower is rapidly lost by the apical meristem under adverse temperature conditions.

This work has also established that there is a well defined cell-cycle in the shoot apical meristem of *Helipterum roseum*. This corroborates research using the same technique in shoot apical meristems of other species eg. *Rudbeckia bicolor* (Jacqmard 1970) and *Sinapis alba* (Gonthier *et al.* 1985, 1987) and other tissues eg. root meristems of *Pyrrhopappus multicaulis* and *Agoseris retrorsa* (Price and Bachmann 1976). The exposure of the apex, and labelling treatments did not result in physiological perturbation at the shoot apical meristem, as shown by continued development of the shoot apex, and unchanged numbers of mitoses between treated and untreated plants.

The duration of the cell-cycle was constant in cells labelled during the pulse at stages 2, 3 and 4 (64, 41, 47.5 h respectively). The longest cell-cycle was observed at stage 2 (large vegetative meristem) and the shortest at stage 3 (doming

of meristem). Cell-cycle duration was also reported to be reduced from that observed in the vegetative meristem in induced meristems of the absolute LDPs *Silene* (Francis and Lyndon 1978 b) and *Sinapis* (Gonthier *et al.* 1987), and in domed meristems of the quantitative LDP *Chrysanthemum segetum* (Nougarède *et al.* 1987), and the DNPs *Datura Stramonium* (Corson 1969) and *Helianthus annuus* (Marc and Palmer 1984). The induced apices of *Silene* and *Sinapis* correspond to a morphological stage between stages 2 and 3, while the domed apices correspond to stage 3 of the morphological scale for *Helipterum roseum*. Reduced cell-cycle duration during the transition from stage 2 to 3, is therefore observed in many plants and may be an integral part of the flowering process. The fact that plants with different photoperiodic requirements respond in a similar manner suggests that the reduction in cell-cycle duration during the floral transition is independent of the photoperiodic control of the flowering response.

Previous research on cell-cycling in the apical meristem has concentrated on the progress of the meristem from stage 2 to 3. It has been established in Part A that the shoot apical meristem of *Helipterum roseum* is not committed to flowering at stage 3. This study also investigates cell-cycling in shoot apical meristems of *Helipterum roseum* which are committed to flowering ie. meristems at stage 4 (appearance of involucral bracts). The length of the cell-cycle at stage 4 is slightly greater than at stage 3, but still considerably less than at stage 2. The only other case where cell-cycle duration has been investigated in meristems beyond stage 3 is reported by Gonthier *et al.* (1987) using meristems of *Silene* at the time of

initiation of the first flower primordia (which corresponds to stage 5 of the morphological scale for *Helipterum roseum*). They concluded that cell-cycle duration was reduced from 38 h in induced meristems to 32 h at stage 5. It appears therefore that the duration of the cell-cycle in the shoot apical meristem changes at all morphological stages from the vegetative to the reproductive state, but that the greatest change is probably the reduction which occurs between the vegetative and partially evoked states (stages 2 and 3 respectively). In this and all comparable investigations, the reduction in duration of the cell-cycle results from a decrease in the length of all three component phases, G1, S and G2 (Francis and Lyndon 1979, Gonthier *et al.* 1985, 1987).

The cells in shoot apical meristems of *Helipterum roseum* at 20 °C, which are labelled during the pulse proceed through the cell-cycle at a constant rate for each stage of morphological development. That is, a "steady-state" system, as defined by Quastler and Sherman (1957), exists for this subpopulation of cells and PLM curves are obtained. Thus all labelled metaphase cells are synchronised in their division. This is not the case with the subpopulation of cells which is labelled during the pulse in shoot apical meristems at 22 and 28 days after planting at 25 °C. Labelled cells do not have the same cell-cycle duration as each other ie. they proceed through the cell-cycle at different rates. Therefore a steady-state system does not exist at either 22 or 28 days after planting and no PLM curves are obtained. The reduction in percent labelled mitoses 84 h after labelling at 22 and 28 days, at 25 °C is probably due to the fact that [6-^{3}H]TdR is diluted with each subsequent cell division, and

therefore labelled cells are more difficult to identify. The fact that steady-state systems are observed at 16, 22 and 28 days after planting at 20 °C, but not at 22 and 28 days after planting at 25 °C, indicates that the loss of steady-state cell-cycling may be a characteristic feature of meristems of *Helipterum roseum* held at 25 °C.

25 °C conditions at a 12 h photoperiod, either throughout development, or from stage 3 of the apical meristem onwards, reduce the number of flowers of *Helipterum roseum* by arresting the development of the shoot apex at stages 2 or 3. Nevertheless, these apices are capable of continued vegetative growth and the production of morphologically normal leaves.

The maximum proportion of labelled mitoses (peak of PLM) curve does not reach 100 % in the apical meristem of *Helipterum roseum* at 20 °C. This implies that there are apical cells which do not comply to the steady-state system and which proceed through the cell-cycle at either a faster or slower rate than those cells labelled during the pulse. The peak of the PLM curve will increase when the proportion of these cells decreases. Gonthier *et al* (1985) confirmed that the apical meristem of *Sinapis* was composed of at least two subpopulations of cells which they termed rapid cycling and very slow cycling cells. There is a greater proportion of cells which conform to the steady-state system at stages 3 and 4 than at stage 2 in *Helipterum roseum*. Gonthier *et al.* (1985, 1987) similarly reported that a greater proportion of apical cells of *Silene* conformed to the system in stage 5 than stage 2 meristems. Therefore the proportion of cells with cell-cycle durations the same as those cells labelled during the pulse, increases during the floral transition and

subsequent floral development. This could occur in two ways; firstly the cell-cycle duration of some of the cells which do not conform to the system at stage 2 could be altered by changes in the duration of one or all of the component phases; or secondly there could be a release of previously non-cycling cells into the system which also have the same cell-cycle duration as the cells labelled during the pulse. Both of these imply an accumulation of cells into a particular phase of the cell cycle and therefore "partial synchrony". Francis and Lyndon (1979) inferred from changes in the proportion of cells with 2 C and 4 C DNA amounts that there was a rise in the proportion of cells in the G2 phase in apical meristems of *Silene* on the ninth day after floral induction. However an increased proportion of G2 cells is not considered to be essential for flowering in this species since plants will flower when no rise is observed (Grose and Lyndon 1984). Recent work by Gonthier *et al.* (1987) with *Sinapis* has provided some evidence that non-cycling cells are released from the G1 phase into the S phase at the time of floral induction. It is possible that the presence of a subpopulation of cells with constant cell-cycle durations is necessary for floral induction to proceed and that the presence of a high proportion of cells with constant cell-cycles in the apical meristem may be an indication of evocation and commitment to flowering. This would explain why floral initiation in *Helipterum roseum* does not proceed in adverse temperature conditions of 25 °C, where no well-defined cell-cycle was observed despite the fact that these plants were capable of continued vegetative development. However, it also implies that at 20 °C, even at stage 2 (large vegetative meristem), due to the existence of a well defined cell-cycle some of the processes for floral evocation may be in

progress. This suggests that floral evocation commences at a very early stage in *Helipterum roseum* (in this case less than 16 days after planting), which would be consistent with the ephemeral nature of this species.

This study has extended existing knowledge on the physiological control of flowering in *Helipterum roseum* by determining both the morphological stage at which inhibition is imposed by high temperature, and the cytological basis of the perturbation. The condition of evocation appears to correspond to a doming of the apical meristem, whereas commitment to flowering is achieved only upon the initiation of involucral bracts. Inhibition by 25 °C arrests apical morphological development at the vegetative stage, and results in a loss of steady-state cell-cycling. As the ability to flower appears to be lost at an early stage under 25 °C conditions, and large vegetative meristems at 20°C have a well defined cell-cycle it is suggested that the flowering process in *Helipterum roseum* is a continuous event from seed germination onwards. This implies that the complex sequence of events leading to flowering are already in train, long before the stage of evocation, which is generally considered to be the first stage of floral development.

CHAPTER 6. PRODUCTION OF *Helipterum roseum* AND *Helichrysum bracteatum* FOR THE CUT-FLOWER MARKET

6.1 Introduction

Many Australian native plant species are successfully produced as cut-flower crops during their natural flowering season. It is, however, desirable to develop and implement management techniques and post-harvest handling procedures which extend this season, to both ensure a continued supply of top quality blooms, and to provide the flexibility to target peak periods in demand. *Chrysanthemum morifolium* is a traditional flower crop which is successfully produced on a year round cycle by the manipulation of photoperiod and temperature (Kofranek 1980). Flowering in this species may be scheduled to coincide with peaks in market demand such as Christmas and Mothers' Day.

Severe water stress is a major factor contributing to the early wilting and senescence of cut-flowers and is thought to be caused by the physical blockage of the xylem vessels by either air embolisms, and/or bacteria (van Doorn 1988, Dixon and Peterson 1989). Post-harvest treatment, with solutions containing a combination of germicide, citric acid and sucrose, are recommended to extend vase-life of traditional flower crops such as carnation and stock (Faragher 1986) and some Australian native flowers eg, *Chamelaucium uncinatum* (Joyce 1988) and *Anigozanthos manglesii* (Heggers 1983). Germicides and citric acid solutions reduce bacterial growth and facilitate water uptake, whilst sucrose is a food source for the cut-flower, and helps maintain respiration and normal cell function (Halevy and Mayak 1981). Two main methods of post-harvest treatment are common; pulsing and

holding. Pulsing is a short-term treatment designed to last the entire shelf-life of the flower, whilst holding solutions are used continuously by the wholesaler and consumer. Ethylene, a natural plant hormone, also contributes to the early senescence of cut-flowers (Halevy and Mayak 1981). A single application of silver thiosulphate (STS) immediately after harvest is beneficial in inhibiting the action of ethylene and is recommended for many traditional flower crops eg. snapdragon, sweet pea and carnation (Faragher 1986).

There is potential to produce blooms of both *Helipterum roseum* and *Helichrysum bracteatum* for the fresh cut-flower market but information on cultural conditions and post-harvest handling procedures are not available. The aims of this section are: (a) to investigate inflorescence production in these species in two commercial growing conditions; glasshouse and outdoors and to develop guidelines for the scheduling of these crops for optimum production, (b) to determine post-harvest handling procedures for maximum vase-life.

6.2 Materials and methods

6.2.1 Experiment 1: commercial production

6.2.1.1 Environmental conditions

Twenty plants of each species were placed in each of two environmental conditions; glasshouse and outdoor, at four planting dates. Plants in the outdoor treatments were grown in the glasshouse for two weeks prior to transfer to the outdoor environment. Preliminary experiments had shown that plants

placed directly outdoors had a low survival rate due to poor establishment at this early stage. Meteorological data for the first three months of growth for each planting date are given in Table 1. Previous work has shown that floral initiation in both species occurs within the first three months of plant growth and development (Table 3.1).

6.2.1.2 Measurements

Data were collected for all experimental treatments in each environment on a minimum of 10 plants, twice weekly from day one after planting until plant death. The time to macroscopic appearance of the apical inflorescence bud and anthesis of the apical inflorescence, plant height and diameter, number of nodes, internode length, diameter of the apical inflorescence and plant dry weight were recorded as described in section 4.2.2. The total number of inflorescences reaching anthesis during the lifespan of each plant and the flowering period of each plant were also recorded. Inflorescence diameter and stem length (measured from the base of the inflorescence to the point of attachment to the main stem) were recorded for all inflorescences when the blooms opened. All inflorescences were removed from the plant after measurements were taken. The proportion of plants which exhibited floral abnormalities (Figs 3.19, 3.20) as described in section 5.3.1.2 was recorded.

Inflorescences reaching anthesis at day one, 21, 42, 49, 63 and 70 from anthesis of the apical inflorescence were collected on three plants of *Helipterum roseum* at planting date 4 in the outdoor environment. The following measurements were

Table 6.1 Growing conditions for *Helipterum roseum* and *Helichrysum bracteatum* at four planting dates in glasshouse and outdoor environments

Values presented are means and standard errors for the first three months of growth

Planting date	Daylength (h)	Daylength trend	Temperature Max (°C)	Temperature Min (°C)	Temperature Average (°C)	Light intensity (Wm^{-2})	Temperature Max (°C)	Temperature Min (°C)	Temperature Average (°C)	Light intensity (Wm^{-2})
			Glasshouse				Outdoor			
1. (4 xii 86) Summer	14.0 ±0.5	decreasing	28.8 ±1.4	16.3 ±1.0	22.6 ±1.0	179.7 ±49.8	25.8 ±1.7	15.0 ±1.2	20.2 ±1.7	482.4 ±133.8
2. (5 iii 86) Autumn	11.5 ±1.0	decreasing	26.2 ±1.7	15.2 ±1.2	20.7 ±0.6	120.0 ±36.3	21.5 ±3.2	13.2 ±1.7	17.4 ±2.6	322.4 ±97.7
3. (1 vi 86) Winter	10.2 ±0.5	increasing	25.1 ±0.8	16.7 ±0.8	20.9 ±0.8	80.4 ±28.2	15.1 ±0.8	8.7 ±0.6	11.9 ±0.8	221.2 ±84.9
4. (27 viii 87) Spring	12.7 ±1.0	increasing	25.4 ±4.0	17.3 ±1.4	21.4 ±2.9	154.8 ±42.9	21.3 ±3.2	11.7 ±1.9	16.5 ±2.6	414.5 ±122.5

recorded; (i) inflorescence diameter, (ii) length of the longest involucral bract, (iii) receptacle diameter, (iv) number of florets per capitulum.

6.2.2 Experiment 2: post-harvest handling

Forty plants of each species were placed in the glasshouse on 1 June 1988. Apical blooms of *Helipterum roseum* were harvested at anthesis. The bloom and entire stem were severed from the plant. Sprays of *Helichrysum bracteatum* were harvested when the apical inflorescence reached anthesis. Each spray (apical inflorescence and stem, complete with 4.0 ± 0.2 [1] developing inflorescence buds) was severed from the plant at its base. Blooms of both species were harvested in the morning, stem length was trimmed to 400 mm and blooms immediately placed in water. Ten blooms of each species were placed in separate vases in each of three treatments; 1, distilled water; 2, 2 % sucrose solution containing 0.08 g/l pool chlorine (60 % sodium dichloroisocyanurate) and 0.25 g/l citric acid (Faragher 1986); 3, 5 % sucrose solution plus above additions as a 24 h pulse followed by distilled water. Blooms were placed at 20.0 ± 2.0 °C in constant fluorescent light (50 W m^{-2}). The blooms were inspected daily and solutions replaced every three days. The number of days from harvest to the end of the vase-life of each bloom was recorded. Blooms were considered to have reached the end of their vase-life when one of the following occured; (a) the bloom began to nod due to loss of turgidity in the inflorescence stem, (b) the involucral bracts reflexed releasing the florets or

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1. Mean ± standard error

(c) wilting commenced in the leaves or stem.

6.2.3 Statistical analysis

6.2.3.1 Experiment 1

The effect of planting date on growth and flowering for each species and environment was determined by a one-way analysis of variance for each growth parameter measured.

The effect of time from anthesis of the apical inflorescence on the diameter of successive inflorescences and stem length, of each species in each environment was determined by a one-way analysis of variance for each planting date.

The effect of time from anthesis of the apical inflorescence on inflorescence size of *Helipterum roseum* was determined by a one-way analysis of variance for each parameter recorded.

6.2.3.2 Experiment 2

A one-way analysis of variance was used to test the effect of post-harvest treatment solutions on vase-life of the blooms.

6.3 Results

6.3.1 Experiment 1

Blooms of *Helipterum roseum* and *Helichrysum bracteatum* were produced under all experimental conditions (Tables 2, 3).

Table 6.2 Effect of planting date (1-4) on growth and flowering of *Helipterum roseum* in glasshouse and outdoor environments

Values presented are means and standard errors of a minimum of 10 replicates

	Glasshouse planting date:				Level of	Outdoor planting date:				Level of
	1	2	3	4	significance	1	2	3	4	significance
Time to macroscopic appearance of apical inflorescence bud (days from planting)	42.7 ±1.5	77.6 ±3.2	63.7 ±2.5	56.8 ±1.7	**	35.8 ±4.0	90.7 ±2.2	30.8 ±1.0	59.5 ±0.3	**
Time from macroscopic appearance to anthesis of the apical inflorescence (days)	23.1 ±1.1	41.4 ±2.9	29.0 ±1.1	30.7 ±1.6	**	27.0 ±1.5	78.0 ±3.5	39.5 ±0.3	17.8 ±0.3	**
Time to anthesis of apical inflorescence (days from planting)	65.8 ±3.0	122.8 ±4.4	92.8 ±1.0	87.5 ±3.6	**	61.1 ±5.5	168.6 ±3.3	70.3 ±0.8	77.3 ±1.1	**
Plant height at anthesis of apical inflorescence (mm)	609.0 ±34.1	820.0 ±49.4	578.0 ±15.0	612.0 ±28.3	**	480.0 ±39.6	710.0 ±35.8	561.0 ±35.5	513.0 ±19.1	**
Number of nodes to apical inflorescence	81.7 ±5.5	154.7 ±12.7	93.4 ±15.0	117.3 ±8.0	**	71.9 ±12.9	126.9 ±5.6	75.8 ±2.6	85.2 ±2.3	**
Internode length at anthesis of apical inflorescence (mm)	7.5 ±0.6	5.1 ±0.2	6.3 ±0.3	5.1 ±0.5	**	6.7 ±0.5	5.7 ±0.4	7.4 ±0.5	5.8 ±0.3	**
Total number of inflorescences produced per plant	16.7 ±3.9	32.1 ±3.9	29.5 ±5.1	6.6 ±1.6	**	25.6 ±8.2	34.4 ±3.3	21.2 ±2.3	30.2 ±2.6	n.s.
Lifespan of plant (days from planting)	210.9 ±3.9	191.9 ±3.7	141.4 ±2.7	112.0 ±0.3		271.1 ±9.6	247.5 ±9.6	108.5 ±0.0	142.4 ±1.5	**
Flowering period (days)	145.1 ±4.1	72.9 ±4.6	48.7 ±2.6	24.5 ±3.6	**	205.3 ±10.6	78.9 ±3.0	38.2 ±0.8	65.2 ±1.7	**
Diameter of apical inflorescence at anthesis (mm)	41.7 ±1.5	42.5 ±1.7	43.0 ±1.5	40.0 ±1.8	n.s.	36.7 ±1.0	51.0 ±2.2	47.7 ±11.3	32.9 ±1.3	**
Plant dry weight at 84 days from planting (g)	18.5 ±2.2	11.0 ±0.5	4.6 ±0.3	18.0 ±1.3	**	13.2 ±1.3	6.1 ±0.1	2.8 ±0.1	2.6 ±0.3	**
% of plants with floral abnormalities	46.6	19.0	7.6	83.3		30.0	18.0	0.0	7.0	

** $P < 0.01$; n.s., not significant.

Table 6.3 Effect of planting date (1-4) on growth and flowering of *Helichrysum bracteatum* in glasshouse and outdoor environments

Values presented are means and standard errors of a minimum of 10 replicates

	Glasshouse planting date: 1	2	3	4	Level of significance	Outdoor planting date: 1	2	3	4	Level of significance
Time to macroscopic appearance of apical inflorescence bud (days from planting)	37.5 ±0.9	60.1 ±3.7	89.7 ±2.0	69.7 ±3.2	**	96.9 ±11.0	154.0 ±1.9	119.5 ±1.9	68.5 ±1.9	**
Time from macroscopic appearance to anthesis of the apical inflorescence (days)	25.6 ±0.5	38.0 ±0.6	31.5 ±0.5	23.6 ±1.0	**	28.0 ±1.1	53.1 ±0.7	34.5 ±0.2	24.5 ±1.2	**
Time to anthesis of apical inflorescence (days from planting)	63.0 ±2.8	100.2 ±5.3	121.2 +2.0	91.9 ±3.2	**	124.9 ±12.5	207.1 ±0.9	154.0 ±1.3	92.8 ±2.7	**
Plant height at anthesis of apical inflorescence (mm)	424.0 ±33.5	415.0 ±10.6	668.0 ±26.6	571.0 ±10.3	**	413.0 ±23.3	622.0 ±56.6	594.0 ±34.7	558.0 ±20.7	**
Plant diameter at anthesis of apical inflorescence (mm)	326.5 ±9.2	350.9 ±33.8	394.6 ±9.6	380.4 ±29.4	**	201.7 ±16.8	271.8 ±16.0	287.9 ±15.4	262.5 ±5.6	**
Numbers of nodes to apical inflorescence	26.1 ±1.7	38.5 ±1.7	43.5 ±1.8	38.9 ±1.6	**	36.7 ±2.7	59.2 ±1.8	39.7 ±0.5	35.9 ±1.4	**
Internode length at anthesis of apical inflorescence (mm)	17.8 ±1.5	10.5 ±1.2	15.6 ±0.8	14.2 ±1.0	**	11.5 ±0.6	11.1 ±0.8	14.4 ±0.8	15.6 ±0.5	**
Total number of inflorescences produced per plant	18.5 ±3.2	17.8 ±1.7	43.2 ±1.8	134.1 ±13.9	**	10.0 ±2.0	26.3 ±2.4	49.5 ±4.7	53.7 ±4.9	**
Lifespan of plant (days from planting)	132.3 ±1.8	165.5 ±3.3	203.3 ±1.6	229.1 ±2.6	**	173.3 ±0.9	272.1 ±2.5	266.5 ±1.1	314.7 ±6.1	**
Flowering period (days)	69.3 ±3.8	69.6 ±5.3	82.1 ±0.8	135.4 ±3.6	**	47.9 ±12.5	64.9 ±2.5	147.2 ±1.8	114.6 ±2.6	**
Diameter of apical inflorescence at anthesis (mm)	31.2 ±1.5	32.0 ±1.6	35.9 ±1.3	34.9 ±1.5	n.s.	29.2 ±1.1	37.0 ±2.0	43.3 ±1.7	37.8 ±0.9	**
Plant dry weight at 84 days from planting (g)	5.8 ±0.3	11.3 ±0.9	9.5 ±0.3	14.3 ±0.6	**	0.80 ±0.1	6.9 ±0.2	6.4 ±0.5	13.2 ±1.8	**

** $P < 0.01$; n.s., not significant.

Floral abnormalities including a thickened peduncle with increased numbers of bract scales, and abnormal growth patterns were observed in *Helipterum roseum* only. The time to macroscopic initiation was reduced in both species when grown during the longest days of the year (planting date 1) and periods of increasing daylength (planting dates 3 and 4) in both outdoor and glasshouse environments. The only exception was *Helichrysum bracteatum* planting date 3 in the glasshouse, where the time to macroscopic appearance of inflorescence buds was retarded (Tables 2, 3). There were significant effects of planting date on the majority of characters measured in both species, under both environments. An exception was the total number of flowers produced per plant of *Helipterum roseum* which did not vary in the outdoor environment, but was very low following planting date 4 in the glasshouse. In contrast, planting date 4 produced the highest number of inflorescences recorded for *Helichrysum bracteatum* in the glasshouse. The diameter of the apical inflorescence did not vary in the glasshouse environment, but the outdoor environment produced very small blooms of *Helichrysum bracteatum* following planting date 1 and very large blooms of *Helipterum roseum* following planting dates 2 and 3. The highest incidence of floral abnormalities in *Helipterum roseum* followed planting date 4 in the glasshouse.

In both species and both environments most inflorescences were produced between the months of September and March irrespective of planting date (Figs 1, 2). The only exceptions were *Helipterum roseum* at planting date 1 in the glasshouse (Fig. 1b) and *Helichrysum bracteatum* at planting date 1 in both environments (Fig. 2) where peak production occurred between

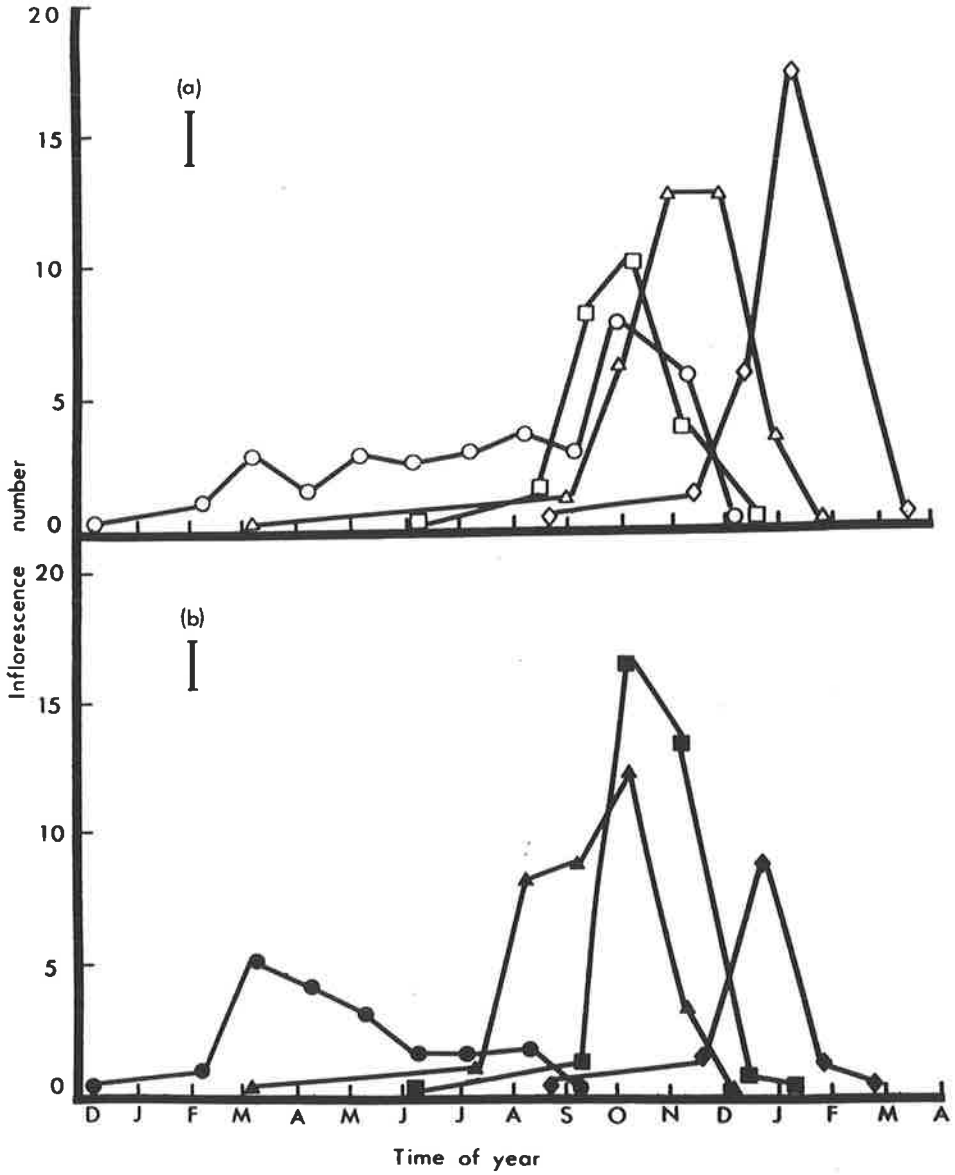


Figure 6.1

Mean number of inflorescences per plant of *Helipterum roseum* following four planting dates in (a) outdoor (○1,△2,□3,◇4) and (b) glasshouse (●1,▲2,■3,◆4) environments. The vertical bar indicates the maximum standard error. The first point of each curve corresponds to the date of planting and the second to the appearance of the apical (first) bloom.

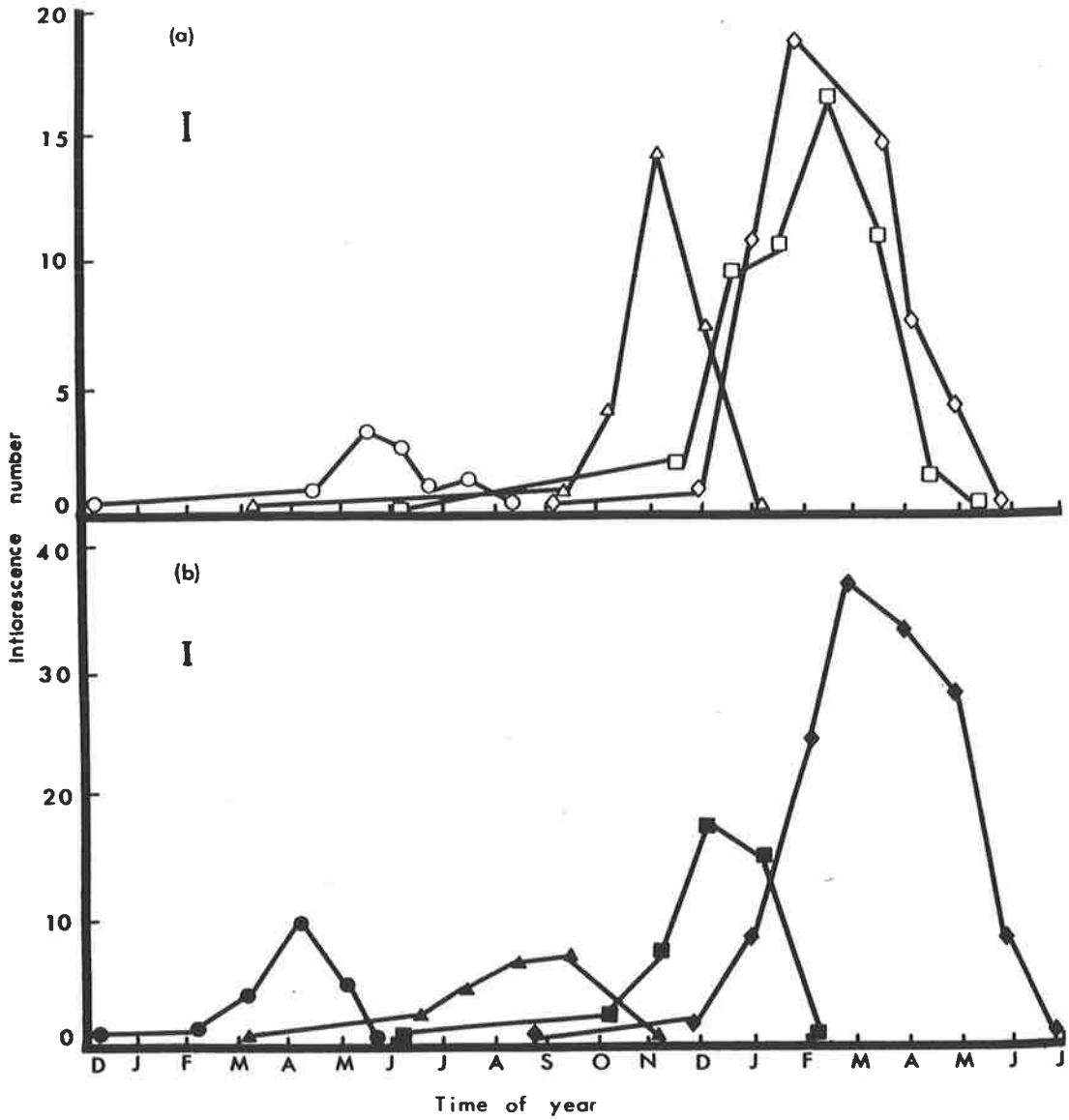


Figure 6.2

Mean number of inflorescences per plant of *Helichrysum bracteatum* following four planting dates in (a) outdoor (○1, △2, □3, ◇4) and (b) glasshouse (●1, ▲2, ■3, ◆4) environments. The vertical bar indicates the maximum standard error. The first point of each curve corresponds to the date of planting and the second to the appearance of the apical (first) bloom.

March and June. In these cases, fewer flowers were produced than in those plants flowering during September to March.

The diameter of axillary inflorescences produced sequentially after the apical inflorescence by both species in the outdoor environment declined with the exception of planting date 1 (Figs 3a, 4a). The reduction in inflorescence diameter of *Helipterum roseum* grown in the outdoor environment was accompanied by significant reductions in the length of the involucral bracts and the number of florets per capitulum (Table 4). In the glasshouse environment, a decline in inflorescence diameter also occurred in *Helipterum roseum* planted at date 3 and in *Helichrysum bracteatum* planted at dates 3 and 4 (Figs 3b, 4b). Stem length of the axillary inflorescences of *Helipterum roseum* decreased with time in all treatments except planting date 1 (Fig. 3). Stems of individual blooms of *Helichrysum bracteatum* were very short due to the high insertion of the lateral branches on the main stem (Fig. 3.47). Stem length in this species did not vary consistently with environment or planting date (Fig. 4).

6.3.2 Experiment 2

Vase-life of *Helipterum roseum* and *Helichrysum bracteatum* was increased by 17 and nine days respectively when blooms were held continuously in 2 % sucrose solution compared with distilled water, (Table 5). Pulsing solutions of 5 % sucrose also extended vase-life in *Helipterum roseum* over that in distilled water, but was not effective in *Helichrysum bracteatum*. The majority of inflorescence buds on sprays of *Helichrysum brateatum* opened during the treatment period. Only the smallest buds failed to

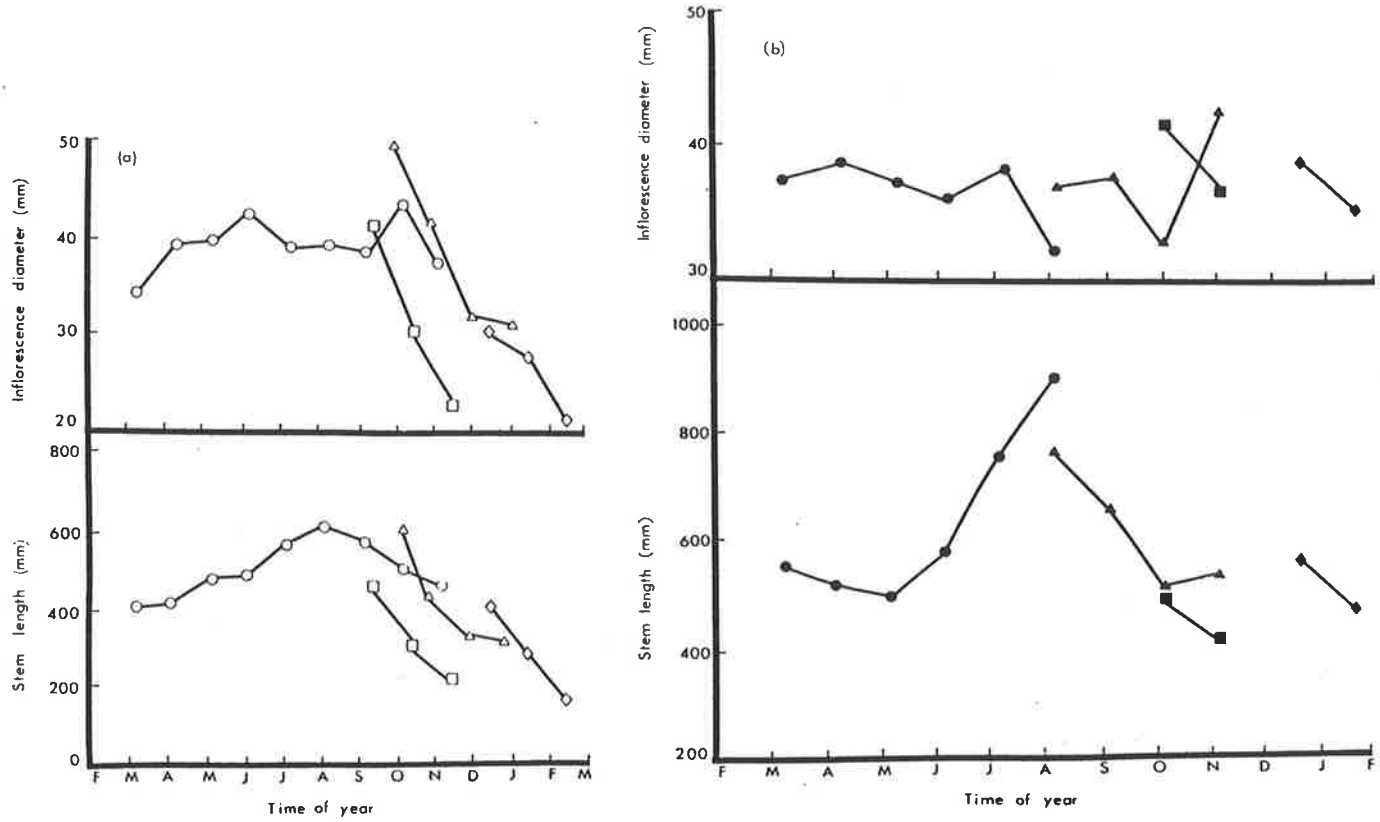


Figure 6.3

Mean inflorescence diameter and mean stem length of *Helipterum roseum* following four planting dates in (a) outdoor (○1, △2, □3, ◇4) and (b) glasshouse (●1, ▲2, ■3, ◆4) environments. Significant differences in mean inflorescence diameter and mean stem length over time ($P<0.01$) were recorded in all treatments except inflorescence diameter at planting date 4 in the glasshouse, which was not significant.

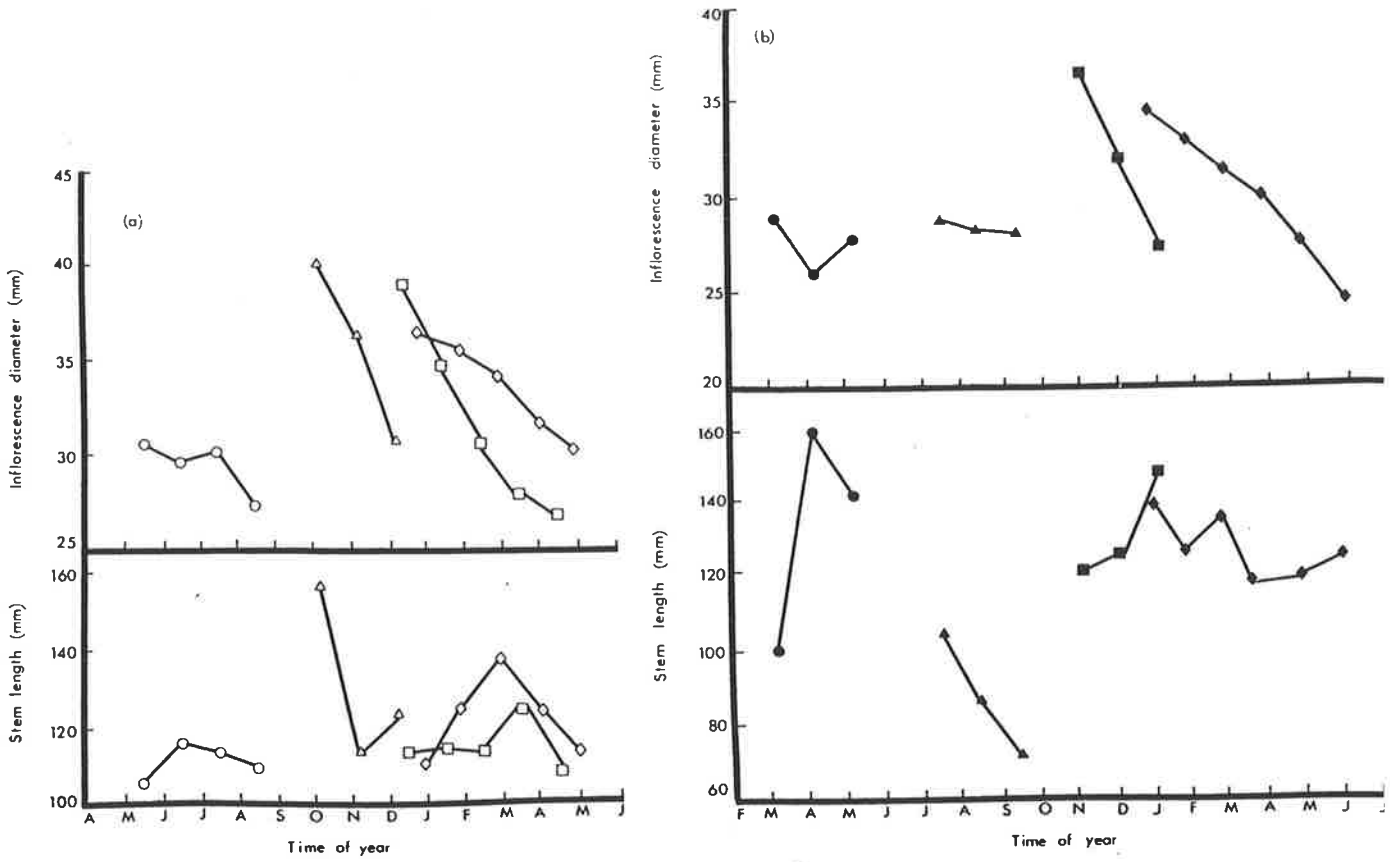


Figure 6.4

Mean inflorescence diameter and mean stem length of *Helichrysum bracteatum* following four planting dates in (a) outdoor (○1, △2, □3, ◇4) and (b) glasshouse (●1, ▲2, ■3, ◆4) environments. Significant differences in mean inflorescence diameter over time ($P<0.01$) were observed in all treatments except planting date 1 outdoors and planting date 2 in the glasshouse, which were not significant. Significant differences in mean stem length over time ($P<0.01$) were recorded for all treatments except planting dates 1 and 3 in the glasshouse and outdoors respectively ($P<0.05$) and planting date 1 outdoors (not significant).

Table 6.4 Effect of time from anthesis of the apical inflorescence on size of axillary inflorescences of *Helipterum roseum*

Values presented are means and standard errors of three replicates

	Days from anthesis of the apical inflorescence						Level of signif-icance
	1	21	42	49	63	70	
Inflorescence diameter (mm)	32.5 ±1.0	30.2 ±1.3	28.2 ±2.0	21.8 ±2.0	21.5 ±1.0	17.5 ±1.3	**
Length of longest involucral bract (mm)	12.6 ±0.4	12.8 ±0.8	11.0 ±0.8	8.2 ±1.1	8.2 ±0.6	6.7 ±0.5	**
Receptacle diameter (mm)	4.3 ±0.5	3.3 ±0.1	3.5 ±0.3	3.3 ±0.4	3.2 ±0.5	3.0 ±0.0	n.s.
Number of florets per capitulum	266.7 ±36.0	120.3 ±3.0	111.0 ±20.7	68.0 ±12.7	68.0 ±13.3	41.7 ±15.6	**

** $P<0.01$; n.s., not significant

Table 6.5 Effect of post-harvest treatment solutions on vase-life (days from harvest) of *Helipterum roseum* and *Helichrysum bracteatum* blooms

Values presented are means of 10 replicates. Means followed by different letters (a,b,c) are significantly different ($P<0.001$)

Species	Treatment solution		
	Distilled water continuous	2 % sucrose continuous	5 % sucrose pulse
Helipterum roseum	17.4 a	34.0 b	22.0 c
Helichrysum bracteatum	11.3 a	20.4 b	11.6 a

open but these still contributed to the overall floral display since yellow involucral bracts were visible.

6.4 Discussion

Blooms of *Helipterum roseum* and *Helichrysum bracteatum* were produced throughout the year by planting at three-monthly intervals, but peak flowering occurred during spring and summer irrespective of planting date. This response is typical of quantitative long day plants (Bernier *et al.* 1981a) and supports the findings reported in Chapter 4 using controlled environment growth cabinets. Floral initiation will occur under any daylength but is accelerated under conditions of increasing daylength. Very few blooms are produced following planting in December and March, until the daylength starts to increase in June. Under natural light conditions the grower is therefore limited to a six-month period where bloom production in reasonable numbers is possible. Within this period the choice of planting time and environment will depend largely upon considerations of the inflorescence number and quality required combined with the time at which peak production is desired.

Blooms must be of a high standard if they are to be accepted in the market place and strict quality criteria regulating the size and floral characteristics of blooms have been established for some crops such as *Chrysanthemum* (McKay 1979). The abnormal blooms observed in *Helipterum roseum* are unsuitable for sale and were most prevalent in treatments planted during the warmest months. Thus economic production of *Helipterum roseum* is effectively restricted to planting during

the cooler months.

The blooms of both species are traditionally dried on wires to give extra length and support to the inflorescence and to provide a pliable stem for the construction of posies (Hillier and Hilton 1986). Both species would also be suitable as cut-flowers. Inflorescences of *Helipterum roseum* could be marketed fresh on their own stems as single blooms. The short stem of *Helichrysum bracteatum* would preclude marketing single blooms although it may be possible to market a fresh, flowering stem or spray. Chrysanthemum and carnation are two flower crops which are successfully produced and marketed as sprays (McKay 1979, Salinger 1987). Each spray is comprised of an apical inflorescence at anthesis and several developing inflorescences on the lateral branches. Fresh cut-flowers of *Helipterum roseum* and fresh sprays of *Helichrysum bracteatum* have a relatively good vase-life in distilled water of 17 and 11 days respectively and this can be further extended by one to two weeks by holding the blooms in 2 % sucrose solution. The possibility exists to further extend vase-life by post-harvest treatment with STS (silver thiosulphate) which inhibits ethylene production. STS successfully extends the vase-life of carnation, sweet pea, snapdragons and stock (Faragher 1986).

At present no quality criteria have been established by the market place for fresh or dried blooms of these two species. The following parameters are proposed for cut-flowers of these species, although they are speculative at this time: a bloom diameter of 30 mm and a stem length of 400 mm for *Helipterum roseum*, amd a bloom diameter of 30 mm for *Helichrysum bracteatum*. Smaller blooms of both species would be acceptable

for drying and wiring, and in this case stem length is not important.

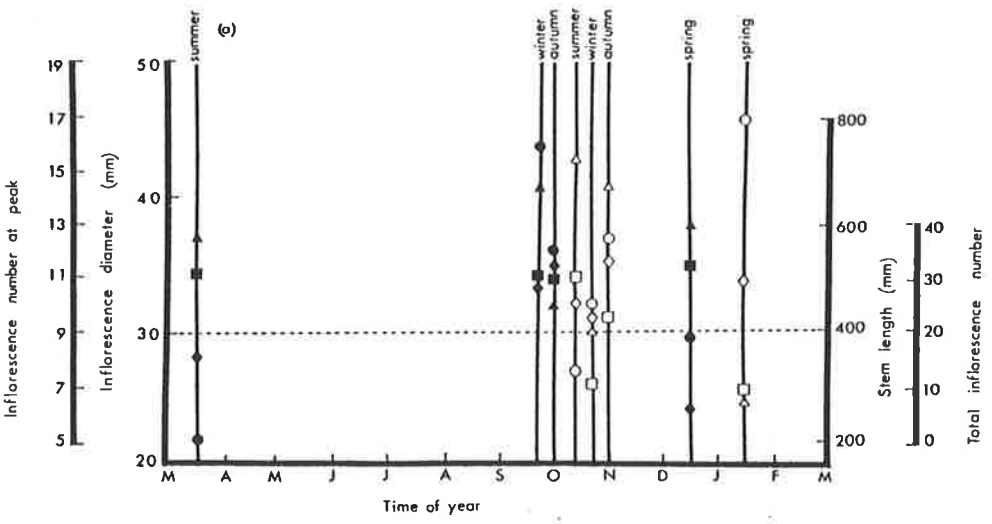
The number of high quality blooms produced per plant at peak production, and total inflorescence number produced during the life of the plant are also important considerations. A peak of 15 and a total of 40 blooms of *Helichrysum bracteatum*, and a peak of nine and a total of 18 blooms of *Helipterum roseum* could be expected, based on the present investigation. Using these criteria, it is clear that prolonged production of fresh cut-flowers is not possible in some circumstances due to the decline in bloom diameter and stem length with plant age. Thus, frequent planting is desirable both to target peak periods of demand such as Christmas and Mothers' Day and to ensure continued production.

All of the criteria for fresh cut-flower production are fulfilled when *Helipterum roseum* is planted during the winter in the glasshouse, and during the autumn outdoors (Fig. 5). Sufficient numbers of high quality blooms are also produced with autumn plantings in the glasshouse but planting at this time may be prohibited by the high cost of production incurred during the extended growing season. Blooms which would be unsuitable for the cut-flower trade due to reduced stem length, but suitable for drying can be produced economically outdoors by planting during winter and spring. A planting schedule producing both fresh and dried flowers would provide peaks in bloom production in October, November and January (Fig. 5, Table 6).

Production levels and bloom quality of *Helichrysum bracteatum* which comply with these criteria are achieved with

Figure 6.5

Synthesis of flowering data for (a) *Helipterum roseum* and (b) *Helichrysum bracteatum* at four planting times; spring, summer, autumn and winter, in outdoor (open symbols) and glasshouse (closed symbols) environments. Symbols represent mean inflorescence number per plant (○, ●), mean inflorescence diameter (△, ▲) and mean stem length (□,■) at peak production, and mean total number of inflorescences produced per plant over the whole flowering period (◇,◆). The horizontal dotted line represents proposed minimum quality criteria for all characters.


(a)
summer
winter
autumn
summer
winter
autumn
spring
spring
Inflorescence number at peak
Inflorescence diameter (mm)
Stem length (mm)
Total inflorescence number
Time of year
M A M J J A S O N D J F M

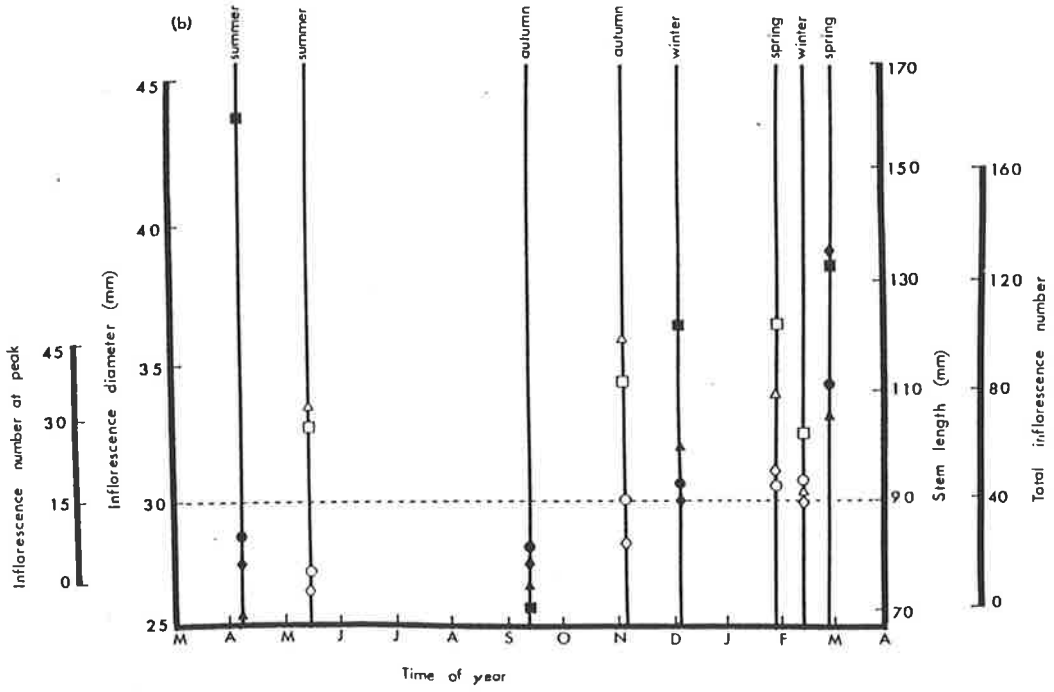

(b)
summer
summer
autumn
autumn
winter
spring
winter
spring
Inflorescence number at peak
Inflorescence diameter (mm)
Stem length (mm)
Total inflorescence number
Time of year
M A M J J A S O N D J F M A

Table 6.6 Planting times for peak production of fresh and dried blooms of *Helipterum roseum* and *Helichrysum bracteatum* at Glen Osmond, South Australia

Peak production	Planting time	Environment	Product
		Helipterum roseum	
October	winter	glasshouse	fresh cut-flower
October	winter	outdoor	dried
November	autumn	outdoor	fresh cut-flower
January	spring	outdoor	dried
		Helichrysum bracteatum	
December	winter	glasshouse	dried
February	winter	outdoor	dried
February	spring	outdoor	dried
March	spring	glasshouse	dried

winter and spring plantings in both environments (Fig. 5). Peaks in bloom production could be expected from December to March using this planting schedule (Fig. 5, Table 6).

To ensure a continuous supply of quality blooms during the production period it may be possible to promote sequential peaks in production by weekly plantings during the recommended seasons, thus filling any existing gaps in production and extending the flowering season. Additionally, work reported in Chapter 4 has shown that floral initiation in both species is promoted by a night-break treatment, the inclusion of one hour of low intensity incandescent light in the middle of the dark period following a short day. It may therefore be possible to produce peaks in production from March to September in *Helipterum roseum* and March to November in *Helichrysum bracteatum* by either imposing a night-break treatment, or artificially extending the daylength with supplementary light following summer and autumn plantings when the daylength is decreasing. These treatments could be implemented in either the glasshouse or outdoor environments. Further research is needed to determine the optimum timing, length and intensity of light break treatments, and also to determine the effect of continuous supplementary light on these species. The findings outlined in this chapter relate to seed collected from the wild and grown in 15 cm pots. The use of genetically improved seed and the implementation of row cropping may influence some of the details of the responses observed, but the results provide basic information for floral production in these species.

This work has shown that high quality blooms of *Helipterum roseum* and *Helichrysum bracteatum* can be produced

commercially under southern Australian conditions. A grower could supply the market with both fresh and dried blooms over a six-month period under ambient conditions and could extend the season with artificial daylength control. Conditions for the extension of vase-life have also been determined. There is now the potential for large-scale commercial production of Australian daisies for the fresh cut-flower market.

CHAPTER 7. GENERAL DISCUSSION

There have been two major themes to this study. Firstly cultural information has been obtained such that commercial cut-flower production of *Helipterum roseum* and *Helichrysum bracteatum* is now possible. Secondly existing knowledge on the physiological control of flowering in these two species has been extended, and the results have implications not only in these species but in flowering plants in general.

The successful exploitation of Australian wild-flowers as new cut-flower crops is dependent upon the development of cultural and management practices in all areas of production from propagation to post-harvest handling. Extensive research has been completed on a number of Australian wild-flowers destined for the cut-flower market eg. Sturts' Desert Pea (*Clianthus formosus*), Geraldton wax-flower (*Chamelaucium uncinatum*) and Kangaroo paw (*Anigozanthus* spp.) (Barth 1989, Lamont 1989, Manning *et al.* 1989). This study has developed two Australian paper daisies, *Helipterum roseum* and *Helichrysum bracteatum*, collected from wild populations, to the point where they too may be exploited as cut-flower crops. The breeding system, seed production, germination requirements, planting schedules for year-round production of top quality blooms, and post-harvest handling procedures for maximum vase-life have all been determined. It is proposed that fresh single blooms of *Helipterum roseum* and fresh sprays of *Helichrysum bracteatum* could be valuable new cut-flower products. The exploitation of many Australian wild-flowers including *Helipterum* and *Helichrysum* spp. is presently hampered by a lack of imagination on the part of flower wholesalers and retailers, with many blooms still being

dried and often wired before sale. Whilst this market is likely to continue it is important to diversify into new sectors. The cut-flower trade is essentially a fashion industry, and it is therefore necessary to constantly introduce novelty items to maintain consumer interest. The promotion of Australian wild-flowers such as *Helipterum roseum* and *Helichrysum bracteatum* as fresh cut-flowers will provide a valuable new alternative in a competitive market.

Seven stages in morphological development of the apical meristem, from the vegetative to the reproductive state, have been defined for both *Helipterum roseum* and *Helichrysum bracteatum* (Chapter 3). It is therefore possible to monitor the progress of these species from germination to anthesis, with very few apical samples. Similar morphological scales have been used to determine the best growing conditions for *Chrysanthemum* (Cathey 1954, Cockshull and Hughes 1971, 1972) and *Campanula carpatica* cv. 'Kark Forster' (Kristiansen 1988) by monitoring the effect of environmental influences such as irradiance and temperature on floral initiation and development. Sophisticated mathematical models for predicting short and long-term crop growth and development have also been developed for *Senecio* using morphological scales (Larsen 1988). The opportunity exists therefore to further extend present research on flowering in *Helipterum roseum* and *Helichrysum bracteatum*, using the morphological scale as a tool, to establish the best growing conditions for flower production and to predict crop development in commercial growing conditions. For example, further work is required to establish whether the quantitative long day response observed in both species (Chapters 4, 6) may be exploited by

using continuous supplementary light to promote flowering, whilst maintaining flower numbers.

Controlled environment studies have shown that floral initiation in *Helipterum roseum* was inhibited at a constant temperature of 25 °C with a photoperiod of 12 h and light intensity of 250 W m^{-2}. That is, the quantitative long day response observed in *Helipterum roseum* appeared to be lost in constant high temperature (25 °C) conditions. Temperature is known to interact with photoperiod such that high temperatures are sufficient in some species to alter the photoperiodic response. For example in the SDP *Hyoscymus* the critical daylength increases from 8.5 to 11.5 h when night temperatures increase from 15.5 to 28.5 °C (Lang 1965). Longer daylengths are therefore required to promote floral initiation at higher temperatures in this species. It is possible that the photoperiodic response of *Helipterum roseum* is also altered at higher temperatures and that daylengths longer or shorter than the 12 h may promote floral initiation at 25 °C. There is some evidence for this, as results from seasonal plantings in the glasshouse and outdoors indicated that inflorescences of *Helipterum roseum* were produced at daylengths of 10 to 14 h duration coupled with temperatures which fluctuated above and below 25 °C. Further work is thus required to determine the nature of the photoperiod/temperature interaction in this species.

A considerable amount of research has been directed towards understanding the physiological controls of flowering, and yet we are still far from understanding the basic internal mechanisms that underlie the floral transition (Bernier *et al.* 1981b). It is generally considered that there is a decisive step

in the development of a young primordium or bud before which it is capable of vegetative or floral development and after which it seems inexorably committed to produce a flower or inflorescence (Bernier 1971). The "point of no return" or stage of commitment to flowering marks the end of evocation during the transition of the meristem. A multitude of morphological and cytological evocational events have been reported in the literature and several of these are observed in *Helipterum roseum* and *Helichrysum bracteatum*; rapid internode elongation or "bolting" (*Helichrysum bracteatum* only), change from production of large vegetative leaves to smaller sessile scarious bracts, enlargement and doming of the apical meristem, reduced cell-cycle duration, and partial synchronisation of cell division. These events are not however considered essential to flowering in other species, since it is possible to inhibit some of them without inhibiting flowering (Lyndon and Francis 1984), and evocational events may also occur in the absence of floral initiation eg. in intermediate meristems, (Lance 1957). This suggests that there is no single evocational event which can set in motion all other events and that the events observed in *Helipterum roseum* and *Helichrysum bracteatum* may be necessary but not determining components of floral evocation.

Inhibition of floral initiation at high temperature in *Helipterum roseum* has, in conjunction with the development of a well-defined morphological scale, provided an ideal opportunity to investigate some of the physiological and cytological events leading to the floral transition, and enabled the identification of the stage of commitment to flowering in this species. It is concluded that in shoot apical meristems of *Helipterum roseum*

some evocational events are in train at stage 2 (large vegetative meristem) and that developmental pathways at stage 3 (doming of meristem) are still optional ie. meristems are partially evoked, whilst stage 4 meristems are committed to the formation of an inflorescence ie. the evocational process is complete. This is supported by the following observations: (1) studies on cell-cycling in the shoot apical meristem have shown that steady-state cell-cycling is only observed in meristems which proceed to flowering and is present as early as stage 2; (2) reversions to vegetative growth are observed after the production of a few bract scales but not after the appearance of involucral bracts with coloured papery laminae; (3) floral development in the majority of shoot apical meristems transferred from 20 to 25 °C conditions at stage 3, is arrested, whilst development is delayed for a short period only, when meristems are transferred at stage 4.

Apices grown at a constant temperature of 25°C do not show steady-state cell-cycling and do not flower, whereas those grown at 20 °C have a well-defined cell-cycle and produce an inflorescence. Thus, it is possible that steady-state cell-cycling may be an evocational event. It is not possible to establish from this study whether steady-state cell-cycling in *Helipterum roseum* is a pre-existing phenomenon which is disrupted, by high temperature, or whether it is an evocational event which develops in the stage 2 meristem. It appears most likely that it is a pre-existing phenomenon, since steady-state cell-cycling is also observed in vegetative meristems (held in non-inductive conditions) of the absolute LDP *Sinapis* (Gonthier *et al.* 1985). It is not, however, a definitive evocational event

since shoot apical meristems of *Sinapis* held in non-inductive photoperiodic conditions do not flower (Gonthier *et al.* 1985) and apical development of *Helipterum roseum* is arrested at stage 3 when the meristems are transferred to high temperature conditions. It would be of interest to establish whether steady-state cell-cycling is lost in meristems of *Helipterum roseum* transferred from 20 to 25 °C at stage 3 and also whether stage 1 meristems at 20 °C exhibit the phenomenon.

An increase in the growth rate at the shoot apical meristem is observed during the floral transition and is often associated with a synchronisation of cell division (Francis 1987). Natural synchrony is not however, a common phenomenon in either animal or plant tissue (Yeoman and Aitchison 1976). Why then do shoot apical cells show a high degree of synchronisation during evocation? Bernier (1971) suggested that mitosis could be a mechanism whereby a cell destroys its nuclear history after which it proceeds in a new direction of differentiation. It is also possible that a sufficient proportion of the cell population may be required in a critical phase of the cycle because only cells in that phase could react properly to floral promoters or produce a substance needed for evocation (Bernier 1988, Francis 1987). The fact that the duration of the cell-cycle varies within a cell population suggests that there are internal controls on the progression of cells through the cycle (Yeoman and Aitchison 1976). Van't Hof *et al.* (1973) proposed that there were two possible control points; one in G1 which determined whether a cell progressed to S, and one in G2 which determined whether it proceeded to M. The synchronisation of cell division may provide a mechanism by which shoot apical cells could become more

organised, in preparation for the more complex events of organ initiation and differentiation which accompanies the floral transition.

There are currently four theories proposed to explain the endogenous control of flowering: (1) the florigen/antiflorigen concept; (2) electrical signals; (3) the nutrient diversion hypothesis with control by assimilates; (4) the model of multifactorial control, generally involving participation of plant growth regulators (Bernier 1988). These theories will be discussed in light of the findings of this research involving *Helichrysum bracteatum* and *Helipterum roseum*.

The florigen/antiflorigen concept proposes that floral evocation proceeds when the balance of florigen (promotor) and antiflorigen (inhibitor) shifts in favour of the promotor, at the meristem. Further to this, it is postulated that these substances are produced in the leaves. In this study the proportion of cells conforming to the steady-state system increases during the floral transition. It is possible that more cells become synchronised as more florigen arrives in the apex. The inhibition of floral initiation at 25 °C could be due to the arrival of greater proportions of the inhibitor (antiflorigen) in the apex, alternatively, the promotor (florigen) may not be translocated or even produced. There is however, no evidence to support the existence of either florigen or antiflorigen and after decades of extensive work the chemical structure of these hypothetical hormones is still unknown.

The electrical signal theory was proposed to account for the fact that stimuli eg. light, applied to one part of a plant

could have an effect in other areas, more rapidly than it would be possible for a chemical to be transported through the symplast or the phloem. In this study it is possible that high temperature (25 °C) is affecting the transfer of signals to the apical meristem and therefore inhibiting flowering. Once again no transmissible signal has been detected and there is therefore, no direct evidence to support the electrical signal concept.

The nutrient diversion hypothesis with control by assimilates postulates that the source/sink relationship is altered in inductive conditions such that the shoot apex receives a higher concentration of assimilates than under non-inductive conditions. It is possible that assimilates are directed towards vegetative growth rather than reproductive growth in high temperature (25°C) conditions in *Helipterum roseum*. This may explain why shoot apical meristems of *Helipterum roseum* at 25°C continue to produce leaves, and why reversions to vegetative growth after the production of a few bract scales are observed.

Evidence from this research lends most support to the multifactorial control of flowering, particularly in the shoot apical meristem of *Helipterum roseum*. This is supported by the fact that the numerous evocational events observed in this study; bolting, change from production of leaves to bracts, enlargement and doming of the apical meristem, reduced cell-cycle duration, and partial synchronisation of cell division, can be considered essential but not definitive evocational processes. It is this complexity of the physiological processes comprising the floral transition which has, almost certainly, hampered the achievement of the understanding of the floral event. This, and other recent research, has emphasised this complexity, and has demonstrated

the importance of further subdividing the floral event into its component physiological processes. This is the approach which will lead in future to the understanding, and subsequent exploitation of the floral transition.

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CUT FLOWER PRODUCTION OF AUSTRALIAN DAISIES, *Helichrysum bracteatum* AND *Helipterum roseum*.

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INTRODUCTION

Both *Helipterum roseum* and *Helichrysum bracteatum* are native annual species. Both are used to a limited extent in the cut flower trade where the blooms are traditionally placed on wires and dried.

Helipterum roseum has single blooms on long stems. The involucral bracts are generally magenta in colour but occasionally white or rose. These blooms would also be attractive marketed as a fresh cut flower. The bloom is an inflorescence. The individual florets arise on the surface of the receptacle (yellow area) and are surrounded by the infertile involucral bracts. These involucral bracts are papery in texture and hence the blooms are often referred to as paper daisies.

Helichrysum bracteatum forms a long apical stem with several lateral branches arising from it. The blooms are at the tips of short shoots. The shortness of these shoots has been one reason for the traditional method of wiring in this species. There is also the possibility of harvesting a flowering stem bearing an apical bloom at anthesis and several developing blooms as a spray, similar to spray carnations. The bloom is also an inflorescence comprised of many florets surrounded by colourful, yellow involucral bracts.

The aim of this work was to obtain a greater understanding of the flowering process in these two species, to relate this to the environmental control of flowering such as temperature and length of day and finally to investigate the effect of commercial growing conditions; glasshouse and outdoors, on the production of quality blooms. A synthesis of all the findings will lead to a possible planting schedule for the production of high quality fresh and dried blooms of these species.

1. Floral initiation and development

The transition of the apical meristem was studied from planting through to anthesis. Plants of both species were grown in the glasshouse at the Waite Institute. Apical meristems were dissected at daily intervals and viewed with a scanning electron microscope. The development of the apical meristem was similar in both species and occurred over a brief period of 2-3 months. Seven stages of morphological development were identified.

Stage 1. A small vegetative meristem with two leaves.

Stage 2. The meristem is larger but still vegetative ie. producing leaves.

Stage 3. The meristem has become domed. This is considered to be the point of floral transition, when the apex stops producing leaves and begins to produce the floral parts of the bloom.

Stage 4. The initiation of involucral bracts has commenced. These become the coloured bracts of the bloom.

Stage 5. Florets are initiated. Both involucral bracts and florets are present at this stage.

Stage 6. Flower buds are now visible to the naked eye.

Stage 7. Anthesis of the inflorescence. The involucral bracts reflex and surround the capitulum covered with florets.

2. Control of flowering

It is well documented that the flowering response in some plants is triggered by such factors as the length of day and temperature. A well known example is the Chrysanthemum which is a quantitative short day plant. The Chrysanthemum can be forced to flower by providing short daylength conditions. This knowledge is exploited in the cut flower and flowering pot plant trades where Chrysanthemums are forced to flower at particular peak periods for example Mothers' Day, by strictly controlling the daylength.

Effect of daylength.

Controlled environment growth cabinets were used to test the effect of varying daylengths on flowering. It is possible to strictly control the environment in these cabinets. 8, 12, 16 and 20 hours of light in a 24 hour cycle were chosen, and the plants grown at a constant temperature of 20 oC. The time to flowering in days from planting and the total number of blooms produced per plant were recorded in each condition. Results were similar for both species. Flowering occurred sooner at longer daylengths, but the most blooms were recorded at short

daylengths. The ideal situation for bloom production would therefore be a compromise between time to flowering and bloom number. This response to daylength is typical of long day plants.

In physiological terms the plant is not responding to the long day but to the short night. Therefore if short night conditions are provided artificially the plants should flower sooner. This can be done by interrupting the middle of a long dark period by light or by providing continuous supplementary light. Interrupting the long night with a short period of light (1 hour of incandescent light) promoted flowering with both *Helipterum roseum* and *Helichrysum bracteatum*. Flowering occurred sooner when the long night was interrupted by light (night break) than when 8 hours of light was followed by a long uninterrupted period of darkness. Therefore in winter when the nights are very long, it is possible to interrupt the night with light to promote flowering.

Effect of temperature.

Experiments were conducted in controlled environment growth cabinets. A constant day length of 12 hours was chosen and three constant temperature conditions imposed, 15, 20 and 25 OC. The time to flowering in days from planting and the total number of blooms produced per plant were recorded. Floral initiation did not take place at 25 oC in *Helipterum roseum*. Production of this species in the summer months would therefore be difficult. The greatest number of blooms were produced at 15 oC in Helipterum roseum and at 20 oC in *Helichrysum bracteatum*.

3. Cut flower production in commercial growing conditions

Two commercial growing conditions were investigated; glasshouse and outdoors at the Waite Agricultural Research Institute. Seedlings of each species were planted into pots at four planting times which coincided with the commencement of the four seasons, December, March, June and September.

The effect of plant age on the number of blooms produced per plant, bloom diameter and stem length were investigated for each planting time. 3,4 and 5. The trends were essentially similar for both species in both environments.

Peaks in bloom production occurred during the months September to March irrespective of the time of planting. Some blooms were produced during the late summer and autumn months but the number was minimal. This is a typical long day response, with plants waiting until the longer days of spring and summer to flower.

The diameter of the blooms decreased with time irrespective of planting time. It is proposed that a bloom diameter of no less than 30 mm would be acceptable for a cut flower, and this means that some treatments produce blooms of lesser quality, eg; planting in September with blooms produced in summer. These smaller blooms would be suitable for drying on wires. Sequential planting would be required to provide continuous production of high quality blooms.

Stem length generally decreased with time irrespective of the planting time. An acceptable stem length for fresh cut flowers is 400 mm. Some treatments produced blooms on short

stems eg planting in September. This would not be a problem if the blooms were dried.

The maximum number of blooms of *Helipterum roseum* were obtained in the glasshouse following planting in March and June. In *Helichrysum bracteatum* the maximum number of blooms were produced in the glasshouse following planting in June and September. Planting during the warmer months December and September produced the most abnormal blooms in *Helipterum roseum*. These blooms had thickened peduncles and an increased number of bract scales and would not be suitable for sale. No abnormalities were recorded in *Helichrysum bracteatum*.

Summary

1. The flowering transition is rapid.

Taking place in 2 to 3 months. Therefore careful management from day 1 is essential.

2. Daylength control is possible.

Both species are long day plants. Flowering can be promoted during the winter months, when the days are short by extending the daylength or with a night break treatment. Blooms can be produced to target key market periods eg. Mothers Day.

3. High temperature inhibits flowering in *Helipterum roseum*.

Temperature control during the summer months would be essential to produce high quality blooms.

4. Under natural light conditions plants will flower during September to March irrespective of planting date.

This is a long day response as the plant waits until the longer days of the year before flowering.

5. Bloom diameter and stem length decrease with time.

Sequential planting would therefore provide a continued production of high quality blooms.

Considering all of these factors it is now possible to provide a planting schedule for the production of high quality fresh and dried blooms of these two species.

Fresh and dried blooms of *Helipterum roseum* may be harvested in October by planting in the glasshouse and outdoors respectively during winter. Fresh blooms may also be obtained in November by planting outside during the autumn months. Planting during the spring outdoors will produce blooms acceptable for drying during January.

Dried blooms of *Helichrysum bracteatum* can be successfully produced from December through to March by planting during winter and spring in both glasshouse and outside environments. It may also be possible to produce sprays during this period.

The possibility exists to promote flowering in both species during the months of March to November by the use of a night break treatment or the addition of continuous supplementary light.

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